A CHEMICAL STUDY OF CERTAIN TISSUES OF SWINE FED THYROPROTEIN IN LOW AND HIGH FAT RATIONS

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INTRODUCTION

Knowledge of the function of the thyroid hormone has been derived chiefly by observing the effect of an excessive amount or a deficiency of this hormone as it occurs spontaneously in disease or is induced by thyroidectomy or the administration of thyroid extracts. The fact that thyroid extracts have been expensive has resulted in limited practical application of the knowledge of thyroid function in growth studies. It is now possible, however, to produce thyroactive iodinated proteins which resemble the thyroid hormone in biological activity. The direct iodination of tyrosine-containing proteins under carefully controlled conditions of temperature and pH has produced synthetic materials having an apparent thyroxine content of 3 percent and a biological activity many times that of dried thyroid gland. Control of thyroid status by the oral administration of these thyroproteins permits the study of possible beneficial effects of mild hyperthyroidism upon rapidity of growth and related economic factors in animal production.

The Oklahoma Agricultural Experiment Station in 1948 undertook a study to determine the effect of thyroprotein on the rate of growth and fattening of swine. The purpose of the present investigation, as part of this project, was to determine the effect of the added thyroprotein on the physical properties and composition of body fat and also its effect on vitamin A storage in the liver and the calcification of bone. Since two basal rations were to be used, one low and the other high in fat, the project offered an opportunity also to obtain information on the effect of dietary fat upon body fat characteristics.

REVIEW OF LITERATURE

Economical meat production depends upon rapid growth and fattening of meat animals. Since the thyroid hormone in intimately related to these processes various techniques have been used in an attempt to alter the amount of circulating hormone in a number of species. There is some evidence that a mild hyperthyroid condition may be conducive to rapid growth. Reineke and McMillen (1) reported that Berkshire pigs receiving from 0.005 to 0.0075 percent thyroprotein in their grain ration gained slightly more than controls during an eight-week period. In further experiments by Beeson et al. (2) purebred Duroc pigs were fed 0.0044 and 0.0088 percent iodinated casein in their rations for 84 days. The iodinated casein fed at the 0.0044 percent level had no effect on growth but the pigs receiving 0.0088 percent gained 26 pounds more and required 10 percent less feed per pound gain than controls. Similar results were reported by Reineke et al. (3) in 1948; Yorkshire and Duroc Jerseys receiving 0.006 percent iodinated casein in their grain rations gained 15.1 and 11.3 pounds more than their controls in a 122 day feeding trial. In all cases the economy of gains was slightly improved.

In similar studies with pigs negative results have been reported by Branda in England (4). Starting with high dosages of 1.5 grams daily and lowering the dosage, this investigator could not demonstrate any significant influence of thyroprotein on the rate of growth. Vander Noot et al. (5) reported similar negative results with pigs fed thyroprotein at levels of 0.075, 0.15, and 0.225 gram per 100 pounds body weight.

These conflicting results may possibly be accounted for by differences in experimental conditions.

In all trials in which increased growth rate resulted, thyroprotein has been given at an early age and as a very small percentage of the ration. Perceptible stimulation of growth did not occur until 6 weeks to 2 months of treatment and no stimulation to growth was noted in pigs placed on iodinated casein for a short time during the fattening period. Influence of thyroprotein on fat metabolism. There is some evidence that the increased growth rate associated with thyroprotein administration is accompanied by an increase in the percentage of body fat. Perry et al.(6) reported that thyroprotein fed to pigs at levels of 0.0088 and 0.0132 percent produced an increased percentage of body fat. Muhrer and co-workers (7) have shown that hypothyroidism induced by thiouracil feeding to growing pigs resulted in 3 percent less body fat.

Fats and sterols are apparently normally absorbed by hyperthyroid subjects. There is evidence that the hormone plays some role, probably indirectly, in the metabolism of these substances. Both free and esterified cholesterol and lipid phosphorus are elevated in hypothyroidism and depressed, though less regularly, in hyperthyroidism. Fatty acids appear less saturated in hyperthyroidism than in normal animals (8). In contrast, reports have been made showing that thyroidectomy had little or variable effect on the lipid metabolism of monkeys and dogs (9). Remington (10) further disputes the theory that there is any specific function of the thyroid gland in the metabolism of fat.

Reports of the relation of thyroprotein feeding to physical

characteristics of the fat produced are meager. Dyrendahl (11) reported that in stomach and kidney fat, iodine number, Reickert Meissel number, and refractometer number increased and saponification number decreased. This is in accord with the report of Duncan (8). Kummerow et al.(12) have shown that feeding either iodinated casein or thiouracil to poultry did not have any appreciable effect on the stability of the fat extracted from the slaughtered birds.

Influence of thyroprotein on vitamin A metabolism. Since 1928 considerable literature has accumulated on the interrelation of thyroid function and vitamin metabolism. Hyperthyroidism has been shown to increase the requirements for certain vitamins (13). Euler and Klussmann (14) obtained a reduction of vitamin A and carotene in the liver of guinea pigs when thyroxine was administered. When fed a vitamin A-free basal diet, administration of limited amounts of carotene led to storage of vitamin A and carotene in the liver, but when thyroxine was given with carotene the liver failed to show storage of either carotene or vitamin A. Greaves and Schmidt (15) found that rats given thyroxine were depleted of vitamin A more rapidly than normal rats and thyrodectomized rats showed a decreased rate of depletion. This would indicate that the vitamin A requirement of the rat may be increased by the administration of thyroxine. Similar results have been obtained in experiments with swine (16).

In contrast, Allen et al.(17), Baumann and Moore (18), Logara and Drummond (19), and Johnson and Baumann (20) observed no reduction of vitamin A in the liver and no increased conversion of carotene to vitamin A when thyroxine was administered to calves and rats. These

conclusions clearly indicate that the problem of vitamin A-thyroid interrelationship is in no sense a simple one.

Influence of thyroprotein on bone calcification. The effect of thyroprotein upon calcium and phosphorus utilization has been observed in calcium-phosphorus balance studies. There is an increased retention of calcium in hypothyroidism and an increased excretion of this element and phosphorus in hyperthyroidism. This increased fecal excretion of calcium appears to be due to overeating and increased peristalsis of the gut for there is no evidence for any failure of calcification in the bones of growing rats or of decalcification in the adult (21). Toxic doses of thyroid extract, however, do cause a retardation or cessation of growth at the epiphyseal junction of the femur and tibia by affecting the endochondral bone formation (22). Dyrendahl (11) also observed profound effects upon bone formation in swine after prolonged feeding of iodinated casein. Stiffness in the leg joints was evident and X-rays of the lower tibia, hock joint, and phalangeal joints of forelegs showed porosity of the bone.

Influence of dietary fat on body fat. Although fat has long been regarded as an optional component of the diet and one which is interchangeable with carbohydrate, a number of recent reports have indicated that it probably should be classed as an essential food stuff. Apparently the value of dietary fat is not entirely explained by the essential fatty acids it contains, but may be related to some additional factor or factors.

Distribution of fat in the animal body is found to be independent of the type of diet which, however, controls the amount and in many species, the character of the fat deposited (23). The influence of body temperature is regarded as a secondary factor which may modify the composition of the depot fat but the specific type of the latter seems to depend primarily

on the species (24).

The fat in the depots is primarily triglyceride of low iodine number and bears a close relationship to the ingested fat. Lovern (25) reported that in eels fed mussels with low fat content, depots remained unaltered whereas, in eels fed herring flesh, depots approximated herring fat in character. Changes in the character of fat in the depots occur in hypo- and hyperthyroidism, but the mode of action of thyroxine in this connection is not known.

Diet influences the type of fatty acids deposited by the animal in its own fatty tissue. The type of fatty acid in fat in turn has a marked influence on the speed with which rancidity will develop during storage. There is a possibility that substances which act to prevent oxidation may be present in body fat. It would appear reasonable that nutritional factors may also effect the anti-oxidant content of the fatty tissue, and it might be expected that changes in the fatty acid composition and variation in anti-oxidant content might result in some correlation between susceptibility to oxidation of the fat and the diet of the animal. Overman (26) observed that under some conditions of diet, fat from thin hogs resisted rancidity better than fat from heavier hogs although the thin hogs had softer fat. It is suggested that fat deposition in the heavier hogs may have outstripped the accumulation of anti-oxidants in the tissue. Overman further noted that diets which reduced the amount of depot fat increased its stability.

Influence of dietary fat on vitamin A metabolism. Fat level of the diet as well as specific fatty acid composition of the dietary fat has been observed to play an important role in the utilization of various vitamins, particularly vitamin A and thiamin. Since vitamin A is fat soluble,

many investigators have suggested that dietary fat may promote its absorption and utilization. Studies of this relationship have been conflicting in results so that there is little agreement about the role which fat may have in the metabolism of vitamin A.

Muelder and Kelly (27) demonstrated that 10 percent fat in the rations of rats aided absorption of vitamin A sufficiently to produce statistically significant gains in weight over a basal ration containing no fat, but not over a basal ration containing 5.0 percent fat. Russell et al. (28) reported that hens absorbed less crystalline carotene from a low-fat ration than from a ration containing 4.0 percent fat. When the level of carotene was increased four-fold there was only a two-fold increase in carotene retention and the quantity retained was considerably less than with the control ration. However, hens seemed to absorb vitamin A as effeciently on low-fat rations as on normal rations but apparently did not retain the vitamin as well on the low-fat ration. Absorption was measured by the quantities found in the droppings and retention was measured by the amounts found in the livers. It has also been shown by Green (29) that excessive amounts of fat in the diet of rats do not make excessive demands on vitamin A reserves such as might be expected if vitamin A were directly related to the metabolism of fatty acids. Other investigators reported that both carotene and vitamin A are more completely absorbed if the diet contains a high percentage of fat (30).

Influence of dietary fat on bone calcification. The importance of the discovery that dietary fat effects bone calcification is evidenced by the numerous reports appearing during the past several years dealing with the influence of fat on the metabolism of calcium and phosphorus. It seems

evident that fats free of vitamin D may exert either a beneficial or harmful effect on calcification. Fat in quantity is harmful when dietary phosphorus is low but is beneficial when dietary phosphorus is optimal. Furthermore, fat does not aid calcification when fed to animals which are receiving a ration high in phosphorus and low in calcium (31, 32).

Booth et al. (33) demonstrated that fat exerts no beneficial calcifying effect for rats when added to a diet high in phosphorus and low in calcium. That fecal excretion of calcium seems independent of dietary fat content in young and middle aged rats was reported by Kane et al. (34). In older animals, however, calcium excretion was proportional to the fat content of the diet. Bunkfeldt and Steenbock (35) have extended these observations and have emphasized further that the effect of dietary fat on calcification is conditioned by the calcium and phosphorus of the diet. In rats fed 0.075 percent phosphorus in a ration having a calcium-phosphorus ratio of 3:1 and increasing amounts of cottonseed oil, a decrease in calcification occurred which was proportional to the amount of oil ingested. Increasing the calcium-phosphorus ratio from 1:1 to 6:1 with a constant intake of phosphorus at 0.075 percent of the diet had no influence on the decrease in calcification produced by the cottonseed oil. When the phosphorus content was increased from 0.075 to 0.25 percent the action of cottonseed oil was beneficial. Jones (36) reported that from 5.0 to 25.0 percent lard in synthetic rachitogenic diets had definite anti-rachitogenic properties not associated with the nonsaponifiable fraction.

EXPERIMENTAL

It was the general plan of this experiment to feed low and high levels of fat to four groups of pigs, two groups receiving a low-fat ration and two groups receiving a high-fat ration. One of the two groups at each fat level was to be fed thyroprotein. In the first trial, begun in the summer of 1949, lard was used as the source of fat. In the second trial, begun in the winter of 1950, beef fat was fed.

Rations and animals. The feeding and slaughter phase of this work was carried out by the Animal Husbandry Department under the direction of J. C. Hillier. Thirty-two young pigs, weighing from 30 to 50 pounds, were selected on the basis of their size, sex, appearance, and litter. One of four rations was assigned to each group of eight pigs. Each pig was placed in an individual pen and fed a mixed ration ad libitum. Individual pig weights were taken every fourteen days. Feed records were also kept on a fourteen day basis.

The animals were slaughtered at an average weight of about 225 pounds. Samples of body fat, one lobe of the liver, and a one inch cross-section of a leg bone from each of the animals were removed and kept frozen until analyses were completed.

Composition of rations.

Table 1. Composition of rations fed to pigs in trial I (1949) and trial II (1950)

Feed	Ration description and number[1]			
	Low fat		High fat	
	I	II[2]	III	IV[2]
Degermed corn -------- percent --	67.00	57.00	55.00	55.00
Soybean oil meal ----- percent --	18.00	18.00	20.00	20.00
Fish meal ---------- percent --	5.00	5.00	5.00	5.00
Alfalfa leaf meal ---- percent --	7.00	7.00	7.00	7.00
Yeast ---------- percent --	1.00	1.00	1.00	1.00
Salt ---------- percent --	1.00	1.00	1.00	1.00
Bone meal ---------- percent --	1.00	1.00	1.00	1.00
Fat ---------- percent --	----	----	10.00	10.00
Delsterol -grams per cwt. feed --	6.00	6.00	6.00	6.00

[1]The ration numbers are identical with the animal group numbers.

[2]Each of the animals in group II (ration II) and group IV (ration IV) received 6 grams of thyroprotein per 100 pounds of feed.

Methods of analysis. Before any analyses were made representative portions of all the fat samples were rendered. Rendering was accomplished by placing small beakers of the frozen fat in an oven at 100 °C for three hours. The melted fat was then filtered through Whatman No. 3 filter paper into brown glass bottles. The rendered samples were kept in cold storage until used.

As a means of indicating the relative degree of unsaturation of the different fat samples iodine numbers were determined by the Hanus method as described in the Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists (38). The iodine number determination is based on the fact that one mole of iodine adds quantitatively to each double bond of an unsaturated fatty acid. A high

iodine number indicates a large number of double bonds in the fat.

The melting points of fats are often used in conjunction with the iodine number to characterize a fat. As a rule the melting points of fats decrease as the unsaturation and iodine numbers increase. Melting points were determined on four samples of fat from each group of animals. The method used was a slight modification of the official A.O.A.C alcohol-water method (37). A mechanical stirrer was used in the water bath in place of the recommended air jets.

Thiocyanogen numbers were determined on all the samples in trial I (1949) and on four samples from each of the four groups in trial II (1950). The procedure as described by Jamieson (38) consisted of the addition of the thiocyanogen solution to a weighed amount of fat and allowing it to stand in the dark for 24 hours. Potassium iodide was then added and the solution titrated with standard sodium thiosulfate. By means of this value and the iodine number, the percentage of oleic, linoleic, and linolenic and saturated acids can be calculated providing other unsaturated acids are absent. The iodine-thiocyanogen method is based on the assumption that one mole of iodine adds quantitatively to each double bond of an unsaturated fatty acid, whereas the thiocyanogen radical, SCN, adds quantitatively to the single bond of a monoethenoid acid, to one of the two double bonds of a diethenoid acid, and to two of the three double bonds of a triethenoid acid. This assumption is valid in the case of the iodine addition but it is not valid for the thiocyanogen addition. The method, however, is satisfactory if the theoretical constants of the original Kaufmann equations are replaced by empirical constants obtained by determining the thiocyanogen number on pure unsaturated acids under standard

conditions (39).

Saponification numbers were determined on fat samples from the same animals used for the melting point and thiocyanogen number (37). Since these samples showed no significant differences in their saponification numbers it did not appear worthwhile to determine this value for all the samples. The saponification number is an indication of the mean molecular weight of the glycerides of the fatty acids present in the fat. Thus a high value indicates that the glycerides are mainly those of acids of low molecular weight.

Natural fats and oils oxidize spontaneously when exposed to the air, oxygen attacking a double bond of the unsaturated fatty acids forming a highly reactive peroxide. The reaction of an auto-oxidizable substance with oxygen is usually characterized by a phase of very slow change which precedes rapid oxidation. One of the most satisfactory means available for following the earlier stages of atmospheric oxidation in fats is by the determination of peroxide oxygen.

For the purpose of comparing the susceptibility of the fats to oxidation a modification of Lea's method of aeration was used (40). Four samples, 1 to 2 grams each, were weighed into 10 cm. evaporating dishes and placed in an oven at 100 °C. Samples of the same fat were removed at zero, two, three, and six hour intervals and the peroxides determined by the method of Lea as described by Markley (41). Determinations were made on duplicate samples on separate dates at the zero, two, and six hour intervals. The samples used in this determination were extracted from the original unrendered fat by blending in a Waring blendor for one minute with 100 milliliters of chloroform and 70 grams of anhydrous sodium sulfate. The extract was filtered through Whatman No. 2 filter paper and

the filtrate dried by bubbling nitrogen through it. The extraction and drying procedures were carried out as rapidly as possible in subdued light.

The amount of vitamin A stored in the livers of the pigs was determined by the method described by Gallup and Hoefer (42). Twenty-five samples were available from trial I (1949) and thirty-two samples from trial II (1950).

The percentage of ash in the dry fat free bone was used as a measure of the degree of calcification. The marrow was removed and the bone ground into small pieces. After drying and extracting with ether for sixteen hours the bone was oven dried in tared crucibles for four hours at 100 °C. Ashing was carried out in a muffle furnace at 600 °C for two hours. The percentage of bone ash was determined only on the available samples of trial II (1950).

RESULTS

The growth made by the pigs in trial I (1949) and trial II (1950) is shown in table 2.

Table 2. Average gains and feed intake of pigs
Trial I (1949)

Items compared	Ration description and number[1]			
	Low fat		High fat	
	I	II[2]	III	IV[2]
Number of pigs	8	8	8	8
Average weight of pigs				
Initial ------- pounds--	38.4	40.3	41.0	39.8
Final --------- pounds--	229.5	226.4	224.5	228.9
Total gain ---------- pounds--	191.1	186.1	183.5	189.1
Length of period --- days----	104.0	110.0	112.0	113.0
Daily gain ---------- pounds--	1.8	1.7	1.7	1.7
Average daily feed - pounds--	5.3	5.6	4.5	4.9
Trial II (1950)				
Number of pigs	8	8	8	8
Average weight of pigs				
Initial ------- pounds--	53.3	52.9	51.8	52.8
Final --------- pounds--	228.7	229.3	232.3	228.7
Total gain ---------- pounds--	175.5	176.4	180.5	175.9
Length of period --- days----	98.0	101.0	100.0	90.0
Daily gain ---------- pounds--	1.8	1.8	1.8	2.0
Average daily feed - pounds--	6.9	6.5	6.2	6.5

[1]The ration numbers are identical with the animal group numbers.

[2]Each of the animals in group II (ration II) and group IV (ration IV) received 6 grams of thyroprotein per 100 pounds of feed.

The figures in table 2 indicate that thyroprotein in the amounts fed had only a very slight effect upon the rate and economy of gains.

The results of all analysis, with the exception of peroxide values, made of tissues from pigs in trial I (1949) are shown in table 3. The figures represent average values obtained for samples from each group. Individual values are given in table 1 of the appendix.

Table 3. Results of analysis of tissues taken from pigs at the end of trial I (1950)

Items compared	Ration description and number[1]			
	Low fat		High fat	
	I	II[2]	III	IV[2]
Fat constants				
Iodine number	51.77	54.02	60.55	62.78
Melting point ---------°C	40.60	39.50	34.40	32.50
Thiocyanogen number	46.32	48.21	52.30	54.83
Olein ----------percent	67.26	70.29	79.10	82.63
Linolein ----------percent	6.47	6.90	9.91	9.52
Saturated glycerides-percent	26.27	22.81	10.99	7.85
Saponification number	194.17	195.09	194.63	193.99
Liver vitamin A --- mcg. per gm.	26.90	32.30	15.40	13.80

[1]The ration numbers are identical with the animal group numbers.

[2]Each of the animals in group II (ration II) and group IV (ration IV) received 6 grams of thyroprotein per 100 pounds of feed.

The results in table 3 show that iodine numbers increased from approximately 52 to 54 when thyroprotein was added to the low-fat rations and from 61 to 63 when added to the high-fat rations. The average melting

point of the fat which was 40.6 for the low-fat ration and 34.4 for the high-fat ration was decreased slightly by thyroprotein. The average thiocyanogen numbers were increased from 46.3 to 48.2 by the addition of thyroprotein to the low-fat ration and from 52.3 to 54.8 by a similar addition to the high-fat ration. The calculated values for the percentages of the glycerides of oleic, linoleic, and saturated fatty acids were 67.3, 6.5, and 26.3, respectively, for the low-fat ration, and 79.1, 9.9, and 11.0, respectively, for the high-fat rations. Thyroprotein had little effect on the calculated percentages of linolein but increased the percentage of olein and decreased the saturated glycerides of fat for both the low- and high-fat rations. Saponification numbers varied only slightly from 194 to 195.

The average vitamin A content of the liver which was 26.9 micrograms per gram for the low-fat group and 15.4 for the high-fat group was effected very little by the addition of thyroprotein to the diet.

The peroxide values obtained for the fat from pigs in trial I (1949) are shown in table 4.

Table 4. Peroxide values obtained for fat from pigs in trial I (1949)

Ration	Animal	Hours			
		0	2	3	6
I	5	36.8	64.7	93.6	115.5
		31.3	58.4	----	106.7
	7	12.6	47.2	50.8	103.7
		23.1	61.9	----	96.6
	average	26.0	58.1	72.2	105.6
II	2	21.6	61.2	65.7	126.4
		27.0	73.4	----	126.2
	5	22.8	78.8	83.2	119.3
		31.4	60.6	----	-----
	average	25.7	68.5	74.5	124.0
III	3	10.9	54.8	44.7	108.1
		11.7	61.0	----	101.5
	4	58.3	79.6	83.7	133.0
		70.7	83.8	----	122.0
	average	37.9	69.8	84.2	116.0
IV	3	66.3	82.8	100.0	134.7
		74.4	82.1	-----	-----
	5	53.7	74.5	98.0	149.0
		62.0	93.7	------	-----
	average	64.1	83.3	99.0	141.9

Average peroxide values for fat with an initial value of approximately 26 increased to 106 at six hours for the low-fat ration and increased from an initial value of 38 to 116 for the high-fat ration. For the low-fat ration containing thyroprotein peroxide values increased from 25.7 to 124 in a six hour period and for the high-fat ration containing thyroprotein these values increased from 64 to 142.

A comparison of the results obtained with the low-fat rations, I and II, and the high-fat rations, III and IV, shows that, in general, the percentage of fat in the ration had more effect on fat constants and liver storage of vitamin A than did thyroprotein. The increase in fat content of the diet increased iodine numbers and decreased melting points; thiocyanogen numbers and percentage of olein increased and saturated glycerides

decreased. Vitamin A was decreased markedly in the livers of pigs receiving the high-fat rations.

The results of analyses of tissues from pigs in trial II (1950) are shown in table 5. Individual values are given in table 2 of the appendix.

Table 5. Results of analysis of tissues taken from pigs at the end of trial II (1950)

Items compared	Ration description and number[1]			
	Low fat		High fat	
	I	II[2]	III	IV[2]
Fat constants				
Iodine number	53.66	54.12	58.68	61.26
Melting point -----°C-----	40.00	42.80	36.30	35.00
Thiocyanogen number	40.90	41.70	44.81	47.68
Olein ----- percent	69.81	70.43	76.58	80.54
Linolein ----- percent	15.61	15.81	16.97	17.04
Saturated glycerides-percent	14.58	14.39	6.47	2.42
Saponification number	195.27	195.34	194.14	194.98
Bone ash[3] ----- percent	69.20	68.91	69.27	69.22
Liver vitamin A ---- mcg. per gm.	30.80	33.10	18.10	20.90

[1]The ration numbers are identical with the animal group numbers.

[2]Each of the animals in group II (ration II) and group IV (ration IV) received 6 grams of thyroprotein per 100 pounds of feed.

[3]Dry fat-free basis.

Results presented in table 5 show, that in general, the percentage of fat and thyroprotein in the rations had the same effect on fat constants and liver storage of vitamin A in trial II (1950) as in trial I (1949). When added to the low-fat rations thyroprotein had no effect on the iodine numbers but when added to the high-fat rations the iodine numbers increased

from approximately 59 to 61. Thyroprotein in the low-fat rations increased the melting points from 40.0 to 43.0 but decreased the melting points from 36 to 33 in the high-fat rations. The average thiocyanogen number, which was 41 for the low-fat ration and 45 for the high-fat ration, was decreased slightly by thyroprotein.

The percentage of ash in bone, determined in trial II (1950), was unaffected by the addition of thyroprotein to either the low- or high-fat rations. The percentage of bone ash was about 69 for all the groups.

The average vitamin A content of the liver, which was about 31 micrograms per gram for the low-fat ration and 18 for the high-fat ration, was affected very slightly by the addition of thyroprotein.

DISCUSSION

From the similarity of results obtained in trial I (1949) and trial II (1950) it appears that although thyroprotein in the amounts fed was less effective than fat in changing fat constants, it did increase slightly the iodine numbers of the fat. Thyroprotein decreased the average melting points of fat in all instances except in trial II (1950) when added to the low-fat ration. It increased thiocyanogen numbers which indicates a greater number of double bonds per unit of fat. Calculations involving both thiocyanogen and iodine numbers make it possible to determine which of the two fats, olein and linolein, account for this increased unsaturation. When total saturated fatty acids are not determined separately from the unsaturated acids, the calculation of the percentages of olein and linolein is based on the assumption that linolinein is absent. This assumption was made since lard normally contains only trace amounts of linolincin or more highly unsaturated acids. From the results of this calculation the increase in the unsaturation of the fats, as shown in tables 3 and 5, was found to be due to greater percentages of olein and linolein while saturated glycerides decreased.

These effects of thyroprotein might be considered undesirable. The production of soft fat, with the increase in the susceptibility to oxidation, increases the problems of storage of the extracted fat as well as the problems of producing a desirable fat more rapidly. While thyroprotein fed at the level of 6 grams per 100 pounds of feed may not have a beneficial effect on the economy of gains, it did produce a softer fat. Thus, if fed in larger amounts to produce more rapid fattening, the undesirable effects would possibly be more pronounced.

If thyroprotein had any effect on the rate of formation of peroxides in the stored fat it was not evident in this study. Saponification numbers were not affected.

Total ash content of dry fat free bone is usually used to show varying degrees of calcification. In this study thyroprotein had no effect on bone ash.

Slight differences in the vitamin A of the livers of pigs on comparable rations, with and without thyroprotein, is evidence that when fed at the level of 6 grams per 100 pounds of feed thyroprotein is without effect on the utilization of vitamin A.

Comparison of low- and high-fat rations shows that fat constants were affected to a greater extent by differences in the fat content of the two rations than by the addition of thyroprotein. Iodine numbers were increased and melting points decreased in all instances. The increase in thiocyanogen numbers in the high-fat rations was apparently due to increased percentages of olein and linolein; saturated glycerides decreased. The fact that saponification numbers showed no significant differences between low- and high-fat rations indicates that the fatty acids making up the glycerides had about the same average molecular weight. These results are in agreement with the general observation of practical feeders that hogs fattened on high carbohydrate rations produce a firm desirable fat.

Apparently the difference in fat level of the two rations was without effect on bone calcification.

There was a marked decrease in the liver vitamin A of pigs fed the high-fat rations. From this it might appear that the level of dietary fat increased the vitamin A requirements of the pigs which resulted in

a decrease in the amounts stored, or that it had some adverse effect on vitamin A storage. Reference to table 2 shows, however, that all groups did not consume the same amounts of carotene. For trial I (1949), groups III and IV, in which there was a decreased vitamin A storage in the liver, consumed approximately 14 percent less feed per day during a slightly longer feeding period than groups I and II on the low-fat rations. In trial II (1950), groups III and IV consumed approximately 5 percent less feed per day than groups I and II. Thus, it appears that the lower vitamin A for pigs on the high-fat rations may be due to their lower carotene intake as well as to the high-fat content of their rations.

CONCLUSIONS

Thyroprotein fed at the level of 6 grams per 100 pounds of feed to young pigs weighing 40 pounds has only a slight adverse effect on the character of the body fat as measured by the iodine and thiocyanogen numbers, melting point, saponification number, and peroxide value. When fed in this amount thyroprotein is without effect on bone calcification or storage of vitamin A in the liver.

The addition of 10 percent lard or beef fat to low-fat rations for pigs has an appreciable effect on the fat constants of the body fat but is without effect on the calcification of bone. When added in this amount, fat decreases the melting point and increases the degree of unsaturation of the body fat as shown by an increase in iodine and thiocyanogen numbers and the percentages of olein and linolein.

A decrease in the amount of vitamin A found in the liver was observed in pigs fed the high-fat rations. This may have been due to the decreased carotene intake as well as to an effect of increased fat intake.

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APPENDIX

Table 1. Individual results of analysis of tissues taken from pigs at the end of trial I (1949).

Ration number	Animal number	Fat				Liver
		Iodine number	Melting point °C	Thiocyanogen number	Sapon. number	Vitamin A mcg. per gm.
I	1-1	51.98	39.1	46.51	193.26	48.3
	2-1	52.55	38.8	-----	196.01	44.9
	4-1	52.72	43.7	47.71	196.32	----
	5-1	51.67	----	45.99	195.83	19.7
	6-1	50.33	----	45.71	194.60	21.0
	7-1	52.59	40.6	53.51	195.09	16.7
	8-1	50.55	----	45.66	------	20.2
	average	51.77	40.6	46.32	194.17	26.9
II	2-2	60.99	32.8	53.78	195.05	40.3
	3-2	56.36	40.5	47.85	------	29.1
	4-2	53.61	40.1	47.35	196.32	14.7
	5-2	53.45	40.5	47.35	194.94	31.7
	6-2	51.16	44.6	46.49	194.06	37.1
	7-2	51.58	----	48.53	------	31.9
	8-2	50.97	----	46.13	195.81	41.2
	average	54.02	39.5	48.21	195.09	32.3
III	1-3	62.88	----	54.49	------	8.8
	2-3	57.89	----	51.26	------	11.6
	3-3	56.06	----	49.08	------	30.0
	4-3	64.66	34.5	-----	195.39	----
	5-3	61.85	38.7	-----	193.93	16.7
	6-3	61.42	30.1	53.38	194.96	----
	7-3	58.22	34.3	51.45	194.24	----
	8-3	61.41	----	54.11	------	10.0
	average	60.55	34.4	52.30	194.63	15.4
IV	2-4	60.69	38.5	-----	194.14	30.4
	3-4	61.69	----	54.90	------	----
	4-4	61.99	----	53.58	------	----
	5-4	66.04	27.6	-----	194.14	13.2
	6-4	64.58	29.2	56.08	193.31	7.7
	7-4	62.86	34.5	56.20	194.38	7.8
	8-4	61.18	----	53.80	------	12.5
	average	62.78	32.5	54.83	193.99	13.8

Table 2. Individual results of analysis of tissues taken from pigs at the end of trial II (1950).

		Fat				Bone	Liver
Ration number	Animal number	Iodine number	Melting point °C	Thiocyanogen number	Sapon. number	Ash percent	Vitamin A mcg. per gm.
I	1-1	55.06	39.3	41.86	193.79	-----	33.1
	2-1	54.60	----	-----	------	66.82	25.3
	3-1	50.91	----	-----	------	70.09	34.5
	4-1	51.52	40.6	38.87	195.56	69.44	43.1
	5-1	55.72	----	-----	------	69.70	21.2
	6-1	54.54	----	-----	------	69.48	33.5
	7-1	54.37	39.9	42.18	196.29	69.46	31.7
	8-1	52.58	40.3	40.70	195.43	69.39	23.9
	average	53.66	40.0	40.90	195.27	69.20	30.8
II	1-2	55.48	----	-----	------	67.63	32.1
	2-2	54.49	----	-----	------	69.76	33.1
	3-2	54.43	----	-----	------	70.25	41.2
	4-2	51.59	54.0	42.44	194.98	70.06	28.8
	5-2	51.35	45.0	41.32	194.91	-----	31.3
	6-2	53.44	39.0	40.89	195.89	69.64	36.0
	7-2	55.88	42.0	42.13	195.59	67.96	37.6
	8-2	56.23	----	-----	------	67.09	24.7
	average	54.12	42.8	41.70	195.34	68.91	33.1
III	1-3	60.47	33.7	42.43	194.06	65.61	20.2
	2-3	59.08	----	-----	------	70.41	29.6
	3-3	59.03	35.5	45.12	194.78	69.84	20.2
	4-3	57.23	43.5	44.39	194.51	69.68	14.5
	5-3	61.15	32.5	47.30	193.22	-----	8.9
	6-3	57.89	----	-----	------	69.38	16.2
	7-3	58.99	----	-----	------	-----	18.0
	8-3	55.55	----	-----	------	70.70	17.0
	average	58.68	36.3	44.81	194.14	69.27	18.1
IV	1-4	57.41	36.5	46.17	195.43	-----	17.2
	2-4	60.25	31.6	49.39	195.54	69.29	24.3
	3-4	64.15	----	-----	------	68.99	9.4
	4-4	61.43	32.3	48.35	195.18	-----	16.7
	5-4	59.89	31.5	46.79	194.76	69.67	23.3
	6-4	63.40	----	-----	------	69.15	30.4
	7-4	62.01	----	-----	------	68.99	22.4
	8-4	61.57	----	-----	------	-----	23.7
	average	61.26	33.0	47.68	194.98	69.22	20.9

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