Chemical Composition of Muscles from Hexestrol-Treated Steers

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Portions of *longissimus dorsi* muscles of 16 Hereford cross steers aged $2^1/_2$ years were chemically analyzed. Twelve animals had been implanted (five of them on two different occasions) with hexestrol. The remaining four were used as controls. Implantation caused a reduction in intramuscular fat. The muscles from the doubly implanted animals had a higher moisture content than those of the controls. The difference was significant at the 5% level. There were no significant differences between treatments in total protein, intramuscular collagen, hexosamine hydrochloride, or Warner Bratzler shear values. The correlations between intramuscular fat and shear values, and between hexosamine hydrochloride and shear values, were low and not significant. Collagen contents correlated significantly at the 1% level with shear values. No evidence indicated that variations in shear values (tenderness) could be attributed to preslaughter implantation or that any deterioration in meat quality was caused by this treatment.

Considerable interest has been shown in the use of synthetic sex hormones for promoting increased live weight gains in fattening animals. Nevertheless, Everitt and Carter (*6*) reported that little had been published concerning the effects of implantation on the chemical composition of the musculature. Brownlie, Stockdale, and Gadd (*3*) discussed estrogen-treated animals and stated that "there is some doubt about tenderness, which like carcass grade, appears to be unaltered or somewhat reduced."

In the work presented here the portions of the *longissimus dorsi* muscles at the 11th and 12th ribs from 16 Hereford cross steers [from a hexestrol implantation trial (*4*) in which 32 steers were used] were examined to determine if there were any significant differences in biochemical composition and or tenderness (as measured objectively by the Warner Bratzler shear machine) between the muscles from the four control animals and those from each of the three treated groups (early, early and late, late implantations). The treatments of the 16 animals were as follows:

Group A (4 animals). Untreated (controls).

Group B (4 animals). 45 mg. of hexestrol (150 days before slaughter) designated earlies.

Group C (5 animals). 45 mg. on each of two occasions (150 and 75 days before slaughter) designated doubles.

Group D (3 animals). 45 mg. (75 days before slaughter) designated lates.

Experimental Procedure

At approximately 720 pounds live weight, nine (groups B and C) of the 16 animals were implanted in the ears with 45 mg. of hexestrol [4,4′-(1,2-diethylethylene)diphenol]. Seventy-five days later, five of the previously implanted steers (group C) and three of the animals not previously implanted (group D), were given 45 mg. each. After a further 75 days, all 16 animals were slaughtered (having reached the age of approximately $2^1/_2$ years). Throughout the 5-month experimental period (May to October) all the steers grazed as a single herd.

The day after slaughter the muscle portions were excised, wrapped in aluminum foil, and stored at −23° C. until it was convenient to carry out the analyses (the first muscle analyzed was frozen for 2 weeks and the last one for 11 months).

Methods of Analysis. After removal from the freezer, the muscle portions were allowed to thaw for 6 days at 2° C. The epimysium was removed and discarded and the drip added to the comminuted sample. The uncomminuted half was wrapped in aluminum foil, roasted to an internal temperature of 65° C., and allowed to cool, and 1-inch cores were sheared in the Warner Bratzler shear machine. Moisture, fat, and protein were determined (*8*). Hydroxyproline (a measure of collagen) was determined by the Moehler and Antonacopoulos (*14*) modification of the Neuman and Logan (*15*) technique.

Hexosamine hydrochloride (an index of ground substance) was determined on the stromal residue of the Helander extract by the method of McIntosh (*13*), which is based on the Boas (*2*) modification of the Elson and Morgan (*5*) method. The stromal residues from 15 grams of muscle, after washing with distilled water and defatting with acetone, were hydrolyzed for 4 hours by 12.5 ml. of 4*N* HCl in a stoppered 50-ml. conical flask, in an autoclave at a pressure of 15 p.s.i. After cooling, the hydrolyzate was filtered and made up to 250 ml. with distilled water. This dilution permitted the Dowex 50, X-8 ion exchange columns (used to remove interfering amino acids and sugars) to be loaded in 0.2*N* HCl (*9*). Five-milliliter aliquots of each hydrolyzate were pipetted on to each of six columns. After percolation, the resin was flushed with 10 ml. of distilled water. The hexosamine was now eluted with 2*N* HCl. All the hexosamine was found to be present in the first 5 ml. of eluate. Three milliliters of eluate were used for each determination. To determine the percentage recovery of hexosamine from the resin, 5-ml. aliquots of glucosamine hydrochloride standard containing 10 μg. per ml. in 0.2*N* HCl were also pipetted onto each of another six columns. Six tubes each containing 30 μg. of glucosamine hydrochloride were also run as standards with each determination. After elution the hexosamines were heated at 89° to 92° C. in alkaline solution with recently distilled acetylacetone. The condensation product was treated with Ehrlich's reagent and the extinction of the red color read at 530 mμ.

Sarcoplasmic, myofibrillar, and nonprotein-soluble nitrogens were determined by Lawrie's modification (*12*) of Helander's (*7*) extraction procedure. Fifteen-gram portions of muscle were used.

Results

Table I shows the average percentage composition and Warner Bratzler shear values of the muscles. One muscle portion (from animal 527) in the early group had a fat content (6.43%) greatly in excess of that of any of the other 15 animals. Although the pellets implanted in No. 527 were completely absorbed, it is possible that the effect had worn off in the 5 months that elapsed between implantation and slaughter. In Table

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Table I. Average Per Cent Composition and Warner Bratzler Shear Values of Muscles[a]

Treatment	No. of Animals	Moisture	Fat	Protein N × 6.25	Collagen	Hexosamine Hydrochloride, Mg./100 G.	Shear Values (Pounds) of 1-Inch Cores
				Whole Tissue Basis			
Control	4	73.64 ± 0.41	2.69 ± 0.44	22.27 ± 0.58	0.35 ± 0.07	4.59 ± 1.87	13.2 ± 4.2
Double	5	74.92 ± 0.36	1.67 ± 0.39	22.30 ± 0.52	0.39 ± 0.02	5.79 ± 1.67	12.3 ± 3.8
Late	3	74.13 ± 0.47	2.13 ± 0.51	21.96 ± 0.67	0.39 ± 0.03	3.29 ± 2.16	13.4 ± 4.6
Early	4	73.45 ± 0.66	3.46 ± 1.47	22.23 ± 0.58	0.40 ± 0.07	4.36 ± 1.87	13.8 ± 4.2
Early[b]	3	74.00 ± 0.22	2.48 ± 0.45	22.62 ± 0.71	0.39 ± 0.04	4.60 ± 1.37	...
		Total Nitrogen	Sarcoplasmic Nitrogen	Myofibrillar Nitrogen	Nonprotein-Soluble Nitrogen	Stroma Nitrogen	
				Fat-Free Basis			
Control	4	3.67 ± 0.07	24.15 ± 0.90	52.20 ± 2.67	11.89 ± 0.48	11.76 ± 1.98	
Double	5	3.61 ± 0.07	22.89 ± 1.42	50.17 ± 1.67	11.78 ± 0.91	15.16 ± 1.17	
Late	3	3.63 ± 0.08	24.62 ± 1.83	50.59 ± 2.16	12.21 ± 0.56	12.58 ± 2.12	
Early	4	3.66 ± 0.07	25.61 ± 1.58	51.08 ± 1.87	12.41 ± 0.48	10.90 ± 1.98	
Grand mean[c]		3.64 ± 0.10	24.21 ± 2.17	50.98 ± 3.88	12.04 ± 0.96	12.77 ± 3.68	

[a] Standard errors of treatment means.
[b] Early group with animal 527 excluded.
[c] Standard errors per value of grand mean.

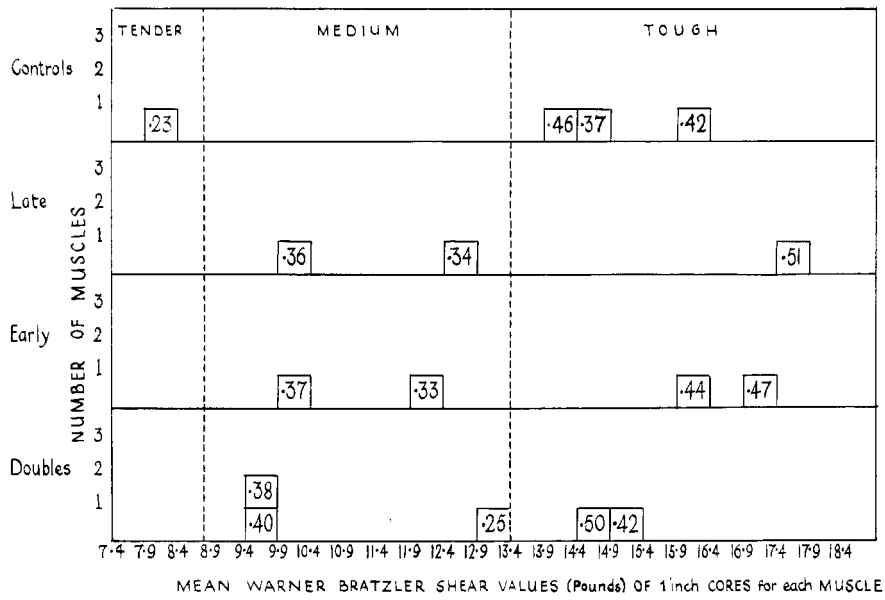

Figure 1. Distribution of shear values

Collagen percentages corresponding to each shear value shown

I, two sets of means are given for the early treatment, one including the muscle from animal 527 and one excluding this muscle.

None of the components showed significant differences between treatments, except moisture. The moisture of the double treatment was significantly greater at the 5% level than that of the control treatment, and also that of the early treatment if animal 527 is excluded.

While differences between treatments in intramuscular fat were not significant, they were nevertheless noteworthy, the average value for the controls (2.69%) being greater than that for the doubles (1.67%), the lates (2.13%), and also the earlies (2.48%), if the muscle from animal 527 is excluded. Within treatments, differences between muscles in intramuscular fat, total protein, and intramuscular collagen were significant at the 0.1% level. Moisture differences between muscles within the control, late, and double treatments were significant at the 0.1% level. Hexosamine hydrochloride values showed differences significant at the 0.1% level between muscles within the control, early, and double groups. Muscles within the late group differed significantly at the 5% level. On a fat-free basis total nitrogen between muscles within groups showed differences significant at the 0.1% level. Fat-free sarcoplasmic values showed significant differences at the 5% level between muscles within the late, early, and double groups. Fat-free nonprotein-soluble nitrogens showed differences significant at the 0.1% level between muscles within the control, late, and early treatments and within the double group differences were significant at the 1% level. Figure 1 shows the distribution of the shear value averages for each muscle in the form of a histogram. There were no significant differences between treatments in shear values, average values for the control, late, early, and double treatments being, respectively, 13.2 ± 4.2, 13.4 ± 4.8, 13.8 ± 4.2, and 12.3 ± 3.8 pounds. Differences between muscles within treatments were significant at the 0.1% level.

Discussion

Curran (*4*) reporting on all of the 32 animals stated that the only obvious side effect in the implanted animals was the development of rudimentary teats. The condition was accentuated to some degree after the second implantation. Over the whole experimental period all the treated groups showed live weight gains significantly higher than the control group, but the differences between the treated groups were not significant. The average daily live weight gains for the 5-month experimental period were 2.45 ± 0.21, 2.92 ± 0.20, 2.79 ± 0.24, and 2.76 ± 0.31 pounds for the control, early, double, and late groups, respectively. The average slaughter weights were 1109 ± 13, 1161 ± 20, 1173 ± 28, and 1144 ± 35 pounds, respectively, for the control, early, double, and late groups. The only significant difference between the carcasses of the implanted animals and those of the controls was in the amount of caul fat, which was significantly higher (at the 1% level) in the control than in the treated groups. Curran (*4*) concluded that in general there is no advantage in reimplanting with hexestrol within 75 days of the original treatment.

Analytical Results for Hexosamine Hydrochloride and Nitrogen Fractionation. The recovery of the glucosamine hydrochloride standards from the Dowex resin was not quantitative and fell within a range of 67.7 to 100% (average 88.7%) for 20 determinations. The average percentage recovery of the glucosamine hydrochloride standards from the six columns was calculated on each occasion and the results for the muscle duplicates were adjusted to 100% recovery. The method of hexosamine determination as carried out in this work would appear to be sensitive to one or more variables other than those mentioned by Boas (*2*)—temperature for condensation with acetylacetone, sodium chloride concentration, and precision of neutralization. The variability of the hexosamine contents from animal to animal was high, as shown by the standard errors. The individual results of the extraction procedures appeared to reflect to some extent denaturation, although the scatter was not nearly so great as in *psoas major* muscles (*11*). The glycolytic rate in beef *longissimus dorsi* is considerably slower than in the *psoas* (*10*), possibly because the *psoas* is more involved in the spontaneous kicking of the hind legs which occurs after death (*1*). This nonaccelerated rate of pH fall in the *longissimus dorsi* is possibly reflected in high extraction yields of the nitrogen fractions. Repeatability in six of the myofibrillar extractions was poor (ranges of 8 to 14% between duplicates being obtained), suggesting perhaps that the extraction technique as carried out here is sensitive to some inherent factor or factors which on occasions become operative and cause reduced extractability.

Values for sarcoplasmic and myofibrillar nitrogens of adult bovine *longissimus dorsi* (at the 4th, 5th, and 6th lumbar vertebrae) are given by Lawrie (*12*) as 25.1 and 51.5%, respectively (averages of 39 muscles). The samples were analyzed 24 hours after slaughter. The corresponding averages for the 16 animals studied here are 24.21 ± 2.17 and 50.98 ± 3.88%. These comparisons suggest that storage at −23°C. (over periods from 2 weeks to 11 months) did not appreciably reduce extractability.

The mean absorptions of the pellets implanted in the 12 animals were 88.3, 99.8, and 58.1% of the weights implanted in the double, early, and late groups, respectively. Regression analysis showed no relationship between the amount of pellet absorbed and the content of intramuscular fat or the contents on a fat-free basis of moisture, protein, collagen, and hexosamine hydrochloride.

According to this work, the only variation in composition of the muscles that can be attributed to hexestrol implantation is the noteworthy, though not significant, trend toward a reduction in intramuscular fat of the treated animals and the significantly greater moisture content of the double-treated muscles when compared to the controls. The trend toward a reduction in intramuscular fat in the muscles from the implanted animals is in agreement with the general leanness superficially observed in the carcasses of implanted animals. The coefficients of correlations for intramuscular fat and hexosamine hydrochloride with the shear values were, respectively, 0.34 and 0.13. Neither coefficient was significant. The correlation coefficient for shear values with intramuscular collagen was 0.68, and was significant at the 1% level. Regression analysis gave the following equation for the relationship between shear values in pounds (Y) and the percentage of intramuscular collagen (X) on a whole tissue basis:

$$Y = 3.34 + 25.09\,X$$

The standard error of the regression coefficient was ±7.19.

It can be concluded from this work (keeping in mind that only two rib portions of *longissimus dorsi* from only 12 implanted and four control animals were examined) that hexestrol treatment tended to cause a reduction in intramuscular fat, which in the case of the double treatment was concomitant with an increase in moisture (significant at the 5% level), and that variations in tenderness (as measured by shear values) cannot be attributed to the preslaughter treatment of implantation.

The investigation did not bring to light any evidence that loss of quality occurred in the muscles from the treated animals.

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