THE EFFECTS OF OVARIECTOMY AND HYPOPHYSECTOMY ON THE PHYSICAL CHARACTERISTICS OF RABBIT MUSCLE TISSUE

by

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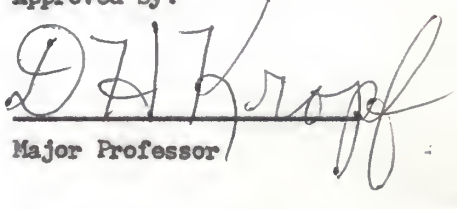

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INTRODUCTION

Expressible moisture of the bovine is more important than either color or tenderness for meat quality. Expressible moisture also affects cooler shrinkage, drip loss, curing yields and cooking loss in beef (Hamm, 1963).

Skeletal muscles from female bovine have a higher expressible moisture than do their male counterparts. Currently cattle feeders are using estrogenic compounds in castrated animals and progestational compounds to eliminate estrus in females, thereby promoting greater efficiency. Estrogens and progesterone have been shown to have marked effects on water and electrolyte content in smooth muscle (Thorn and Engel, 1938; Talbot et al., 1940). Little literature pertaining to the effects of hypophysectomy on muscle tissue is available. The effect of the trophic hormones demands more research.

This study was designed to elucidate whether or not these same effects might occur in skeletal muscle. To study this problem female rabbits (being more economically feasible) were (1) ovariectomized (absence of ovarian steroids) and (2) ovariectomized plus supplemented with exogenous progesterone (effect of progesterone alone). Hypophysectomized animals were studied to eliminate hormones other than gonadal and elucidate possible effects.

Each of these treatments embraced an extreme and in total, a wide variation of progesterone-estrogenic inclusion or exclusion.

In order to more closely study the effects of female ovarian hormones and their interrelationships on muscle water, energy and electrolytes, the following measurements were carried out on rabbit longissimus dorsi muscle: expressible moisture, glycogen, glucose, total moisture, protein, fat, potassium, and

sodium. In addition, starting weight and 14 day body weight changes were measured.

REVIEW OF LITERATURE

Little information is available on ovarian hormone effects on skeletal muscle. Variations in natural levels of progesterone and estrogens often occur in meat animals, but little is known about their effect on muscle water holding capacity and related factors. The effect of ovarian hormones on smooth muscle tissue has been quite widely reported and perhaps this might provide a clue toward possible skeletal muscle effects.

A. OVARIAN STEROID EFFECTS.

A retention of water occurs in the pregnant mouse (Brooksby & Newton, 1938; Dewar, 1957) and the weight loss after parturition or pseudo-parturition largely consists of water. The quantity of this water bears no apparent relation to the loss of nitrogen that may occur at any time, which is proportional to the sodium loss (Dewar, 1957). Progesterone treatment has been noted to result in an increased water turnover in rats (Selye & Bassett, 1940) and nitrogen loss due to the effect of progesterone in humans appears to be clearly established (Kyle & Hess, 1956).

Many have observed that progesterone can cause retention of salt and water (Thorn & Engel, 1938; Thorn et al., 1938). Caspo (1959) reported the administration of progesterone increased the loss of sodium and chloride in uterine tissue. This, Caspo stated, was not a direct progesterone effect but due to the inhibition of certain adrenal cortical hormones. Thorn et al. (1938) stated, "All hypotheses that water retention is responsible for weight gain are clouded by the observation of an increase in food intake, in progesterone treated mice, as well as during pregnancy." It was also noted that women during the premenstrual phase had an increase in appetite.

Dewar (1957) stated the mouse body may be richer in per cent protein after pregnancy. Bourdel and Jacquat (1965) found injections of 3-6 mg of progesterone for 20 days had no effect on nitrogen metabolism in recently castrated or 1 month castrated female rats fed a nitrogen deficient diet. The gain of weight of normally fed 1 month old castrated rats was more marked for progesterone treated rats than for the control. Bourdel et al. (1960) tried to imitate the weight changes of pregnancy in the non-pregnant animal and found that progesterone alone caused a 15-20% increase in body weight. No attempt was made to determine the cause of the weight increase.

Thomson and Hytten (1961) have produced evidence that part of the weight gain in pregnancy of woman is due to an increased deposition of body fat. Hammond and Lawrie (1967) studied the longissimus dorsi muscles from intact and castrated male ferrets. Implantation of progesterone pellets increased the body and muscle weights of intact males (but not of castrated animals) and also increased intramuscular fat. The water content of the muscle decreased only reciprocally in relation to the increased fat content. Progesterone had no discernable effect on the composition of muscle in castrated ferrets. In summer the castrated animals had more intramuscular fat which was more highly unsaturated than the intact males.

Talbot et al. (1940) studied the nature of water and electrolyte changes during uterine proliferation in ovariectomized rats. These workers found electrolytes in the uterus increased 6 hours after estradiol administration with an increase of intracellular as well as extracellular water. Spaziani et al. (1958) indicated that early accumulation of water and electrolytes following estrogenic stimulation of the uterus are secondary manifestations of the hyperemia and increased capillary permeability resulting from estrogenic-

induced release of endogenous histamine.

Subcutaneous injections of diethylstilbesterol (0.33 γ to 333.0 γ) and estradiol (0.9 γ to 20.0 γ), over a 10 day period increased perineal muscle weight in castrated immature mice. Diethylstilbesterol also increased dry weight and total nitrogen content rather than water content in the perineal muscle which indicated a myotrophic effect by estrogens. Response of perineal muscles in mice to estrogens may have a correlation with the anabolic effect of estrogens in domestic animals (Hershberger et al., 1966).

Chick oviducts displayed very large increases (1000 to 10,000 %) in total protein on the administration of 0.75 to 10 mg of estradiol propionate for 10 days (Brown et al., 1961; Dewar, 1957). Robinson and Singleton (1966) studied the effect of Norbolethone (an anabolic steroid) on pigs and found the steroid significantly improved the percentage of muscle and longissimus dorsi muscle area.

Bitman et al. (1965), studied in vivo glycogen synthesis in the rat uterus after estrogen administration. Glycogen synthesis was induced by 1 γ of estradiol. With this dosage the glycogen synthesis increased about 12% each hour.

The acid mucopolysaccharides are important for electrolyte regulation, water binding and other physiological processes (Rasmussen, 1967). Rasmussen (1967) stated that it is generally known that in woman varying amounts of water are bound in the tissues during the menstrual cycle and pregnancy caused edema. This is believed due to the variations in the endocrine secretion of estrogenic hormones. Schmidt (1958) demonstrated increased contents of hexosamine and water in the skin of estrogen treated mice and this may partially explain the edema.

Hawk et al. (1961) studied 10 estrus (estrogen predominant), 7 luteal (progesterone predominant) and 5 ovariectomized ewe myometriums and found greater proportions of extracellular water in the estrus and luteal phases. These workers also found higher sodium and chloride concentrations and found endometrial water to decrease with age. Yochim and DeFeo (1962) concluded that progesterone alone was incapable of duplicating the weight and water content of the deciduomata of intact psuedopregnant rats. Combinations of estrogens and progesterone that were effective were similar to those reported for optimal maintenance of gestation.

Leonard et al. (1953) injected estradiol benzoate and cortisone acetate into normal, gonadectomized and hypophysectomized rats of both sexes, and determined the effect of glycogen levels in the following skeletal muscles: rectus femoris, abdominal muscles of both sexes, the cremaster of the male and the diaphragm of the female. Estradiol increased the storage of glycogen in all three muscles of the spayed female. No increase in glycogen was observed in the muscles of the hypophysectomized rats treated with estradiol except for a small but statistically significant increase in the rectus femoris. Results indicate not all skeletal muscles respond alike in depositing glycogen under hormonal influence.

Edelman (1963) stated, "Any process that interferes seriously with metabolism such as cold, anoxia, or the presence of metabolic inhibitors will accelerate the loss of potassium from cells usually with a concomitant gain of sodium." Evidence suggests that adenosinetriphosphate (ATP) is the proximate energy donor for potassium accumulation and sodium extrusion in a variety of cells including those of skeletal muscle.

B. EXPRESSIBLE MOISTURE

Expressible moisture of muscle affected the quality of meat during almost all processing operations (Hamm, 1960). To define water retention, he stated that beef contains 60% water, 4% to 5% of which is true hydration water bound by muscle proteins. The amount of true hydration water is scarcely influenced by structure and electrical charges of muscle protein. The great difference in water holding capacity is due to the extent free water is immobolized within the microstructure of the tissues. The structural protein of the muscle is responsible for binding hydration water and for immobilization of free water. No relationship existed between water holding capacity and total moisture of protein content in beef (Hamm, 1960). Hamm also related intramuscular fat content of meat was influenced by water holding capacity. Wanderstock and Miller (1948) stated as carcass finish increases expressible juice decreases.

Addition of salt increased the water holding capacity of muscle protein as long as the salt concentration did not exceed 4% to 5% (Hamm, 1963). Salt linkages may be split off by the binding of chloride ions causing an increase in meat hydration (Hamm, 1959).

Swift and Berman (1959) studied eight beef muscles. The longissimus dorsi, psoas, semimembranosus, serratus ventralis, rectus abdominius, semitendinosus, latissimus dorsi, and the trapezius were used for analyses. These workers reported statistically significant correlations between water retention and pH, fat content and the ratio of moisture to protein. A highly significant negative correlation was calculated between water retention and protein content (Nitrogen x 6.25). This inverse relationship was contrary to opinion since protein is the component of muscle assumed to be responsible for water

retention (Swift & Berman, 1959).

Swift and Berman (1960) found glycogen and buffering capacity to vary among muscles. This variation had a definite pattern with regard to pH and water retention. A significant correlation was found between buffering capacity and pH. All muscles contained residual glycogen at the ultimate pH so the high pH of certain muscles could not be attributed to the lack of glycogen.

C. MUSCLE COMPOSITION

Swift and Berman (1960) found average values for total moisture, ash, protein (N x 6.25), fat and electrolytes. The averages were 72.3% moisture, 1.21% ash, 23.1% protein and 2.1% fat. Long (1960) reported the skeletal muscles of rabbits had a range of 0.08 to 0.15 mg/gm of glycogen and 0.382 mg/100 gms of glucose.

Kirton et al. (1963) studied the separable lean of lamb carcasses and reported potassium levels of 313-316 mg/100 gms in fresh muscle. Elkinton and Widdowson (1959) studied rat skeletal muscle and found 487 ± 0.58 mg potassium/100 gms and 66.4 mg sodium/100 gms of muscle. Swift and Berman (1959) reported an average potassium level of 395 mg/100 gms muscle and a range in sodium content of 36-85 mg/100 gms on eight beef muscles.

EXPERIMENTAL METHODS

A. ANIMALS AND TREATMENTS

Thirty, four month old estrous female rabbits of the New Zealand breed were randomly alloted into four treatment groups. The treatments were (1) control (laparotomized), (2) ovariectomized, (3) ovarietomized plus exogenous progesterone, and (4) hypophysectomized. Twelve rabbits were assigned to treatment 4 because of the high mortality rate expected; 6 rabbits each were used in the other treatments. The pituitary gland was excised by the parapharyngeal approached as outlined by Zarrow (1964). The ovaries were excised by the procedure as reported by Zarrow (1964).

The animals were weighed and anesthesized with dilute Nembutal through the marginal ear vein, at the rate of 0.5 gr./Kg. of body weight. The Nembutal was diluted 1:1 with physiological saline.

Immediately after the animals in treatment 4 were hypophysectomized 1 ml. of cortisone was injected intramuscularly. These animals were then placed in an incubator at 40° C. until conscious. All treatments received 1 ml. of combiotic (Pfizer) after the operation.

Treatment 3 received 1 mg of progesterone (Upjohn) in oil daily. The progesterone was injected subcutaneously each day from day 1 to day 13 of the experiment.

The animal laboratory was held at 26 ± .5° C. and 50 ± 1% relative humidity. On day 7 (after the operation) another weight was taken and a final weight was taken upon termination of the experiment on day 14.

Upon termination of the experiment the animals were put in deep anesthesia. While in anesthesia the left and right longissimus dorsi muscles,

from the anterior edge of the pelvic girdle to the 3rd rib were excised for analyses. The left longissimus dorsi was ground for expressible water determinations, sodium and potassium analysis. Intact sections of the right longissimus dorsi were taken for glycogen and glucose determinations and proximate analysis.

The samples for glycogen and glucose determinations were placed in a liquid nitrogen tank (- 300° C.) and held at (- 30° C.) until analysis. All analyses were reported on a fresh longissimus dorsi muscle tissue basis.

B. DETERMINATION OF EXPRESSIBLE MOISTURE

Expressible moisture was determined by a modification of the centrifuge method of Wierbicki et al. (1957). A 10 to 15 gm ground meat sample was placed in a polyethylene centrifuge tube, stoppered with a rubber stopper that was fitted with a capillary tube and placed in a 70° C. water bath for 30 minutes. The cooked meat sample was removed and placed in a crucible with a fritted glass bottom. The crucible was taped to the top of the polyethylene tube. The tubes were centrifuged at 3,000 RPM for five minutes and the expressed juice was poured off into a volumetric cylinder and the volume recorded. Water content of the juice was determined and results were recorded as percent expressible moisture. The crucibles were kept in a dessicator containing 60 gm of KCl and 100 ml H_2O for 12 hours before use in an effort to stabilize moisture in the fritted glass area.

C. GLYCOGEN DETERMINATION

Glycogen was determined by a modified Anthrone procedure (Hassid and Abraham, 1957). A 50-200 mg sample of tissue was weighed in centrifuge tubes to which 2 ml of potassium hydroxide had been added. The samples were digested in boiling water (100° C.) for 20 minutes, cooled in 4° C. water, and

2.5 ml of 25% ethanol was added. Tube contents were then brought to a boil, cooled, and centrifuged for 15 minutes at 3,000 RPM. After centrifugication, the supernatent was decanted and the tubes were drained on paper for 1 or 2 minutes. The glycogen was then dissolved in 10 ml of water.

Ten ml of anthrone reagent was added to colorimeter tubes containing 0.5 ml of the unknown. During the addition of the anthrone, tubes were submerged in a cold water bath (4° C.).

The tubes were then put in a boiling water bath for 10 minutes and marbles (which acted as a condenser) were placed on each individual tube. The tubes were then cooled and read at 620 mu on a Beckman DU-2 Spectrophotometer. Standards were made up by using 0.1 mg/ml of Nelsons standard. Results were read off the standard curve and reported as mg glycogen per gm of tissue weight. The Anthrone reagent contained 500 ml of 95% sulfuric acid, 1 gm anthrone and 5 gms of thiourea.

D. GLUCOSE DETERMINATION

Glucose was determined by a modified Nelson (1944) procedure using polyethylene centrifuge tubes. A 0.2-0.3 gm sample of tissue was added to 7.5 ml of water, mixed, and 1 ml of barium hydroxide was added. After additional mixing and the solution had turned brown, 1 ml of zinc sulfate was added. The solution was then centrifuged at 3,000 RPM for 10 minutes.

Into labeled Folin-Wu sugar tubes, 1 ml of the unknown and 1 ml of Nelsons reagent (Fisher) A & B were added. The tubes were then placed in actively boiling water for twenty minutes, cooled in a beaker and 2 ml of arsenomolybdate reagent was added. The solution was then diluted to 25 ml with distilled deionized water; stoppered and mixed.

The unknowns and standards were read at 520 mu on a Beckman DU-2

Spectrophotometer. Readings were recorded as optical density.

Standards that were made ranged from 0.02-0.1 mg glucose and each sample was read off the standard curve. Results were recorded as mg glucose per gm of tissue.

E. MOISTURE AND ETHER EXTRACT DETERMINATION

Total moisture and fat content were determined with a modified AOAC (1966) procedure. Extraction thimbles were dried at 100° C. under a vacuum of minus 15 lbs. inch^2, cooled in a dessicator and weighed. Approximately 2 gm samples were then weighed into each thimble to the nearest 0.1 mg.

Samples were run in triplicate and dried in a vacuum oven at 100° C. for sixteen hours, cooled in a dessicator, weighed and total water was expressed as a loss in weight. These same thimbles were then extracted for 12-16 hours in a Soxhlet extractor using petroleum ether and redried. The thimbles were then reweighed and the additional weight loss was expressed as percent ether extract.

F. PROTEIN DETERMINATION

Nitrogen was determined with the AOAC (1966) Kjeldahl nitrogen procedure. Nitrogen times 6.25 was considered to be percent protein.

G. ELECTROLYTE DETERMINATION

Sodium and potassium were determined by a modified Trichloroacetic Acid (T.C.A.) method (Kirton and Pearson, 1963). This method consisted of weighing 1.5 to 3.5 gms of tissue in a Virtis homogenizing bottle to which 75 ml of 2% T.C.A. had been added. The mixture was then homogenized for 2 minutes at the high setting, rinsed with 2 ml T.C.A. and poured into a tared polyethylene bottle.

The volume was taken to 100 gms and the sample was then stored at 5° C.

overnight. The homogenized samples were then centrifuged at 3,000 RPM for 5 minutes, the supernatent decanted and the sample diluted volumetrically (3:1) with deionized water.

Sodium and potassium determinations were made with a Beckman DU-2 Spectrophotometer equipped with a flame attachment. The wave length settings for sodium and potassium were 590 mu and 768 mu respectively.

The results were recorded as mg of electrolyte per 100 gms of sample weight.

H. STATISTICAL ANALYSES

Analysis of variance was used on each factor to determine if treatment differences existed. If a significant variance ratio was calculated, Duncans Multiple Range Test was used to determine where these differences existed. Since no differences existed between treatments, except in the case of muscle protein content, data was pooled for correlation analysis. Correlations of 0.433 and 0.549 are required for significance at the 0.05 and 0.01 percent levels with 19 degrees of freedom (Snedecor, 1962). Correlations were also calculated within each treatment group.

RESULTS AND DISCUSSION

A. TREATMENT DIFFERENCES

Of the 30 rabbits originally assigned to the experiment only 21 survived the 14 day period following surgery. Seven of the deaths occurred in Treatment 4 (hypophysectomy), while one death each occurred in Treatments 2 (ovariectomy) and 3 (ovariectomy + progesterone).

Analysis of variance was used to determine if significant differences existed between treatments (see Table I). No significant differences occurred in *longissimus dorsi* characteristics except for total protein which was significantly higher in Treatment 3 (ovariectomy + progesterone).

1. Expressible Moisture (Water Holding Capacity)

Although Treatment 3 (ovariectomy + progesterone) tended to have a higher expressible moisture average, no significant differences existed between treatments. The averages for the Treatments (1 to 4) were 30.6, 31.9, 32.4 and 30.9 percent respectively. Since two transfers of the expressed moisture were made, some of the moisture could have been lost. However, all 21 samples were run identically and this error should have been constant between samples.

2. Glycogen and Glucose

Average glycogen values were 0.125, 0.143, 0.152, and 0.084 mg/gm respectively for Treatments 1 through 4, which compares favorably with the values reported by Long (1960). Glucose averages were 0.091, 0.078, 0.079, and 0.064 mg/gm, respectively. Although no treatment differences were significant, a trend existed for lower glycogen and glucose values in treatment 4.

3. Total Moisture

No significant difference occurred between treatments. Values of 73.5, 74.5, 74.3 and 74.3 percent were found for Treatments 1 through 4, respectively.

4. Protein

Treatment 3 (ovariectomy + progesterone) had higher percent muscle protein, a contradiction to existing reports, since progesterone is supposedly catabolic whereas estrogens are anabolic (Turner, 1962). Also connective tissue protein was increased by the effects of estrogens and not progesterone (Rasmussen, 1967; Schmidt, 1958; Schiff, 1961).

5. Ether Extract

No significant difference existed in the percentage of ether extract between treatments. Average values of 2.4, 1.9, 1.4, and 1.8 percent were found respectively for Treatments 1 to 4.

Since Treatment 3 (ovariectomy + progesterone) had the highest percent protein, a lower fat percentage was expected and a trend existed in this direction.

6. Potassium and Sodium

Average values were 367.1, 374.4, 361.9, and 313.0 mg/100 gms wet tissue for potassium and 31.4, 26.3, 25.2, and 30.2 mg/100 gms wet tissue for sodium. Significant differences did not exist between treatments for either potassium or sodium level. Treatment 4 had the lowest apparent average concentration of potassium and the second highest concentration of sodium. This tends toward agreement with Edleman (1963) who reported that any process that interferes seriously with metabolism, such as cold, anoxia or the presence of metabolic inhibitors will accelerate the loss of potassium, usually with a gain of sodium.

TABLE 1

EFFECT OF OVARIECTOMY, OVARIECTOMY + PROGESTERONE INJECTION AND HYPOPHYSECTOMY UPON RABBIT LONGISSIMUS DORSI CHARACTERISTICS±

	Control (Laparotomy)	Ovariectomy	Ovariectomy + Procesterone	Hypophysectomy	F Value (Variance Ratio)
Expressible Moisture, % ±±	30.63 ± 2.02	31.88 ± 1.45	32.40 ± 0.75	30.90 ± 2.66	1.03
Glycogen, mg/gm	0.125	0.143	0.152	0.084	0.85
Glucose, mg/gm	0.091	0.078	0.079	0.064	1.71
Total Moisture, %	73.51 ± 0.70	74.54 ± 0.78	74.32 ± 0.50	74.32 ± 0.84	2.22
Protein (6.25 x N), %	23.13(b) ± 0.6	23.28(b) ± 0.3	24.06(a) ± 0.1	23.42(b) ± 0.5	3.93*
Ether Extract, %	2.42 ± 0.7	1.88 ± 0.7	1.42 ± 0.5	1.84 ± 0.6	2.11
Potassium, mg/100 gms	367.10 ± 28.4	374.38 ± 19.2	361.94 ± 49.0	313.04 ± 58.0	2.31
Sodium, mg/ 100 gms	31.38 ± 8.48	26.32 ± 6.13	25.18 ± 3.67	30.16 ± 9.46	0.87
Starting Weight, Kg.	3.36 ± 0.34	3.17 ± 0.45	3.34 ± 0.57	3.40 ± 0.41	0.27
14 Day Weight - Starting Weight, Kg.	- 0.07 ± 0.18	- 0.02 ± 0.18	0.09 ± 0.09	- 0.527 ± 0.86	1.88

* $P < .05$

(a), (b) = All lot means with identical or no superscript are not significantly different at the 5% level of probability.

± All muscle components are expressed on a fresh tissue basis.

±± Expressible moisture is % of total moisture expelled with Wierbicki centrifuge method.

The sodium content tended to be lower in Treatment 3. This parallels results of Caspo (1959) who reported that upon the administration of progesterone, there occurred a loss of sodium and chloride in uterine tissue. This, Caspo stated, was not a direct progesterone effect, but due to the inhibition of certain adrenal cortical hormones.

7. Body Weight

Fourteen day body weight changes were not significantly different (- 0.07, - 0.02, 0.09, and - 0.53 Kg for Treatments 1 to 4 respectively). Treatment 3 (ovariectomy + progesterone) was the only treatment with a mean weight gain during the experiment. Bourdel and Jacquat (1965) found that injections of 3 - 6 mg of progesterone to castrated female rats caused them to gain more weight in a 20 day period than control (normal) animals. Hervey and Hervey (1965) treated intact female rats with progesterone and produced an increase in body weight. These workers state the substance gained was lean tissue and fat. However, castrated rats of either sex did not show this response, which would be a contradiction to the results of the study reported in this thesis.

B. SIMPLE CORRELATION COEFFICIENTS (POOLED DATA)

Simple correlation coefficients were calculated between all longissimus dorsi characteristics measured on the 21 individual animals (See Table II). Correlations were also determined between muscle characteristics within each of the four treatments to determine if different relationships might exist in different treatments.

Since Treatment four (hypophysectomy) contained only one animal with complete stalk removal, correlation coefficients were also calculated on data collected from hypophysectomized rabbits with incomplete stalk removal. The

remaining animals in this group appeared to have residual pituitary tissue remaining at necropsy.

The animals used in this experiment were under varying degrees of "stress" and relationships calculated could conceivably differ with those in the literature. Literature dealing with relationships of muscle characteristics deals with intact, apparently normal animals.

When the animal populations was pooled, expressible moisture (reciprocal of water holding capacity) was significantly correlated with total moisture ($r = 0.51$), and highly significantly correlated with fat (ether extract) ($r = -0.55$), sodium ($r = -0.56$), and 14 day body weight changes ($r = 0.62$). This disagrees with Hamm (1960) who stated expressible moisture was "not correlated" to total moisture. Expressible moisture was not significantly correlated with either muscle glycogen or glucose. The expressible moisture versus protein correlation was negative and nonsignificant; this disagrees with Swift and Berman (1960) who reported a high negative correlation existed between expressible moisture and protein. Expressible moisture had a significant negative ($r = -0.55$) correlation with percent fat, which agrees with Wanderstock and Miller (1948), who reported as carcass intramuscular fat increases, expressible moisture decreases. Expressible moisture had a nonsignificant correlation ($r = 0.32$) with potassium. However, a significant negative correlation ($r = -0.56$) existed between expressible moisture and sodium. Hamm (1959) stated sodium had little effect on the binding ability of water by muscle. Expressible moisture had a significant positive correlation ($r = 0.62$) with 14 days body weight gain. Therefore, the more weight the animal gained during the experiment, the more expressible moisture weight found in the longissimus dorsi muscle; and conversely, the more weight lost, the less expressible moisture

TABLE II. CORRELATION COEFFICIENTS BETWEEN RABBIT LONGISSIMUS DORSI CHARACTERISTICS ON POOLED DATA

	Glycogen	Glucose	Total Moisture	Protein	Ether Extract	Potassium	Sodium	Starting Weight	14 Day Weight - Starting Weight
Expressible Moisture	- .15	- .28	.51*	- .30	- .55**	.32	- .56**	- .38	.62**
Glycogen		- .05	.03	.16	- .30	- .11	.19	.03	- .07
Glucose			- .23	- .00	.38	.05	.02	.43*	- .01
Total Moisture				.10	- .29	.17	- .09	- .24	.15
Protein					.03	- .10	- .09	.15	- .31
Ether Extract						.00	.34	.28	- .20
Potassium							- .10	- .52*	.52*
Sodium								- .05	- .46
Starting Weight									- .37

* $P < .05$

** $P < .01$

remained in this muscle.

Glycogen and glucose were not correlated significantly with any factor except for a small significant correlation (r = 0.43) between glucose and starting weight.

Low insignificant correlations were calculated for total moisture with protein (r = 0.10), fat (r = - 0.29), potassium (r = 0.17), and sodium (r = - 0.09). Usually stronger relationships of moisture to ether extract and protein exist (Brown et al., 1951).

Potassium had a significant positive correlation (r = 0.52), while sodium had a significant negative correlation (r = - 0.46) with 14 day body weight changes. Potassium was higher in animals that encountered less weight loss during the 14 day period while sodium content of muscles was higher for those rabbits exhibiting a greater weight loss.

C. CORRELATIONS COEFFICIENTS BETWEEN FACTORS WITHIN TREATMENTS

No significant correlations were found between expressible water and the nine variables within Treatments 1. (control), 2. (ovariectomy), 3. (ovariectomy + progesterone), or 4a (group 4 minus 1 rabbit with complete hypophysectomy) (See Table III). However, in Treatment 4 (hypophysectomy), significant correlations were calculated between expressible moisture and total moisture and a negative correlation (r = - 0.93) with protein. Hamm (1960) stated expressible moisture was not correlated with either total moisture or protein content of beef muscle. However, Hamm was reporting on "normal" intact animals, whereas this study deals with hypophysectomized animals that were severely "stressed".

Hypophysectomy, while eliminating the release of follicle stimulating hormone (FSH) and luteninizing hormone (LH) for ovarian function, also

eliminated the release of all the other hormones produced or stimulated by the pituitary. Since complete hypophysectomy very likely upset the body's physiological balance, compositional changes may have taken place. The significant correlations that were reported for Treatment 4 may also have been caused by the high variation that occurred within this treatment.

The significant negative correlation between expressible water and protein ($r = 0.93$) in Treatment 4 (hypophysectomy) agreed with Swift and Berman (1960). Conceivably more protein per cent helps to bind water more effectively. However, this relationship could be clouded by such factors as stereo effect (amount of space available for water) and also the contractile state of the protein.

Expressible water had a significant negative correlation ($r = - 0.92$) with sodium in this one treatment. Also a significant positive correlation ($r = 0.93$) existed between expressible water and 14 day body weight changes in Treatment 4. Expressible moisture was not significantly correlated with glucose, glycogen, fat or potassium within any of the treatments.

In Treatment 4 glycogen had a significant negative correlation ($r = - 0.94$) with potassium and a significant positive correlation ($r = 0.94$) with starting weight. Glycogen was not significantly correlated with glucose, total moisture, protein, fat, sodium, starting weight or 14 day body weight changes as correlations varied considerably between treatment groups.

In Treatment 4 (hypophysectomy) glucose was positively correlated ($r = 0.92$) with fat (ether extract). Glucose was not significantly correlated with any other factor, as inconsistent trends occurred between treatments for its relationship to total moisture, protein, potassium and sodium.

Total moisture was negatively correlated with protein ($r = - 0.92$) and

sodium ($r = -0.96$) in Treatment 4. No other significant correlations existed between variables within any other treatment. It is of interest that expressible water was also negatively correlated with protein and sodium in Treatment 4 (hypophysectomy).

Protein was positively correlated ($r = 0.88$) with fat (ether extract) in Treatment 1 (control). The correlations between protein and the other factors in all treatments were inconsistent and no definite trend seemed to exist. The small numbers within groups may have been a contributing factor to the inconsistency within treatments.

Fat (ether extract) was positively correlated ($r = 0.90$) with starting weight in Treatment 3 (ovariectomy and progesterone). Fat content was not significantly correlated with either potassium or sodium within any treatment.

Potassium and sodium were not correlated with each other or with either weight factor within treatments.

Of the 14 significant correlations within treatments, 10 correlations existed in Treatment 4 (hypophysectomy). The definite trend for significance within Treatment 4 could have been due to either the variability within the treatment or to the fact the numbers within treatments were small.

TABLE III. CORRELATION COEFFICIENTS BETWEEN RABBIT LONGISSIMUS DORSI CHARACTERISTICS AND LIVE WEIGHT WITHIN TREATMENT BASIS

	Glycogen	Glucose	Total Moisture	Protein	Ether Extract	Potassium	Sodium	Starting Weight	14 Day Body Weight Changes
Expressible Moisture									
1	- .66	.16	.66	- .33	- .12	.17	- .07	- .09	.04
2	.27	- .48	.64	.22	- .75	.59	.10	- .22	- .28
3	.38	- .27	.73	.76	- .33	- .18	- .72	- .64	.32
4	- .72	- .75	.97**	- .93*	- .67	.60	- .92*	- .68	.93*
4A	.40	- .56	.86	- .71	- .81	- .21	- .89	.81	.39
Glycogen									
1		- .07	.12	.06	- .24	- .07	.72	- .48	- .46
2		- .36	.43	- .35	- .70	- .46	- .16	.08	- .01
3		- .14	.86	.18	- .35	.37	.33	- .64	.64
4		.28	- .76	.66	.20	- .94*	.64	.94*	- .60
4A		- .59	- .11	- .32	- .41	- .92	- .07	.82	.71
Glucose									
1			.02	- .49	- .32	- .11	.31	.22	.65
2			- .70	.67	.34	- .44	- .78	.71	- .50
3			- .44	.24	.80	- .49	.08	.69	- .84
4			- .68	.53	.92*	- .03	.80	.34	- .60
4A			- .25	- .10	.91	.74	.60	- .51	.04
Total Moisture									
1				- .36	- .39	.14	.58	- .63	- .48
2				- .48	- .77	.55	.20	- .76	.50
3				.31	- .48	.20	- .12	- .80	.74
4				- .92*	- .68	.70	- .96**	- .65	.84
4A				- .63	- .63	.29	- .92	.44	.06
Protein									
1					.88*	.77	- .04	.23	- .32
2					.02	.01	- .57	.69	- .88*
3					- .14	- .14	- .57	- .36	- .11
4					.51	- .61	.82	.51	.95*
4A					.17	- .07	.40	- .75	- .78

TABLE III. Continued

	Glycogen	Glucose	Total Moisture	Protein	Ether Extract	Potassium	Sodium	Starting Weight	14 Day Body Weight Changes
Ether Extract									
1						.69	- .20	.18	.07
2						- .17	.30	.18	.08
3						- .81	- .09	.90*	- .86
4						- .04	.85	.14	- .45
4A						.46	.88	- .58	.03
Potassium									
1							.31	.17	- .34
2							.43	- .70	.26
3							.63	- .62	.69
4							- .54	- .80	.51
4A							.00	- .56	- .39
Sodium									
1								- .53	- .37
2								- .57	- .46
3								- .05	.22
4								.52	- .73
4A								- .48	.07
Starting Weight									
1									.32
2									- .85
3									- .89*
4									- .61
4A									.79

* $P < .05$
** $P < .01$

SUMMARY

In this study, thirty, four month old estrous female rabbits of the New Zealand breed were randomly alloted into four treatment groups. The treatments were control (laparotomized) Treatment 1, (ovariectomized) Treatment 2, (ovariectomized + exogenous progesterone) Treatment 3, and (hypophysectomized) Treatment 4.

Upon necropsy the left and right longissimus dorsi muscles were excised to obtain expressible water, glycogen, glucose, total moisture, protein, ether extract, potassium and sodium values. Simple correlation coefficients and analyses of variance were calculated to see if treatment differences existed between these characteristics.

One significant difference was found when using analysis of variance to determine treatment differences. Per cent longissimus dorsi protein was significantly higher in Treatment 3 (ovariectomy + progesterone). All other factors between treatments were non-significant.

Correlation coefficients on the pooled population showed expressible moisture to have a significant positive correlation with total moisture, and a significant negative correlation with fat and sodium. Glucose had a significant positive correlation with starting weight and potassium had a significant negative correlation with starting weight and a significant positive correlation with 14 day weight minus starting weight.

When calculating correlation coefficients on longissimus dorsi characteristics to determine differences within treatments, 10 of the 14 significant differences were found in Treatment 4 (hypophysectomy). This may have been due to either the variation within treatments or small numbers used in each treatment.

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THE EFFECTS OF OVARIECTOMY AND HYPOPHYSECTOMY ON THE PHYSICAL CHARACTERISTICS OF RABBIT MUSCLE TISSUE

by

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Water holding capacity of muscle is an economically important trait to all segments of the livestock and meat industry. In this study, wide variations in estrogens and progesterone were studied to determine effects on skeletal muscle water holding capacity and related characteristics.

Thirty, estrous female rabbits were randomly alloted into four treatment groups. The treatments were Lot 1 (control, laparotomy), Lot 2 (ovariectomy), Lot 3 (ovariectomy + exogenous progesterone injected subcutaneously at the rate of 1 mg/Kg), and Lot 4 (hypophysectomy by parapharyngeal approach). Twelve rabbits were assigned to Treatment 4 because of the high mortality rate expected; 6 rabbits each were used in the other treatments. Animals were sacrificed 14 days after surgery.

Longissimus dorsi muscle was excised immediately post mortem and frozen with liquid nitrogen. Samples were analyzed for moisture, ether extract, protein, glucose, glycogen, sodium and potassium. Fresh longissimus dorsi muscle was used to determine expressible moisture by centrifuge method.

Analysis of variance was used to test for differences between treatments. Correlation coefficients were calculated on the pooled population and within each treatment group. The only significant treatment effect was a higher longissimus dorsi protein percentage in the ovariectomized group which received exogenous progesterone.

When using pooled populations from all treatments, correlation coefficients between rabbit longissimus dorsi muscle characteristics, showed expressible water to be highly significantly related with ether extract ($r = -.55$), sodium ($r = -.56$), body weight change ($r = .62$) and significantly correlated with total moisture ($r = .51$). Significance was not observed for relationships between the other muscle characteristics.

When correlation coefficients were calculated within treatment groups, results were inconsistent. Of the 14 significant correlation coefficients 10 occurred in Treatment 4 (hypophysectomy), due to the greater variation in the muscle characteristics studied.