

## Interrelationships of Gross Chemical Components of Pork Muscle

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Loin and ham muscles from 471 pork carcasses representing nine chronological age groups, four weight classifications, and five levels of intramuscular fat were analyzed for gross chemical components. The protein-moisture ratio (P/M) was compared to chronological age, sex, and muscle type. The P/M did not vary among barrow, gilt, and sow muscles, but was consistently lower for ham muscles (0.276) as compared to the *longissimus dorsi* (0.287). From birth to 3½ months, the P/M increased rapidly and then leveled off from 3½ to 6½ months. The ratio remained constant throughout the older age groups. Fat could be accurately estimated from the moisture content of pork muscle, while total protein could be estimated by difference, if chemically determined fat and moisture were known.

THE CONSTANCY of the protein-moisture ratio (P/M) in muscle tissue is of interest when gross compositional changes are the result of an experimental treatment. However, during early and rapid growth, the P/M may change and thus confuse the results. According to Moulton (17), carcass nitrogen content of the pig increased with a concurrent decrease in water during the first 300 days of life, after which the P/M remained constant. The carcass ash content remained constant after the first 75 days; thus, lipid was the primary constituent subject to quantitative change after "cellular maturity." McMeekan (14-16) reported that the P/M in porcine muscles changed up to 196 days. He also showed, as did Kropf (13), that the plane of nutrition affected the P/M during early stages of growth. However, if the nutritive levels were altered after cellular maturity, the P/M did not change. This would substantiate current theories which imply that elevated nutritive levels hasten maturity. Callow's studies (4-6) also indicate that the P/M is constant once the animal reaches physiological maturity. Unpublished data of Kastelic (17) show that mice fed varying levels and ratios of calcium and phosphorus, and ranging in age from 43 to 338 days, had a constant body

P/M of 0.30. Results of Ogilvie *et al.* (18) indicate that administration of stilbestrol to cattle throughout a 168-day fattening period did not change P/M even though protein and moisture increased at the expense of fat. Fat content for various wholesale cuts ranged from 1 to 29%, yet P/M remained constant at 0.29. These investigations also showed no difference in P/M due to sex; however, Dreyer (7) concluded that a significant difference in P/M existed between male and female albino rats. Dreyer also reported an increase of P/M with advancing age, as did Bender and Doell (2).

Assuming that P/M was constant for pork muscle, Karmas *et al.* (10) estimated from moisture analysis the proportion of lean in products composed of at least 60% lean. Bieber *et al.* (3) showed that protein content calculated from moisture content of ground beef was highly correlated with protein content assessed by Kjeldahl determination ( $r = 0.90$ ). A correlation coefficient of similar magnitude was found between fat calculated from moisture analysis and fat determined by ether extraction. However, Grau (8) indicated that the interrelation between moisture and fat is not always linear. Therefore, it may be necessary to determine all three major chemical components when evaluating meat products such as sausage that usually contain added amounts of both fat and moisture.

The purpose of this investigation was to ascertain the interrelationships among the protein, fat, and moisture contents of pork muscle. Special attention was

given to the relationship between P/M and chronological age. Since there is considerable interest in rapid estimations of protein, fat, and water content of meat, the relationships between the actual determination of these constituents and the estimation of protein and fat content from known moisture values was studied.

### Experimental Procedures

A total of 439 pork carcasses was selected to represent five levels of loin intramuscular fat within each of four carcass weight groups (<125-pound, 155- to 170-pound, >200-pound, and 280- to 320-pound packer sows) or five chronological age classifications (4- to 4½-month, 6- to 7-month, 9- to 11-month, 15- to 18-month sows, and 36 to 42-month sows). These carcasses were originally chosen to determine the influences of intramuscular fat and chronological age on pork quality (12). An additional 32 pork carcasses representing equal numbers of males and females were selected from four younger chronological age groups (1 day, 1 month, 2 months, and 3½ months). These carcasses were selected to complete the pattern of P/M change from birth to maturity.

The carcasses were fabricated into wholesale cuts. A five-rib section of the *longissimus dorsi* was excised, defatted, and frozen. A 1-inch thick cross-sectional sample containing eight muscles was removed from the center of the ham. External fat was removed and the sample was frozen. The loin and ham samples

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were ground three times in a food grinder, and an aliquot from each sample was analyzed for protein, moisture, fat, and ash content (7). Composition was expressed on a fresh-weight and/or dry-weight basis. P/M ratios were determined from fresh weight percentages of protein and moisture.

### Results and Discussion

**Protein-Moisture Ratio.** For both the *longissimus dorsi* and the composite ham muscles, the P/M values remained essentially constant within all groups compared irrespective of intramuscular fat content. However, within each age or weight group studied, the *longissimus dorsi* muscle maintained a significantly higher P/M value than the composite sample from the ham. This suggests that anatomical, physiological, and/or biochemical differences in various muscles may affect P/M. Studies by Karmas *et al.* (9) have clearly demonstrated differences in P/M between the *gluteus medius* and *biceps femoris*. These authors have suggested that the P/M variations may be attributed to differences in the water-holding capacity of the proteins involved.

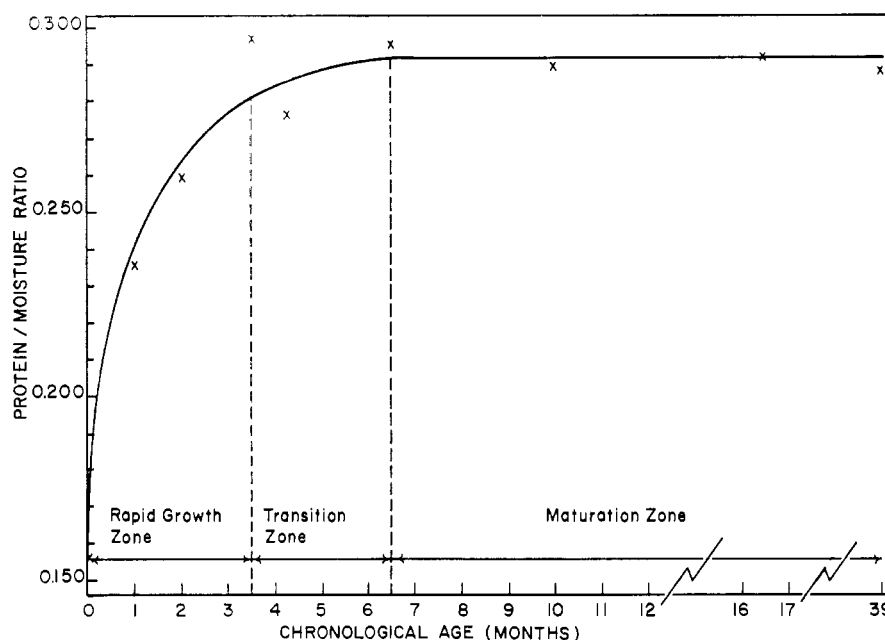
Table I shows the means and standard deviations of the P/M for each age and sex group and also indicates the level of significance for differences between each of these groups. As chronological age increased from birth to 3½ months, the P/M of both muscle samples at first increased rapidly and then more slowly. The P/M plateaued from 6 to 7 to 42 months of age. The exact age of cellular maturity cannot be assessed because P/M of the 4 to 4½ month group was significantly lower than that group immediately on either side of it. This may be partially explained by the differences of breed lines of swine between Groups I and II, and because environmental conditions may have varied regardless of the precautions taken to prevent this. Also, only eight animals were used in each of the four younger age groups. Table I also indicates that sex did not affect the P/M and thus permitted the pooling of results within age groups.

Figure 1 graphically illustrates the change in P/M from birth to an advanced age. The three zones have been arbitrarily designated. The rapid growth zone is designated as that phase of growth in which the P/M in muscle is increasing rapidly. This may indicate either that the biochemical structure of the individual muscle cell is in a state of change or that cell division is still in process at a decreasing rate—the difference in P/M resulting from the proportionate number of daughter cells existing at a particular age. The transitional zone is defined as that phase of growth in which the P/M is increasing

**Table I. Protein-Moisture Ratios of Pork Muscles from Nine Age Groups and Three Sex Groups**

Chronological Age, Months	Number	<i>Longissimus dorsi</i>			Composite of 8 Ham Muscles			
		Mean	Std. dev.	Significance level	Mean	Std. dev.	Significance level	
GROUP II <sup>a</sup>								
0 (1 day)	8	0.156	0.008	0.001	0.138	0.016	0.001	
1	8	0.236	0.016		0.025	0.234		0.017
2	8	0.259	0.010		0.001	0.264		0.013
3½	8	0.297	0.019			0.286		0.018
GROUP I <sup>b</sup>								
4 to 4½	49	0.277	0.017	0.010	0.268	0.011	0.010	
6 to 7	54	0.296	0.014	0.001	0.281	0.014	0.001	
9 to 11	44	0.289	0.017	0.100	0.279	0.019	0.600	
15 to 18	26	0.292	0.016	0.600	0.281	0.015	0.700	
36 to 42	35	0.288	0.025	0.500	0.276	0.018	0.300	
Sex Group								
Barrows	174	0.287	0.019	0.700	0.275	0.017	∞	
Gilts	158	0.286	0.019	0.300	0.275	0.016	0.200	
Sows	107	0.289	0.021		0.278	0.018		

<sup>a</sup> Group II data collected at University of Illinois. <sup>b</sup> Group I data collected at University of Wisconsin.



**Figure 1. Relationship of protein-moisture ratio of *longissimus dorsi* to cellular maturity as based on chronological age**

**Table II. Some Interrelationships of Protein, Moisture, and Fat for 439 Pork Loin and Ham Muscles**

Comparison	Longissimus dorsi		Composite of 8 Ham Muscles	
	Known <sup>a</sup> history	Unknown <sup>b</sup> history	Known <sup>a</sup> history	Unknown <sup>b</sup> history
Protein × moisture (simple correlation)	0.09	0.31 <sup>c</sup>	0.11	0.13
Protein × fat (simple correlation)	-0.38 <sup>c</sup>	-0.52 <sup>c</sup>	-0.36 <sup>c</sup>	-0.37 <sup>c</sup>
Moisture × fat (simple correlation)	-0.88 <sup>c</sup>	-0.91 <sup>c</sup>	-0.91 <sup>c</sup>	-0.89 <sup>c</sup>
Protein-moisture ratio (mean)	0.288	0.285	0.276	0.274
Composite total % of protein, moisture, and fat (mean)	98.2	98.6	98.4	98.6

<sup>a</sup> Classified according to chronological age, 206 samples.

<sup>b</sup> Classified according to carcass weight, 233 samples.

<sup>c</sup>  $P < 0.01$ .

**Table III. Means and Standard Deviations for Various Methods Used in Calculating Protein and Fat Content of Muscles from 439 Pork Carcasses**

Analysis	Longissimus dorsi		Composite of 8 Ham Muscles	
	Mean	Std. dev.	Mean	Std. dev.
% Moisture	72.6	2.2	70.9	2.0
% Fat (fresh basis)				
By chemical analysis	5.0	2.8	8.1	2.2
By regression equation				
Ham = $87.0 - (1.11) (\% \text{ moisture})$	...	...	8.2	2.2
Loin = $87.1 - (1.13) (\% \text{ moisture})$	5.0	2.5	...	...
% Protein (fresh basis)				
By chemical analysis	20.8	1.4	19.5	1.2
By difference				
$100 - (\% \text{ moisture} + \% \text{ fat} + 1.5\%)$	21.4	1.2	19.9	1.1
$100 - (\% \text{ moisture} + \text{calculated } \% \text{ fat} + 1.5\%)$	20.8	0.3	19.3	0.2
By P/M constant				
Ham = $0.275 \times \% \text{ moisture}$	...	...	19.5	0.6
Loin = $0.287 \times \% \text{ moisture}$	20.8	0.6	...	...
By regression equation				
Ham = $16.2 + (0.04) (\% \text{ moisture})$	...	...	19.0	0.8
Loin = $7.0 + (0.19) (\% \text{ moisture})$	20.7	0.4	...	...

**Table IV. Simple Correlations between Calculated Estimates and Chemical Analyses of Protein and Fat**

Analyses	Representative Groups of Carcasses			
	155- to 170- Pound carcass weight group	6- to 7- Month age group	3-Marbling <sup>a</sup> score	Total Carcasses in Study
Number	59	54	50	439
% Fat calculated by regression equation				
Ham = $87.0 - (1.11 \times \% \text{ moisture})$	0.90 <sup>b</sup>	0.86 <sup>b</sup>	0.84 <sup>b</sup>	0.90 <sup>b</sup>
Loin = $87.1 - (1.13 \times \% \text{ moisture})$	0.94 <sup>b</sup>	0.88 <sup>b</sup>	0.74 <sup>b</sup>	0.90 <sup>b</sup>
% Protein calculated by:				
Regression equation				
Ham = $16.2 + (0.04 \times \% \text{ moisture})$	0.28 <sup>c</sup>	0.17	-0.04	0.15 <sup>b</sup>
Loin = $7.0 + (0.19 \times \% \text{ moisture})$	0.48 <sup>b</sup>	0.35 <sup>b</sup>	-0.18	0.24 <sup>b</sup>
P/M constant				
Ham = $0.275 \times \% \text{ moisture}$	0.30 <sup>c</sup>	0.12	0.00	0.15 <sup>b</sup>
Loin = $0.287 \times \% \text{ moisture}$	0.47 <sup>b</sup>	0.36 <sup>b</sup>	-0.17	0.25 <sup>b</sup>
Difference				
Ham = $100 - (\% \text{ moisture} + \% \text{ fat} + 1.5\%)$	0.51 <sup>b</sup>	0.64 <sup>b</sup>	0.60 <sup>b</sup>	0.62 <sup>b</sup>
Ham = $100 - (\% \text{ moisture} + \% \text{ calcd. fat} + 1.5\%)$	0.30 <sup>c</sup>	0.10	0.01	0.15 <sup>b</sup>
Loin = $100 - (\% \text{ moisture} + \% \text{ fat} + 1.5\%)$	0.64 <sup>b</sup>	0.53 <sup>b</sup>	0.48 <sup>b</sup>	0.65 <sup>b</sup>
Loin = $100 - (\% \text{ moisture} + \text{calcd. } \% \text{ fat} + 1.5\%)$	0.47 <sup>b</sup>	0.35 <sup>b</sup>	-0.19	0.25 <sup>b</sup>

<sup>a</sup> Includes carcasses in the four weight classifications of the unknown history group that contained modest amounts of intramuscular fat in the loin.

<sup>b</sup>  $P \geq 0.01$ .

<sup>c</sup>  $P < 0.05$ .

at a decreasing rate and differences found may be the direct result of biological variation. Cellular maturity was probably reached sometime between 3½ and 6½ months of age. The maturation zone is defined as the phase of growth in which the P/M does not change statistically, and may support the postulation that, in this more advanced age range muscle cells may increase in physical size and do not necessarily undergo cell division.

The change in P/M from birth to maturity may be partially the result of an increase in nitrogen content of the muscle proteins. Since the procedure used to determine protein assumes a constant 16% nitrogen, any increase in nitrogen due to an alteration of amino acid composition, or any increase in non-protein nitrogen would reflect a higher P/M. Nevertheless, it is very unlikely that such an alteration explains the wide P/M variation shown in Table I.

**Component Relationships and Estimations.** Relationships between muscle protein, fat, and moisture content for all but the four youngest age groups are presented in Table II. Regardless of the major group or muscle type analyzed, apparently the variation in protein could not necessarily be accounted for by the variations of moisture. This may be partially explained by the lower and changing P/M of muscles originating from pigs which are within the transitional zone of maturity as defined by P/M. A lower correlation between protein and moisture would therefore be expected for the 4- to 4½-month group as compared to older groups. This is indicated by the protein-moisture correlations for carcasses under 125 pounds, 155 to 170 pounds, and over 200 pounds, which were 0.21, 0.47, and 0.54, respectively. The low correlations between moisture and protein may be due to the small variation in protein as compared to the greater variations of both moisture and fat, or to the differences existing in the water-binding capacity of these proteins.

When intramuscular fat was compared with either protein or moisture, the relationship was significant as anticipated. Statistically, more than 80% of the variation in moisture content and 13% of the variation in protein content could be explained by variations in fat content. This obviously illustrates the well established fact that an increased protein and moisture content must be accompanied by a decrease in intramuscular fat deposition, and vice versa since the three components represent over 98% of the total composition of muscle as shown in Table II. As calculated by difference, ash and other minor constituents would represent about 1.5% of the muscle mass.

Because a definite relationship existed between the fat and moisture contents,

nd because the P/M appeared to be relatively constant for butcher weight pigs, a number of calculations were made to estimate protein and fat on the basis of known moisture content of the muscles. To ascertain the validity of these estimates, they were statistically compared with values obtained from actual chemical analyses.

Table III presents means and standard deviations for the comparison of chemical analyses of fat and protein to estimations of these two components. Protein was estimated by a regression equation, multiplication of the P/M constant by per cent moisture, and difference when actual moisture was known and when it was either chemically determined or estimated by moisture. Fat was calculated by a regression equation only when moisture was known. The values represented in Table III indicate that for the 439 carcasses, all estimated values for fat and protein are similar to those determined chemically. However, these averages should not be construed to indicate that variations do not exist among individuals. Such evidence is clearly indicated by the accompanying standard deviations and by the correlation coefficients in Table IV.

As shown in Table IV, correlations between extracted fat and fat estimated from moisture content were highly significant. Because the coefficients were of high magnitude, confidence was given to this indirect method of estimation. Conversely, the direct and indirect protein estimations were not as highly correlated. When the regression equation was employed, some of the relationships were significant but were of such

low magnitude that only minimum variation could be accounted for. Occasional negative coefficients for the 3-marbling score group cannot be explained except that this group included data from heavier weight carcasses as well as lighter weight pork carcasses. The low correlations are believed to be caused by the very narrow range in chemically determined protein and the probable overlap of values due to the difficulty in arriving at absolute values even with the greatest care in carrying out the determinations.

The estimations of protein by the use of the P/M constant are quite similar to the results of the regression equation and would therefore be of little merit for practical application. The protein estimate by difference was, however, more meaningful, and this technique may be useful to approximate protein content indirectly. Subtraction of fat content (calculated from moisture content), actual moisture content, and a constant (for minor muscle components) from 100 gave estimated protein values which were not as highly correlated with chemically determined protein as when the actual extracted fat value was used. Approximately 36% of the accountable variation was lost.

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## MEAT PRESERVATION

# Oxidative Changes in Cured and Uncured Frozen Cooked Pork

**A** PRIMARY OBJECTIVE of this paper is a comparison of patterns of lipid oxidation in frozen cooked meats versus frozen cured meats. Recent work from his laboratory on the freezing preservation of roast beef slices (2) and of pre-cooked mullet (18) reveals a similar pattern of lipid oxidation as measured by the thiobarbituric acid (TBA) test.

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Both the meat and fish had moderately high TBA numbers throughout the period of frozen storage, with no significant changes related to storage time. The results were interpreted as a rapid oxidation of the meat lipids during preparation for freezing and upon subsequent thawing. The oxidation was believed to be catalyzed by the ferric cooked meat pigment. The reaction evidently was arrested in the freezer, but proceeded rapidly upon thawing.

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The addition of sodium nitrite and salt to meat before heat treatment would be expected to change this pattern. Nitrite converts the meat pigments to the catalytically inactive ferrous nitric oxide hemochromogen; consequently, the stability of refrigerated cured meats to lipid oxidation is high as compared to the stability of cooked meats under the same conditions (17). On the other hand, sodium chloride used in curing brines is known to accelerate lipid