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THE EFFECT OF SUCROSE FEEDING ON HIOSPHORUS CONTENT, PROTEIN CONTENT, AND COLOR OF TURKEY LIVERS AND MUSCLES

by

Mary Tai

A thesis submitted in partial fulfillment of the requirements for the degree

of

MASTER OF SCIENCE

in

Foods and Nutrition

UTAH STATE AGRICULTURAL COLLEGE * Logan, Utah

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Mary Tai

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INTRODUCTION

All carbohydrates metabolized in the body must first be phosphorylated. As a result of Lundsgaard's (1930) and Lohmann's (1934) discoveries, the focal interest of muscle biochemistry has passed from the carbohydrate molecules to the phosphorus compounds. For glucose. the first reaction is the formation of a phosphate ester, glucose-6phosphate. D. M. Needham (1938) found that ATP acts as a phosphorus donor. Glucose-6-phosphate may be transformed into glucose-1-phosphate or into fructose-6-phosphate. In liver, it may be hydrolized back to free glucose and inorganic phosphorus. Bate-Smith (1948) indicated that the free phosphate must be present before any breakdown of glycogen occurs and that the breakdown of glycogen will occur to the extent that free phosphate becomes available. Analysis of total and inorganic phosphorus in the liver is a measure of how much sugar is phosphorylated. This is related to the utilization of sugar. As the liver is an organ vitally concerned with the mechanism of carbohydrate, protein, and fat metabolism, it stores, transforms, and regulates food materials. T. B. Osborne, et al. (1919) suggested that economy in nutrition during growth depends upon the correct adjustment between the proportions of protein and total energy supplied. Quo (1955) found that total carbohydrate content of turkey livers increased slightly with the addition of sucrose to the ration. The effect of sucrose in the ration on the protein content of turkey livers has not been investigated. The purpose of this study was to determine the influence of short pre-slaughter feeding of various levels of sucrose to turkeys on total and inorganic phosphorus

and protein content of turkey liver and muscle.

This thesis is part of a larger project which includes the feeding of sucrose to various farm animals and poultry. This work has been under investigation at Utah Agricultural Experiment Station since 1950. Weight gains, dressing percentages, liver weights, and total carbohydrate content, pH, total solids, ether extract and color of liver and muscle of the sucrose-fed animal or bird has been determined. This thesis includes the total, inorganic, and organic phosphorus and protein values on both liver and muscle and measurement of color of liver of the sucrose-fed turkeys.

REVIEW OF LITERATURE

Molasses is usually a cheap source of energy for poultry and other farm animals. The use of molasses as a substitute for cereal grains in the ration for poultry has been reported by several workers (Scott, 1954; Rosenberg, 1955; and McGinnis, et al., 1948). Additional protein was added to some of the diets. Levels of 5 to 34 per cent molasses of the total ration have been fed. However, the higher levels of molasses feeding have a laxative effect on the birds. McGinnis, et al. (1948) found that levels up to 20 per cent did not have this laxative effect.

Research workers at Utah State Agricultural College have been investigating the use of high levels of crystalline sucrose in poultry rations since 1952. The disadvantages noted with molasses have been absent. Result of feeding sucrose to turkeys has been reported by Quo (1955). He found that the addition of sucrose to the turkey ration tended to increase weight gains and carbohydrate content of the liver. Liver weights increased as the level of sucrose in the ration increased. Feed consumption and dressing percentage tended to decrease with addition of sucrose to the turkey ration.

More extensive research work has been done on the feeding of sugar to farm animals.

Madsen (1943) studied the effect of feeding sugar containing foodstuffs on the quality of pork and liver. He found that both muscle and liver contained a higher glycogen content than those from animals that had not been fed for 2 days before slaughter. The color of the muscle was slightly improved.

Gibbon and Rose (1950) reported that the keeping quality of meat was directly affected by the acid collected in meat, and the lactic acid produced from this glycogen alters the quality of the meat. Bate-Smith (1948) found that sucrose feeding did improve the keeping quality of meat.

Several reports have been made by the Utah Agricultural Experiment Station on their findings with sucrose feeding.

Merkley (1952), Wilcox, <u>et al</u>. (1953), and Greenwood, <u>et al</u>. (1953) have noted benefits of feeding sucrose to swine and beef cattle prior to slaughter. Slight increases in dressing percentages, liver weights, and total carbohydrate content of the muscle and liver of beef and swine were noted after sucrose feeding. Livers from animals fed sucrose were often lighter in color and more tender.

METHOD OF PROCEDURE

Description and feeding of birds

Two groups of Broad Brested Bronze turkeys were used in this study, 240 in Experiment I and 150 in Experiment II. The birds were raised on the range for about 7 months at the Utah State Agricultural College turkey farm.

In each experiment the turkeys were distributed equally by sex, age, and original weights into 30 pens. These pens were divided into 3 replications. In Experiment I each pen contained 4 males and 4 females, and in Experiment II, 3 males and 2 females. The treatments were applied at random within each replicate.

The experimental design is given in table 1.

Per cent of sucrose	Number of pens	fed rations
in ration	For 3 days	For 6 days
None	3	3
10	3	3
20	3	3
30	3	3
40	3	3

Table 1. Experimental design

The basal ration used for both series is shown in table 2. Four other rations containing 10, 20, 30, and 40 per cent sucrose were prepared by mixing the basal ration with commercial sucrose. Each ration Table 2. Basal ration for turkeys

Ingredients	Per cent in ration
Ground corn	10
Ground milo	20
Ground barley	30
Fish meal (74 per cent protein)	4
Meat and bone meal (50 per cent protein)	12.5
Soybean meal (44 per cent protein)	7.5
Cottonseed meal (41 per cent protein)	7.5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5
Alfalfa, sun-cured	5
Whey	3
Limestone	1.5 1
Bone meal	1
Salt	0.5
MnSO),	+
Vitamin A and D supplements (2,000 A300 D)) +
Choline Cl 25 per cent	+
Penicillin supplement (4 gm./1b.)	+
Riboflavin supplement (8 mg./mg.)	+

was fed to 1 pen of turkeys in each replicate for 6 days and another for 3 days prior to slaughtering. Birds fed the experimental ration only during the last 3 days received the basal ration for the first 3 days of the experiment. Fresh water was available at all times.

Results of chemical analyses of the rations have been given by Quo (1955). The protein content varied from 13 to 21 per cent with the following percentages: basal ration, 21; basal + 10 per cent sucrose, 18; basal + 20 per cent sucrose, 16; basal + 30 per cent sucrose, 15; and basal + 40 per cent sucrose, 13 per cent protein.

The weight of each bird was determined at the beginning of the experiment, 3 days later, and at the termination of the feeding. Feed consumption was recorded by pens.

Experiment I began November 18, 1954, and ended November 23, 1954. Experiment II started December 2, 1954, and ended December 7, 1954. Feed was removed from the pens the night before the birds were killed. The birds were trucked to Ogden, Utah, where they were killed at a commercial plant after about 20 hours of fasting. The dressed weight of each bird was determined after the removal of blood, feathers, shanks, head, oil gland, and all internal organs except the kidney and the gizzard. The livers were weighed as they were removed from the bird in the processing line and frozen for later analysis.

Phosphorus determination

Phosphorus was determined by a modification of the method of Summer (1944). For total phosphorus, 5 ml. of 7.5 N H_2SO_4 was used to digest between 50 and 100 mg. of the liver or muscle. After digestion and cooling, 30 per cent H_2O_2 was added. This was heated again for 10 minutes to decompose the H_2O_2 . Five ml. of water was then added, the mixture boiled, and 2 ml. of 7.5 N H_2SO_4 together with 5 ml. of 6.6 per

cent of ammonium molybdate and 5 ml. ferrous sulfate (μ gm. FeSO₄ *7H₂O in 50 ml. water and 1 ml. of 7.5 <u>N</u> H₂SO₄) added. Color was then read on a Junior Coleman spectrophotometer. Inorganic phosphorus was determined by grinding the sample in a glass homogenizer and centrifuged at 2500 r.p.m. for 5 minutes. Two ml. of 10 per cent trichloroacetic was added to precipitate the protein. The sample was then centrifuged for 5 minutes. Color was developed in the same manner as for total phosphorus. Organic phosphorus was obtained by subtracting the values for inorganic from the values for total phosphorus.

Protein determination

Protein was determined by a modification of the micro-Kjeldahl method for determining nitrogen of Bock and Benedict as described by Hawk, Oser, and Summerson (1954). Between 50 and 100 mg. samples of liver or muscle were used. Three hundred mg. of Kjeldahl salt (3 per cent $CuSO_{l_1} + 97$ per cent $Na_2SO_{l_1}$) was added together with 2 ml. of concentrated sulphuric acid. The distillate was collected in l_1 per cent boric acid and titrated with 0.15 N HCl. The factor 6.25 was used in these experiments to give protein.

Color determination

Color of the liver was measured by a reflectance attachment to the Beckman spectrophotometer, a method described by Eastmond (1950). Absorbance was plotted against wave length in millimicrons (500-600). Ground liver was used in the color measurements.

Statistical analysis of data

In the analysis of variance, the F- value for replication, feeding period, treatments, and feeding period x treatment interactions are the ratio of each mean square to that of experimental error variance of the "pens" with 18 degrees of freedom (error a in both experiments). The

mean square for sex x treatment x period, sampling, replication x sex x treatment x period were not significantly different so they were pooled for an estimate of error (b). The F value for sex, sex x feeding period, and sex x treatment interactions are the ratio of each mean square to that of experimental error variance of individual birds with 204 degrees of freedom (error b) in Experiment I and 114 degrees of freedom in Experiment II.

Experimental error

Experimental error is estimated by calculating the mean values and deviations from the means of results of duplicated samples. It is expressed as per cent deviation.

RESULTS AND DISCUSSION

A summary of the average total, inorganic, and organic phosphorus and protein content of turkey liver is given in tables 3 and 4. The effect of sucrose feeding on the phosphorus and protein content is more easily seen in figures 1 to 4. In these figures the values for Experiments I and II were averaged. The statistical analysis of the variance of the data for each experiment is given separately in tables 5 and 6.

Liver

Total phosphorus. There was a significant linear decrease of total phosphorus in turkey livers with increasing sucrose in the ration (figure 1, tables 5 and 6). The total phosphorus of females decreased more with increasing sucrose than did the males. According to Quo's findings (1955), hens stored more carbohydrate in the liver than did toms. He offered a possible explanation for this difference in sexes. The female is apt to rest while the male moves around. At the age of these birds, hens were almost mature while toms were still growing. It is probable that the same explanation may account for the difference between the total phosphorus values in the two sexes.

Total phosphorus content of livers of turkeys in both experiments fed 3 days and 6 days were similar. The birds fed the control ration in either experiment had slightly higher total phosphorus values. This averaged 3.24 and 3.28 mg. per gm. in Experiments I and II as compared to 3.15 and 3.10 in experiments I and II for the sucrose-fed birds and the controls, respectively.

Sucrose				Protein					
in	То	tal	Inor	ganic	Orga	nic*			
ration	Exp. I Exp.		Exp. I	Exp. II	Exp. I	Exp. II	Exp. I	Exp. II	
per cent	mg./gm.	mg./gm.	mg./gm.	mg./gm.	mg./gm.	mg./gm.	h	%	
0	3.24	3.28	1.14	1.27	2.10	2.01	18.42	20.55	
10	3.18	3.17	1.08	1.23	2.10	1.94	18.07	19.66	
20	3.16	3.11	1.10	1.21	2.06	1.90	17.77	18.04	
30 1	3.21	3.09	1.14	1.20	2.07	1.89	18.26	19.06	
40	3.06	3.05	1.04	1.15	2.02	1.90	17.46	16.87	
(avg. of									
10 to 40)	3.15	3.10	1.09	1.20	2.06	1.91	17.89	18.41	

Table 3. Summary of the effect of feeding sucrose on total, inorganic, and organic phosphorus and protein content of turkey liver

* Organic phosphorus values were obtained by difference.

Table 4. Summary of the effect of sex on total, inorganic, and organic phosphorus and protein content of turkey liver

			Deser	6 t					
Sex	То	tal	Inor	ganic	Orga	nic*	Protein		
	Exp. I	Exp. II	Exp. I	Exp. II	Exp. I	Exp. II	Exp. I	Exp. II	
	mg./gm.	mg./gm.	mg./gm.	mg./gm.	mg./gm.	mg./gm.	per cent	per cent	
M	3.23	3.18	1.10	1.23	2.13	1.95	18.79	19.25	
F	3.11	3.08	1.09	1.19	2.02	1.89	17.20	18.21	

* Organic phosphorus values were obtained by difference.

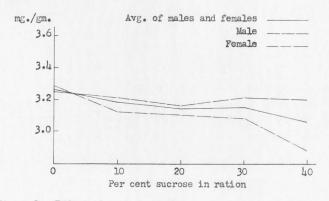


Figure 1. Effect of sucrose feeding on the content of total phosphorus in turkey liver

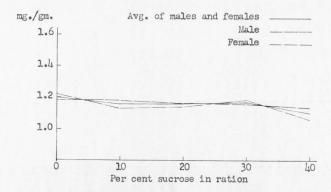
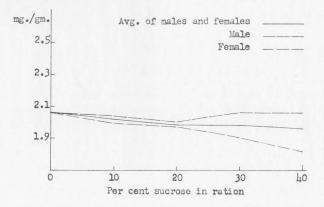
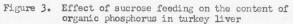


Figure 2. Effect of sucrose feeding on the content of inorganic phosphorus in turkey liver





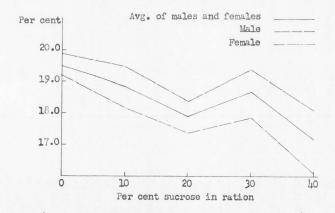


Figure 4. Effect of sucrose feeding on the content of protein in turkey liver

Source of variation	ce of variation d.f.		Inorganic phosphorus	Protein	
Replication	2	0.18	0.01	30.03	
Feeding period Treatment	ĩ	0.09	0.01	12.21 6.08	
Linear Quadratic	1	0.56(.05) 0.04	0.11(.1) 0.01	14.16	
Cubic Quartic	l	0.27(.2)	0.24(.01)	8.58	
Treatment x feeding Period	4	0.09	0.06(.1)		
Error a	18	0.12	0.03	20.97 15.11	
Sex	1	0.89(.001)	0.01	152.44(.001)	
Sex x feeding period	1	0.05	0.02	0.09	
Sex x treatment Error b	4 204	0.31(.01) 0.07	0.04(.1) 0.02	3.85 4.24	

Table 5.	Summary of	f mean squares	obtained by	analysis (of variance of
	data on li	ivers for Exper	ment I sum	narized in	tables 3 and h*

* The numbers in parantheses are the level of significance.

Source of variation	d.f.	Total phosphorus	Inorganic phosphorus	Protein	
Replication	2	0.06	0.005	16.13	
Feeding period Treatment	1	0	0.03(.1)	0.24	
Linear	1	0.85(.01)	0.21(.001)	189.78(.001)	
Quadratic	1	0.05	0.0004	0.003	
Cubic	1	0.02	0.01	18.37	
Quartic	1	0.0007	0.003	36.75(.2)	
Treatment x feeding			0.000	20012(02)	
Period	1.	0.08	0.01	2.64	
Error a	18	0.12	0.009	14.82	
Sex	1	0.36(.05)	0.03	38.90(.1)	
Sex x feeding period	ī	0.17(.2)	0.02	3.79	
Sex x treatment	24	0.15(.2)	0.02	6.70	
Error b	114	0.08	0.02	13.76	

Table 6. Summary of mean squares obtained by analysis of variance of data on livers for Experiment II summarized in tables 3 and 4*

* The numbers in parantheses are the level of significance.

<u>Inorganic phosphorus</u>. There was a significant linear decrease of inorganic phosphorus with increasing sucrose (figure 2, tables 5 and 6). Differences between sexes were not significant.

In both experiments, the inorganic phosphorus content of the liver of birds fed for 6 days was similar to those fed for 3 days. This averaged 1.14 mg. per gm. for the 3-day group and 1.17 for the 6-day group. Inorganic phosphorus values for the control vs. sucrose-fed groups were also similar (1.14 and 1.27 vs. 1.09 and 1.20 mg. per gm. for Experiments I and IL respectively).

Organic phosphorus. The organic phosphorus values were obtained by subtracting the values of inorganic phosphorus from those for total phosphorus.

The influence of sex on the organic phosphorus values is shown in figure 3. Sucrose feeding had little effect on the values for the males. However, in the case of the females, there was a general decrease of organic phosphorus with increasing sucrose in the ration. As compared to males, females had lower values for organic phosphorus at all levels of sucrose feeding.

Not much difference was seen in the organic phosphorus values for the 3-day and 6-day feeding groups. Values for the birds fed the control ration were similar to that of the birds fed different sucrose rations (2.10 and 2.01 vs. 2.06 and 1.91 mg. per gm. for Experiments I and II).

<u>Protein</u>. The decrease in protein content with increasing sucrose percentage was not significant in Experiment I. However, in Experiment II the linear decrease was significant (figure 4, tables 5 and 6). The control birds had 18.42 and 20.55 per cent protein in liver as compared to 17.89 and 18.41 for the average of the sucrose-fed birds in

Experiments 1 and II, respectively. Differences between sexes were significant with females having a lower percentage of protein at all levels of sucrose. According to the analysis of the rations (Quo, 1955), the protein content in the ration decreased as the sucrose level in the ration increased. The complete digestion of dietary protein liberates amino acids. During the absorption of a protein diet, the amino acids are rapidly taken up by the tissue, particularly in the liver. They are not stored in the liver to any great extent; rather, they are metabolized by incorporation into protein by deamination and further oxidation. Reserves of protein accumulate in the liver and muscle can be called upon when the protein intake is inadequate.

In this research, the fact that the protein content of liver decreases with increasing sucrose percentage is difficult to explain. Color. The color of the ground liver was read on the reflectance attachment of the Beckman spectrophotometer. The curves which were obtained for the different levels of sucrose feeding during each of the 3- and 6-day feeding periods are presented in figures 5 to 12. Curves for average values for the color of livers from male and female turkeys fed 3 days in Experiments I and II showed that, in general, as the percentage of sucrose in the ration increased the livers became increasingly lighter in color, except for the 30 per cent sucrose level. The control group, in general, had the highest curve and the group fed 40 per cent sucrose the lowest curve. It is of interest to note that Quo (1955) reported that the livers increased in size, in solids content, and in ether extract content as the percentage of sucrose in the diet increased. Hence, the lighter colored livers tended to be heavier and to contain more solids and fat (ether extract substances) than the darker colored livers.

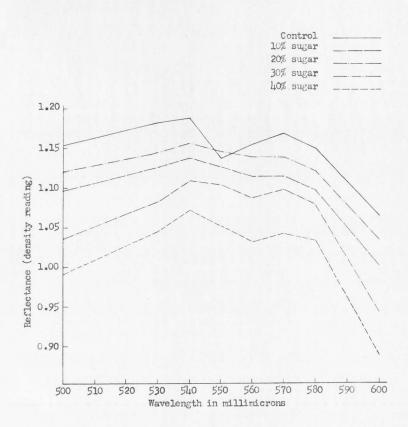


Figure 5. Effect of sugar feeding for 3 days in experiment I on the reflectance curve of male turkey liver

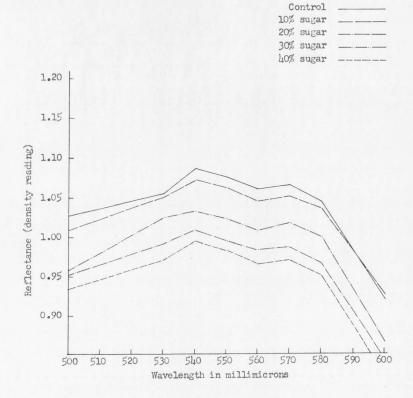


Figure 6. Effect of sucrose feeding for 3 days in experiment I on the reflectance curve of female turkey liver

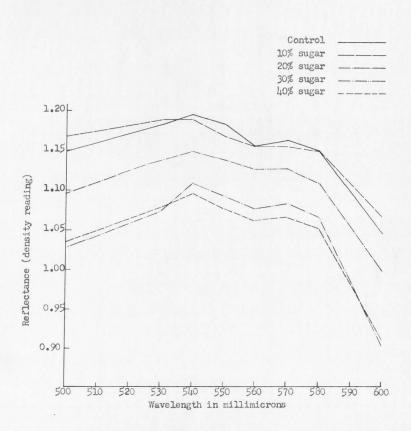


Figure 7. Effect of sucrose feeding for 3 days in experiment II on the reflectance curve of male turkey liver

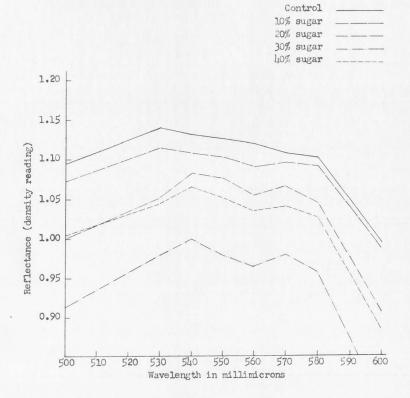


Figure 8. Effect of sucrose feeding for 3 days in experiment II on the reflectance curve of female turkey liver

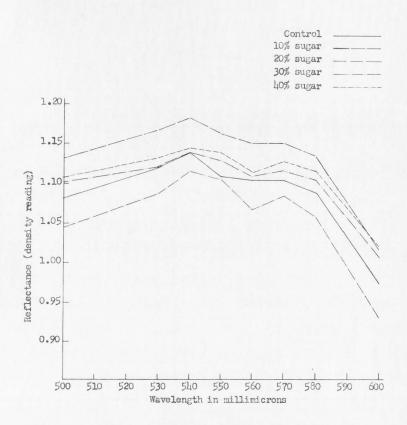


Figure 9. Effect of sucrose feeding for 6 days in experiment I on the reflectance curve of male turkey liver

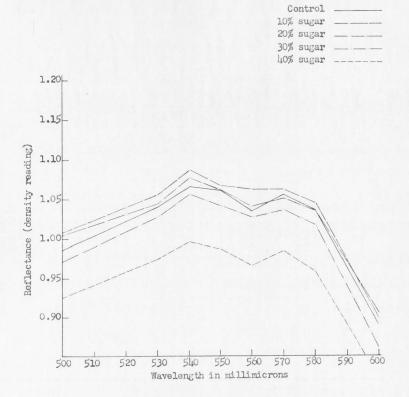


Figure 10. Effect of sucrose feeding for 6 days in experiment I on the reflectance curve of female turkey liver

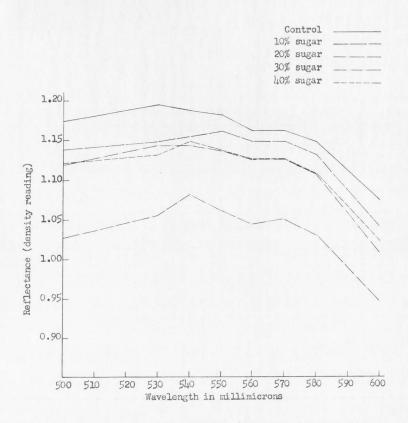


Figure 11. Effect of sucrose feeding for 6 days in experiment II on the reflectance curve of male turkey liver

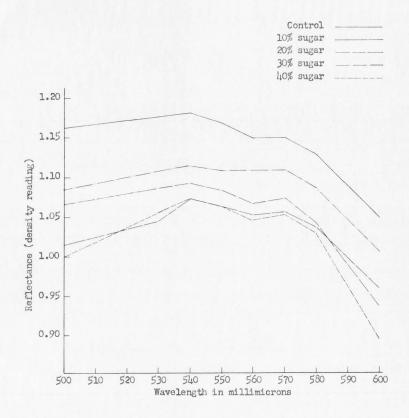


Figure 12. Effect of sucrose feeding for 6 days in experiment Π on the reflectance curve of female turkey liver

Results were not as consistent for the 6-day feeding period. The control groups in Experiment II and the 10 per cent groups in Experiment I for both males and females fed 6 days gave the highest curves, that is, the darkest colored livers. The lowest curves were shown for the female groups in either experiment receiving 40 per cent sucrose and for the male groups receiving 20 per cent sucrose.

Hence, the findings for the 2 feeding periods indicate that the feeding of sucrose and to a lesser extent the feeding of increasing amounts of sucrose result in a trend toward lighter colored livers. <u>Muscle</u>. Leg and breast muscle was taken for analyses from representative turkey toms from each treatment in Experiment I.

Breast muscle had a higher content of total phosphorus, inorganic phosphorus, and protein than the leg muscle (table 7 and figure 13). The difference was great in the protein content, and slight in the total phosphorus content. The organic phosphorus content showed the opposite trend with breast muscle values being lower than that of the leg muscle.

Birds fed a control ration had a somewhat higher content of total phosphorus, inorganic phosphorus, organic phosphorus, and protein than the birds fed the sucrose rations; an exception was the protein content of the breast muscle.

There was a significant linear decrease of total phosphorus and inorganic phosphorus content in both muscles with increasing sucrose percentage in the ration (table 8). Values for organic phosphorus were similar.

Differences in protein content of turkey muscle were not significant. The protein content of muscle of birds fed 10 per cent and 30 per cent sucrose rations had slightly higher protein content than those

Sucrose		Phosphorus									
in	Total		Inor	ganic	Org	anic*	Protein				
ration	Breast	Leg	Breast	Leg	Breast	Leg	Breast	Leg			
5/e	mg./gm.	mg./gm.	mg./gm.	mg./gm.	mg./gm.	mg./gm.	80	%			
0 10 20 30 40 (avg. of	2.58 2.54 2.51 2.39 2.42	2.56 2.34 2.56 2.26 2.28	1.82 1.79 1.78 1.78 1.68	1.58 1.50 1.38 1.43 1.31	0.76 0.75 0.73 0.61 0.74	0.98 0.84 1.18 0.83 0.97	21.08 22.95 21.47 22.04 21.31	19.56 19.89 17.33 20.39 17.64			
10 to 40)	2.46	2.36	1.76	1.40	0.71	0.96	21.94	18.81			

Table 7. Summary of the effect of feeding sucrose on total, inorganic, and organic phosphorus and protein content of turkey muscle in Experiment I

* Organic phosphorus values were obtained by difference.

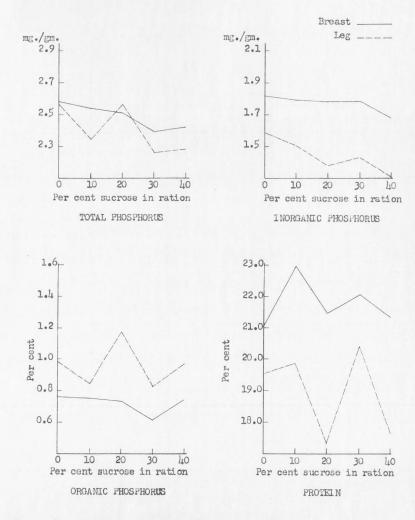


Figure 13. Effect of sucrose feeding on the content of phosphorus and protein in turkey muscle

Source of variation		Total phosphorus		Inorganic	phosphorus	Protein		
	d.f.	Breast	Leg	Breast	Leg	Breast	Leg	
Replication	2	0.04	0.08	0.005	0.02	7.80(.1)	7.48	
Period	1 .	0.01	0	0.02	0.01	0.11	7.96	
Treatment								
Linear	1	0.14(.05)	0.23(.1)	0.04(.1)	0.21(.01)	0.12	6.70	
Quadratic	1	0.002	0.0005	0.006	0.004	4.25	0.12	
Cubic	1	0.01	0.009	0.008	0.01	2.51	5.10	
Quartic	1	0.01	0.29(.1)	0.0009	0.02	6.64(.2)	34.00(.05)	
Treatment x period	4	0.01	0.03	0.005	0.005	1.12	3.63	
Error	18	0.03	0.07	0.01	0.02	2.40	5.99	

Table 8. Summary of mean squares obtained by analysis of variance of data on muscle summarized in table 7*

* The numbers in parantheses are the level of significance.

fed the control, 20 per cent, and 40 per cent sucrose rations. This finding was the same as that obtained for liver protein except that in the liver, the protein content for birds fed the control ration was slightly higher than for those fed 10 and 30 per cent sucrose ration. Experimental error

The range of values of deviation from the mean for duplicate samples, the average deviation from the mean, and percentage deviation from the mean for total phosphorus, inorganic phosphorus, and protein are shown in table 9.

These average percentage deviations from the means for total phosphorus is 2.52, inorganic phosphorus, 1.63, and protein, 4.10.

Table 9.	Summary of	range o	f deviation	from	the	means	of	duplicate	samples	for	total	phosphorus,	
	inorganic	phosphor	us, and pro	tein					1		00 out	priosprior us ;	

	Range of deviation from the mean of duplicate samples	Average deviation from the mean	Average per cent deviation from the mean
Total phosphorus	0.01-0.16	0.08	2.52
Inorganic phosphorus	0.005-0.04	0.02	1.63
Protein	0.44-1.13	0.83	4.10

SUMMARY

Three hundred and ninety turkeys were used in 2 experiments to determine the effect of feeding sucrose for short periods prior to slaughter on total phosphorus content, inorganic phosphorus content, organic phosphorus content, protein content of liver and muscle, and color of liver. Birds fed the basal ration were compared with birds fed the same ration and 10, 20, 30, or 40 per cent sucrose. Three and 6-day feeding periods were used.

There was a significant linear decrease of total and inorganic phosphorus content in turkey livers with increasing sucrose in the ration. Organic phosphorus values decreased slightly with increased sucrose feeding.

The livers of female turkeys contained significantly less total phosphorus than that of the males. Sex differences in organic phosphorus values were somewhat less than in the case of total phosphorus. Inorganic phosphorus values were similar for either sex.

Phosphorus values in all three forms were similar for the 2 feeding periods.

Protein content decreased significantly with increasing sucrose percentage in the ration in experiment II. Females had significantly lower protein values than the males.

The findings on color of liver indicate that, in general, the feeding of sucrose, and, to a lesser extent, the feeding of increasing amounts of sucrose resulted in lighter colored livers.

Breast muscle had slightly higher total phosphorus, inorganic

phosphorus, and protein values than did the leg muscle. Organic phosphorus showed the opposite trend. There was a general decrease of total and inorganic phosphorus in both muscles with increasing sucrose. Organic phosphorus values were similar in both muscles. Differences in protein content of turkey muscle were not significant.

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