

# Factors Affecting the Water Retention of Beef

## I. Variations in Composition and Properties among Eight Muscles<sup>a</sup>

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CONSIDERABLE INTEREST in identifying the factors which affect the water retention of meat has recently been in evidence in the meats field. The practical goal of this research is the development of means of controlling and, when desired, enhancing the capacity of meat to retain water or juice. Present knowledge permits this to only a limited extent.

Results of previous studies indicate that pH markedly affects water retention and, also that electrolytes exert an influence. The pronounced effect of pH value has been reported as a result of studies dealing with meat only (8, 9, 15, 18), and with meat containing additives (12, 22, 30). Known causes for variation in the pH values of meat include differences in lactic acid formation after slaughter (3, 19), in beef from animals of different age (20), and during the aging of meat (19, 31). Muscles of both the pig (27) and the horse (19) have been found to have different ultimate pH values, "ultimate" pH being the low pH attained in the post rigor muscle. It has been reported that the effect of calcium and zinc on the hydration of meat proteins is to decrease their water retention (11). The effect of the ions appears to be appreciably influenced by pH, which has been reported to determine the amount and direction of ion effects (11), and by the presence of ion-complexing agents such as the polyphosphates (10). It should be noted that muscle and meat pH values in this paper and in the literature cited are the pH values of extracts or juice given opportunity to equilibrate to some degree with muscles or meat proper. A basis for assuming that the movement of ions during post mortem aging is related to the hydration and tenderness of meat has been reported (1). The information available falls short of explaining differences in meat as reflected by variations in water retention, juiciness, and tenderness which, in part, is also thought to be affected by water retention. New light shed on variations in meat as regards both composition and properties is needed to improve this situation.

Previous investigations of water retention were made by different methods and generally have involved determination of the effects of variations in composition indirectly by the addition of salt solu-

tions, adjustment of pH with acid, alkali, or buffers, and, on occasion, adjustment of ion content with complexing agents. In the present study a different approach was used, involving a comparative study of the properties of muscles from the same and different animals. By this means, variables related to the history of the samples were eliminated and variation in composition and properties was obtained, without introducing extraneous materials, with the exception of water. The experiments involved determining water retention, pH values, electrolyte composition (calcium, magnesium, zinc, iron, phosphorus, chloride, sodium, and potassium), and the proximate composition of eight muscles from each of four animals and pH values and water retention of eight muscles from an additional animal. These results provide new information on variations to be expected in the composition and properties of muscles from the same, as well as from different, animals.

The results were also used in investigating the pattern of the pH values and water retention of the series of muscles and in investigating relations between water retention and composition. The inability of the correlations attempted to indicate the independent effects of variation of components was recognized, as was the fact that interpretation of trends based on analyses of total contents has obvious limitations that will be dealt with later in detailed studies. However, by considering both the degree and direction of the correlations of the relationships investigated, the results provided new information on the relations between meat composition and water retention and indicated promising areas in need of further investigation.

### EXPERIMENTAL AND RESULTS

**Animals and muscles used.** Carcasses of two cows, a bull, a heifer, and a steer were obtained immediately after slaughter, and the 8 muscles named in Table 1 were separated. Separation of the muscles from the carcasses and removal of separable fat and connective tissue required from 1 to 2 hours, measured from the time of slaughter. Each muscle was cut into cubes approximately 16 cc. in size, and the cubes were thoroughly mixed.

**Determination of water retention.** The procedure applied to samples of each muscle was as follows: A suitable number of cubes was ground through a grinder equipped with a plate having approximately 5 mm. openings. Each of two 15 g. portions of sample was weighed into tared cellulose centrifuge

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TABLE 1  
Muscles selected and locations

Number	Muscle	Location	
		Fore quarter	Hind quarter
1	<i>Longissimus dorsi</i>	Rib, chuck	Sirloin, porterhouse
2	<i>Psoas</i>		Sirloin, porterhouse
3	<i>Semimembranosus</i>		Round
4	<i>Serratus ventralis</i> (thoracic part)	Rib, chuck	
5	<i>Rectus abdominus</i>	Plate	Flank
6	<i>Semitendinosus</i>		Round
7	<i>Latissimus dorsi</i>	Rib, chuck	
8	<i>Trapezius</i>	Rib, chuck	

tubes (40 ml. capacity) and 15 ml. of cold, distilled water (0° C.) were added to each tube. Tissue and water were well mixed with a stirring rod and the mixtures were stored overnight at 0° C. Caps were placed on the tubes, approximately 8 ml. of distilled water were added to each tube, and the mixtures were centrifuged in a Spinco Model L,<sup>b</sup> fitted with head No. 30, for 20 minutes at 15,000 r.p.m. The supernatant liquid was decanted from the tubes which were inverted to drain for 5 minutes. The pH values of the supernatant fractions were determined using a Beckman Model GS pH meter. The drained tubes and meat were weighed and the differences between the original and final weights were calculated and averaged. In the calculations loss of extractives in the supernatant liquid were disregarded. The mean change in weight of the samples is reported as water retention,  $\Delta$  wt./15 g. meat.

**Analytical methods.** Modifications of the basic analytical methods used will be described in a separate report (6). The following describes the general methods employed in analyses of muscles from all the animals, except those of the heifer, which were only used in the investigation of the relation of pH and water retention.

A portion of the cubed sample obtained from each muscle was finely comminuted and mixed in a Servall Omni-mixer. The comminuted sample resembled a fine paste. Fifteen-gram aliquots of the paste were weighed into quartz crucibles and dried to constant weight by heating them for approximately 16 hours at 105° C. The samples were then charred by heating at 350° C. and ashed overnight at 550° C. (21). The ash was dissolved in 6 N HCl and diluted to volume. Aliquots of the stock solution were used in determining sodium, potassium, calcium, magnesium, zinc, iron, and phosphorus as follows: Sodium and potassium were determined by flame photometry (4), zinc as the dithizonate in carbon tetrachloride solution (26), iron as the ortho-phenanthroline complex (2), phosphorus as molybdivanadophosphoric acid (17), calcium as the oxalate (5, 29), and magnesium with EDTA (16, 23) subtracting the contribution of calcium. Fat was determined by a modified Babcock procedure on separate 9-g. samples of meat paste (25). Chloride was determined on separate 10-g. samples of meat paste using a modified Volhard method (7, 32). Moisture content was determined by the oven drying method (33).

Average values obtained from pooled data on the fat, moisture, and protein (N% x 6.25) content of the muscles and the results of calculations of moisture-to-protein ratios based on these data are shown in Table 2. Averages of data obtained in determinations of electrolytes are summarized in Tables 3 and 4. The data obtained on determining water retention and the pH values of water extracts of muscles from 5 animals are shown in Table 5, along with the results of a statistical analysis of differences between individual muscles. The results of calculations of the correlation coefficients of relationships between components and water-holding capacity are given in Table 6.

## DISCUSSION

Muscles used in the investigation are named and their location in ordinary meat cuts is given in Table

<sup>b</sup> The mention of specific trade names throughout this paper does not constitute endorsement of the product used over comparable equipment.

1. At the outset of the work the only indication of possible differences in the muscles was the various degrees of tenderness attributed to them by Ramsbottom (24). While differences in tenderness need not indicate differences in water-holding capacity and other properties, the limited guidance this provided proved useful as a basis for selection. As the present results indicate, sufficient variation in properties existed to satisfy the needs of the investigation.

The results of the proximate analysis and the calculations of moisture: protein ratios for the different muscles are given in Table 2. As indicated by the data, the muscles were low in fat and high in protein and moisture content, and tended towards leanness with the exception of the *serratus ventralis* muscles, which had some visible, but not readily separable, fat between bundles of muscle fibers. The results of the determination of calcium, magnesium, zinc, sodium, potassium, iron, phosphorus, and chloride are reported in Table 3. As the results indicate, the content of electrolytes of individual muscles ranged widely. A comparison of the average content of electrolytes in the muscles with values reported in the literature for meat is given in Table 4. The values previously reported and those found are substantially in agreement.

The 8 muscles of given animals are arranged in Table 5 in order of decreasing average pH values and water retention. The differences between the average pH values of the *serratus ventralis* (muscle 4) and the *rectus abdominus* (muscle 5), and between the pH values of these two muscles and those of the other six, were statistically significant. The average water retention of the *serratus ventralis* muscles was significantly larger than the average values obtained for the other muscles. Otherwise, differences between pairs of muscle pH values or between the water retention of pairs of muscles were not significant.

Analyses of trends of relations among the properties of the eight muscles were made to provide a statistical basis for interpreting results. The animals were slaughtered over a period of several months. As work on muscles of the individual animals was completed the data obtained were analyzed. The statistical analyses involved calculating linear regression equations and coefficients, and testing the significance of correlation coefficients. The results of calculations based on the data obtained on individual animals indicated that the same relationships were significant in each case. Subsequently, the data on each of the 8

TABLE 2  
Average moisture, fat, and protein content and moisture/protein ratios of 8 muscles from 4 animals

Muscle	Moisture content	Fat content	Protein (N x 6.25) content	Moisture: protein ratio
	%	%	%	
1. <i>Longissimus dorsi</i>	74.15	2.48	22.60	3.28
2. <i>Psoas</i>	72.95	3.83	20.60	3.54
3. <i>Semimembranosus</i>	73.90	1.85	22.10	3.34
4. <i>Serratus ventralis</i>	70.90	8.30	17.90	3.96
5. <i>Rectus abdominus</i>	73.20	4.80	21.13	3.47
6. <i>Semitendinosus</i>	74.20	2.10	21.95	3.38
7. <i>Latissimus dorsi</i>	74.75	2.53	21.18	3.53
8. <i>Trapezius</i>	73.90	2.45	20.50	3.61

TABLE 3

Average content of electrolytes of 8 beef muscles from 4 bovine animals, mg./100 g.

Muscle	Ca	Mg	Zn	Na	K	Fe	P	Cl
<i>Longissimus dorsi</i> .....	4.30	21.99	3.63	43.0	415	2.73	172	40.3
<i>Psoas</i> .....	4.03	23.13	2.13	43.8	383	2.60	179	44.2
<i>Semimembranosus</i> .....	4.14	23.12	3.68	39.5	411	2.85	170	42.9
<i>Serratus ventralis</i> .....	3.26	17.09	7.37	52.7	336	3.51	154	55.0
<i>Rectus abdominus</i> .....	4.07	19.99	5.71	53.2	393	2.55	176	55.7
<i>Semitendinosus</i> .....	4.40	22.47	2.83	42.7	436	2.16	170	48.5
<i>Latissimus dorsi</i> .....	3.74	21.71	4.66	49.0	405	2.56	184	53.7
<i>Trapezius</i> .....	4.28	19.32	3.64	64.2	381	2.60	164	72.9

TABLE 4

Content of electrolytes in beef muscles

	Ion content, mg./100 g. muscle							
	Ca	Mg	Zn	Na	K	Fe	P	Cl
Literature, beef, general.....	(1, 6)	(1, 2, 3)	(1, 5)	(1, 2)	(1, 2)	(1, 4)	(6)	
Found, range, individual muscles.....	2.6-12.0	19.0-31.8	4.7-6.1	41-67	300-338	1.5-3.7	131-186	
Found, average, all muscles.....	2.7-5.6	14.0-25.7	1.2-8.3	36-85	297-451	1.6-5.5	109-213	34-91
	4.0	21.1	4.2	38.5	395	2.7	171	51.6

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muscles of the 4 animals (5 animals in the case of the pH values and the values for water retention reported in Table 5) were averaged, and the averaged data were statistically analyzed. The relationships derived from the averaged data completely agreed with those

based on data obtained on the muscles of individual animals, except that the correlation coefficients obtained on analysis were somewhat higher. These averaged data would yield significant correlations only if the relative order of the muscles, with regard to water retention and to other properties, tended to follow the same pattern from animal to animal. Following this procedure, a number of significant correlations between properties of the muscles were found; hence, the results indicate that certain differences in the muscles of the animals followed a pattern.

TABLE 5

pH values and water retention of muscles

Muscle no.	pH		Water retention	
	Average value	Statistical significance	Average wt./15 g. meat	Statistical significance
4	5.79		-.85	
5	5.68		-1.74	
8	5.60		-2.51	
7	5.59		-2.59	
2	5.53		-2.92	
1	5.53		-2.99	
3	5.51		-3.01	
6	5.50		-3.13	

<sup>1</sup>The values (pH and water retention) for muscles opposite a bar are different from those not opposite the same bar (calculated from data on five animals, statistically significant at the 5% level, 8 degrees of freedom).

TABLE 6

Results of statistical analyses of relationships between properties of 8 muscles

Component	Correlation with water retention		Correlation with protein (N% x 6.25) content	
	r	P <sup>1</sup>	r	P <sup>1</sup>
pH	+0.947 ± .028	.01	-0.892 ± .056	.01
Zn	+0.912 ± .046	.01	-0.631 ± .165	.10
Fe	+0.648 ± .160	.10	-0.709 ± .178	.05
Na	+0.634 ± .165	.10	-0.525 ± .199	n.s.
Moisture	-0.718 ± .133	.05	.....	.....
Protein	-0.854 ± .074	.01	.....	.....
Moisture, %/ protein, %	+0.861 ± .071	.01	.....	.....
Fat	+0.868 ± .067	.01	.....	.....
Cl	+0.519 ± .201	n.s.	-0.456 ± .218	n.s.
Ca	-0.779 ± .108	.05	+0.827 ± .087	.01
Mg	-0.952 ± .038	.01	+0.806 ± .097	.02
K	-0.811 ± .094	.01	+0.937 ± .033	.01
P	-0.544 ± .194	n.s.	+0.604 ± .175	.10

<sup>1</sup> 6 degrees of freedom.

The data in Table 6 show the results of statistical analyses of the relations between the water retention and components of the muscles, and between protein content (N% x 6.25) and the content of other components. The results indicate that water retention was inversely related to protein content (N% x 6.25). Further, as is shown, the correlations could be separated into two groups; one, of components which varied positively with variations in water retention and negatively with variations in protein content, and another, of components which varied negatively with variations of water retention and positively with variations of protein content. The data generally indicate that components positively associated with increasing water retention were negatively associated with increasing protein content and *vice versa*. A second generality of possible significance is that components which may be considered as intracellular components, including calcium, magnesium, potassium, phosphorus, and the bulk of the protein, were found to be inversely associated with water retention, while the trend of sodium and chloride, which are mainly expected to be components of extra-cellular fluids, were found to be directly associated with water retention.

The negative correlation between water-reten-

tion and protein content, in which protein content was calculated using the generally based relation, per cent N x 6.25 equals per cent protein, was highly significant ( $r = 0.854 \pm .074$ ,  $P < .01$ ). This inverse relation is paradoxical, inasmuch as protein is the component of muscle assumed to be principally responsible for water retention. Interpretation of the results involves consideration of the validity of the assumption that content of nitrogen and protein in the 8 muscles had a constant relation, whether by a factor of 6.25 or by any other. Inasmuch as minor variation in the proportion of protein to non-protein nitrogenous substances would not affect the inverse relation found between water retention and protein content, and the occurrence of wide variations has not, as yet, been reported, the validity of expressing per cent N x 6.25 as per cent protein, in this case has been tentatively accepted. In rigorous aspect it should, however, be noted that nitrogen, not protein, was determined.

As shown by the data given in Table 6, increasing pH values were found to be closely correlated with increasing water retention ( $r = .947 \pm .028$ ,  $P < .01$ ). This confirms the results previously reported (8, 9, 15). Assuming pH to be the significant parameter and the pH of the series of muscles to be inversely related to protein content provides an explanation for the paradoxical relation of protein content to water retention. Investigations are in progress to obtain information on the relative proportions of different constituents of the muscles affecting pH, such as glycogen, lactic acid, and buffers, as is needed to determine the validity of this assumption. Consideration of the relation of pH to water retention requires recognition of its complexity, specifically noting that pH is reported to affect the binding of ions to proteins and, consequently, influences water retention (10) and, conversely, that ion-binding may be expected to influence the buffering capacity of protein, thereby influencing pH (28).

To the limited extent that zinc is mentioned in the literature concerned with water retention (10), its action has been considered to be similar to that of calcium in that its binding to the structural proteins of meat has been assumed to have an adverse effect on water retention. Although zinc may, in part, have this tendency, it is parallel to pH in its relation to water retention suggesting that zinc may participate as a component, or an activator, of an enzyme the action of which may partly determine pH differentials. The formation of lactic acid from glycogen apparently varies, adding to the complexity of the system which determines pH (19).

The data in Table 6 show that increasing moisture-protein ratios were directly associated with increasing water retention. As can be seen from even cursory inspection of the results given in Table 2, the moisture content of the muscles did not vary to the extent that protein content varied, producing ratios of moisture to protein ranging from 3.28 to 3.96. An extensive discussion of variation of water-to-protein ratios in different meats has been published revealing vari-

ations among cuts of meat and differences related to the age, condition, and finish of animals from which meat is obtained (14). The problem in controlling the composition of sausage arising from these variations was cited. The problem would be increased, if differences in water-to-protein ratios in meats are accompanied by variation in water retention as in the case of the series of muscles presently discussed. Also, the data in Table 6 show that the tendency of increasing water retention to be accompanied by increasing fat content was marked ( $r = .868 \pm .067$ ,  $P < .01$ ). This would appear to be a reflection of some other factor, or factors, directly influencing water retention, inasmuch as fat itself is recognized as having little tendency to hold water. In beef fatty tissue water-to-protein ratios ranging from 2.8 to 4.6 have been reported (14).

The data in Table 6 also show that calcium, magnesium, and potassium content varied inversely with water retention, and, on the other hand, increased with increasing content of protein. The protein content and calcium, magnesium, and potassium content were found to be directly and closely related ( $r = .827 \pm .087$ ,  $P < .01$ ;  $r = .806 \pm .097$ ,  $P < .02$ ; and  $r = .937 \pm .033$ ,  $P < .01$ , respectively), indicating that the content of these ions was related to protein content in relatively constant proportions. Phosphorus content also tended towards a similar relation to protein content, but the relation was significant only at the 10% level ( $r = .604 \pm .175$ ).

The data show that iron, sodium, and chloride content tended to be directly related to water retention and to be inversely related to protein content; these relations, however, were not statistically significant, except that the content of iron was inversely related to protein content (significant at the 5% level). As previously pointed out, the correlations attempted do not indicate the independent effects of variation of single components, as has been the goal in previous work (10, 11, 12, 30). The data do show that, in a series of muscles arranged in order of increasing water retention, there was found to be positive correlation with increasing pH, zinc and fat content, and ratios of moisture to protein, and negative correlation with components, including protein content ( $N\% \times 6.25$ ), and calcium, magnesium, and potassium content.

#### SUMMARY

A comparative study of the composition and properties of eight different muscles from bovine animals was conducted in an investigation of factors which influence water retention. Water retention is defined as the change in the weight of the experimental meat samples after mixing the meat with distilled water (1:1) and centrifuging the meat-water mixtures for 20 minutes at 15,000 r.p.m., following overnight storage of the mixtures at 0° C.

The results indicate that considerable variation existed with respect to water retention and pH and the nitrogen, fat, moisture and electrolyte content of the muscles. The results further indicate that the relative order of the muscles of the different animals

followed a pattern with regard to water retention, pH and a number of other properties.

Statistically significant correlations were obtained between water retention and directly related components, including pH, zinc and fat content, and ratios of moisture to protein, and inversely related components, including protein content ( $N\% \times 6.25$ ), and calcium, magnesium, and potassium content.

A direct, highly significant correlation was found between water retention and zinc content, in contrast to the inverse relation found between water retention and either calcium or magnesium content. This information indicates that zinc differs in an important aspect from the two other ions. The possibility that zinc may participate in determining pH as a component of an enzyme system is pointed out.

The ratio, moisture/protein content, was found to be directly related to water retention, hence, the results show that the relative ability of the muscles to hold added moisture was predictable on the basis of the original proportions of moisture and protein present.

The analysis of factors influencing water retention is a complex problem. The present results have opened new channels for investigation; specifically, one of these concerns determination of factors influencing pH, including zinc containing enzymes, and the other, the paradoxical relation noted between protein content and water retention.

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