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To the Graduate Council:

I am submitting herewith a thesis written by Robert Y. T. Tsai entitled "The Effect of Fasting on Tissue Shrinkage and Some of Its Mechanisms in Rats." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

Major Professor

We have read this thesis and recommend its acceptance:

Accepted for the Council:

Vice President for
Graduate Studies and Research

THE EFFECT OF FASTING ON TISSUE SHRINKAGE AND SOME OF ITS MECHANISMS IN RATS

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Robert Y. T. Tsai

December 1967

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Robert Y. T. Tsai

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CHAPTER I

INTRODUCTION

Ante-mortem fasting long has been recognized as a sound practice for meat animals. However, the length of a feasible fasting time is not well established. There are two advantages of fasting meat animals before slaughter. First, it is more difficult to thoroughly bleed and eviscerate the animal if it has been fed, watered and/or severely excited immediately prior to slaughter. In addition, fiery fat and dark-colored muscle may occur. Secondly, from a monetary standpoint, annual feed consumption, particularly in large meat packing companies, may be quite costly in preslaughter periods. In fact, the expenditure for feed and labor totals millions of dollars in the United States. A feasible fasting time, which would not produce tissue shrinkage or serious loss of meat quality, should be determined.

Laboratory animals are desirable subjects in many biochemical, physiological and biological experiments. The response of laboratory animals to fasting regimes could yield useful and desirable information. Whether such information can be transposed to meat animal species should be investigated.

Two kinds of shrinkage (excretory and tissue) occur during the fasting period. Excretory shrinkage does not affect the yield of edible meat, but it is important to investigate tissue shrinkage, as it does affect yields. The two most important tissues in the meat animal

carcass are fat and muscle. Fat tissue is considered to be of minimum economic importance in the United States, and is only important insofar as it affects eating quality and cutability. Knowledge of muscle shrinkage during the fasting period is far more essential because of the possitive relation of muscle yield to the total value of the carcass.

The main objectives of this experiment were to determine the length of fasting time necessary to effect tissue shrinkage and to determine which tissues were affected most by fasting.

CHAPTER II

REVIEW OF LITERATURE

Extensive research has been done on the physiology, biology and biochemistry of fasting effects on laboratory animals. This review will deal primarily with the effects of fasting on tissue shrinkage and some of their physiological, biological and biochemical reactions in rats and meat animals.

Pomeroy (1941) reared five pig littermates to a weight of 330 pounds. One hog then was killed and the other four were fed a submaintenance ration of straw and water. The four hogs were killed at approximately equal weight intervals from 330 pounds to the reduced weight of 200 pounds. He found that tissues were affected in inverse order of their development. Fat was used as an energy source before muscle and bone. The fatty tissue affected first was kidney fat, followed by subcutaneous fat and then early developing caul, mesenteric and intermuscular fat. Shrinkage of muscle tissue was followed by bone shrinkage which was affected the least.

Bowland and Standish (1966), using 72 market weight pigs, found that withholding feed for 24 hours resulted in a decreased dressing percentage (77.6 percent) as compared to the controls (78.6 percent). This was an apparent carcass shrink of 0.9 kg. Withholding feed for 48 hours resulted in a further reduction in yield to 75.5 percent or an apparent carcass shrink of 2.8 kg.

Ingram (1964) reported losses of 7 percent in carcass weight for animals fasted for 72 hours. A tendency for average backfat thickness to be reduced with fasting was found. This reduction was significant after 48 hours of fasting. Carcasses from fasted pigs had 3.34 cm. of average backfat thickness as compared to 3.66 cm. for nonfasted pigs.

Saffle and Cole (1960), using 84 hogs with an average off-feed weight of 217 pounds, found that one-half the total live shrinkage occurred during the first 24 hours of fasting, and only 12 percent of the total shrinkage occurred during the last 48 hours of fasting. An average total shrinkage of 8.37 percent occurred after 96 hours of fasting. No significant differences among fasting periods were found in percent wholesale cuts; however, highly significant differences were found among litters for percent yield of the wholesale cuts. Thus, carcass cut out, based on off-feed weight, was affected more by type and breed differences than by the length of the fasting period.

Bowland and Standish (1966) reported that, when food or water was withheld from 104 rats prior to slaughter, liveweight shrinkage and carcass shrinkage were greater on a percentage basis for rats than for pigs. Weight of the gastrointestinal tract and its contents decreased progressively from 22.8 to 11.5 gm. as food and/or water were withheld for 24 hours, or food was withheld for 48 hours. This loss of weight represented 56 to 72 percent of the total weight loss of the live animals during this period. Liver weight also decreased progressively from 9.2 to 5.7 gm. as food and water were withheld for longer periods.

Rixon and Stevenson (1956) used male Sprague-Dawley rats varying in weight from 200 to 450 gm. and found that the individual duration of survival of rats in complete fasting varied considerably. The range at an environmental temperature of 22°C. was 6-16 days; at 2-5°C., 1-7 days; and in thyroidectomized animals at 22°C., 15-25 days. This variation in survival time was not closely related to the initial body weight but was related to the individual proportionate weight loss per day. The daily mean rate of weight loss was 4.0 gm./100 gm. of prefasting weight at a temperature of 22°C. The thyroidectomized rats lost body weight at a daily rate of only 2.0 gm./100 gm. at 22°C. The cold environment increased the rate of weight loss to 8.8 gm./100 gm.

Marilyn and Victor (1957), using ground squirrels, found that liver glycogen increased 45 percent ($P < .01$) during a three-day fasting period, whereas, the glycogen of skeletal and cardiac muscle decreased ($P < .10$) 67 percent and 31 percent, respectively. The glycogen of skeletal muscle remained significantly below the control.

Rose and Peterson (1951) reported that reserve carbohydrate in both muscle and liver of rats was depleted somewhat by starvation and seriously depleted by exercise. Maximum depletion resulted from a combination of the above.

Wilcox et al. (1953) studied five swine which were fasted for 16 hours before slaughter and reported an average liver weight of 3.0 pounds. They found that the liver had an average of 0.86 percent glycogen while the muscle contained only 0.18 percent.

Wool and Krahl (1959), using male albino rats of the Sprague-Dawley strain weighing 110 to 130 gm., studied incorporation of C^{14} histidine into protein of isolated diaphragms. They found that a fasting period of either 12 or 24 hours led to a small but definite decrease in histidine-2-C^{14} incorporation into muscle protein. In neither the 12- nor 24-hour fasted group was histidine incorporation influenced by the glucose concentration in the medium. Diaphragms isolated from rats, which have been fasted 48 hours, showed a markedly depressed rate of incorporation of histidine-2-C^{14} into muscle protein which was significantly modified when a sufficiency of glucose was present in the incubation medium.

Bernhard and Schoenheimer (1940) used stable bound deuterium in fatty acids of animals as an indicator of fat formation. They found that the deuterium content of the depot fats was lowest while those from the intestinal wall and kidney were intermediate between liver and depot fats. Thus, fat formation in liver was more rapid than in the other organs.

Caspersson and Bracket (1950), who worked independently and with different histochemical techniques, demonstrated that RNA is particularly abundant in cells engaged in the synthesis of protein either for growth or secretion. The observations have been repeatedly confirmed. Thus, RNA is abundant in embryonic tissues, tumors, rapidly growing bacterial cultures and exocrine cells of the pancreas.

Davidson (1960) in quoting from the work of Hevesy and collaborators (1951) revealed negligible incorporation of P^{32} labeled adenine into the

nuclei of nondividing cells. Subsequent work with C^{14} formate as a precursor has made it clear that the resting rat or rabbit liver *in vivo* or *in vitro* incorporates only negligible amounts of the isotope into DNA purines (Sibatani, 1957). Brues *et al*. (1944), using the hepatectomy technique in rat liver, found that P^{32} and C^{14} adenine incorporated into DNA during the regeneration period are retained over long periods of time in a manner which suggests pronounced biochemical stability. The balance of evidence at present suggests that, although DNA may not be metabolically completely inert, it is much more stable than other components of the cell.

CHAPTER III

EXPERIMENTAL PROCEDURE

Forty-two young, male, white rats were used in this experiment. They were castrated at the age of three weeks and were provided a standard commercial feed plus water ad libitum until the beginning of the experiment. The rats were weighed at eight weeks of age and divided into seven groups of six each by using the stratification sampling method. The total weight of each group of six ranged from 958 to 961 gm.

The experiment started two weeks after initial grouping. Each of the seven groups was assigned to a treatment by complete random sampling techniques. Group 1 was the control and was slaughtered immediately. Groups 3, 4 and 6 were deprived of feed but were fed water ad libitum for 24, 48 and 72 hours, respectively, and then slaughtered. Groups 2, 5 and 7 received feed and water for 24, 48 and 72 hours, respectively, before they were slaughtered.

The carcass of each rat and many of its organs were weighed to the nearest 0.01 gm. The following organs and tissues were weighed: unskinned head (removed at the atlas joint), skin plus most of the subcutaneous fatty tissue and tail, thymus gland, lungs, heart, liver, gastrointestinal (G.I.) tract, spleen, kidneys and empty warm carcass.

The carcass of each rat was boned and all muscles were removed and ground thoroughly with a hand grinder. An aliquot of the freshly

ground muscle was weighed and processed for the extraction of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

DNA and RNA were separated by a modified Schmidt-Thannhauser procedure (Schmidt and Thannhauser, 1945), and individual fractions were assayed for the respective substances. DNA was determined by Burton's modification of the diphenylamine reaction (Burton, 1956), and RNA was determined by the orcinol reaction (Dische, 1955). Protein was determined by a macro-Kjeldahl nitrogen determination. Ether extract and moisture content were determined by methods recommended by the A.O.A.C. (1960). Analyses was performed in duplicate.

Method for calculating tissue shrinkage. Since the amount of DNA supposedly is constant within a single diploid cell in any species (Boivin, 1948), the number of diploid cells in a unit weight of muscle can be calculated. This can be accomplished by determining the amount of DNA per unit of muscle and dividing by the amount of DNA per cell (6.2 μμg. in the rat; Enesco and Leblond, 1962).

After the approximate number of cells is ascertained, the weight of protein per cell can be calculated. A comparison of the amount of protein per cell in animals that have been fasted with that in control animals may reveal a shrinkage in muscle cell size. This is based on the assumption that animals will have been fasted long enough to use all readily available energy such as glucose, glycogen and fat.

Analysis of the data was conducted according to the method of Snedecor (1956). Duncan's Multiple Range Test was used to test the

differences between means when a significant effect was observed in the main factor.

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CHAPTER IV

RESULT AND DISCUSSION

Forty-two rats, randomly divided into seven treatment groups of uniform total weight, were used in this experiment. As shown in Tables I and II, whole body weight was not significantly increased by feeding for 72 hours. However, empty warm carcass, skin (with most of the subcutaneous fat and the tail) and liver significantly ($P < .05$) increased in weight as the rats continued to receive feed. Based on off-feed weight of the 0 hour group, the percent increase in weight of warm carcass was 4.66; skin, 2.37; and liver, 0.96 during the 72-hour feeding period. There was no significant change in the other seven components studied, although the G.I. tract, heart, lungs and kidneys tended to increase in weight with each 24-hour feeding period.

One of the objectives of this study was to determine the source of energy during fasting periods. As shown in Tables III and IV, all of the body components studied except lungs showed significant shrinkage during fasting. A significant loss in weight of the warm carcass, spleen, heart and thymus did not occur until the rats had been fasted 48 hours. However, skin, head, G.I. tract, liver and kidneys were significantly affected in only 24 hours. The loss of weight in these components probably was due to loss of adipose tissue with the exception of the G.I. tract. The shrinkage of the G.I. tract was essentially due to the disappearance of feed. Of the total shrinkage in liver, skin

TABLE I

MEANS FOR THE EFFECTS OF FEEDING ON THE WEIGHT OF SELECTED BODY COMPONENTS

Body Component	Length of Feeding Period, Hr.			
	0	24	48	72
	gm.	gm.	gm.	gm.
Whole body				
At 0 hr.	202.20	199.50	203.00	202.50
At slaughter	202.20	206.00	215.16	220.00
Warm carcass	83.33	87.10	90.12	92.89
Skin, subcutaneous fat and tail	45.86	46.37	50.21	50.74
Head	18.29	18.93	18.41	18.70
G.I. tract	24.21	24.02	24.88	26.53
Liver	10.00	10.52	11.45	11.96
Spleen	0.64	0.60	0.67	0.67
Heart	0.72	0.79	0.82	0.88
Lungs	1.32	1.33	1.41	1.26
Kidneys	1.84	1.96	2.03	2.06
Thymus	0.93	1.03	0.92	1.02
Blood	5.57	6.35	7.65	7.57
Loss during slaughter	9.49	7.00	6.59	5.12

TABLE II

MEANS FOR THE EFFECTS OF FEEDING ON THE YIELD OF SELECTED BODY COMPONENTS[a]

Body Component	Length of Feeding Period, Hr.			
	0	24	48	72
	%	%	%	%
Whole body	100.00	103.25	105.99	108.64
Warm carcass	41.22[b]	43.66[c]	44.40[c,d]	45.88[d]
Skin, subcutaneous fat and tail	22.69[b]	23.24[b]	24.73[c]	25.06[c]
Head	9.05	9.49	9.07	9.24
G.I. tract	11.98	12.49	12.25	13.10
Liver	4.95[b]	5.28[c]	5.64[c,d]	5.91[d]
Spleen	0.32	0.30	0.33	0.33
Heart	0.36	0.39	0.40	0.43
Lungs	0.66	0.67	0.69	0.72
Kidneys	0.91	0.98	1.00	1.02
Thymus	0.46	0.52	0.45	0.51
Blood[e]	2.75	3.18	3.76	3.73
Loss during slaughter[e]	4.68	3.51	3.24	2.52

[a]Calculated as percent of the whole body weight of the 0-hr. group. N = 6 rats per treatment.

[b,c,d]Means on the same line bearing different superscripts differ significantly ($P < .05$). Means with the same or no superscripts do not differ significantly.

[e]Not statistically analyzed.

TABLE III

MEANS FOR THE EFFECTS OF FASTING ON THE WEIGHT OF SELECTED BODY COMPONENTS

Body Component	Length of Fasting Period, Hr.			
	0	24	48	72
	gm.	gm.	gm.	gm.
Whole body				
At 0 hr.	202.20	203.66	201.00	201.50
At slaughter	202.20	182.16	167.16	159.16
Warm carcass	83.33	82.95	78.47	75.73
Skin, subcutaneous fat and tail	45.86	43.83	39.69	37.84
Head	18.29	17.67	16.48	15.86
G.I. tract	24.21	13.64	11.57	9.91
Liver	10.00	6.94	5.65	5.12
Spleen	0.64	0.57	0.44	0.42
Heart	0.72	0.76	0.71	0.65
Lungs	1.32	1.27	1.04	1.16
Kidneys	1.84	1.75	1.53	1.43
Thymus	0.93	0.96	0.76	0.71
Blood	5.57	6.80	5.44	5.01
Loss during slaughter	9.49	4.94	5.38	5.32

TABLE IV

MEANS FOR THE EFFECTS OF FASTING ON THE YIELD OF SELECTED BODY COMPONENTS[a]

Body Component	Length of Fasting Period, Hr.			
	0	24	48	72
	%	%	%	%
Whole body	100.00[b]	89.44[c]	83.16[d]	78.98[d]
Warm carcass	41.22[b]	40.73[b]	39.04[c]	37.59[c]
Skin, subcutaneous fat and tail	22.69[b]	21.52[c]	19.75[d]	18.77[e]
Head	9.05[b]	8.68[c]	8.20[d]	7.87[d]
G.I. tract	11.98[b]	6.70[c]	5.76[c]	4.92[d]
Liver	4.95[b]	3.41[c]	2.81[d]	2.54[d]
Spleen	0.32[b]	0.28[b]	0.22[c]	0.21[c]
Heart	0.39[b]	0.38[b,c]	0.35[c,d]	0.33[d]
Lungs	0.66	0.62	0.52	0.58
Kidneys	0.91[b]	0.86[c]	0.77[d]	0.71[d]
Thymus	0.48[b]	0.48[b]	0.38[c]	0.35[c]
Blood[f]	2.75	3.33	2.71	2.48
Loss during slaughter[f]	4.68	2.71	3.48	3.34

[a]Calculated as percent of the whole body weight of the 0-hr. group. N = 6 rats per treatment.

[b,c,d,e]Means on the same line bearing different superscripts differ significantly ($P < .05$). Means with the same or no superscripts do not differ significantly.

[f]Not statistically analyzed.

and warm carcass about 63.90 percent, 29.84 percent and 13.49 percent, respectively, occurred in 24 hours. During the second 24 hours of fasting, about 24.89 percent, 45.15 percent and 45.55 percent of the total shrinkage occurred in liver, skin and warm carcass weight, respectively. During the last 24-hour fasting period the shrinkage was less. The liver, skin and warm carcass shrank 11.20 percent, 25.12 percent and 39.96 percent of the total shrinkage, respectively. These results are graphically depicted in Figure 1.

Table V shows the percent shrinkage each trait contributed to the total shrinkage. After 24 hours, the G.I. tract contributed 50.86 percent, liver 14.83 percent, skin 11.27 percent, carcass 4.72 percent and unskinned head 3.56 percent to make up most of the total loss. At the end of 48 hours of fasting, G.I. tract decreased from 50.86 to 38.65 percent and the liver from 14.83 to 13.30 percent, but the carcass increased from 4.72 percent to 13.54 percent, skin increased from 11.27 percent to 18.27 percent, and the head increased from 3.56 percent to 5.28 percent. After the 72-hour fasting period, tissue shrinkage of the empty carcass and skin (with subcutaneous fatty tissue) were almost equal, 17.80 percent and 19.22 percent, respectively. It would appear that the maximum fasting time for rats without serious tissue loss is 24 hours.

Bowland and Standish (1966) reported that, when feed and water were withheld from rats prior to slaughter, liveweight shrink and carcass shrink were greater on a percent basis for rats than pigs. Saffle and Cole (1960) reported no significant difference between dressing

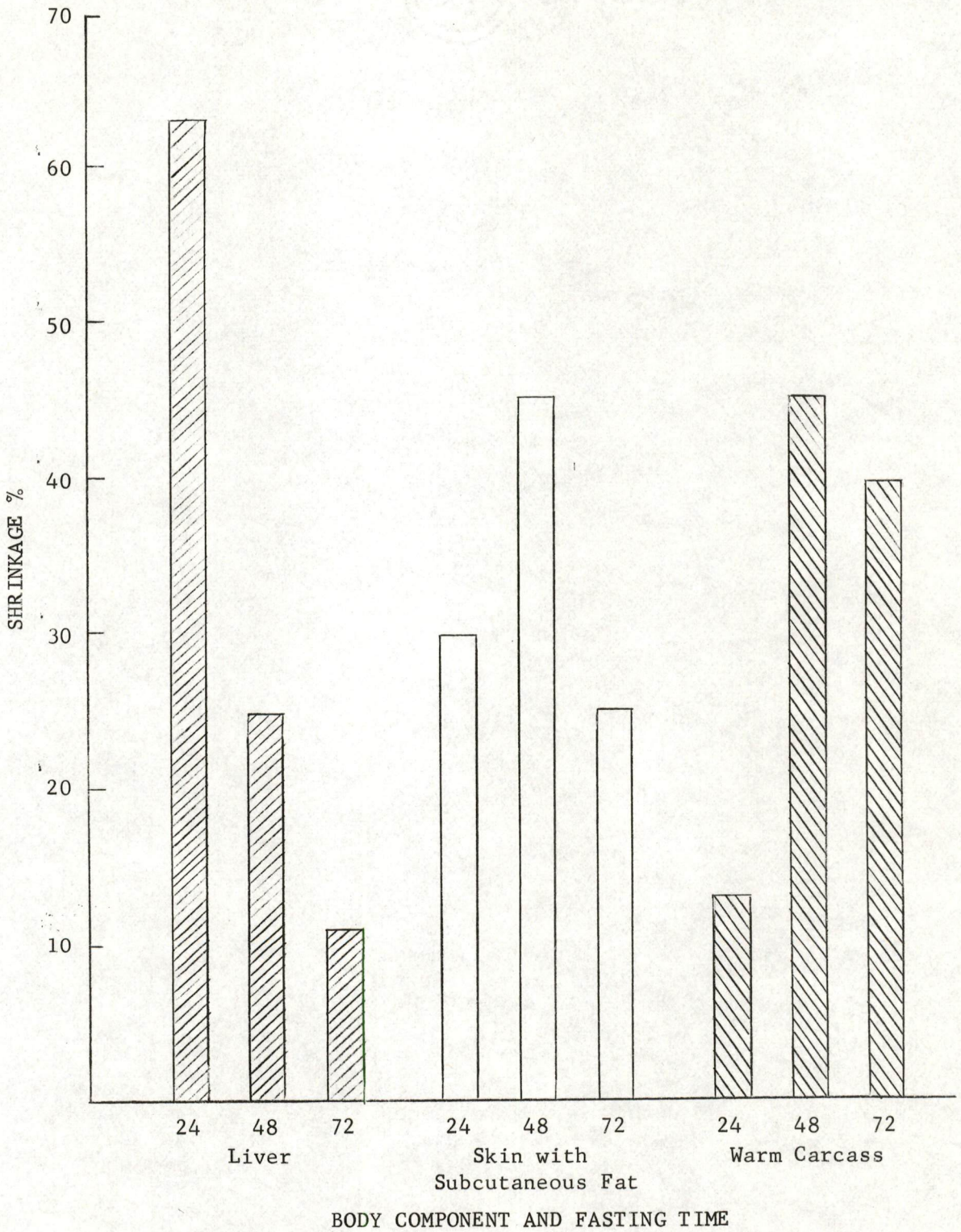


Figure 1. Effect of fasting time on percent of shrinkage of liver, skin (with subcutaneous fat) and warm carcass weight.

TABLE V

MEANS FOR THE SHRINKAGE DURING FASTING OF EACH BODY COMPONENT AS A PERCENT OF TOTAL SHRINKAGE

Body Component	Length of Fasting Period, Hr.			
	0	24	48	72
	%	%	%	%
Warm carcass	0	4.72	13.54	17.80
Skin, subcutaneous fat and tail	0	11.27	18.27	19.22
Head	0	3.56	5.28	5.78
G.I. tract	0	50.86	38.65	34.62
Liver	0	14.83	13.30	11.81
Spleen	0	0.38	0.62	0.54
Heart	0	0.09	0.25	0.29
Lungs	0	0.38	0.87	0.39
Kidneys	0	0.48	0.87	0.98
Thymus	0	0	0.62	0.64
Blood	0	-5.58[a]	0.25	1.32
Loss during slaughter	0	18.97[b]	7.45	6.57

[a]Negative value was due to an increase in blood weight after 24 hours of fasting.

[b]Relatively large percent loss during slaughter was due to the small amount of total shrinkage.

percentage and shrinkage time in pigs. Rixon and Stevenson (1956) reported that the individual proportionate rate of weight loss was correlated with the metabolic rate. The rat has much more body surface area exposed per unit of body weight than the pig. Therefore, rats tend to have a higher metabolic rate than pigs. It is doubtful if the shrinkage data obtained from rats should be transposed to pigs without some modification.

In this study liver weight was highly affected in the first 24 hours of fasting, which supports the findings of similar research with swine. Saffle and Cole (1960) reported that livers of swine were significantly affected ($P < .01$) by fasting. Ingrahm (1948), Wilcox et al. (1953), Rose and Peterson (1951), Callow (1938, 1939) and Bate-Smith (1938, 1948) found the liver to be a storehouse of glycogen which became a primary energy source when animals were exercised or fasted. Marily and Victor (1957) reported that liver glycogen increased during fasting, as compared to the fed controls, apparently as a result of glycolysis in both skeletal and cardiac muscle. They stated that, during the three-day fasting period,, glycogen in the liver increased 45 percent ($P < .01$); whereas, skeletal ($P < .01$) and cardiac muscle ($P < .1$) decreased 67 percent and 13 percent, respectively, in glycogen content. All of the liver shrinkage probably was not due to depletion of glycogen. Glycogenesis occurs both from lactic acid by glycolysis in skeletal and cardiac muscle and by glyconeogenesis, primarily from fat but possibly from protein as indicated by loss in body weight.

As shown in Tables IV and V, pages 15 and 18, the weight of blood lost at slaughter increased after the first 24 hours of fasting as compared to the control group. This may be due to a more active circulatory system of the control rats caused by a higher assimilation rate. The blood in smaller capillaries fails to be pumped out before the rat expires. Following 48 and 72 hours of fasting, the weight of blood decreased gradually as a possible result of the lowered amount of nutrients necessary to maintain and/or increase the total blood supply.

The effects of delayed slaughter on the moisture content of the liver, spleen and muscle are shown in Table VI. The moisture content of spleen and muscle showed no significant decrease with increased fasting or feeding. Liver showed a significant decrease in moisture after 72 hours of fasting. This was a contributing factor to the total tissue shrinkage of liver as the fasting periods were extended.

Table VII shows that about two-thirds of the fat loss in the liver occurred in the first 24 hours of fasting. Bernhard and Schoenheimer (1940) reported that the rate of fatty acid regeneration in liver was much faster than in the fat tissue. They found that the half life of the average saturated fatty acid mole in the liver was very short, probably about one day. Therefore, the rapid shrinkage of liver probably was due partially to the depletion of glycogen as well as a fast fatty acid turnover.

Following 48-hour and 72-hour fasting periods, visual observation indicated that the main shrinkage of the G.I. tract was due to loss in weight of caul and mesenteric fat. Pomeroy (1941) reported that fat was

TABLE VI

THE EFFECT OF SLAUGHTER TIME WITH AND WITHOUT FEED ON PERCENT MOISTURE OF THE LIVER, SPLEEN AND MUSCLE

Item	Slaughter Period, Hr.			
	0	24	48	72
	%	%	%	%
Liver				
Fed	65.62	65.53	65.55	66.65
Fasted	65.62[a]	65.22[a]	66.41[a]	63.17[b]
Spleen				
Fed	76.66	75.63	76.81	76.86
Fasted	76.66	76.92	75.50	75.65
Muscle				
Fed	74.70	74.50	73.84	73.84
Fasted	74.70	74.89	74.13	73.80

[a,b]Means on the same line bearing different superscripts differ significantly ($P < .05$). Means with the same or no superscripts do not differ significantly.

TABLE VII

THE EFFECT OF SLAUGHTER TIME WITH AND WITHOUT FEED ON YIELD OF ETHER EXTRACT FROM LIVER AND MUSCLE

Item	Slaughter Period, Hr.			
	0	24	48	72
Liver				
Fed--wet basis,[a] mg.	189.40	152.76	166.83	140.06
Fed--dry basis,[e] %	55.05	42.09	42.26	35.02
Fasted--wet basis,[a] mg.	189.40[c]	65.81[d]	40.65[d]	51.04[d]
Fasted--dry basis,[e] %	55.05	27.26	21.40	27.04
Muscle,[b] %				
Fed	2.92	2.93	3.02	3.09
Fasted	2.92[c]	2.53[c]	1.62[c]	1.33[d]

[a]Total extracted from the entire liver.

[b]Based on total muscle weight.

[c,d]Means on the same line bearing different superscripts differ significantly ($P < .05$). Means with the same or no superscripts do not differ significantly.

[e]Not statistically analyzed.

used as an energy source before muscle and bone. He further observed that the tissue affected first was kidney fat; next subcutaneous fat, then early developing caul, mesenteric and intramuscular fat. The muscle tissue was next followed by bone, which was affected the least. As Figure 2 shows, the rate of liveweight gain increased proportionally with length of feeding time. The fasting effects on weight loss tended to be more severe during the first 24 hours of fasting and tended to slow down as the fasting time was extended.

The mechanism of fasting appeared to be getting more complicated as the length of fasting period increased. Up to 48 hours of fasting, most of the selected carcass traits seemed to shrink proportionally (Tables IV and V, pages 15 and 18). The loss in empty carcass weight was greatest after the 48-hour period. Wool and Krahl (1959) reported that diaphragms isolated from donor rats, which have been fasted 48 hours, showed a markedly depressed rate of incorporation of histidine-2-C^{14} into muscle protein, which was significantly modified when a sufficient amount of glucose was present in the incubation medium. They gave a possible explanation for glucose requirement after fasting. Glucose may favor peptide synthesis solely because of its ability to serve as an energy source, or it is possible that the energy available from glycogen and nonglucose sources may be insufficient for, or improperly coupled to, peptide synthesis. The initial metabolic products of glucose may be required to form some critical intermediate, such as one of the nucleotides, polynucleotides or nucleic acids now known to participate in

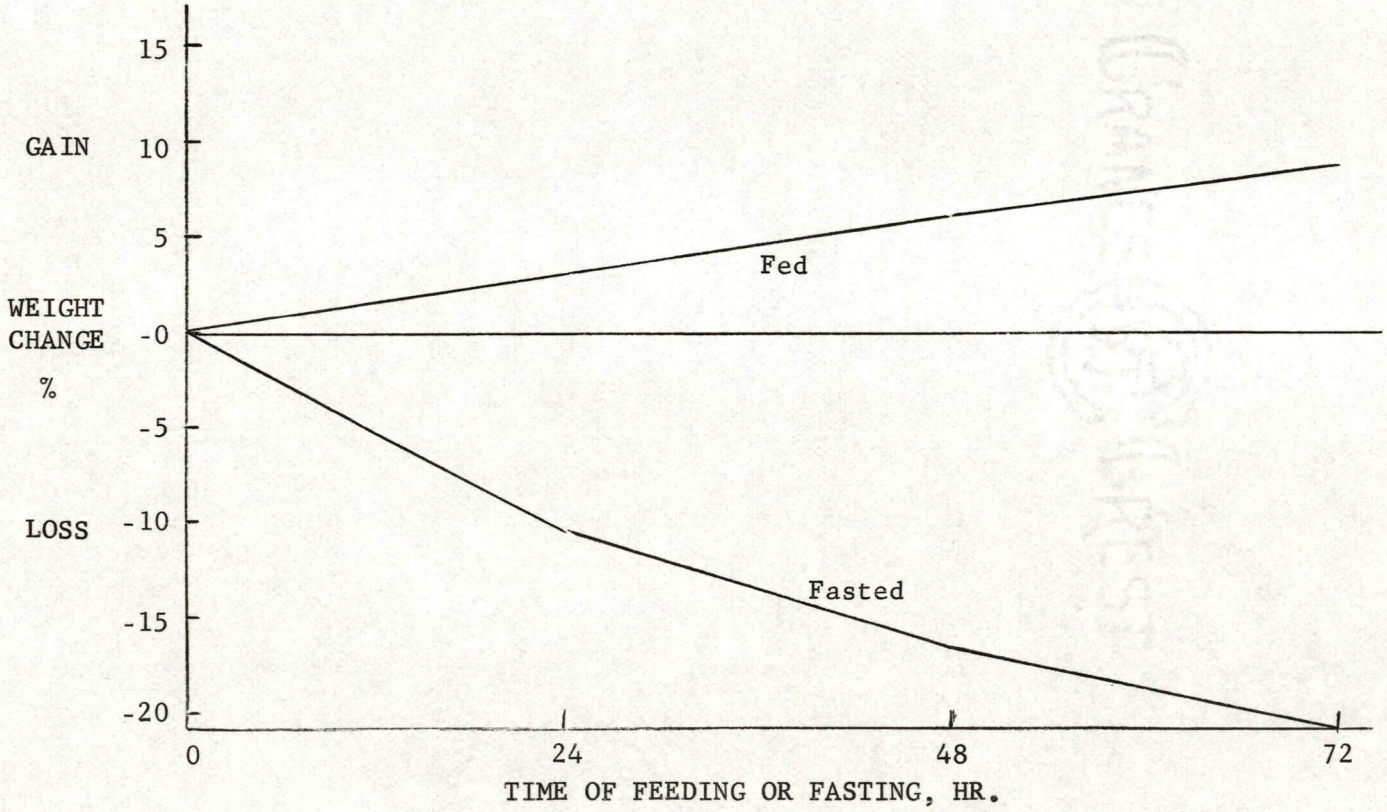


Figure 2. The effect of feeding and fasting on the percent of liveweight change.

peptide synthesis in mammalian systems. The glucose available from residual glucose after fasting may be insufficient for this purpose.

As shown in Table VII, page 22, and Table VIII there was no significant decrease in muscle ether extract or protein until after 48 hours of fasting. Muscle protein significantly increased while intermuscular and intramuscular fat significantly decreased when the rats were fasted 72 hours. This protein increase probably was due to the significant loss of fat in the muscle. It appears that fat was used as the main source of energy in the first 48 hours of fasting.

The RNA content of muscle did not reveal a consistent trend as shown in Table IX. A significant decrease in RNA content of 1 gm. of muscle of the fasted groups can be noted; however, the rats which were fed showed no significant difference among treatments. Caspersson and Bracket (1950) reported that RNA is particularly abundant in cells engaged in synthesis of protein for either growth or secretion. The observed decrease in RNA content indicated that the rate of protein synthesis decreased in groups receiving no feed as the slaughter time was delayed.

Table IX shows the DNA content in 1 gm. of muscle. DNA increased from 547.55 to 737.85 μg. during 72 hours of fasting. This probably was due to a gradual shrinkage in cell mass as muscle protein and fat were used, and effected a simultaneous increase in the total number of cells per gram of muscle. Since the amount of DNA is constant within a single diploid cell in any species, the total number of cells can be calculated. Enesco (1962) found that the amount of DNA per cell

TABLE VIII

THE EFFECT OF SLAUGHTER TIME WITH AND WITHOUT FEED ON PERCENT OF MUSCLE PROTEIN AND THE CALCULATED PROTEIN CONTENT PER CELL[e]

Item	Slaughter Period, Hr.			
	0	24	48	72
Muscle protein,[a] %				
Fed	21.35	21.64	20.86	21.04
Fasted	21.35[c]	21.56[c]	21.76[c]	22.25[d]
Protein/cell,[b] µg.				
Fed	2.42[c]	2.30[c]	2.05[d]	1.98[d]
Fasted	2.42[c]	2.39[c]	1.95[d]	1.87[d]

[a]Based on total muscle weight.

[b]Calculated muscle protein in µg. per cell.

[c,d]Means on the same line bearing different superscripts differ significantly ($P < .05$). Means with the same or no superscripts do not differ significantly.

[e]Formula for the calculated protein content per cell:

$$\text{Cell number in 1 gm. of muscle} = \frac{\text{DNA content in 1 gm. of muscle}}{6.2\ \mu\mu\text{g.}},$$

$$\text{Protein content per cell} = \frac{\text{Protein content in 1 gm. of muscle}}{\text{No. of cells in 1 gm. of muscle}}.$$

TABLE IX

THE EFFECT OF SLAUGHTER TIME ON THE CONTENT[a] OF DNA AND RNA IN ONE GRAM OF MUSCLE

Item	Slaughter Period, Hr.			
	0	24	48	72
DNA, μg.				
Fed	547.55[b]	582.70[b]	634.77[c]	657.47[c]
Fasted	547.55[b]	559.85[b]	692.92[c]	737.85[c]
RNA, μg.				
Fed	2,366.26	2,327.79	2,499.26	2,462.39
Fasted	2,366.26[b]	2,135.68[b,c]	2,308.28[b]	2,078.96[c]

[a]DNA and RNA are expressed as μg. per gram of muscle.

[b,c]Means on the same line bearing different superscripts differ significantly ($P < .05$). Means with the same or no superscripts do not differ significantly.

was 6.2 μμg. in the rat. It is feasible to estimate the total number of cells in a given weight of muscle along with the protein content per cell. With this combined information, an estimation of cell size is possible.

Table VIII shows that after 48 hours of fasting, the protein content per cell was reduced significantly ($P < .05$) from 2.42 to 1.95 μg. It appears that some protein is utilized in the fasting rats after 24 hours, which would contribute to the overall tissue shrinkage.

As shown in Table IX and Figure 3 the DNA content of rats which received feed showed a significant increase ($P < 0.05$) from 547.55 to 657.47 μg. during 72 hours. This effected a significant decrease in protein per cell from 2.42 to 1.98 μg. The DNA increase was probably the result of normal growth of the rats and indicated that the number of muscle cells per gram of muscle tissue increased with feeding.

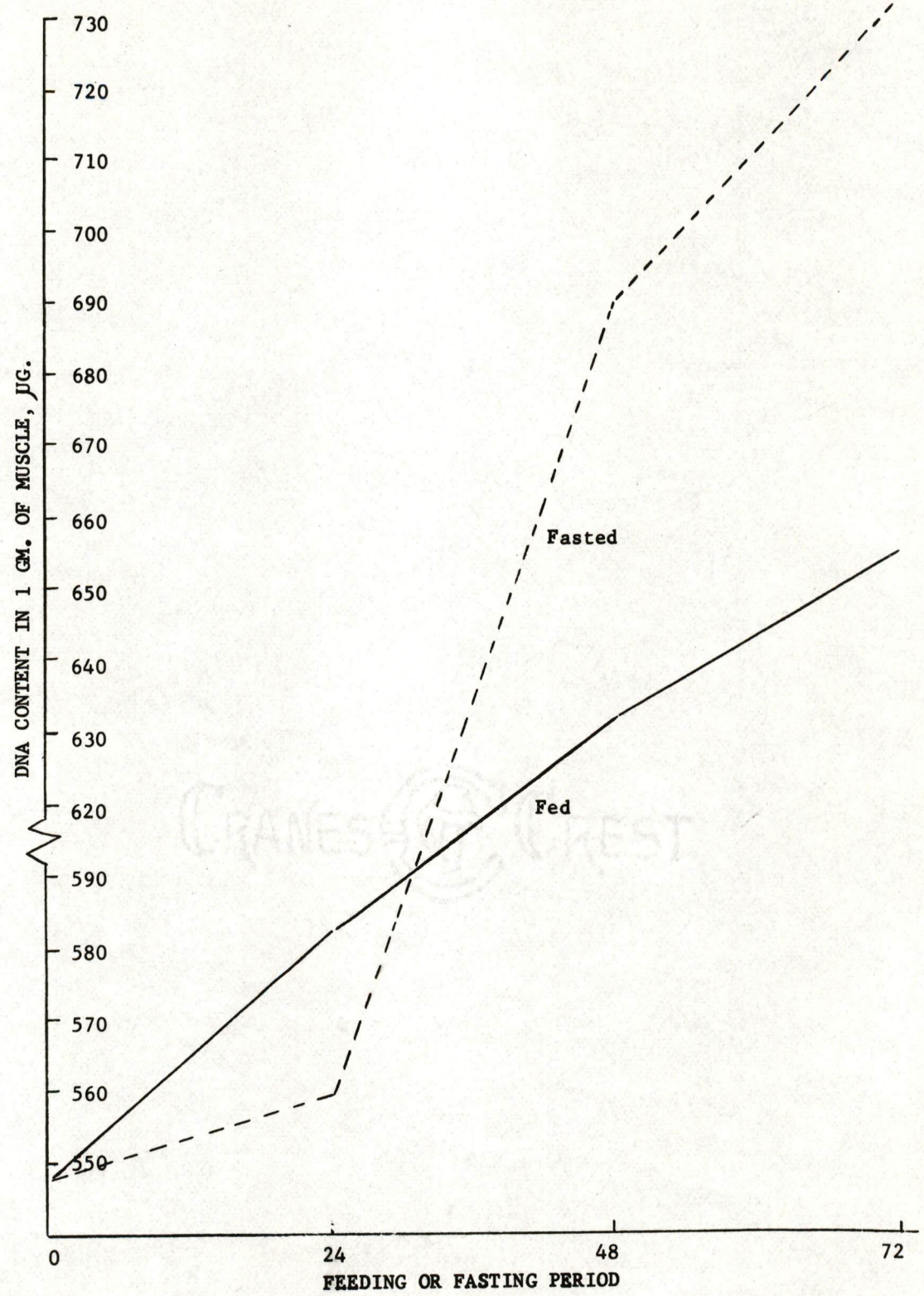


Figure 3. The effect of feeding or fasting on the content of DNA in 1 gm. of muscle.

CHAPTER V

SUMMARY AND CONCLUSIONS

In analysing the data presented in this thesis, the following summary and observations are made.

Empty warm carcass, skin (with most of the subcutaneous fat and tail) and liver significantly ($P < .05$) increased in weight as the rats continued to receive feed. There was no significant change in the other seven components studied.

All of the body components studied except lungs showed significant shrinkage during fasting. A significant loss in weight of the warm carcass, spleen, heart and thymus gland did not occur until the rats had been fasted 48 hours. Skin, head, G.I. tract, liver and kidneys were significantly affected in 24 hours. Of the total shrinkage in liver, skin and warm carcass, about 63.90 percent, 29.83 percent and 13.49 percent, respectively, occurred in 24 hours. About 24.89 percent, 45.15 percent and 46.55 percent of the total shrinkage occurred in liver, skin and warm carcass weight, respectively, during the second 24 hours of fasting. During the last 24 hours of fasting, these three traits shrank 11.20 percent, 25.12 percent and 39.96 percent, respectively.

The rapid shrinkage of liver probably was due to the depletion of glycogen, a fast turnover rate of fatty acids and the loss of moisture. Fat and protein continued to be utilized after 24 hours of fasting. The

empty warm carcass weight did not show a significant shrinkage during 24 hours of fasting.

The DNA content in 1 gm. of muscle of fasting rats increased from 547.55 to 737.85 μg. after 72 hours of fasting, and the protein content per cell decreased significantly from 2.42 to 1.87 μg., indicating an overall tissue shrinkage.

The RNA content of muscle significantly decreased from 2366.26 to 2078.96 μg. as the fasting time extended to 72 hours. This relationship was not linear but indicated that the rate of protein synthesis did decrease.

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