15385

NATIONAL LIBRARY
OTTAWA

CANADA

BIBLIOTHÈQUE NATIONALE
OTTAWA

NAME OF AUTHOR...SHARON JOY YOUNG.....

TITLE OF THESIS...STUDIES OF THE KETOGENICITY OF SATURATED AND UNSATURATED FATS FOR THE CHICK.....

UNIVERSITY...OF ALBERTA (EDMONTON).....

DEGREE FOR WHICH THESIS WAS PRESENTED..M.Sc.....

YEAR THIS DEGREE GRANTED...1973.....

Permission is hereby granted to THE NATIONAL LIBRARY OF CANADA to microfilm this thesis and to lend or sell copies of the film.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

(Signed) Sharon J. Young

PERMANENT ADDRESS:

12340 - 89 STREET

EDMONTON, ALBERTA

CANADA

DATED March 1.....1973

NL-91 (10-68)

THE UNIVERSITY OF ALBERTA

STUDIES OF THE KETOGENICITY OF SATURATED AND UNSATURATED FATS FOR THE CHICK

by

© SHARON JOY YOUNG

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

SCHOOL OF HOUSEHOLD ECONOMICS

EDMONTON, ALBERTA

SPRING, 1973

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Studies of the ketogenicity of saturated and unsaturated fats for the chick" submitted by Sharon Joy Young, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

.....
Supervisor

.....

.....

Date.....

ABSTRACT

Experiments were conducted to study the ketogenicity of various fats for the chick. Initially experiments were conducted to compare the ketogenicity of a saturated fat, coconut oil, and an unsaturated fat, soybean oil. Subsequently the effect of a vitamin B_{12} deficiency on the ketogenicity of beef tallow and safflower oil was studied.

Results of these experiments showed that substitution of the polyunsaturated fats, safflower oil and soybean oil, for the saturated fats, beef tallow and coconut oil, did not reduce levels of blood ketone bodies nor increase levels of liver glycogen in chicks fed "carbohydrate-free" diets. These results show that polyunsaturated fats are just as ketogenic as saturated fats and do not indicate that propionyl CoA is formed in the oxidation of polyunsaturated fatty acids. In subsequent studies, the failure of a vitamin B_{12} deficiency, produced by deleting vitamin B_{12} from a diet low in one carbon units, to increase the ketogenicity of beef tallow and safflower oil also suggests that propionyl CoA is not formed in the oxidation of polyunsaturated fatty acids.

The effects of supplementing chick diets with sodium propionate were also studied. Results showed that the addition of sodium propionate to "carbohydrate-free" diets in which non-protein energy was supplied by either safflower oil or beef tallow did not affect level of blood lactic acid but caused a reduction in levels of both blood ketone bodies and liver glycogen which was most marked in the absence of supplementary vitamin B_{12}.

In the course of these experiments it was observed that chicks fed "carbohydrate-free" diets containing coconut oil grew slightly but

significantly slower and had higher levels of liver glycogen than chicks fed "carbohydrate-free" diets containing soybean oil. The growth depressing property of coconut oil was accentuated when glycerol was deleted from the diet by substituting coconut fatty acids for coconut oil and, in contrast to soybean fatty acids, the growth depressing property of coconut fatty acids was not overcome by the addition of glucose. Studies showed that the growth depressing property of coconut oil and coconut fatty acids was not due to reduced absorbability since these lipids were shown to be absorbed to 99 and 92%, respectively. Whether lauric and myristic acids contributed to the growth depression observed when chicks were fed diets containing coconut oil and coconut fatty acids is unknown.

Results of these studies do not support the hypothesis that polyunsaturated fats are metabolized via propionyl CoA and negate the possibility that the increased requirement for vitamin B_{12} observed when chicks are fed high fat-"carbohydrate-free" diets is due to an increased need for the cofactor for methylmalonyl CoA mutase. Thus the question of why the chick's requirement for vitamin B_{12} is increased when fat is substituted isocalorically for glucose remains unanswered.

ACKNOWLEDGMENTS

The author wishes to thank Dr. Ruth Renner, Professor of Nutrition, School of Household Economics, for her invaluable guidance which was freely given at all times. Thanks are also extended to Dr. E. A. Donald, Dr. Z. Hawrysh and Dr. M. E. Stiles whose interest and constructive discussion was much appreciated.

The technical assistance of Mr. A. Cheung, of the School of Household Economics, and Mr. W. Giese, of the Department of Animal Science, is also gratefully acknowledged. The assistance of Mrs. C. Land in the typing of this manuscript is greatly appreciated.

Special thanks is due to the author's husband for his patience and understanding.

Financial assistance from the National Research Council of Canada is also gratefully acknowledged.

TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Previous investigations have shown that the chick can sustain normal growth on a high fat, "carbohydrate-free" diet if nutrient balance is maintained (Renner, 1964; Renner and Elcombe, 1964, 1967; Brambila and Hill, 1966, 1967; Scott et al., 1969). Looi (1971), studying the chick's requirement for vitamin B_{12}, observed that chicks fed a high fat diet containing hydrogenated fat (Crisco) required three times as much vitamin B_{12} in their diet to achieve maximum growth as that required by chicks fed a high carbohydrate diet. Only two functions of vitamin B_{12} in animal metabolism have been well established. One of these is the role of vitamin B_{12} in the biosynthesis of labile methyl groups by which methionine is synthesized from homocysteine. Looi's experiments showed that the increased requirement for vitamin B_{12} that she observed was not a reflection of an increased need for methionine.

The other known function of vitamin B_{12} in animal tissues is its role in the metabolism of propionate. The conversion of propionyl CoA to succinyl CoA is a two-step process in which vitamin B_{12} is an essential coenzyme. Sinclair (1964) proposed a pathway for unsaturated fatty acid metabolism which involved propionyl CoA as an intermediate.

Whether production of propionyl CoA during oxidation of unsaturated fatty acids contributes to the increased requirement for vitamin B_{12} observed by Looi (1971) when fat was substituted isocalorically for carbohydrate forms the basis of the following experiments. Initially experiments were conducted to compare the ketogenicity of saturated and unsaturated fats for the chick. Subsequently the effect of a vitamin B_{12} deficiency on the ketogenicity of a saturated and an unsaturated fat was studied.

LITERATURE REVIEW

Recent studies (Looi, 1971) have shown that the requirement of the chick for vitamin B_{12} was increased from 9 µg/kg of diet to 27 µg/kg of diet when hydrogenated fat (Crisco) was substituted isocalorically for glucose. In studies to determine why the chick's requirement for vitamin B_{12} was increased when non-protein energy was supplied by fat, Looi (1971) showed that the increased requirement was not due to an increased requirement for methionine. She also showed that vitamin B_{12} was not involved in either fat digestion or fat absorption and her studies suggested that vitamin B_{12} was not involved in the oxidation of acetate. Thus, the reason why the isocaloric substitution of fat for carbohydrate increases the chick's requirement for vitamin B_{12} remains to be elucidated.

Previous studies had implicated vitamin B_{12} in the metabolism of fat. Thus, Moore and Doran (1962) showed that vitamin B_{12} deficiency interfered with the utilization of triglycerides in the liver of chick embryos. Similarly, Williams et al. (1937) found that the utilization of plasma triglycerides was impaired in patients suffering from pernicious anemia.

One of the known functions of vitamin B_{12} in animal tissues is its role as coenzyme in the isomerization of methylmalonyl CoA to succinyl CoA (Lengyel et al., 1960). That the formation of methylmalonyl CoA in catabolism increases the rat's requirement for vitamin B_{12} was shown by Dryden and Hartman (1971) in studies to determine why vitamin B_{12} requirement was increased 7.5-fold when dietary protein was increased from 25 to 65%. Results of their studies indicated that the

amino acids isoleucine, threonine and valine contributed to the growth depression observed when rats were fed high casein, vitamin B_{12}-deficient rations. Since methylmalonyl CoA is formed in the normal catabolism of isoleucine, threonine and valine, these results suggest an explanation for the increased vitamin B_{12} requirement of rats fed high protein diets.

Methylmalonyl CoA is also formed during the metabolism of propionate (Flavin and Ochoa, 1957). Known sources of propionate in animal metabolism are fatty acids containing an uneven number of carbon atoms (Kaziro and Ochoa, 1964), the pyrimidine bases uracil and thymine (Contreras and Giorgio, 1972), and, in the case of ruminants, bacterial fermentation of carbohydrates in the digestive tract. Propionic acid has also been found in the digestive tract of the fowl, mostly in the ceca, as a product of microbial metabolism (Annison et al., 1968). Sinclair (1964) has suggested that propionate may also be formed in the oxidation of unsaturated fatty acids through the hydration of fatty acyl CoAs containing β-γ unsaturated double bonds and their subsequent thiolytic cleavage following dehydrogenation. He proposed that in the oxidation of linolenic acid, after the removal of three molecules of acetyl CoA, the dodecatrienyl CoA formed is oxidized to yield four molecules of propionyl CoA.

That the double bonds in unsaturated fatty acids may also shift from the β-γ to the α-β position has now been shown. An enzyme which catalyzes this reaction has been isolated from rat liver mitochondria and has been named $\Delta^{3,4}$cis-$\Delta^{2,3}$-trans-enoyl CoA isomerase (Stoffel et al., 1964). This enzyme together with 3-hydroxy fatty acyl CoA epimerase (Stoffel et al., 1964; Stern et al., 1955) are required,

in addition to the enzymes normally needed for β-oxidation, to complete the oxidation of polyunsaturated fatty acids. Thus unsaturated fatty acids, like saturated fatty acids, may be oxidized to yield only acetyl CoA.

Indirect evidence in support of Sinclair's hypothesis of an alternate route of fatty acid oxidation has been furnished by Dupont and Mathias (1969). They showed that, when rats fed diets containing 20% corn oil with and without vitamin B_{12} were injected with U-^{14}C-linoleate intraperitoneally, ^{14}C-methylmalonate was excreted in the urine. Vitamin B_{12} nutriture did not affect the amount excreted in the first 12 hours. During the second 12 hours and during the second day excretion of ^{14}C-methylmalonate was higher in vitamin B_{12}-deficient rats than in rats receiving supplemental vitamin B_{12}. These results suggest that one pathway of linoleate oxidation has methylmalonate as an intermediate. In addition, Dupont and Mathias (1969) also observed that rats fed 20% corn oil grew more slowly on vitamin B_{12}-deficient than on vitamin B_{12}-supplemented diets. No such effect was observed in rats fed beef tallow. These results suggest that highly unsaturated fat enhances a vitamin B_{12} deficiency.

Several studies have been reported showing that saturated fats are more ketogenic than unsaturated fats. These studies may be interpreted as indicating that unsaturated fatty acids have the potential to supply oxaloacetate (Hahn et al., 1963). In this regard, Brahmankar and Nath (1963) found that a greater accumulation of ketone bodies in plasma, liver, kidney and heart muscle occurred when rats were fed a diet containing 35% saturated fat (butter fat, coconut oil, or hydrogenated fat) than when diets containing a similar level of unsaturated

fat (groundnut oil or sesame oil) were fed. Similar results have been reported by Tidwell et al., (1966). They found that rats fed lard had approximately 40% higher levels of blood ketone bodies than rats fed linseed oil. In addition they found that the livers of rats fed linseed oil and safflower oil contained up to 2.5 times as much glycogen as did the livers of rats fed diets containing lard.

More recently, Chung and Dupont (1968) fed high fat diets containing beef tallow or corn oil to female rats. They observed that the concentration of acetoacetate in the plasma of corn oil-fed rats was half that of the level in rats fed beef tallow. They concluded that dietary lipid containing a high concentration of polyunsaturated fatty acids was not ketogenic and that the kind of fat not the amount appeared to affect ketogenicity.

Krebs (1970) disagrees with Sinclair's hypothesis. Krebs (1970) studied the formation of ketone bodies and glucose in isolated perfused rat liver after the addition of fatty acids. The rate of ketone body production from the fatty acids tested was approximately equal and independent of chain length except for pentanoate which produced ketone bodies at one-third the rate of other fatty acids. Glucose was formed from all odd-numbered fatty acids tested. Arachidonate was almost quantitatively converted to ketone bodies and yielded no glucose. Krebs concluded that gluconeogenesis from polyunsaturated fatty acids with an even number of carbon atoms does not occur. Winkler (1970) also reported no significant differences in the production of ketone bodies during the oxidation of palmitate and linoleate by rat tissue slices and mitochondria.

Some investigators (Tidwell et al., 1966 and Dupont and Mathias, 1969) cite the finding of Björntorp (1968) that unsaturated fatty acids are oxidized more rapidly than saturated fatty acids of the same chain length as evidence in favor of an alternative route for the oxidation of unsaturated fatty acids. Numerous studies have shown that unsaturated fatty acids are oxidized more rapidly than saturated fatty acids. For example, Mead et al. (1956) reported that more oleate and linoleate were collected as expired $^{14}CO_2$ than stearate in 24 hours when ^{14}C-labelled fatty acids were fed to mice. In addition, Göransson and Olivecrona (1965) and Göransson (1965a,b,c) reported that in 320 hours after an intravenous dose of ^{14}C-labelled fatty acids to rats, labelled oleic, palmitoleic and linoleic acids were oxidized more rapidly than palmitic acid. Furthermore, Lynn and Brown (1959) reported that labelled linoleate was oxidized more rapidly than labelled stearate when they were fed to rats. Dupont (1966) reported similar results when linoleate and stearate were injected into rats intraperitoneally. Whether the increased rate of oxidation of unsaturated fatty acids can be attributed to an alternative oxidative pathway in which propionyl CoA is produced is unknown.

Since information on the ketogenicity of various fats for the chick is lacking, the following studies were conducted to compare the ketogenicity of saturated and unsaturated fats when chicks were fed high fat, "carbohydrate-free" diets. Subsequently, studies were conducted to determine the effect of a vitamin B_{12} deficiency on the ketogenicity of fat. It was hoped that these studies would cast some light on why the vitamin B_{12} requirement of chicks fed high fat diets is increased.

EXPERIMENT I

Recent studies (Brahmankar and Math, 1963; Tidwell et al., 1966; Chung and Dupont, 1968) have shown that saturated fats are more ketogenic than unsaturated fats when incorporated into the diets of rats. In order to determine whether fats vary in their ketogenicity for chicks the following study was conducted to compare the ketogenicity of coconut oil and soybean oil when these fats served as the sole source of non-protein energy in "carbohydrate-free" diets. In addition, in order to maximize differences, comparisons were made of the ketogenicity of these fats when fed in the absence of glycerol as coconut fatty acids and soybean fatty acids.

Materials and Methods

Diets in which non-protein energy was supplied by coconut oil, coconut fatty acids, coconut fatty acids plus glucose, soybean oil, soybean fatty acids and soybean fatty acids plus glucose were formulated from the high carbohydrate diet shown in Table 1. Fats and fatty acids were substituted isocalorically for glucose assuming the metabolizable energy content of glucose, coconut oil, coconut fatty acids, soybean oil and soybean fatty acids to be 3.64, 9.21, 7.83, 9.21 and 7.83 kilocalories per gram, respectively. In the fatty acid diets supplemented with glucose, 0.105 g glucose/g fatty acids were added (Renner and Elcombe, 1964); this is the amount of glucose required for the theoretical conversion of soybean fatty acids to triglyceride.

The composition of the diets fed is shown in Table 2. All diets were formulated to contain 14.2 kilocalories of metabolizable

Table 1

Composition of high carbohydrate diet

Ingredients	%
Constants	
Soybean protein[1]	23.59
Methionine	0.81
Glycine	0.63
Soybean oil	2.00
Limestone	1.49
Dicalcium phosphate	1.70
Sodium chloride	0.60
Mineral mix[2]	1.243
Vitamin mix[3]	0.58
Antioxidant[4]	0.025
Chromic oxide	0.3
Source of non-protein energy	
Glucose	67.03

[1]Promine, Central Soya, Chemurgy Division, Chicago 39, Illinois.

[2]Mineral mixture supplies in grams per kilogram of diet: KH_2PO_4, 9.30; $MgSO_4$, 2.42; KI, 0.0029; $FeSO_4 \cdot 7H_2O$, 0.278; $CuSO_4 \cdot 5H_2O$, 0.0078; $ZnCO_3$, 0.113; $CoCl_2 \cdot 6H_2O$, 0.0017; $Na_2MoO_4 \cdot 2H_2O$, 0.0083; Na_2SeO_3, 0.00022; $MnSO_4 \cdot H_2O$, 0.33.

[3]Vitamin mixture supplies in grams per kilogram of diet: thiamine HCl, 0.01; riboflavin, 0.01; calcium pantothenate, 0.04; biotin, 0.0004; pyridoxine, 0.02; niacin, 0.08; folacin, 0.003; menadione, 0.003; vitamin A, 10,000 I.U.; vitamin D, 1,500 I.U.; vitamin E, 33.1 I.U.; choline chloride, 3.0; vitamin B_{12}, 0.00005.

[4]Ethoxyquin.

Table 2

Composition of diets

Source(s) of energy (Diet)	Constant ingredients g	Glucose g	Soybean oil g	S.F.A.[1] g	Coconut oil g	C.F.A.[2] g	Cellulose[3] g	Total g
Glucose	32.96	67.03	--	--	--	--	--	99.99
Soybean oil[4]	32.96	--	26.49	--	--	--	10.00	69.45
S.F.A.	32.96	--	--	28.21	--	--	10.00	71.17
S.F.A. + 0.105 g glucose/g S.F.A.	32.96	2.83	--	27.01	--	--	10.00	72.80
Coconut oil[5]	32.96	--	--	--	26.49	--	10.00	69.45
C.F.A.	32.96	--	--	--	--	31.16	10.00	74.12
C.F.A. + 0.105 g glucose/g C.F.A.	32.96	3.12	--	--	--	29.71	10.00	75.79

[1]Soybean fatty acids.

[2]Coconut fatty acids.

[3]Alpha Floc SW40, Lee Chemicals Ltd., 1119 Yonge Street, Toronto 5, Ontario.

[4]Composition in g/100 g fatty acids: Palmitic acid, 7.1; Stearic acid, 3.8; Oleic acid, 39.8; Linoleic acid, 39.2; Linolenic acid, 8.5; Gadoleic acid, 1.6.

[5]Composition in g/100 g fatty acids: Caproic acid, 0.1; Caprylic acid, 3.1; Capric acid, 5.8; Lauric acid, 45.2; Myristic acid, 19.0; Palmitic acid, 10.9; Stearic acid, 3.8; Oleic acid, 9.3; Linoleic acid, 2.7.

energy per gram of protein. Cellulose was added to improve the texture of the high fat diets in an amount to maintain their caloric density approximately equal to the high carbohydrate control diet. Vitamin B_{12} was incorporated in the high carbohydrate diet at a level of 50 µg per kilogram of feed. Because the "carbohydrate-free" diets do not total to 100, their content of vitamin B_{12} and other dietary constituents will be referred to as the level present in the carbohydrate-containing diets from which they were derived.

Coconut fatty acids and soybean fatty acids were prepared by alkaline hydrolysis of coconut oil and soybean oil. After acidification the fatty acids were separated from the acid seat by decantation and washed with water to remove acid and glycerol.

Each diet was fed to duplicate groups of 10 male crossbred (Dominant White x White Plymouth Rock) chicks from 8 to 29 days of age. During the first 7 days of life the chicks were fed a semipurified "carbohydrate-free" diet containing soybean oil. They were then assigned to the experimental groups on the basis of body weight, equalizing both mean body weight and weight distribution among the groups. The chicks were housed in electrically heated, thermostatically controlled battery brooders with raised wire-screen floors in a temperature-controlled laboratory. Feed and water were supplied ad libitum. Data on growth and feed consumption were obtained weekly.

During the fourth week of the experiment, excreta were collected from duplicate groups of chicks fed diets in which non-protein energy was supplied by glucose, coconut oil and coconut fatty acids so that the absorbability and metabolizable energy of coconut oil and coconut fatty

acids could be determined. Chromic oxide was incorporated into all rations as an index substance in order to eliminate the need for quantitative collection of excreta and quantitative measurement of feed intake. The excreta were collected at 24-hour intervals for three consecutive days and kept in the frozen state until processed. The methods for processing excreta and determining moisture and chromic oxide were described by Hill and Anderson (1958). Fecal fat was determined using the method of Fowweather and Anderson (1946) as modified by Renner and Hill (1960). Dietary fat was determined using diethyl ether and a Goldfisch apparatus.

At 29 days of age, blood samples were obtained from the jugular vein of 5 chicks in each group using 3.75 mg sodium oxalate per ml of blood to prevent coagulation and 0.5 mg sodium fluoride per ml of blood to inhibit glycolysis. Protein-free blood filtrates were prepared using barium hydroxide and zinc sulfate (Nelson, 1944; Somogyi, 1945). The filtrates were frozen and stored at -29°C until analyzed. Blood glucose levels were determined using the method of Folin and Malmros (1929). Blood ketone bodies were determined using a modification of the method of Bakker and White (1957). This modification consisted of heating the tubes in a glycerol bath at 110-120°C for 15 minutes and then heating in an autoclave at 15 lbs pressure (121°C) for 30 minutes after the addition of potassium dichromate.

Liver samples were taken from the remaining 5 chicks in each group immediately after killing with sodium pentobarbital. These samples were frozen immediately using dry ice, wrapped in aluminum foil, and stored in plastic bags at -29°C until analyzed. Liver glycogen was precipitated using the method of Good et al. (1933). The precipitate was

washed with 65% ethanol as suggested by Fong et al. (1953). The glycogen was then dissolved in water and estimated using the method of Seifter et al. (1950).

Results and Discussion

Summarized in Table 3 are data showing the average weight gains of chicks fed diets in which non-protein energy was supplied by glucose, soybean oil, coconut oil and their respective fatty acids with and without added glucose. Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the growth data showed that chicks fed diets in which non-protein energy was supplied by soybean oil or coconut oil grew at the same rate and at a rate equal to that of chicks fed diets in which non-protein energy was supplied by glucose. Deletion of glycerol from the diet by substituting soybean fatty acids for soybean oil depressed growth, significantly ($P < 0.05$). The substitution of coconut fatty acids for coconut oil also depressed growth but to a much greater extent than did soybean fatty acids. The addition of glucose in an amount required for theoretical conversion of fatty acids to triglycerides (0.105 g/g fatty acids) alleviated the growth depression in chicks fed soybean fatty acids but only partially alleviated the depression in growth of chicks fed coconut fatty acids.

That the isocaloric substitution of soybean fatty acids for soybean oil depresses growth is now well established (Renner and Elcombe, 1964; Brambila and Hill, 1967). The effectiveness of glucose in overcoming the growth depression of chicks fed soybean fatty acids has also been demonstrated (Renner and Elcombe, 1964; Brambila and Hill, 1967). Thus the failure of glucose (0.105 g/g fatty acids) to overcome the

Table 3

Growth and caloric efficiency of chicks fed carbohydrate-containing and "carbohydrate-free" diets

Treatment	Average weight gain g	Kcal consumed/ g gain[1]
Glucose	481[2,a]	5.28[b]
Soybean oil	502[a]	5.09[b]
S.F.A.[3]	327[c]	5.56[b]
S.F.A. + 0.105 g glucose/g S.F.A.	480[a]	5.04[b]
Coconut oil	478[a]	4.98[b]
C.F.A.[4]	197[d]	6.89[a]
C.F.A. + 0.105 g glucose/g C.F.A.	366[b]	5.32[b]

[1]Calculated from calculated metabolizable energy values for the diets.

[2]Values are averages of duplicate groups each containing 10 chicks. Values without a common letter in their superscripts are significantly different ($P < 0.05$).

[3]Soybean fatty acids.

[4]Coconut fatty acids.

growth depression in chicks fed coconut fatty acids was unexpected. One possible explanation is that the glucose requirement of chicks fed coconut fatty acids is greater than that of chicks fed soybean fatty acids. On the other hand, studies have shown that lauric and myristic acids, when fed singly at a level of 20% in the diet, depress chick growth (Renner and Hill, 1961). Calculations indicate that the coconut fatty acid diet contained 14% lauric acid and 6% myristic acid. Thus, the failure of glucose to overcome the growth depression when coconut fatty acids were fed may be due to the growth depressing properties of lauric and myristic acids in unesterified form. Since coconut oil and coconut fatty acids were found to be absorbed to 99 and 92%, respectively, in this experiment, the depressed growth of chicks fed coconut fatty acids cannot be attributed to low fat absorbability.

Results showing that chicks fed diets containing coconut oil grew at the same rate as chicks fed soybean oil are in contrast to results reported by Menge (1971). He observed that chicks fed a diet containing 12% coconut oil grew less than chicks fed diets containing 7% coconut oil or 0.15, 0.27, 2 or 4% safflower oil.

Data on caloric efficiencies summarized in Table 3 show that replacing glucose isocalorically by soybean oil, coconut oil, soybean fatty acids or soybean fatty acids plus glucose did not affect caloric efficiency, significantly ($P > 0.05$). In contrast, chicks fed diets containing coconut fatty acids utilized energy less efficiently than chicks fed the glucose diet; however, caloric efficiency was restored by the addition of 0.105 g glucose/g coconut fatty acids.

Other evidence that fatty acids in coconut oil may be utilized

differently than fatty acids in other vegetable oils is provided in the studies of Carew et al. (1964). They observed that chicks fed diets containing 20% coconut oil did not increase growth rate, energy intake, tissue fat gain or total energy gain above that obtained with chicks fed a basal, low fat diet. In contrast, Carew et al. (1964) found that chicks fed diets containing corn oil, beef tallow, soybean oil, or a lightly hydrogenated olive oil deposited more energy than chicks fed the low fat basal diet.

Summarized in Table 4 are data showing the metabolic effects of feeding chicks diets in which non-protein energy was supplied by glucose, soybean oil, coconut oil and their respective fatty acids with and without added glucose. Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the data on blood glucose showed that the substitution of soybean oil or coconut oil for the glucose in a high carbohydrate diet had no effect on level of blood glucose ($P > 0.05$). Deletion of glycerol from the diet by substituting soybean fatty acids or coconut fatty acids for their respective oils caused a significant reduction in level of blood glucose ($P < 0.05$). The addition of glucose to the fatty acids diets did not increase level of blood glucose, significantly ($P > 0.05$).

Previous studies with chicks have shown that normal blood glucose levels are maintained when chicks are fed "carbohydrate-free" diets containing neutral fat (Renner and Elcombe, 1967; Brambila and Hill, 1967). Variable effects on level of blood glucose have been observed when glycerol is deleted from the diet by substituting soybean fatty acids for soybean oil. Renner and Elcombe (1967) found levels of blood glucose to

Table 4

Metabolic effects of feeding various fats to the chick

Treatment	Blood glucose mg%	Blood ketone bodies[1] mg%	Liver glycogen content g%
Glucose	190[2,a,b]	1.8[2,b]	3.53[2,a]
Soybean oil	186[a,b,c]	7.8[b]	0.44[c]
S.F.A.[3]	147[d]	50.8[a]	0.10[d]
S.F.A. + 0.105 g glucose/g S.F.A.	159[c,d]	9.2[b]	0.54[b,c]
Coconut oil	202[a]	6.0[b]	0.68[b,c]
C.F.A.[4]	152[d]	57.8[a]	0.12[d]
C.F.A. + 0.105 g glucose/g C.F.A.	173[b,c,d]	9.6[b]	0.81[b]

[1]Total ketone bodies as acetone.

[2]Values are averages of duplicate groups each containing 5 chicks. Values without a common letter in their superscripts are significantly different ($P < 0.05$).

[3]Soybean fatty acids.

[4]Coconut fatty acids.

be maintained while Brambila and Hill (1967) and Allred (1969) found blood glucose levels to be decreased. My results in this experiment using soybean fatty acids and coconut fatty acids as sources of non-protein energy support the findings of Brambila and Hill (1967) and Allred (1969) in that levels of blood glucose were decreased when non-protein energy was supplied by fatty acids.

Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the data on blood ketone bodies showed that the substitution of soybean oil or coconut oil for the glucose in a high carbohydrate diet did not increase levels of blood ketone bodies, significantly ($P > 0.05$). The data also show that soybean oil and coconut oil, when serving as the sole source of non-protein energy in the diet of chicks, did not differ in ketogenicity. Deletion of glycerol from the diet by substituting soybean fatty acids and coconut fatty acids for soybean oil and coconut oil, respectively, caused a marked but similar increase ($P < 0.05$) in level of blood ketone bodies which was alleviated in both cases by the addition of 0.105 g glucose/g fatty acids.

These results agree with those reported previously from this laboratory (Renner and Elcombe, 1967); however, the finding that the isocaloric substitution of soybean oil for glucose did not increase blood ketones is in contrast to the results of Brambila and Hill (1966, 1967) and Allred (1969).

My results indicating that degree of saturation of dietary fat does not affect level of blood ketone bodies in chicks is in contrast to results reported for the rat (Brahmankar and Nath, 1963; Tidwell et al. 1966; and Chung and Dupont, 1968). These investigators showed that rats

fed such saturated fats as coconut oil, lard, beef tallow or butter fat had higher levels of blood ketones than rats fed such unsaturated fats as safflower oil, linseed oil, sesame oil or groundnut oil. Sinclair (1964) suggested, and Dupont and Mathias (1969) have provided evidence in the rat, that unsaturated fats are metabolized via propionyl CoA and are, therefore, more glucogenic and less ketogenic than saturated fats. The results reported in this experiment do not indicate the existence of such a pathway in the chick. Krebs (1970), using perfused rat livers, concluded that polyunsaturated fatty acids with an even number of carbon atoms were not glucogenic.

Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the data on liver glycogen (Table 4) showed that the substitution of soybean oil or coconut oil for glucose in a high carbohydrate diet significantly decreased the concentration of liver glycogen ($P < 0.05$). Deletion of glycerol from the diet by substituting soybean fatty acids and coconut fatty acids for soybean oil and coconut oil, respectively, caused a further reduction in level of liver glycogen ($P < 0.05$). The addition of glucose (0.105 g/g fatty acids) to the fatty acid diets increased level of liver glycogen ($P < 0.05$) permitting glycogen stores not significantly different from those present in the livers of chicks fed the respective oil.

The depression in liver glycogen observed when calories from fat were substituted for calories from carbohydrate agrees with the findings of Renner and Elcombe (1967) in the chick and Zaragoza and Felber (1970) in the rat. The finding that in this experiment chicks fed coconut oil deposited amounts of glycogen in their livers similar to chicks

fed soybean oil is in contrast to results reported by Tidwell et al. (1966). They observed that rats fed the polyunsaturated fats, safflower oil and linseed oil, deposited more glycogen than rats fed the more saturated fat, lard; however, they also found that rats fed coconut oil, which is more saturated than lard, deposited approximately twice as much glycogen as rats fed safflower oil or linseed oil. They suggested that this unexpectedly high value for coconut oil might be explained as the result of carbohydrate sparing during the more rapid oxidation of the short chain fatty acids (Carroll, 1964). Kowale et al. (1966) also have reported that glycogen levels are higher in the livers of rabbits fed butterfat, coconut oil, sesame oil and groundnut oil than those fed a normal carbohydrate diet.

EXPERIMENT II

This experiment was conducted to confirm that coconut oil and soybean oil do not differ in ketogenicity for the chick and to determine whether the growth depressing effects of coconut fatty acids could be overcome by the addition of a higher level of glucose (0.210 g glucose/g fatty acids).

Materials and Methods

Diets in which non-protein energy was supplied by coconut oil, coconut fatty acids, coconut fatty acids plus glucose, soybean oil, soybean fatty acids and soybean fatty acids plus glucose were formulated from the high carbohydrate diet shown in Table 1. Fats and fatty acids were substituted isocalorically for glucose assuming the metabolizable energy content of glucose, coconut oil, coconut fatty acids, soybean oil and soybean fatty acids to be 3.64, 9.09, 8.42, 9.21 and 7.83 kilocalories per gram, respectively. Metabolizable energy values for coconut oil and coconut fatty acids were calculated using the determined absorbability values of 99 and 92%, respectively (Experiment I) and assuming coconut oil to have a gross energy of 9.2 kilocalories per gram. Addition of glucose to the fatty acid diets was in an amount to provide 0.105 g glucose/g fatty acids in the case of soybean fatty acids and 0.105 and 0.210 g glucose/g fatty acids in the case of coconut fatty acids. Otherwise the diets are as described in Experiment I.

Chicks were fed a "carbohydrate-free" diet containing soybean oil for one week and then allotted to the experimental groups on the basis of body weight. Each diet was fed to duplicate groups of 10 male

crossbred (Dominant White x White Plymouth Rock) chicks from 8 to 29 days of age. Feed and water were supplied ad libitum. Growth and feed consumption were determined weekly. The methods of housing and collection of blood and liver samples were as described in Experiment I. Determinations of blood glucose, blood ketone bodies and liver glycogen were performed as described previously.

Results and Discussion

Data on weight gain and caloric efficiency (Table 5) show that, as in Experiment I, chicks fed coconut oil grew at the same rate as chicks fed soybean oil and utilized their feed just as efficiently. As in the preceding experiment, coconut fatty acids were more growth depressing than soybean fatty acids when serving as the sole source of dietary non-protein energy. However, in contrast to Experiment I, chicks utilized the coconut fatty acid diet just as efficiently as the soybean fatty acid diet. Results of this experiment show that the addition of 0.210 g glucose/g fatty acids was no more effective than the addition of 0.105 g glucose/g fatty acids in alleviating the growth depressing effects of coconut fatty acids. These results show that the carbohydrate requirement of chicks fed coconut fatty acids is no greater than 0.105 g glucose/g fatty acids when growth is used as the criterion. Whether the glucose requirement is greater than 0.042 to 0.054 g glucose/g fatty acids, that is, the glucose requirement of chicks fed soybean fatty acids (Lodhi et al., 1969) is unknown. The failure of glucose to overcome the growth depressing property of coconut fatty acids may be due to the fatty acid composition of the mixture derived from coconut oil. Previous studies (Renner and Hill, 1961) have shown that the incorporation of 20 parts lauric or

Table 5

Growth and caloric efficiency of chicks fed carbohydrate-containing and "carbohydrate-free" diets

Treatment	Average weight gain g	Kcal consumed/ g gain[1]
Glucose	460[2,a]	5.02[c]
Soybean oil	487[a]	4.88[c]
S.F.A.[3]	279[b]	5.62[a,b]
S.F.A. + 0.105 g glucose/g S.F.A.	435[a]	4.94[c]
Coconut oil	435[a]	4.94[c]
C.F.A.[4]	206[c]	5.98[a]
C.F.A. + 0.105 g glucose/g C.F.A.	298[b]	5.32[b,c]
C.F.A. + 0.210 g glucose/g C.F.A.	322[b]	5.10[c]

[1]Calculated from calculated metabolizable energy values for the diets.

[2]Values are averages of duplicate groups each containing 10 chicks. Values without a common letter in their superscripts are significantly different ($P < 0.05$).

[3]Soybean fatty acids.

[4]Coconut fatty acids.

myristic acid in a semi-purified chick diet caused a significant depression in rate of growth.

Statistical analysis of the combined data on weight gains and caloric efficiencies from Experiments I and II supported the findings observed in individual experiments with one exception. The data in Table 6 show that chicks fed coconut oil grew at a significantly slower rate than chicks fed soybean oil ($P < 0.05$). This trend was noted in both experiments but the difference was not large enough to be significant until the data were combined. Thus, if results of both experiments are considered, my results are in agreement with the studies of Menge (1971) which showed that chicks fed diets containing 12% coconut oil grew significantly slower than chicks fed 7% coconut oil or 0.15, 0.27, 2 or 4% safflower oil. In addition, it should be noted that results summarized in Table 6 indicate that the diet containing coconut fatty acids was utilized less efficiently than diets containing soybean fatty acids. This was observed in Experiment I but in Experiment II the differences were not large enough to be significant.

Summarized in Table 7 are data showing the metabolic effects of feeding chicks diets in which non-protein energy was supplied by glucose, soybean oil, coconut oil and their respective fatty acids with and without added glucose. Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the data on blood glucose (Table 7) showed that source of non-protein calories did not affect blood glucose levels. These results indicated that the chick maintained level of blood glucose in the absence of dietary carbohydrate and glycerol and agrees with the results of Renner and Elcombe (1967). This is in contrast

Table 6

Growth and caloric efficiency of chicks fed carbohydrate-containing and "carbohydrate-free diets

Treatment	Experiment Number	Average weight gain g		Kcal consumed/ g gain	
Glucose	I	481[2]		5.28[2]	
	II	460		5.02	
			470[a,b]		5.15[c,d]
Soybean oil	I	502		5.09	
	II	487		4.88	
			495[a]		4.98[d]
S.F.A.[3]	I	327		5.56	
	II	279		5.62	
			303[c]		5.59[b]
S.F.A. + 0.105 g glucose/g S.F.A.	I	480		5.04	
	II	435		4.94	
			458[a,b]		4.99[d]
Coconut oil	I	478		4.98	
	II	435		4.94	
			456[b]		4.96[d]
C.F.A.[4]	I	197		6.89	
	II	206		5.98	
			202[d]		6.43[a]
C.F.A. + 0.105 g glucose/g C.F.A.	I	366		5.32	
	II	298		5.32	
			332[c]		5.32[b,c]

[1]Calculated from calculated metabolizable energy values for the diets.

[2]Values are averages of duplicate groups each containing 10 chicks. Values without a common letter in their superscripts are significantly different ($P < 0.05$). Underlined values are averages of duplicate experiments.

[3]Soybean fatty acids.

[4]Coconut fatty acids.

Table 7

Metabolic effects of feeding various fats to the chick

Treatment	Blood glucose mg%	Blood ketone bodies[1] mg%	Liver glycogen content g%
Glucose	212[4,a]	2.4[2,d]	4.88[2,a]
Soybean oil	188[a]	11.4[b,c]	0.50[b,c]
S.F.A.[3]	171[a]	54.9[a]	0.21[c]
S.F.A. + 0.105 g glucose/g S.F.A.	171[a]	9.2[b,c]	0.51[b,c]
Coconut oil	177[a]	9.8[b,c]	0.90[b]
C.F.A.[4]	167[a]	57.6[a]	0.12[c]
C.F.A. + 0.105 g glucose/g C.F.A.	183[a]	14.2[b]	0.88[b]
C.F.A. + 0.210 g glucose/g C.F.A.	186[a]	6.0[c,d]	0.77[b]

[1]Total ketone bodies as acetone.

[2]Values are averages of duplicate groups each containing 5 chicks, with the exception of coconut oil in which one value of 106.2 was discarded. Values without a common letter in their superscripts are significantly different ($P < 0.05$).

[3]Soybean fatty acids.

[4]Coconut fatty acids.

to the results of Experiment I which showed that deleting glycerol from the diet caused hypoglycemia in the chick. The reason for this discrepancy between the results of Experiments I and II is unknown. The data do show that fatty acid composition of the fat does not affect level of blood glucose. Analysis of variance (Steel and Torrie, 1960) of the combined results of Experiments I and II (Table 8) support this conclusion.

Similar statistical treatment of the data on level of blood ketone bodies (Table 7) showed that the substitution of soybean oil or coconut oil for the glucose in a high carbohydrate diet increased levels of blood ketones, significantly ($P < 0.05$). As in Experiment I, deletion of glycerol from the diet by substituting coconut fatty acids and soybean fatty acids for coconut oil and soybean oil, respectively, caused a further significant increase in level of blood ketone bodies ($P < 0.05$) which was alleviated by the addition of glucose. When 0.210 g glucose/g fatty acids were added to the coconut fatty acid diet, levels of blood ketones were reduced still further and were not significantly higher than those of chicks fed the high carbohydrate diet ($P > 0.05$). These results indicate that neither of these two oils nor their fatty acids differ in their ketogenicity for the chick. Analysis of the combined results of both experiments support this conclusion (Table 8).

Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the data on liver glycogen showed that, as in Experiment I, the substitution of coconut oil and soybean oil for the glucose in a high carbohydrate diet caused a significant reduction in concentration of liver glycogen ($P < 0.05$). Deletion of glycerol from the diet by substituting soybean fatty acids for soybean oil did not

Table 8

Metabolic effects of feeding various fats to the chick

Treatment	Experiment Number	Blood glucose mg%	Blood ketone bodies[1] mg%	Liver glycogen content g%
Glucose	I	190[2]	1.8[2]	3.53[2]
	II	212	2.4	4.88
		201[a]	2.1[d]	4.20[a]
Soybean oil	I	186	7.8	0.44
	II	188	11.4	0.50
		187[a,b]	9.6[b]	0.47[d]
S.F.A.[3]	I	147	50.8	0.10
	II	171	54.9	0.21
		159[c]	52.8[a]	0.15[e]
S.F.A. + 0.105 g glucose/g C.F.A.	I	159	9.2	0.54
	II	171	9.2	0.51
		165[b,c]	9.2[b]	0.53[c,d]
Coconut oil	I	202	6.0	0.68
	II	177	9.8	0.90
		190[a]	7.9[b]	0.79[b,c]
C.F.A.[4]	I	152	57.8	0.12
	II	167	57.6	0.12
		160[c]	57.7[a]	0.12[e]
C.F.A. + 0.105 g glucose/g C.F.A.	I	173	9.6	0.81
	II	183	14.2	0.88
		178[a,b,c]	11.9[b]	0.84[b]

[1]Total ketone bodies as acetone.
[2]Values are averages of duplicate groups each containing 5 chicks. Values without a common letter in their superscripts are significantly different ($P < 0.05$). Underlined values are averages of duplicate experiments.
[3]Soybean fatty acids.
[4]Coconut fatty acids.

cause any further reduction in level of liver glycogen ($P > 0.05$); however, as in Experiment I, substitution of coconut fatty acids for coconut oil caused a further reduction in the level of liver glycogen ($P < 0.05$). Supplementation of the fatty acid diets with 0.105 g glucose/g fatty acids resulted in liver glycogen levels not significantly different from those observed in the livers of chicks fed diets containing coconut oil or soybean oil; the addition of 0.210 g glucose/g fatty acids to the coconut fatty acid diet did not cause any further increase in level of liver glycogen.

Comparison of levels of liver glycogen in chicks fed "carbohydrate-free" diets indicated a trend towards higher levels of liver glycogen when non-protein energy was supplied by coconut oil than when non-protein energy was supplied by soybean oil but the difference was not great enough to be significant in either this or the preceding experiment. Analysis of the combined results of both experiments (Table 8) indicated that levels of liver glycogen were significantly higher ($P < 0.05$) for chicks fed diets in which non-protein energy was supplied by coconut oil than when non-protein energy was supplied by soybean oil. Previously Tidwell et al. (1966) observed that levels of liver glycogen were higher in rats fed coconut oil than in rats fed safflower oil, linseed oil or lard. They suggest that this unexpectedly high value for coconut oil might be explained as the result of carbohydrate sparing during the more rapid oxidation of short chain fatty acids.

Results of these experiments (Experiments I and II) show that coconut oil and soybean oil do not differ in ketogenicity for the chick. However, conclusions as to whether more carbohydrate precursors are

produced in the oxidation of a polyunsaturated fat than in the oxidation of a saturated fat are not justified because (1) the medium chain triglycerides in coconut oil have been shown to resemble carbohydrates in some respects in both rats and humans (Senior, 1968) and in these experiments chicks fed coconut oil deposited more glycogen than chicks fed diets containing soybean oil, and (2) the mixture of fatty acids in coconut oil has been shown in the present study to contain a growth depressing factor (factors) when fed in unesterified form which is (are) not responsive to glucose. Thus the choice of coconut oil as the saturated fat in these experiments was unfortunate.

Whether differences in ketogenicity exist between saturated fats containing a preponderance of long chain saturated fatty acids and unsaturated fats containing a preponderance of polyunsaturated fatty acids when fed to chicks forms the basis of the following experiment.

EXPERIMENT III

This experiment was conducted to compare the ketogenicity of a saturated fat (beef tallow) and an unsaturated fat (safflower oil) when fed to chicks as the sole source of non-protein energy in diets with and without supplementary vitamin B_{12}. It was proposed that since vitamin B_{12} is involved in the conversion of propionyl CoA to succinyl CoA, a vitamin B_{12} deficiency might decrease glucogenicity and thus increase ketogenicity of polyunsaturated fats if significant amounts of propionyl CoA are formed in the oxidation of polyunsaturated fatty acids. Sodium propionate was incorporated into half of the diets to accentuate the vitamin B_{12} deficiency since the chicks were from non-depleted hens. That the addition of sodium propionate to the diets of rats and chicks increases the requirement for vitamin B_{12} has been shown by Dryden and Hartman (1971) and Venkataraman et al. (1967), respectively.

Materials and Methods

A factorial design was used involving three sources of non-protein energy, two levels of vitamin B_{12} and two levels of sodium propionate. Glucose, beef tallow and safflower oil served as sources of non-protein energy. Safflower oil was chosen as the unsaturated fat since it is a rich source of polyunsaturated fatty acids, while beef tallow was chosen as the saturated fat since it is fairly well absorbed and contains only small amounts of short and medium chain fatty acids.

The composition of the high carbohydrate diet used in this experiment is shown in Table 9. The diet was formulated to contain 14.5 kilocalories of metabolizable energy per gram of protein and to be low in

Table 9

Composition of high carbohydrate diet

Ingredients	%
Constants	
Soybean protein[1]	23.59
Glycine	0.63
Methionine	0.10
Cystine	0.25
Soybean oil	2.00
Limestone	0.98
Dicalcium phosphate	1.70
Mineral mix[2]	1.253
Vitamin mix[3]	0.58
Antioxidant[4]	0.025
Chromic oxide	0.3
Calcium chloride ($CaCl_2 \cdot 2H_2O$)	0.75
Source of non-protein energy	
Glucose	66.09
Sodium bicarbonate	1.75

[1]Promine, Central Soya, Chemurgy Division, Chicago 39, Illinois.

[2]As in Experiment I except that content of $FeSO_4 \cdot 7H_2O$ was increased from 0.278 to 0.400 g/kg diet to meet the 1971 poultry requirements published by the Subcommittee on Poultry Nutrition of the National Research Council.

[3]As in Experiment I except that vitamin B_{12} was deleted from the vitamin mix.

[4]Ethoxyquin.

methionine, containing only 0.1% supplemental methionine. Diets containing supplementary vitamin B_{12} and/or sodium propionate were formulated by the addition of 100 µg vitamin B_{12}/kg of diet and 2% sodium propionate, respectively. Sodium propionate was added at the expense of glucose. The sodium content of the diets was maintained constant by deleting the appropriate amount of sodium bicarbonate.

"Carbohydrate-free" diets in which non-protein energy was supplied by safflower oil and beef tallow (Table 10) were formulated from the respective high carbohydrate control diet by substituting beef tallow and safflower oil isocalorically for glucose using the values 3.64, 6.78 and 9.21 kilocalories metabolizable energy per gram for glucose, beef tallow and safflower oil, respectively. The caloric density of the "carbohydrate-free" diet was maintained approximately equal to the high carbohydrate diet by the addition of cellulose.

Each diet was fed to duplicate groups of 10 male crossbred (Dominant White x White Plymouth Rock) chicks from 8 to 29 days of age. During the initial one-week feeding period, chicks were fed a "carbohydrate-free" diet containing soybean oil, 0.1% methionine and no added vitamin B_{12}. The method of allotment, feeding and housing were described previously in Experiment I. At 29 days of age blood samples were taken from 5 chicks per group. The remaining chicks in each group were killed with sodium pentobarbital and their livers excised as in Experiments I and II. The preparation of blood filtrates and the analytical methods for determining metabolites have been described previously (Experiment I). In addition blood lactic acid was determined using the method of Barker and Summerson (1941). Blood was analyzed for lactic acid in order to

Table 10

Composition of diets

Diet	Constant ingredients g	Glucose g	Safflower oil g	Beef tallow g	Cellulose[1] g	Vitamin B_{12} µg	Na propionate g	Na bicarbonate g	Totals g
Safflower oil[2] + B_{12}	32.16	--	26.12	--	10.00	10.00	--	1.75	70.03
Safflower oil + B_{12} + Na propionate	32.16	--	26.02	--	10.00	10.00	2.0	--	70.18
Safflower oil - B_{12}	32.16	--	26.12	--	10.00	--	--	1.75	70.03
Safflower oil - B_{12} + Na propionate	32.16	--	26.02	--	10.00	--	2.0	--	70.18
Beef tallow[3] + B_{12}	32.16	--	--	35.48	10.00	10.00	--	1.75	79.39
Beef tallow + B_{12} + Na propionate	32.16	--	--	35.35	10.00	10.00	2.0	--	79.51
Beef tallow - B_{12}	32.16	--	--	35.48	10.00	--	--	1.75	79.39
Beef tallow - B_{12} + Na propionate	32.16	--	--	35.35	10.00	--	2.0	--	79.51
Glucose + B_{12}	32.16	66.09	--	--	--	10.00	--	1.75	100.00
Glucose + B_{12} + Na propionate	32.16	65.84	--	--	--	10.00	2.0	--	100.00
Glucose - B_{12}	32.16	66.09	--	--	--	--	--	1.75	100.00
Glucose - B_{12} + Na propionate	32.16	65.84	--	--	--	--	2.0	--	100.00

[1] Alpha Floc SW40, Lee Chemicals Ltd., 1119 Yonge Street, Toronto 5, Ontario

[2] Composition in g/100 g fatty acids: Palmitic acid, 6.6; Stearic acid, 2.8; Oleic acid, 12.7; Linoleic, 77.9.

[3] Composition in g/100 g fatty acids: Myristic acid, 2.7; Palmitic acid, 24.6; Palmitoleic acid, 3.5; Stearic acid, 24.7; Oleic acid, 41.7; Linoleic acid, 2.9.

determine whether vitamin B_{12}-deficient chicks like vitamin B_{12}-deficient rats responded to sodium propionate by increasing levels of lactic acid in the blood (Williams et al., 1971).

Results and Discussion

Data showing average weight gains and caloric efficiencies of chicks fed carbohydrate-containing and "carbohydrate-free" diets with and without supplementary vitamin B_{12} and containing two levels of sodium propionate are summarized in Table 11. Analysis of variance of the factorial arrangement of treatments (Steel and Torrie, 1960) showed that chicks grew at the same rate irrespective of whether non-protein calories were supplied by glucose, safflower oil, or beef tallow ($P > 0.05$). Supplementation of diets containing 0.1% added methionine with vitamin B_{12} did not increase rate of growth, irrespective of source of non-protein energy ($P > 0.05$). Thus, it can be concluded that in this experiment a vitamin B_{12} deficiency was not produced by curtailing intake of vitamin B_{12}. These results are in contrast to those reported by Looi (1971) who observed that supplementation of a similar diet containing 0.1% methionine with vitamin B_{12} increased the growth of chicks fed diets in which non-protein energy was supplied by glucose or hydrogenated fat (Crisco). Results show that a vitamin B_{12} deficiency was produced by the addition of 2% sodium propionate to diets in which non-protein energy was provided by safflower oil, beef tallow or glucose. The growth depression was overcome by the addition of vitamin B_{12} (100 µg/kg) irrespective of source of non-protein energy. Previously, Venkataraman et al. (1967) and Dryden and Hartman (1971) showed that the growth depressing properties of sodium propionate for rats and chicks, respectively, were overcome by the addition

Table 11

Growth and caloric efficiency of chicks fed carbohydrate-containing and "carbohydrate-free" diets containing two levels of dietary vitamin B_{12} and two levels of Na propionate

Treatment	Average weight gain g	Kcal consumed/ g gain[1]
Safflower oil + B_{12}	378[2,a,b]	5.70[2,d,e]
Safflower oil + B_{12} + Na propionate	424[a]	5.59[e]
Safflower oil - B_{12}	364[a,b]	5.98[b,c,d]
Safflower oil - B_{12} + Na propionate	258[c]	6.81[a]
Beef tallow + B_{12}	370[a,b]	5.84[c,d,e]
Beef tallow + B_{12} + Na propionate	340[b]	5.84[c,d,e]
Beef tallow - B_{12}	358[b]	5.83[c,d,e]
Beef tallow - B_{12} + Na propionate	226[c]	6.92[a]
Glucose + B_{12}	395[a,b]	5.92[c,d]
Glucose + B_{12} + Na propionate	344[b]	6.03[b,c]
Glucose - B_{12}	346[b]	6.24[b]
Glucose - B_{12} + Na propionate	265[c]	6.74[a]

[1]Calculated from calculated metabolizable energy values for the diets.

[2]Values are averages for duplicate groups each containing 10 chicks. Values without a common letter in their superscripts are significantly different ($P < 0.05$).

of vitamin B_{12}. The data also show that the vitamin B_{12} deficiency produced by the addition of sodium propionate to diets containing no added vitamin B_{12} reduced caloric efficiency of chicks fed diets in which non-protein energy was supplied by safflower oil, beef tallow and glucose.

Summarized in Table 12 are data showing levels of blood glucose, blood ketone bodies, blood lactic acid and liver glycogen. Analysis of variance of the factorial arrangement of treatments (Steel and Torrie, 1960) of the data on blood glucose showed that neither source of energy, level of vitamin B_{12} nor level of sodium propionate affected level of blood glucose, significantly ($P > 0.05$). These results indicate that blood glucose levels were maintained in chicks made vitamin B_{12} deficient by the addition of sodium propionate. The finding that level of blood glucose in chicks fed "carbohydrate-free" diets is not affected by the fatty acid composition of the fat is in agreement with the results obtained in Experiments I and II.

Similar statistical treatment of the data on blood ketones showed that the fatty acid composition of the fat affected level of blood ketone bodies with safflower oil producing significantly higher levels of ketone bodies than beef tallow ($P < 0.05$). These results are in contrast to the results of Experiments I and II which showed no significant difference in level of blood ketone bodies when non-protein energy was supplied by coconut oil and soybean oil. These results are also in contrast to results reported for the rat (Brahmankar and Nath, 1963; Tidwell et al., 1966; Chung and Dupont, 1968) which showed that saturated fats were more ketogenic than unsaturated fats. Recently, it has been

Table 12

Metabolic effects of feeding diets containing various fats, two levels of vitamin B_{12} and two levels of Na propionate to the chick

Treatment	Blood glucose mg%	Blood ketone bodies[1] mg%	Blood lactic acid mg%	Liver glycogen content g%
Safflower oil + B_{12}	$196^{2,a,b}$	$12.4^{2,a}$	$30.2^{2,a}$	$0.55^{2,c}$
Safflower oil + B_{12} + Na propionate	$188^{a,b}$	7.6^{b}	$25.8^{a,b}$	$0.78^{b,c}$
Safflower oil - B_{12}	$198^{a,b}$	11.6^{a}	$28.6^{a,b}$	0.51^{c}
Safflower oil - B_{12} + Na propionate	$205^{a,b}$	$4.2^{c,d}$	$20.8^{a,b}$	0.15^{c}
Beef tallow + B_{12}	$206^{a,b}$	8.4^{b}	$28.3^{a,b}$	$0.86^{b,c}$
Beef tallow + B_{12} + Na propionate	$187^{a,b}$	$6.1^{b,c}$	19.1^{b}	0.59^{c}
Beef tallow - B_{12}	$192^{a,b}$	7.1^{b}	$24.8^{a,b}$	0.51^{c}
Beef tallow - B_{12} + Na propionate	182^{b}	$3.1^{d,e}$	$21.8^{a,b}$	0.10^{c}
Glucose + B_{12}	208^{a}	1.2^{e}	30.6^{a}	4.49^{a}
Glucose + B_{12} + Na propionate	$200^{a,b}$	$1.8^{d,e}$	$28.0^{a,b}$	4.10^{a}
Glucose - B_{12}	$199^{a,b}$	1.2^{e}	30.0^{a}	$3.69^{a,b}$
Glucose - B_{12} + Na propionate	$207^{a,b}$	0.8^{e}	$29.4^{a,b}$	$2.66^{a,b,c}$

[1]Total ketone bodies as acetone.

[2]Values are averages of duplicate groups each containing 5 chicks. Values without a common letter in their superscripts are significantly different ($P < 0.05$).

observed in this laboratory (Renner, unpublished data) that sunflower oil decreases lipogenesis. If safflower oil also reduces lipogenesis then increased levels of ketone bodies might be expected.

The data also show that the addition of sodium propionate to "carbohydrate-free" diets caused a significant reduction in level of blood ketone bodies with the reduction being more marked in chicks receiving diets containing no added vitamin B_{12} than in those receiving supplementary vitamin B_{12}. The finding that vitamin B_{12} deficiency, induced by feeding sodium propionate, decreased rather than increased the ketogenicity of fats suggests that the function of vitamin B_{12} as a cofactor for methylmalonyl CoA mutase takes precedent over its methionine sparing action (Briggs et al., 1950; Patrick, 1950; Fox et al., 1957) and/or its involvement in protein synthesis (Chang and Kaiser, 1972), thus causing more dietary protein to be utilized for energy which is reflected in decreased growth and reduced caloric efficiency. Recently, Williams et al. (1971) have also observed that after injection of sodium propionate total ketone bodies were reduced in vitamin B_{12}-deficient rats that had been starved for 48 hours. In contrast to the reduction in ketone bodies observed in chicks fed diets adequate in vitamin B_{12} in this experiment, Williams et al. (1971) found that blood ketones in normal starved rats were not altered significantly after injection of sodium propionate.

Analysis of variance of the factorial arrangement of treatments (Steel and Torrie, 1960) of the data on blood lactic acid showed that neither source of non-protein energy nor level of vitamin B_{12} had an effect on levels of blood lactate; however, results showed that supplementation with sodium propionate caused a significant reduction in levels of blood lactate ($P < 0.05$). This finding is reflected in the significantly

lower level of liver glycogen ($P < 0.05$) in chicks fed diets supplemented with sodium propionate.

In agreement with results of this experiment, Williams et al. (1971) did observe a slight decrease in level of blood lactate in normal rats after administration of propionate. However, they found a twofold increase of lactate in blood of vitamin B_{12}-deficient rats rather than a decrease as observed in the present study with chicks.

Analysis of variance of the factorial arrangement of treatments (Steel and Torrie, 1960) of the data on liver glycogen showed that, as in Experiments I and II, the isocaloric substitution of fat for glucose significantly decreased level of liver glycogen ($P < 0.05$). Since levels of liver glycogen were similar in chicks fed "carbohydrate-free" diets containing safflower oil and tallow, it would appear that the fatty acid composition of these fats did not affect liver glycogen. These results are in contrast to the finding of Tidwell et al. (1966) which showed that levels of liver glycogen in rats fed safflower oil and linseed oil were higher than when lard was fed.

Analysis of variance of the factorial arrangement of treatments also showed that supplementation with sodium propionate significantly decreased level of liver glycogen ($P < 0.05$) while supplementation with vitamin B_{12} significantly increased level of liver glycogen ($P < 0.01$). Average values for glycogen in the liver of chicks fed diets with and without sodium propionate were 1.39 and 1.77 g%, respectively, while average values for chicks fed diets with and without vitamin B_{12} were 1.89 and 1.27 g%, respectively. The finding that supplementation with sodium propionate decreased level of liver glycogen was unexpected since

propionate is glucogenic at least in the presence of vitamin B_{12}. It should be noted that the decrease in level of liver glycogen on the addition of sodium propionate was most marked in diets containing no added vitamin B_{12}. Recently, Williams et al. (1971) showed that the conversion of lactate into glucose is inhibited in vitamin B_{12}-deficient rats after sodium propionate administration. They concluded that the effect is due to inhibition of the pyruvate carboxylase step resulting from a decrease in acetyl CoA concentration and a postulated increase in methylmalonyl CoA concentration. Whether pyruvate carboxylase is inhibited on administration of sodium propionate to chicks is unknown. Blood lactate was not observed to increase in chicks in the present study; however, the possibility exists that if the conversion of glucose precursors into glucose is inhibited, they may be diverted to replenish intermediates in the citric acid cycle, thus permitting fatty acids to be completely oxidized. In support of this suggestion is the finding that blood ketone bodies were reduced in chicks fed diets with and without supplementary vitamin B_{12} on the administration of sodium propionate.

EXPERIMENT IV

In the preceding experiments, the effect of a vitamin B_{12} deficiency on the ketogenicity of dietary fat was determined in chicks in which the vitamin B_{12} deficiency was produced by supplementing a diet low in vitamin B_{12} and one carbon units with sodium propionate. The object of this experiment was to determine the effect of a vitamin B_{12} deficiency on the ketogenicity of fats when chicks were fed diets sufficiently low in one carbon units so that in the absence of supplemental vitamin B_{12} a deficiency would be produced without the need for accentuating the deficiency with sodium propionate.

Materials and Methods

The experimental design and procedures used were the same as in Experiment III with the exception that all diets contained 0.074% supplemental methionine rather than 0.1% as shown in Table 9. This level of supplemental methionine was based on the observation of Looi (1971) that 0.074% supplemental methionine in a vitamin B_{12} deficient diet induced the symptoms of a vitamin B_{12} deficiency in the chick.

Results and Discussion

Summarized in Table 13 are the weight gains and caloric efficiencies of chicks fed carbohydrate-containing and "carbohydrate-free" diets with and without supplemental vitamin B_{12} and containing two levels of sodium propionate. Analysis of variance of the factorial arrangement of treatments (Steel and Torrie, 1960) showed that chicks fed diets containing safflower oil grew significantly faster than chicks fed diets containing beef tallow or glucose ($P < 0.05$). A vitamin B_{12} deficiency

Table 13

Growth and caloric efficiency of chicks fed carbohydrate-containing and "carbohydrate-free" diets containing two levels of dietary vitamin B_{12} and two levels of Na propionate

Treatment	Average weight gain g	Kcal consumed/ g gain[1]
Safflower oil + B_{12}	$408^{2,a}$	$6.12^{2,e,f}$
Safflower oil + B_{12} + Na propionate	415^{a}	5.92^{f}
Safflower oil - B_{12}	$286^{c,d,e}$	$6.89^{b,c,d}$
Safflower oil - B_{12} + Na propionate	$274^{d,e,f}$	$6.96^{b,c}$
Beef tallow + B_{12}	$334^{b,c,d}$	$6.18^{e,f}$
Beef tallow + B_{12} + Na propionate	$354^{a,b}$	$6.12^{e,f}$
Beef tallow - B_{12}	$268^{e,f}$	$6.55^{c,d,e}$
Beef tallow - B_{12} + Na propionate	216^{f}	$7.28^{a,b}$
Glucose + B_{12}	$364^{a,b}$	$6.39^{d,e,f}$
Glucose + B_{12} + Na propionate	$340^{b,c}$	$6.50^{c,d,e}$
Glucose - B_{12}	$279^{c,d,e}$	$6.58^{c,d,e}$
Glucose - B_{12} + Na propionate	$254^{e,f}$	7.56^{a}

[1] Calculated from calculated metabolizable energy values for the diets.

[2] Values are averages for duplicate groups each containing 10 chicks. Values without a common letter in their superscripts are significantly different ($P < 0.05$).

was produced as shown by the significantly increased rate of growth on the addition of vitamin B_{12} to diets in which non-protein energy was supplied by safflower oil, beef tallow and glucose ($P < 0.01$). The addition of sodium propionate to the vitamin B_{12}-deficient diet did not alter rate of growth irrespective of source of non-protein energy. The failure of sodium propionate to reduce growth of chicks fed diets containing no added vitamin B_{12} is in contrast to the results of Experiment III in which chicks showed a marked reduction in rate of growth when sodium propionate was added to diets containing no added vitamin B_{12}. However, in Experiment III, all diets contained 0.1% methionine, and in the absence of supplemental vitamin B_{12} the addition of 2% sodium propionate produced a vitamin B_{12} deficiency. Since a vitamin B_{12} deficiency was observed in this experiment when diets containing 0.074% methionine were fed, these results suggest that the vitamin B_{12}-sparing action of 0.026% methionine is offset by 2% sodium propionate. Although sodium propionate did not accentuate a vitamin B_{12} deficiency using growth as the criterion, caloric efficiency was reduced by the addition of sodium propionate to a vitamin B_{12}-deficient diet in which non-protein energy was supplied by beef tallow or glucose but remained unchanged when non-protein energy was supplied by safflower oil.

Summarized in Table 14 are data showing the metabolic effects of feeding carbohydrate-containing and "carbohydrate-free" diets with and without supplemental vitamin B_{12} and containing two levels of sodium propionate. Analysis of variance of the factorial arrangement of treatments (Steel and Torrie, 1960) showed that source of non-protein energy, level of supplemental vitamin B_{12} and level of sodium propionate in the

Table 14

Metabolic effects of feeding diets containing various fats, two levels of vitamin B_{12} and two levels of Na propionate to the chick

Treatment	Blood glucose mg%	Blood ketone bodies[1] mg%	Blood lactic acid mg%	Liver glycogen content g%
Safflower oil + B_{12}	186[2,a]	12.6[2,a]	24.3[2,a,b]	0.99[2,c]
Safflower oil + B_{12} + Na propionate	186[a]	8.2[b,c]	28.8[a,b]	0.85[c]
Safflower oil - B_{12}	196[a]	11.3[a,b]	30.6[a]	0.62[c]
Safflower oil - B_{12} + Na propionate	214[a]	6.2[c]	26.2[a,b]	0.22[c]
Beef tallow + B_{12}	190[a]	9.9[a,b]	27.6[a,b]	0.48[c]
Beef tallow + B_{12} + Na propionate	192[a]	5.4[c,d]	23.6[a,b]	0.57[c]
Beef tallow - B_{12}	191[a]	10.1[a,b]	22.5[a,b]	0.44[c]
Beef tallow - B_{12} + Na propionate	198[a]	6.0[c]	20.3[b]	0.22[c]
Glucose + B_{12}	209[a]	2.0[d,e]	26.0[a,b]	4.78[a]
Glucose + B_{12} + Na propionate	198[a]	1.7[e]	32.6[a]	3.29[b]
Glucose - B_{12}	196[a]	1.5[e]	29.1[a,b]	4.16[a,b]
Glucose - B_{12} + Na propionate	196[a]	1.2[e]	26.8[a,b]	3.64[b]

[1]Total ketone bodies as acetone.

[2]Values are averages of duplicate groups each containing 5 chicks. Values without a common letter in their superscripts are significantly different ($P < 0.05$).

diet had no significant effect on blood glucose levels ($P > 0.05$). These results are in agreement with the results obtained in Experiment III and show that in the chick neither vitamin B_{12} deficiency nor fatty acid composition of the fat fed affect level of blood glucose.

Similar statistical treatment of the data on blood ketones showed that the isocaloric substitution of safflower oil or beef tallow for glucose significantly increased ($P < 0.05$) levels of blood ketones with safflower oil being more ketogenic than beef tallow. Similar results were obtained in Experiment III.

Results also show that a vitamin B_{12} deficiency produced by curtailing intake of both one carbon units and the vitamin did not affect level of blood ketones, irrespective of source of energy and fatty acid composition of the fat. The failure of vitamin B_{12} deficiency to increase the ketogenicity of safflower oil indicates either that the amount of propionyl CoA formed in the oxidation of polyunsaturated fatty acids is insignificant or that vitamin B_{12} is still present in amounts sufficient to serve as a cofactor for methylmalonyl CoA mutase.

The antiketogenic effect of sodium propionate when added to "carbohydrate-free" diets was again observed. In contrast to Experiment III, in this experiment the antiketogenic effects of sodium propionate were similar in the presence and absence of vitamin B_{12}. Since the addition of sodium propionate did not accentuate the growth depression in vitamin B_{12}-deficient chicks, it would appear that if propionate is metabolized the amount of vitamin B_{12} used in its metabolism is not great enough to depress growth.

Analysis of variance of the factorial arrangement of treatments

(Steel and Torrie, 1960) showed that as in the three previous experiments, substituting neutral fat for the glucose in a high carbohydrate diet significantly ($P < 0.05$) decreased level of liver glycogen. As in Experiment III, level of liver glycogen was not affected by the fatty acid composition of the fat fed.

Similar statistical treatment also showed that vitamin B_{12} deficiency produced by curtailing intake of one carbon units and the vitamin did not affect level of liver glycogen, irrespective of source of energy or fatty acid composition of the fat fed.

The addition of sodium propionate caused a significant overall reduction in levels of liver glycogen ($P < 0.05$), the effect being most marked when sodium propionate was added to vitamin B_{12}-deficient diets. The question thus arises as to whether the reduction in levels of ketone bodies on addition of sodium propionate to diets of vitamin B_{12}-deficient chicks is due to the metabolism of propionate or to the diversion of carbohydrate from storage to other uses.

Statistical treatment of the data on blood lactic acid showed that levels of blood lactic acid were not affected by source of non-protein energy, level of supplemental vitamin B_{12} or level of added sodium propionate. These results are in contrast to results of Williams et al. (1971) and Williams and Spray (1972) which showed that injecting sodium propionate into vitamin B_{12}-deficient rats caused a twofold increase in blood lactic acid concentration.

GENERAL DISCUSSION

Comparison of levels of blood ketone bodies and liver glycogen in chicks fed diets containing saturated and polyunsaturated fats both in the presence and absence of supplemental vitamin B_{12} have failed to produce evidence in support of Sinclair's hypothesis that propionyl CoA is formed in the oxidation of polyunsaturated fatty acids. Studies have shown that levels of blood ketone bodies were as high or higher when non-protein energy was provided by the polyunsaturated fats, soybean oil and safflower oil, as when non-protein energy was provided by the saturated fats, beef tallow and coconut oil. In addition, levels of blood ketone bodies have been shown to be unaffected by vitamin B_{12} deficiency irrespective of degree of unsaturation of the fat. Results also have shown that levels of liver glycogen were as high or higher when chicks were fed diets containing saturated fats as when polyunsaturated fats were fed and that levels of liver glycogen were unaffected by a vitamin B_{12} deficiency (Experiment IV).

These findings are in contrast to results reported for the rat which showed that saturated fats were more ketogenic than polyunsaturated fats when levels of blood ketone bodies (Tidwell et al., 1966; Chung and Dupont, 1968; Dupont and Mathias, 1969) or levels of liver glycogen (Tidwell et al., 1966) were used as the criteria. These investigators suggested that the finding that saturated fats were more ketogenic than unsaturated fats supports the premise that polyunsaturated fats have the potential to supply glucose. Thus, although the rat may metabolize unsaturated fat via propionyl CoA, results of the present studies do not support the evidence of such a pathway in the chick.

Evidence has been accumulated to show that chicks and rats do differ in some other aspects of their metabolism of fat. For example, the chick absorbs lauric, myristic and palmitic acids to a lesser extent than the rat (Renner and Hill, 1961). Studies have also shown that the chick absorbs fatty acids, esterified or unesterified, short or long chain, by the portal system (Scott et al., 1969). In addition, Feigenbaum and Fisher (1963) have shown that the chick, unlike the rat, does not develop a fatty liver upon starvation and that, while polyunsaturated fatty acids predominate in the liver fat of starved chicks, saturated fatty acids predominate in the liver fat of starved rats. Sites of lipogenesis have also been shown to differ in the two species. O'Hea and Leveille (1969) showed that between 90 - 95% of de novo fatty acid synthesis in the chick appears to take place in the liver. In contrast, de novo fatty acid synthesis in the rat occurs predominantly in the adipose tissue (Leveille, 1967). Finally, Pearce (1971) has reported that fat-feeding has no effect on hepatic pyruvate kinase activity in the chicken whereas in the rat dietary lipid represses it which suggests that chicken liver is more restricted in its response to dietary modification than rat liver.

The failure to obtain evidence for formation of propionyl CoA during the oxidation of polyunsaturated fatty acids negates the possibility that the increased requirement for vitamin B_{12} when chicks are fed high fat diets (Looi, 1971) is due to increased need for the cofactor for methylmalonyl CoA mutase. Thus the question of why the chick's requirement for vitamin B_{12} is increased when fat is substituted isocalorically for glucose remains unanswered.

Previous investigators have implicated vitamin B_{12} in the maintenance of blood levels of soluble sulfhydryl compounds, particularly glutathione (Ling and Chow, 1953, 1954; Hsu et al., 1959). Recently Kowale et al. (1966) observed significantly lower levels of blood glutathione in rabbits fed diets containing saturated fats than in those fed diets containing unsaturated fats. They proposed that the lower level of blood glutathione in rabbits fed diets containing saturated fats might be associated with the higher levels of blood ketones observed when saturated fats were fed. Previously, Nath and Natwalne (1950) and Nath et al. (1953) had reported that ketone bodies caused a decrease in level of blood reduced glutathione. Biswas and Johnson (1964) observed that, in the livers of vitamin B_{12}-deficient rats and chicks, the activities of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, isocitrate dehydrogenase, and glutathione reductase were significantly depressed. These investigators concluded that the effect of a vitamin B_{12} deficiency on dehydrogenases and carbohydrate metabolism was secondary to the effect on glutathione reductase. More recently, Nath and Nath (1967) observed that growth of rats and levels of vitamin B_{12} in blood and liver were reduced when ketone bodies were administered in vivo.

Whether the increased levels of blood ketone bodies in chicks fed "carbohydrate-free" diets causes a reduction in levels of vitamin B_{12} in liver and blood and a reduction in blood reduced glutathione is unknown. Further studies might be conducted to compare levels of vitamin B_{12}, blood reduced glutathione, and glutathione reductase in chicks fed diets with and without supplemental vitamin B_{12} in which non-protein energy is provided by fat and carbohydrate.

SUMMARY

1. Studies to compare the ketogenicity of various fats for the chick have shown that substitution of the polyunsaturated fats, safflower oil and soybean oil, for the saturated fats, beef tallow and coconut oil, did not reduce levels of blood ketone bodies nor increase levels of liver glycogen in chicks fed "carbohydrate-free" diets. These results indicate that the chick does not metabolize polyunsaturated fatty acids via propionyl CoA.

2. Levels of blood ketone bodies and liver glycogen have been shown to be unaffected by a vitamin B_{12} deficiency, irregardless of the degree of unsaturation of the fat used to formulate the "carbohydrate-free" diet.

3. Studies have shown that the addition of sodium propionate to "carbohydrate-free" diets in which non-protein energy was supplied by either safflower oil or beef tallow did not affect level of blood lactic acid but caused a reduction in levels of both blood ketone bodies and liver glycogen which was most marked in the absence of supplementary vitamin B_{12}.

4. Chicks fed "carbohydrate-free" diets containing coconut oil grew slightly but significantly slower and had higher levels of liver glycogen than chicks fed "carbohydrate-free" diets containing soybean oil. The growth depressing property of coconut oil was accentuated when glycerol was deleted from the diet by substituting coconut fatty acids for coconut oil and, in contrast to soybean fatty acids,

the growth depressing property of coconut fatty acids was not overcome by the addition of glucose. Studies showed that the growth depressing property of coconut oil and coconut fatty acids was not due to decreased absorbability since these lipids were shown to be absorbed to 99 and 92%, respectively.

5. Results of these studies indicate that the increased requirement of the chick for vitamin B_{12} when fed high fat diets (Looi, 1971) is not due to formation of propionyl CoA during the oxidation of polyunsaturated fatty acids.

BIBLIOGRAPHY

Allred, J. B. 1969. Relationships between the concentration of liver metabolites and ketogenesis in chicks fed "carbohydrate-free" diets. J. Nutr., 99:101.

Annison, E. F., K. J. Hill and R. Kenworthy. 1968. Volatile fatty acids in the digestive tract of the fowl. Br. J. Nutr., 22:207.

Bakker, N. and R. R. White. 1957. Simplified micromethod for the colorimetric determination of total acetone bodies in blood. New Zealand J. Sci. and Technol., 38:1001.

Barker, S. B. and W. H. Summerson. 1941. Colorimetric determination of lactic acid in biological material. J. Biol. Chem., 138:535.

Biswas, D. K. and B. C. Johnson. 1964. Glutathione reductase and dehydrogenase activity in vitamin B_{12} deficiency. Arch. Biochem. Biophys., 104:375.

Björntorp, P. 1968. Rates of oxidation of different fatty acids by isolated rat liver mitochondria. J. Biol. Chem., 243:2130.

Brahmankar, D. M. and M. C. Nath. 1963. Effect of food fats on concentration of ketone bodies and citric acid level in blood and tissues. Proc. Soc. Exp. Biol. Med., 112:670.

Brambila, S. and F. W. Hill. 1966. Comparison of neutral fat and free fatty acids in high lipid-low carbohydrate diets for the growing chicken. J. Nutr., 88:84.

Brambila, S. and F. W. Hill. 1967. Effects of glucose supplementation of high lipid diets based on free fatty acids for the growing chicken. J. Nutr., 91:261.

Briggs, G. M., E. G. Hill and M. J. Giles. 1950. Vitamin B_{12} in all-plant rations for chicks and sparing activity of methionine and choline. Poultry Sci., 29:723.

Carew, L. B., Jr., D. T. Hopkins and M. C. Nesheim. 1964. Influence of amount and type of fat on metabolic efficiency of energy utilization by the chick. J. Nutr., 83:300.

Carroll, C. 1964. Influences of dietary carbohydrate-fat combinations on various functions associated with glycolysis and lipogenesis in rats. II. Glucose vs. sucrose with corn oil and two hydrogenated oils. J. Nutr., 82:163.

Chang, Y. and I. I. Kaiser. 1972. Free and membrane bound ribosomes in experimental animals during vitamin B_{12} and folic acid deficiency. Internat. J. Vit. Nutr. Res., 42:482.

Chung, L. H. and J. Dupont. 1968. Acetoacetate metabolism of rats fed high fat or restricted calorie diets. Lipids, 3:545.

Contreras, E. and A. J. Giorgio. 1972. Leukocyte methylmalonyl CoA mutase. I. Vitamin B_{12} deficiency. Amer. J. Clin. Nutr., 25:695.

Dryden, L. P. and A. M. Hartman. 1971. Vitamin B_{12} deficiency in the rat fed high protein rations. J. Nutr., 101:579.

Dupont, J. 1966. Fatty acid oxidation in relation to cholesterol biosynthesis in rats. Lipids, 1:415.

Dupont, J. and M. M. Mathias. 1969. Bio-oxidation of linoleic acid via methylmalonyl CoA. Lipids, 4:478.

Feigenbaum, A. S. and H. Fisher. 1963. Changes in fatty acid composition in nutritional fatty degeneration of the liver. I. Effect of starvation. Br. J. Nutr., 17:31.

Flavin, M. and S. Ochoa. 1957. Metabolism of propionic acid in animal tissues. I. Enzymatic conversion of propionate to succinate. J. Biol. Chem., 229:965.

Folin, O. and H. Malmros. 1929. An improved form of Folin's micromethod for blood sugar determinations. J. Biol. Chem., 83:115.

Fong, J., F. L. Schaffer and P. L. Kirk. 1953. Ultramicro determination of glycogen in liver. A comparison of the anthrone and reducing-sugar methods. Arch. Biochem. Biophys., 45:319.

Fowweather, F. S. and W. N. Anderson. 1946. A method for the determination of fat in feces. Biochem. J., 40:350.

Fox, M. R. S., G. M. Briggs and L. O. Ortiz. 1957. Nutrients affecting the vitamin B_{12} requirement of chicks. J. Nutr., 62:539.

Good, C. A., H. Kramer and M. Somogyi. 1933. Determination of glycogen. J. Biol. Chem., 100:485.

Göransson, G. 1965a. Metabolism of fatty acids in the rat. IV. Stearic acid. Acta Physiol. Scand., 63:423.

Göransson, G. 1965b. The metabolism of fatty acids in the rat. V. Palmitoleic acid. Acta Physiol. Scand., 63:428.

Göransson, G. 1965c. The metabolism of fatty acids in the rat. VII. Linoleic acid. Acta Physiol. Scand., 64:204.

Göransson, G. and T. Olivecrona. 1965. The metabolism of fatty acids in the rat. II. Oleic acid. Acta Physiol. Scand., 63:121.

Hahn, P., O. Koldovsky, V. Melichar and M. Novak. 1963. Fat metabolism as related to carbohydrate metabolism in infant rats. p. 385. In A. C. Frazer, Problems of Lipids. Vol. I. Elsevier Publishing Co., Amsterdam, London and New York.

Hill, F. W. and D. L. Anderson. 1958. Comparison of metabolizable energy and productive energy determinations with chicks. J. Nutr., 64:587.

Hsu, J. M., B. F. Chow and K. Ochuda. 1959. Vitamin B_{12} deficiency and glutathione in the erythrocytes and livers of chicks. Arch. Biochem. Biophys., 84:15.

Kaziro, Y. and S. Ochoa. 1964. Metabolism of propionic acid. Adv. Enzymol., 26:283.

Kowale, B. N., D. M. Brahmankar, N. Nath and M. C. Nath. 1966. Effect of different dietary fats on carbohydrate metabolism. J. Nutr. and Dietet., 3:33.

Krebs, H. A. 1970. Fatty acid metabolism in the perfused rat liver. Biochem. J., 119:525.

Lengyel, P., R. Mazumder and S. Ochoa. 1960. Mammalian methylmalonyl isomerase and vitamin B_{12} coenzymes. Proc. Natn. Acad. Sci. U.S.A., 46:1312.

Leveille, G. A. 1967. In vivo fatty acid synthesis in adipose tissue and liver of meal-fed rats. Proc. Soc. Exp. Biol. Med., 125:85.

Ling, C. T. and B. F. Chow. 1953. Effect of vitamin B_{12} on the levels of soluble sulfhydryl compounds in blood. J. Biol. Chem., 202:445.

Ling, C. T. and B. F. Chow. 1954. Influence of vitamin B_{12} on carbohydrate and lipid metabolism. J. Biol. Chem., 206:797.

Lodhi, G. N., R. Renner and D. R. Clandinin. 1969. Available carbohydrate in rapeseed meal and soybean meal as determined by a chemical method and a chick bioassay. J. Nutr., 99:413.

Looi, S. H. 1971. Nutrient requirements of chicks fed "carbohydrate-free" diets. Ph.D. Thesis, University of Alberta.

Lynn, W. S. and R. H. Brown. 1959. Oxidation and activation of unsaturated fatty acids. Arch. Biochem. Biophys., 81:353.

Mead, J. F., W. H. Seaton, Jr. and A. B. Decker. 1956. Metabolism of the essential fatty acids. II. The metabolism of stearate, oleate and linoleate by fat-deficient and normal mice. J. Biol. Chem., 218:401.

Menge, H. 1971. Influence of dietary oils on chick growth rate. Poultry Sci., 50:261.

Moore, J. H. and B. M. Doran. 1962. Lipid metabolism in the normal and vitamin B_{12}-deficient chick embryo. Biochem. J., 84:506.

Nath, M. C. and V. G. Hatwalne. 1950. Effect of acetoacetic acid on reduced glutathione content of blood in rabbits. Nature, 166:692.

Nath, M. C, V. G. Hatwalne and J. S. Gadgil. 1953. Progressive depletion in the reduced glutathione content of the blood following acetoacetate injections. Biochem. J., 53:479.

Nath, N. and M. C. Nath. 1967. Effect of acetoacetate and β-hydroxybutyrate on vitamin B_{12} in rats. Proc. Soc. Exp. Biol. Med., 124:210.

National Research Council, Subcommittee on Poultry Nutrition. 1971. Nutrient requirements of poultry, pub. 1345. National Academy of Sciences - National Research Council, Washington, D.C. 20025.

Nelson, N. 1944. Photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem., 153:375.

O'Hea, E. K. and G. A. Leveille. 1969. Lipid biosynthesis and transport in the domestic chick. Comp. Biochem. Physiol., 30:149.

Patrick, H. 1950. Growth-promoting effect of methionine and vitamin B_{12} on chicks. Poultry Sci., 29:923.

Pearce, J. 1971. Carbohydrate metabolism in the domestic fowl. Nutr. Soc. Proc., 30:254.

Renner, R. 1964. Factors affecting the utilization of "carbohydrate-free" diets by the chick. I. Level of protein. J. Nutr. 84:322.

Renner, R. 1972. Unpublished data.

Renner, R. and A. M. Elcombe. 1964. Factors affecting the utilization of "carbohydrate-free" diets by the chick. II. Level of glycerol. J. Nutr., 84:327.

Renner, R. and A. M. Elcombe. 1967. Metabolic effects of feeding "carbohydrate-free" diets to chicks. J. Nutr., 93:31.

Renner, R. and F. W. Hill, 1960. Utilization of corn oil, lard and tallow by chickens of various ages. Poultry Sci., 39:849.

Renner, R. and F. W. Hill. 1961. Utilization of fatty acids by the chicken. J. Nutr., 74:259.

Scott, M. L., M. C. Nesheim and R. J. Young. 1969. Nutrition of the chicken. M. L. Scott and Associates, Ithaca, New York.

Seifter, S., S. Dayton, B. Novic and E. Muntwyler. 1950. Estimation of glycogen with the anthrone reagent. Arch. Biochem. Biophys., 25:191.

Senior, J. R. 1968. Medium Chain Triglycerides. University of Pennsylvania Press, Philadelphia.

Sinclair, H. M. 1964. Carbohydrates and fats. p. 41. In G. H. Beaton and E. W. McHenry, Nutrition - a Comprehensive Treatise. Vol. I. Academic Press, New York and London.

Somogyi, M. 1945. New reagent for the determination of sugars. J. Biol. Chem., 160:61.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics with Special Reference to the Biological Sciences. McGraw-Hill Book Company, New York.

Stern, J. R., A. del Campillo and A. L. Lehninger. 1955. Enzymatic racemization of β-hydroxybutyryl-S-CoA and the stereospecificity of enzymes of the fatty acid cycle. J. Am. Chem. Soc., 77:1073.

Stoffel, W., R. Ditzer and H. Caeser. 1964. Der stoffwechsel der ungesättigten fettsäuren. III. Zur β-oxydation der mono- und polyenfettsäuren der mechanismus der enzymatischen reaktionen an Δ^{3cis}-enoyl-CoA-verbindungen. Z. Physiol. Chem., 339:167. (Wakil, S. J. 1970. Fatty acid metabolism. p. 1. In S. J. Wakil, Lipid Metabolism. Academic Press, New York and London.

Tidwell, H. C., J. L. Pope and P. Gifford. 1966. Metabolism of poly-unsaturated fatty acids and serum cholesterol levels in the rat. J. Nutr., 88:111.

Venkataraman, S., D. K. Biswas and B. C. Johnson. 1967. Effect of propionate on the induction of vitamin B_{12} deficiency in chicks and rats. J. Nutr., 93:131.

Williams, D. L. and G. H. Spray. 1972. Metabolism of propionate in the vitamin B_{12}-deficient rat. Br. J. Nutr., 28:263.

Williams, D. L., G. H. Spray, R. Hems and D. H. Williamson. 1971. Metabolic effects of propionate in normal and vitamin B_{12}-deficient rats. Biochem. J., 124:501.

Williams, H. H., B. N. Erickson, S. Bernstein, F. C. Hummel and I. G. Macy. 1937. Lipid and mineral distribution of the serum and erythrocytes in pernicious anemia - Before and after therapy. J. Biol. Chem., 118:599.

Winkler, V. W. 1970. Comparative studies on mobilization and mitochondrial oxidation of saturated and unsaturated fatty acids. Diss. Abs., 31:2474B.

Zaragoza, N. and J. P. Felber. 1970. Studies on the metabolic effects induced in the rat by a high fat diet. I. Carbohydrate metabolism in vivo. Horm. Metab. Res., 2:323.