THE UNIVERSITY OF MANITOBA

DIETARY FACTORS CONTRIBUTING TO SUDDEN DEATH SYNDROME IN BROILERS

BY

BARBARA ALEKSANDRA ROTTER

A THESIS SUBMITTED TO THE FACULTY
OF GRADUATE STUDIES IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF ANI' \L SCIENCE

WINNIPEG, MANITOBA

1985

ISBN Ø-315-33573-4

DIETARY FACTORS CONTRIBUTING TO SUDDEN DEATH SYNDROME IN BROILERS

BY

BARBARA ALEKSANDRA ROTTER

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

ABSTRACT

Sudden death syndrome (SDS) has become a cause of a significant amount of mortality in rapidly growing broiler chickens. The exact etiology of SDS has not been established, but it appears to be a metabolic disorder with a combination of nutritional, environmental and genetic factors.

Five experiments were conducted to investigate possible nutritional factors. Factors examined involved changes in diet composition by using different fat sources [tallow, sunflower oil (SFO) and hydrogenated coconut oil (HCO)] with or without supplemental biotin and stocking density.

In two out of four experiments, the total SDS incidence was found to be significantly ($P<0.05$) lower when birds were fed diets supplemented with SFO as opposed to tallow. In the third study, the mortality due to SDS was only reduced ($P<0.05$) between 0 to 4 weeks of age on a SFO diet when compared with a tallow plus biotin diet, however the total mortality due to SDS was not affected ($P>0.05$). The general improvement in weight gain and feed conversion on the SFO diet was assumed to be due to a better absorption of SFO over tallow. When increased concentrations of tallow were fed to replace cornstach, a tendency for a reduction in SDS incidence on tallow diet between 0 to 4 weeks of age was noted ($P = 0.08$). However, dietary supplementation with HCO resulted in lower mortality due to SDS when compared with SFO between 0 to 4 weeks, while total mortality was not affected ($P>0.05$). At present the cause for the difference in response in the incidence of SDS is unknown.

Analysis of fatty acids of the heart tissue revealed a reduction in arachidonic acid concentration in SDS birds compared with their controls

(matching penmates), which could indicate an inadequate conversion of linoleic to arachidonic acid. Mineral analysis of the heart tissue showed that SDS birds had increased ($P<0.05$) concentration of sodium, while potassium decreased in concentration as opposed to controls. Biotin analysis indicated that SDS birds had adequate amount of the vitamin in the liver.

Addition of SFO to the diet as a means of reducing SDS incidence can only be conditionally recommended, due to some inconsistency in the results and the cost of dietary fat. Dietary supplementation with biotin does not seem to alleviate SDS. Under our experimental conditions stocking density was not found to have a significant ($P>0.05$) effect on SDS incidence.

ACKNOWLEDGEMENTS

I would like to extend my sincere gratitude to my supervisor Dr. W. Guenter for his wise guidance throughout my graduate program, reviewing of this manuscript and personal support.

Appreciation and thanks are extended to Dr. G.D. Phillips for giving me the opportunity and encouraging me to face the scientific challenge; to Dr. G.H. Crow for statistical advice; to Dr. B.R. Boycott for doing the post-mortem examination on considerable number of birds and overall support; and to the Department of Animal Science for providing experimental animals and facilities.

I would like to thank the members of my examining committee, Drs. R.R. Marquardt, S.C. Stothers and J.R. Ingalls, Department of Animal Science, University of Manitoba; Dr. R.D. Hill, Department of Plant Science, University of Manitoba and Dr. J.R. Hunt, Agriculture Canada Research Station, Agassiz, B.C. for their valuable criticism in reviewing this manuscript. Additional thanks go to Mr. C. Batenchuk for help in mineral analysis and to Mrs. M. Funk for time, effort and patience during typing the manuscript, as well as her editing skills.

Appreciation is also extended to Dr. K. Dakshinamurti, Department of Biochemistry, University of Manitoba for providing the facilities for the biotin analysis.

I would like to acknowledge the poultry barn staff for all the work done during my experiments, numerous graduate students for their assistance and Mr. H. Muc for his help and understanding.

This thesis is dedicated to my parents, Gertruda and Leszek Staszak, for their love and support over the years. I especially want to thank my husband Roland, for his interest, encouragement and help during the pursuit of this doctoral degree.

FOREWARD

This thesis is written in manuscript style. The first manuscript was published in 1985 in "Poultry Science"; the second was submitted to "Poultry Science" and the third and fourth will be submitted to "Poultry Science".

The authors of these manuscripts are: B. Rotter and W. Guenter, Department of Animal Science, University of Manitoba, Winnipeg and B.R. Boycott, Manitoba Department of Agriculture, Winnipeg.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

General Introduction

In recent years sudden death syndrome (SDS) has become a cause of a significant amount of mortality in rapidly growing broiler chickens. SDS is also known as flip-over, acute death syndrome (ADS) and/or lung edema.

Birds dying from SDS are apparently healthy-looking and in good condition. The mortality starts at 5 to 10 days of age and continues until the birds are marketed. All broiler strains commonly used in North America seem to be susceptible. SDS has also been recognized in several other countries other than Canada and USA, as a cause of mortality, affecting an average of 1% to 2% of a population. Although SDS contributes at least 30-40% of the total mortality, limited information is available as to possible causes or prevention of death and chemical characteristics of SDS birds.

Economically, SDS is a very important problem in the poultry industry, although there is no current estimation of economic loss. Brigden and Riddell (1975) in a survey of 38,212 birds determined the loss due to SDS to be $293.56, representing 0.77 cents per chick started. If this value is related to the 25 million broilers raised annually in the prairie provinces of western Canada, the total annual loss due to SDS could be as high as $192,500. Reports from the prairies indicate about 2% SDS mortality in most flocks of broiler chickens, which considering Manitoba's production, is approximately 1/4 million birds per year.

Therefore, it is of economic importance to identify the factors influencing the incidence of SDS, its mechanism(s) and develop practical preventive measures for the industry.

Previous findings by Mollison (1983) suggested a possible interaction between fat metabolism and SDS, and so a series of experiments was initiated to examine this relationship.

Major drawbacks are the irregular low incidence of the disorder and the lack of identifiable signs prior to death. Therefore, this study was based on the hypothesis that SDS is a metabolic disorder affected by nutritional and/or environmental factors.

The objectives were as follows:

1) Examine the relationship between type of dietary fat and SDS mortality by:
   a. changing diet composition (fat type, biotin)
   b. establishing the effect of different fat sources on tissue composition of SDS and control birds
2) Determine if stocking density would affect SDS incidence
3) Provide chemical and morphological characterization of tissue (heart, liver) for SDS and control birds
4) Record general performance characteristics (body weight gain, feed/gain ratio) during the course of the experiments.

Based on the above, a theory to explain the syndrome was to be developed.

LITERATURE REVIEW

I. Incidence of SDS

Sudden death syndrome (SDS) has been observed in many countries throughout the world, including Canada, USA, England and Australia. Table 1 provides an overview of total and SDS mortalities for different broiler flocks from various countries and regions. Brigden and Riddell (1975) reported 3.8% overall mortality in four broiler flocks in Alberta and Saskatchewan, while the average mortality due to SDS was 1.13%. More recently a survey of 51 broiler flocks in western Canada indicated an SDS incidence of 1.95% (Riddell and Springer, 1985). Reports from the USA are very limited, averaging 1.0% in California and Georgia (Riddell, personal communication, 1984). Hemsley (1965) observed that SDS occurred more often in males and mortality was more pronounced below 5 and above 7 weeks of age. Many other researchers reported a peak incidence usually at 3 to 5 weeks of age and a decline to market age at 7 to 8 weeks (Brigden and Riddell, 1975; Ononiwu et al., 1979a; Riddell and Springer, 1985). Similar findings regarding sex differences have been reported elsewhere (Jackson et al., 1972; Brigden and Riddell, 1975; Volk et al., 1974; Hulan et al., 1980; Steele and Edgar, 1982) and some are indicated in Table 1. On the average, 70% to 80% of the affected birds are males. Whether the higher incidence in males is related to faster growth or some physiological (blood pressure)/endocrinological parameters is not known. Although the mortality due to SDS is relatively small, it can account for a significant portion of the overall mortality. Mollison (1983) noted that it

Table 1. Summary of the incidence of sudden death syndrome in broilers

Year	Location	No. of birds surveyed	Total mortality (%)	SDS mortality		Source
				Total (%)	% Males	
1965	England	100,000	2.04	0.46	-	Hemsley, 1965
1972	Canada	-	-	3.00	-	Howell, 1972
1972	Australia	69,068	4.16	0.65	72	Jackson et al., 1972
1975	Western Canada	38,212	3.8	1.13	74-79	Brigden and Riddell 1975
1975	Nova Scotia	20,000	-	2.16	3.22 (male) 1.11 (female)	Cassidy et al., 1975
1978-79	Canada (3 Central Canada broiler tests)	-	-	2.46	-	Riddell and Orr, 1980
1979	Ontario	47,200	2.97	1.10	61	Ononiwu et al., 1979a
1982	Australia	64,000	6.74	2.24	74	Steele and Edgar, 1982
1985	Western Canada	51 broiler flocks	5.59 (2.97-9.42)	1.95 (0.71-4.07)	78	Riddell and Springer, 1985
1982	California	-	-	1.0	-	(Riddell (personal (communication,
1984	Georgia	-	-	1.0	-	(1984)

affected up to 6.5% of the population in six separate experiments and this accounted for over 60% of total mortality. The survey initiated by Riddell and Springer (1985) confirmed that SDS in addition to skeletal deformities is a major cause for losses in broiler flocks.

II. Diagnosis of SDS

1. Gross appearance and post-mortem findings

Birds affected by SDS are usually found lying on their backs with feet and neck extended. Julian (in Ononiwu et al., 1979a) found 80% of broilers on their back, 15% on their sides and 5% on their abdomen. There is no visual evidence of disorder as indicator of the syndrome. The birds are in good flesh with body weights at or slightly above the growth curve of the flock (Brigden and Riddell, 1975; Ononiwu et al., 1979a; Hulan et al., 1980; Steele and Edgar, 1982).

Brigden and Riddell (1975) classified birds as dying from SDS if they were in good flesh with full gastrointestinal tract and no other significant post-mortem signs except congestion or edema of the lungs. Later, Riddell and Orr (1980) reported that congestion or edema of the lungs was not a consistent feature. It was absent or very slight in birds collected shortly after death and this may develop as a post-mortem artifact.

Ononiwu et al. (1979b) made several observations at necropsy of SDS birds, including: feed in the digestive tract, generalized pulmonary congestion, enlarged and contracted ventricles, blood clots in the atria, dilated intestines with pale contents, slightly enlarged liver, dis-

colored and empty gallbladder, pale kidneys and congested thyroid, spleen and thymus.

Since then, a set of criteria to diagnose SDS as described by Brigden and Riddell (1975) or Ononiwu et al. (1979b) has been used by several authors (Hulan et al., 1980; Steele and Edgar, 1982; Steele et al., 1982).

Julian (personal communication, 1984) noted that contracted hearts along with lung congestion and full intestine are considered to be the most important diagnostic criteria while an empty gallbladder is of little significance.

2. Histological and biochemical findings

Ononiwu et al. (1979b) performed histological examinations of several tissues from 142 SDS birds and compared them with control birds. Significant findings from the lungs, hearts and livers of these birds are summarized in Table 2. The lungs showed signs of vascular engorgment and edema of interstitial and interlobular connective tissue in 85% of the cases whereas 70% of the heart specimens revealed degeneration of myofibers, separation by edema fluid and leukocytic infiltration of the interstitium. Similar findings were observed by Steele et al. (1982). In contrast, Volk et al. (1974) observed no marked macroscopic changes in the hearts of SDS birds. However, during the histological examination circulatory disorders were noted with resulting regressive changes in myocardium. Riddell and Orr (1980) performed chemical studies of the blood and histological examinations of hearts of SDS birds, but no significant differences in the heart tissue between the SDS and control birds were observed. The authors did not rule out the possibility of ultra-

Table 2. Detailed histopathological lesions of lungs, hearts and livers of SDS and control birds

Lesions	Groups		
	CC[a]	NCC[b]	SDS[c]
Lung: total tissues	10	60	120
Vascular congestion	0	0	120
Mononuclear cell aggregates	2	2	4
Leukocytic infiltration and inflammation of mucosa of secondary bronchiole	2	5	64
Edema of lung tissue	0	0	106
Heart: total tissues	10	60	99
Vascular congestion	2	4	20
Myocardial degeneration	0	0	8
Mononuclear cell aggregates	2	4	71
Heterophilic infiltration	0	0	70
Bursal dependent nodes	2	6	7
Myocardial separation	0	0	50
Liver: total tissues	10	60	100
Sinusoidal congestion	0	0	52
Bile duct hyperplasia	0	0	64
Mononuclear cell aggregates	6	18	71
Heterophilic infiltration	0	0	9
Constricted bile ducts	3	2	59
Periportal hepatitis	4	0	98
Fatty degeneration	0	0	9

[a]CC - contact control

[b]NCC - noncontact control

[c]SDS - sudden death syndrome

Modified from Ononiwu et al. (1979b)

structural or biochemical lesions, which could lead to SDS. Changes in blood composition were more dramatic as the period between time of death to collection increased (possibly due to post-mortem movements of ions). There were no significant changes in the electrolyte concentrations of recently dead birds. Some of the SDS birds had elevated total serum lipid, suggesting the possibility of altered fat metabolism.

Mollison (1983) examined the mineral composition (calcium, sodium, potassium and copper) of the heart tissues of SDS birds and other mortalities and reported significant lower copper concentration for the SDS birds. He implied the possibility of copper deficiency in SDS susceptible individuals.

III. Possible physiological events leading to SDS

The exact etiology of the syndrome has not yet been established. Several researchers implied it is a metabolic disorder with a combination of nutritional, genetic and environmental factors (Brigden and Riddell, 1975; Riddell and Orr, 1980; Steele and Edgar, 1982). Volk et al. (1974) suggested the circulatory changes with resulting regressive changes in the myocardium may be related to cardiac shock and death. The possibility of blood clots in the heart commonly seen in SDS birds and being the immediate cause of death was ruled out by Cassidy et al. (1975). The histological and histochemical examination of the clots failed to confirm them as a thrombi, but rather of post-mortem origin.

Ononiwu et al. (1979b) proposed a possible sequence of events in order to explain some of the characteristic features of SDS. They sug-

gested that the sequence starts with circulatory lesions manifested by increased permeability of the peripheral circulatory systems. This physiological permeability, caused by short-term increase in blood pressure generally is reversible, however when the stimulus surpasses the tolerance level, vessel permeability is irreversible. In the case of SDS, the damage becomes permanent, both to the capillaries and the tissues they supply. Thus death could be the result of heart damage causing lung edema. The fluid lost from the circulatory system into the lung tissue can result in peripheral circulatory failure and shock.

Lung edema is generally accepted as being an indicator of SDS. The lungs are often congested and edematous, but after removal from the thoracic cavity a straw colored fluid remains between the ribs (Riddell, personal communication, 1984). If birds are examined immediately after death those changes in the lungs are often not found. It can be speculated that they may be due in part to hypostasis in the dead bird lying on its back. Riddell (personal communication, 1984) has reproduced similar lung changes by killing a healthy bird and leaving it lying on its back in a warm room for several hours.

In view of these findings one can question if the fluid from the circulatory system going into the lung tissue is more likely to be a part of post-mortem changes and not a factor predisposing to death.

Finally, Julian (personal communication, 1984) suggested left ventricular fibrillation as a possible cause of death.

IV. Factors associated with SDS

In recent years several research programs have been initiated to identify dietary or other components involved in SDS. Growth rate was first implicated as a major cause of death. Since selection for rapid growth in meat-type chickens resulted in more stress (pressure) on a young growing bird, one could also expect necessary adaptations in dietary requirements, which are unknown at the present time.

1. Growth rate and pelleted diets

SDS has been associated with flock growth rates by a number of researchers. Gasperdone (1981), studying effects of various feeding schedules on growth rate and SDS mortality, observed that the 8 hour program, feeding on alternate days and low protein starter resulted in growth rate reduction. Total and SDS mortalities were not found to be related to feeding treatment, although total mortality was highest for the chicks on 17% starter mash. Mollison et al. (1984) also reported that feed restriction caused a reduced weight gain over 7 weeks, but no decrease in SDS incidence was observed. The authors stated, however, that a greater difference in rate of gain may be necessary to produce a difference in SDS mortality. In another study, the incidence was found to be significantly reduced on high protein finisher (24% vs 19%) between 29 to 56 days of age (Mollison et al., 1984). The weight gain over 7 weeks was not significantly different. In contrast, Julian (personal communication, 1984) obtained 3% SDS mortality on high protein (28%), high caloric (3100 ME kcal/kg) and no mortality on low protein

(18%) and low caloric (2400 ME kcal/kg) pelleted rations. Similarly, Classen et al. (1982) observed a trend to lower SDS incidence with low density diets (2600 vs 3200 ME kcal/kg). These observations are in contrast to a field survey in which no correlation could be found between growth rate and the incidence of SDS (Riddell and Springer, 1985).

Proudfoot and Hulan (1982) and Proudfoot et al. (1982, 1984) reported data dealing with restricted feeding schedules and different dietary textures (mash, pellet, crumbles) in relation to the incidence of SDS. Birds fed crumble-pellet-diet (steam-pressure-die) outperformed the birds on all mash regimens (Proudfoot and Hulan, 1982). Mortality due to SDS was reported to be significantly higher in males on the former dietary regimen. Birds fed the crumble pellet diet grew at a significantly ($P<.05$) faster rate compared with birds fed either the mash or reground crumble pellet diets (Proudfoot et al., 1982). However, the incidence of SDS for crumble-pellet diets in the normal and ground forms was similar, but greater than for the mash form. These observations provide indirect evidence that the association of a higher incidence of SDS is not caused by the stress of rapid growth itself but due to some unidentified factor(s) involved with the crumble-pelleting process. Further studies demonstrated that the pelleted micronutrients or fat were not associated with increased SDS (Proudfoot et al., 1984). When dietary protein supplements (soybean meal, canola meal, fish meal) bypassed the pelleting process, a significant reduction in SDS mortality was observed. The authors suggested that toxic components are produced

when protein supplements are subjected to the pelleting process and they may be a major causative agent of SDS.

2. Vitamins

An association between the availability of biotin and the incidence of SDS was noted by Scott (1981). Hulan et al. (1980) reported that the addition of a vitamin mixture of biotin, pyridoxine and/or thiamine, not normally added to broiler diets, gave some indication of reduced total mortality. However, the inclusion of biotin alone (0.3 mg/kg of diet) resulted in significantly decreased total and SDS mortality. The authors indicated the need for further experimentation on the dose-response effect of biotin. In contrast, Hunt and Gardiner (1982) compared various dietary factors (wheat-based vs corn-based diet, supplemental potassium and supplemental biotin, pyridoxine and thiamine) and their effects on SDS. Neither the total mortality nor mortality attributed to SDS was influenced by these diets. Similarly Mollison et al. (1984) found that fortification of the diet with additional vitamins and trace minerals had no effect on the number of SDS mortalities. In an extensive study Steele et al. (1982) supplied biotin via drinking water to a commercial flock of 64,000 broilers (1.2-5x to 6-25x NRC levels depending on growth stage). The uptake of biotin was confirmed by measuring liver biotin content. Liver levels indicated adequate amounts of the vitamin in SDS and clinically normal birds. They concluded that biotin supplementation neither prevented SDS nor reduced total mortality in the flock.

Some research has drawn attention to the similarity between SDS and fatty liver kidney syndrome (FLKS) of apparently healthy and fast growing

birds (Hood, 1980; Buenrostro and Kratzer, 1982; Whitehead and Randall, 1982). Both conditions have similarities in their incidence and symptoms, while biotin deficiency has been suggested as a common metabolic alteration. Buenostro and Kratzer (1982) found that SDS birds were biotin deficient, but the expected fatty acid alterations observed in FLKS (increased palmitoleic and decreased stearic acids) were not seen. They noted an increase in oleic and a decrease in arachidonic acids when compared to other non-SDS broilers from the same flock.

Whitehead and Randall (1982) observed that some birds showed postmortem signs characteristic of both FLKS and SDS. The occurrence of both syndromes simultaneously was highly variable but in general, correlated with the biotin content of the diet. However, they also found that the incidence of SDS alone was unaffected by dietary biotin concentration. It is likely that the apparent association between the two conditions suggests that an abnormality occurring as a result of FLKS may contribute to the initiation of SDS.

3. Stress

The amount of research conducted to explain the role of environmental stresses (sudden movement, lighting and stocking density) and the use of anti-stress compounds on the incidence of SDS is scant.

Ononiwu et al. (1979a) observed the effect of continuous as opposed to intermittent lighting on the incidence. Continuous lighting resulted in higher SDS mortality, but the process through which light intensity exerts its effect on broiler chickens is unknown. The authors postulated

however, that light intensity above optimum could result in increased stress (by inducing cannibalism, excitement, fighting) and so produce higher incidence of SDS. The above observations were not supported by a field survey (Riddell and Springer, 1985).

Since SDS occurs in chickens which have existed as anonymous flock members until the onset of sudden death, Newberry et al. (1985) conducted a study to identify birds prior to death based on behavioural parameters. They concluded that SDS birds could not be distinguished from other flock members by behavioural characteristics.

In order to reduce the stress effect on the birds a few studies were initiated with anti-stress compounds. Proudfoot and Hulan (1983) conducted four experiments to evaluate the effects of aspirin (ASA) in diets and its effect on decreasing the SDS incidence. The ASA supplementation had a deleterious effect on overall mortality (significant $P<0.01$ in two of four experiments) and no beneficial effect on SDS mortality. Addition of 0.16% ASA to the diet resulted in reduced body weights.

Gardiner and Hunt (1984) used reserpine (an anti-hypertensive and tranquilizing agent) at low levels 0-3 mg/kg to determine the effect on the incidence of SDS. Dietary reserpine significantly reduced growth, indicating that it has a physiological effect on the chickens, however, no effect on either total or SDS mortality was observed.

4. Fat metabolism

Several researchers have implicated lipid metabolism as having a possible role in the syndrome (Hulan et al., 1980; Riddell and Orr,

1980). Mollison et al. (1984) reported that a wheat-soy diet fed ad libitum or restricted had a higher incidence of SDS (1.9%) than birds fed corn-soy diets (0.95%), although the growth rate was greater for the corn-soy diet fed birds. It was also noted that the incidence was higher in males than females. Since those observations were made, wheat-soy diets and male broiler chickens were utilized in subsequent experiments. Mollison (1983) reported that wheat-soy diets with B-complex vitamins had no effect on reducing the incidence of SDS, but supplementation with fat-soluble vitamins A, D and E, indicated a trend towards reduced mortality from 2.86% to 1.66%. These findings further suggest that fat metabolism may be involved in the prevention or reduction of the SDS incidence.

Summary

At present there is little information in the literature which indicates a cause and/or prevention of SDS. Factors such as growth rate, vitamins and pelleting of feed have been investigated in isolated studies with no follow-up.

The problem of SDS is difficult to study due to the apparent complexity of the condition, which is related to its irregular incidence and variability in the mortality data, as well as to the lack of visual signs prior to death. Therefore, the chemical and morphological findings obtained from necropsy materials could be complicated with considerable post-mortem changes. This in turn is complicated by little progress in understanding the pathogenesis and etiology of SDS. Few studies have

succeeded in demonstrating increased and/or decreased SDS mortality and in many cases with non-consistent results, and so further research in this area is warranted.

GENERAL MATERIALS AND METHODS

Five experiments were initiated to study the incidence, possible causes and methods of reducing SDS in broiler chickens.

All studies used one-day old, vaccinated (Marek's) male broiler chicks of commercial (Cobb x Cobb) parentage. The birds were raised in 1.54 x 4.31 m floor pens at a stocking rate of 11 birds/m^2 (70 birds/pen) or 12 birds/m^2 (80 birds/pen). Pens were located in an environmentally-controlled house providing continuous lighting at an intensity of about 5 lux at feeder level. The temperature in the barn for the first week was maintained at 27^{o}C (35^{o}C under the canopy brooder with heat lamps). Thereafter, the temperature was decreased by 3^{o}C per week (raising the brooder, removing bulbs) until it reached 21^{o}C. During the first two weeks each pen was equipped with two trough feeders and a water fountain. From 14 days until the end of the experiment, feed was supplied in tube type hanging feeders (two/pen, 40 cm diameter) and water was supplied by an automatic cup waterer (15 cm diameter).

All feed (mash form) and water was supplied ad libitum. Daily management included cleaning wet spots, stirring litter, shaking down feeders, dusting equipment and culling sick birds. Mortalities occurring during the first 3 days of experiments were replaced by spare birds. Subsequently, mortalities were recorded by pen number, treatment number, wing band and dead weight. Each day they were submitted for necropsy to the poultry pathology laboratory, Manitoba Department of Agriculture.

Birds dying from SDS were diagnosed according to the following criteria: good body condition; well fleshed, of average weight (on

either side of the growth curve); full digestive tract; small gall bladder; lung congestion and edema in the thoracic cavity, enlarged, mottled, soft or wet liver; severe congestion of atria and ventricular systole.

In all experiments the initial pen weight (group weight) of the birds was recorded, followed by weighing at 2, 4 (group weight) and 7 weeks of age (individual or group weight). The average body weights for each pen were calculated by dividing the total weight of the live birds by the total number of birds at the time of weighing.

$$\text{Average body weight/bird} = \frac{\text{Total live pen weight}}{\text{Total no. of live birds}}$$

Average pen gain was calculated by substracting the average initial weight of live birds from the average weight of live birds at the time of weighing (present weight).

$$\text{Average gain} = \text{Average present weight} - \text{Average initial weight}$$

Feed:gain ratios were calculated by dividing total feed consumed per pen during the period of concern of the total weight gain of birds within a pen, including weight of mortalities. That is, total weight gain was calculated by subtracting the initial pen weight from the present weight plus the weight of mortalities that occurred within the particular period.

$$\text{Feed:gain} = \frac{\text{Feed consumption}}{\text{Total pen weight gain}}$$

All performance and mortality data were calculated on pen basis.

Data collected (performance and mortality data - on pen basis) were statistically analysed as completely randomized designs, using analysis

of variance procedures of the Statistical Analysis System (SAS Institute, Inc., 1982). A detailed method of statistical analysis is given in each manuscript. Mortality data were converted by arc sine transformation before statistical analysis was performed, however for interpretation the results are expressed as percentages. Differences among means were detected using the Student-Neuman-Keul's (SNK) test ($\alpha = 0.05$) (Snedecor and Cochran, 1980).

Analytical procedures

1. Mineral analysis

All glassware used for the analysis was immersed in boiling distilled water and detergent for three hours, then washed and rinsed three times in distilled H_2O. It was then soaked in 20% HNO_3 overnight, rinsed four times with deionized distilled water and oven dried at $60^{o}C$.

The heart and liver samples were wet ashed according to the method by Thompson and Blanchflower (1971). The procedure is based on wet ashing with a mixture of nitric and perchloric acids, taking to dryness, dissolving ash in a fixed volume of dilute acid, followed by final measurement on the atomic absorption spectrophotometer. The modification consisted of overnight predigestion of the samples in the acid mixture vs immediate digestion. Ash residues from samples were dissolved with 5 ml of 5% HCl and analysed for copper (Cu), zinc (Zn), sodium (Na), potassium (K), magnesium (Mg) and calcium (Ca) using Instrumentation Laboratory #551 flame (acetylene and air, respectively) atomic absorption spectrophotometer, according to the standard procedures of the Association of Official Analytical Chemists (A.O.A.C., 1980). Accuracy of this wet ashing procedure was checked by inclusion of a standard biological sample obtained from the U.S. Department of Commerce, National Bureau of Standards (bovine liver #1577a) (Table 3). The sensitivity values for different elements are given in Table 4.

2. Isotope dilution assay for biotin

The analysis was done according to the method described by Bhullar et al. (1985).

Table 3. Expected and determined values of minerals for standard bovine liver

Material	Element	Content	Determined
		µg/g	µg/g
Bovine liver #1577a			
	Cu	158±7	153±5
	Zn	123±8	121±9
	Zn	123±8	121±9
	Ca	120±7	124±5
	Mg	600±15	601±12
		-- wt. % --	-- wt. % --
	Na	0.243±0.013	0.246±0.014
	K	0.996±0.007	0.993±0.009

Table 4. Sensitivity values for different elements at recommended wavelengths

Element	Wavelength (nm)	Sensitivity (µg/ml)
Ca	422.7	0.05
Cu	324.7	0.03
K	766.5	0.01
Mg	285.1	0.003
Na	589.0	0.001
Zn	213.9	0.008

Biotin calibration plot. To a series of Eppendorf centrifuge tubes were added 300 µℓ of 0.2 M $(NH_4)_2CO_3$, 100 µℓ of 1.55 M Na_2SO_4, 25 µℓ of ^3H biotin and increasing amounts (0-1.5 ng) of cold biotin. After mixing, 80 µℓ of the avidin working standard solution were added. After mixing in a shaker for 15 min, 0.3 ml of bentonite suspension was added and mixed for a further 15 min. The precipitate of the avidin-biotin complex adsorbed on the bentonite was filtered through the Milipore filter (GSWP 02500) GS with a pore size of 0.22 µm. The filter was washed with 5 ml of 0.2 $(NH_4)_2CO_3$, dried and transferred to a scintillation vial containing 15 ml of the scintillator. The vials were left overnight in the dark and radioactivity determined using a Beckman LS2800 Liquid Scintillation Spectrometer.

Hydrolysis of tissue to release biotin. Various amounts (150-200 mg) of freeze dried tissue were hydrolyzed with 1 ml of 4.5 N H_2SO_4 by autoclaving at 15 psi for 1 hour. The hydrolyzate was neutralized with 1 ml of 4.5 N NaOH, and filtered on a coarse-grade filter paper. When the concentration of biotin in the original sample was too high appropriate dilutions were prepared using 0.2 M $(NH_4)_2CO_3$.

The concentration of biotin in the sample was obtained from the standard curve and used for calculation.

$$\text{ng biotin/g of tissue} = \text{ng of biotin (standard curve)} \times \text{dilution factor} \times \frac{\text{total volume of hydrolyzate}}{\text{aliquot used for the assay}} \times \frac{1}{\text{tissue weight}}$$

3. Total lipid

The total lipid content of the tissues was determined according to the procedure of Folch et al. (1957). The method consists of homogenizing the tissue with a 2:1 chloroform-methanol mixture and washing the extract with the addition of 0.2 its volume of appropriate salt solution or water. The resulting mixture separates into two phases, the lower phase being the total pure lipid extract.

4. Fatty acid methyl esters

Methyl esters of fatty acids of liver and heart tissue were prepared using a modified procedure of Metcalfe and Schmitz (1961). Fifty to 100 mg of fatty acid and 3 ml of BF_3-methanol reagent were placed into a screw-cap tube. The tube (with a loosely fitted cap) was put into a hot water bath at 65° to 70°C for 10 minutes. It was then removed and screw-cap tightened. The sample was incubated in an oven overnight at 65 to 70°C, followed by cooling to room temperature. Subsequently, 10 ml of petroleum ether (b.p. 40-60°C, reagent grade) and 5 ml of distilled water were added. The samples were shaken vigorously and allowed to stand until clear supernatant was separated. The supernatant, containing the mixed methyl esters was transferred to another vial and evaporated in a 35-40°C water bath under continuous nitrogen flow to a constant volume. The fatty acid composition was determined using a Varian Vista 6000 gas chromatograph equipped with 8'x¼"x2 mm glass column packed with 3% SP-2310 and 2% SP-2300 on 100/120 Chromosorb WAW and a Vista 4042 data system. The operating temperature of the column ranged from 190°C to

205°C rising by 1°C per minute. The injector temperature was 230°C. Peaks were identified by comparison with retention times of authentic fatty acid methyl ester mixtures (Nu Chek Prep, Inc. Elysion, MN) and results expressed as percentage of total fatty acid methyl esters.

5. Determination of the linoleic acid content of the feed samples

The linoleic acid content was determined using the fat extraction method of Bligh and Dyer (1959) and the methylation procedure of Metcalfe et al. (1966).

6. Determination of glycogen

Glycogen was determined using the procedure described by Seifer et al. (1950). The method is based on the extraction of glycogen with KOH, and precipitation with ethanol, followed by hydrolysis of glycogen to glucose with sulfuric acid. The anthrone reagent reacts with glucose and the complex is measured spectrophotometrically at 610-615 nm.

7. Crude protein (Macro Kjeldahl)

Protein content was determined according to the method of the Association of Official Analytical Chemists (A.O.A.C., 1984).

8. Blood analysis procedures

a) Sample preparation

Blood samples were centrifuged at 1,500 r.p.m. for 15-20 minutes in an Adams table top centrifuge. The plasma layer was withdrawn by

pipette and stored frozen (-20°C) in 2 separate 5 ml disposable culture tubes.

b) Chemical analysis

All analyses were run in duplicate. Total lipid was determined using the method of Frings and Dunn (1970), while triglyceride and cholesterol concentrations were determined by the method of Moses et al. (1975).

Manuscript I: Sudden Death Syndrome in Broilers: Dietary Fat Supplementation and its Effect on Tissue Composition[1]

[1]Published in Poultry Science 64:1128-1136 (1985).

ABSTRACT: The effects of dietary fat sources on the general performance of broiler chickens and the incidence of sudden death syndrome (SDS) were investigated in two experiments. In Experiment 1, a wheat-soy diet supplemented with sunflower oil was found to significantly ($P<0.05$) improve performance characteristics and reduce the mortality attributed to SDS as compared to the same diet supplemented with tallow. The blood lipid parameters: total lipids, triglycerides and cholesterol at 4 and 7 weeks of age were not affected ($P>0.05$) by the type of fat used in the diet. In Experiment 2, wheat-soy diets were supplemented with either tallow or sunflower oil at the same dietary levels and two levels of stocking density (0.09 m^2/bird or 0.08 m^2/bird). The sunflower oil diet significantly ($P<0.05$) improved the general performance of the birds and reduced the SDS mortality. Bird density had no significant effect. Fatty acid composition of the heart and liver tissues from SDS birds showed differences when compared to culled birds (leg problems) from the same experimental period. In the heart tissue, increased levels of palmitic (16:0) and oleic (18:1) acids were observed, while linoleic (18:2) and arachidonic (20:4) acids were lower. Liver tissue showed increased levels of 18:1 and lower levels for 18:2 and 20:4. Analysis for copper (Cu) and zinc (Zn) in the heart tissue did not indicate significant ($P>0.05$) treatment effects, but calcium (Ca) concentration was significantly ($P<0.05$) higher in SDS than culled birds. Analysis performed on the liver tissue showed significant ($P<0.05$) differences in Cu and Zn levels between these two groups of birds.

Key words: sudden death syndrome, broilers, dietary fat, fatty acid composition, mineral analysis.

INTRODUCTION

Sudden death syndrome (SDS) has become an increasingly important cause of mortality in rapidly growing broiler chickens. Its occurrence is widespread throughout England (Hemsley, 1965), Canada (Brigden and Riddell, 1975; Howell, 1972; Volk et al., 1974) and Australia (Jackson et al., 1972) ranging from 0.5% to 5% mortality. Pathological observations of SDS are generally associated with a failure of the cardiovasculary system, although this has not been exclusively demonstrated (Cassidy et al., 1975: Hulan et al., 1980). A similar condition referred to as cardiomyopathy (cardiac failure, myocardial lesions) was reported by Pass (1983) in broiler breeder hens and egg laying strains from 24 to 30 weeks of age.

Limited information is available in the literature to implicate a specific cause for SDS or a definitive method of its prevention. Even less information is available on biochemical alterations occurring in SDS birds. However, Ononiwu et al. (1979b) proposed a plausible hypothesis that SDS could be caused by heart damage resulting in lung edema and rendering the bird unable to breath. Sufficient fluid from the circulatory system enters lung tissue causing peripheral failure or shock.

Recent data reported by Mollison (1983) suggest that the level and type of dietary fats with various degrees of unsaturation may be associated with the disease. The involvement of fat metabolism was implicated earlier by Riddell and Orr (1980) who noted that birds dying from SDS had an increased total lipid content in serum. Mollison (1983) reported that wheat-soy diets fed ad libitum or restricted resulted in a higher (1.9%) incidence of SDS than corn-soy diets (0.95%). Supplementation of

wheat-soy diets with B-complex vitamins had no effect on the incidence of SDS, whereas supplementation with fat-soluble vitamins A, D and E reduced SDS from 2.86% (control) to 1.66%.

A further observation made by Mollison et al. (1984) was that birds fed high protein (25%) diets tended to be leaner and this was associated with a significant ($P<0.05$) reduction in SDS mortality. The authors speculated that a reduction in fatness could reduce stress on the cardiovascular system. Buenrostro and Kratzer (1982) reported differences in fatty acid composition of liver tissue of SDS birds and those that died of other causes.

These findings suggest that fat metabolism may be involved in the etiology of SDS and that dietary fat type may be involved in prevention or reduction of its incidence.

Two experiments were conducted to investigate the involvement of dietary fat sources. Experiment 1 compared the effect of supplementation of wheat-soy diets with tallow, tallow:sunflower oil and sunflower oil on SDS mortality and lipid parameters at 4 and 7 weeks of age. Experiment 2 was designed to examine the effects of dietary fat on SDS mortality and fatty acid and mineral content of heart and liver tissues.

MATERIALS AND METHODS

Both experiments used one-day old male, vaccinated (Marek's) broiler chicks of commercial (Cobb x Cobb) parentage. The birds were raised in 1.54 x 4.31 m floor pens, at a stocking rate of 0.09 m^2 per bird (70 birds/pen) or 0.08 m^2 per bird (80 birds/pen), located in an environmentally controlled house providing continuous lighting at low intensity. Diets were fed in mash form and water was supplied ad libitum. Mortalities and culls were submitted for post mortem necropsy. Birds dying from SDS were diagnosed according to the following criteria: good bodily condition; well fleshed (with weights on either side of the growth curve); full digestive tract; small gall bladder; lung congestion and edema in the thoracic cavity, pericardial sac and/or abdominal cavity; enlarged, mottled, soft, wet liver; severe congestion of the atria and ventricular systole.

Experiment 1 was conducted to investigate the effect of two different fat sources (tallow and sunflower oil) on the general performance characteristics, mortality due to SDS and blood lipids parameters of young broilers.

Sixteen hundred and eighty (1680) one-day old broilers were randomly allocated to 24 pens. Each diet was fed to 560 birds housed in 8 randomly assigned pens. The wheat-soy diets were supplemented either with tallow, equal amount of tallow and sunflower oil (SFO) or SFO. Starter (0-4 weeks) and finisher (4-7 weeks) formulations are presented in Table 5.

Weight gains and feed conversion were calculated for the experimental periods 0-2, 2-4 and 4-7 weeks.

Table 5. Composition and calculated analysis of starter and finisher diets - Experiments 1 and 2.

Ingredients	Starter	Finisher
	g/kg	
Wheat	582.0	704.6
Soybean meal (47.5%)	293.5	183.6
Fish meal (70%)	28.0	21.8
Fat source[1]	54.0	46.0
Limestone	12.5	12.0
Dicalcium-phosphate	14.0	16.0
Vitamin mix (standard)[2]	10.0	10.0
Mineral mix (standard)[3]	5.0	5.0
DL-methionine	1.0	1.0
	1000.0	1000.0
Calculated analysis: (Chemical analysis)		
Metabolizable energy, (kcal/kg)[4]	3020-3110	3060-3140
Crude protein, (%)	23.5	19.4
Linoleic acid (%)[5]	1.03-3.55	1.05-3.20
	(0.91-3.25)	(0.86-2.87)

[1]Supplies tallow (diet 1), tallow:sunflower oil (50:50) (diet 2) or sunflower oil (diet 3).

[2]Supplies the following per kg of complete feed: vitamin A, 8250 I.U.; vitamin D_3, 870 I.C.U.; vitamin E, 5.5 I.U.; riboflavin 5.5 mg; Ca pantothenate 11 mg; niacin, 16.5 mg; choline chloride, 275 mg; menadione 1.1 mg; santoquin, 250 mg.

[3]Supplies the following in mg per kg of complete feed: manganese oxide, 166; zinc oxide, 14.5; ferrous sulfate·$7H_2O$, 31; copper sulfate·$5H_2O$, 25.5; iodized salt, 4760 mg.

[4]ME values for tallow, tallow:sunflower oil and sunflower oil diets were calculated to be 3020, 3065 and 3110 kcal/kg for the starter diets and 3060, 3100 and 3140 for the finisher diets.

[5]Determined linoleic acid values were 0.91 (1), 2.14 (2) and 3.25 (3) percent for the starter diets and 0.86 (1), 1.78 (2) and 2.87 (3) for the finisher diets.

At 4 and 7 weeks of age, two birds from each pen (16 birds/treatment) were selected at random and 5 cc of blood collected via cardiac puncture. The samples were placed into 10 cc heparinized Vacutainer (Becton Dickinson,Canada) and put on ice until centrifuged. Total serum lipids were determined by the method described by Frings and Dunn (1970), while serum triglycerides and cholesterol were analyzed using the method of Moses et al. (1975). All data were analyzed as a completely randomized design using a one way analysis of variance procedure. Mortality data were converted by arc sine transformation before statistical analysis was done, however, for interpretation are presented as percents. Differences between the means were determined using the Student-Neuman-Keuls (SNK) Test (Snedecor and Cochran, 1980).

In Experiment 2 wheat-soy diets supplemented with either tallow or sunflower oil at the same dietary levels were fed to birds housed at two stocking rates (70 and 80 birds/pen). The composition of the experimental diets was the same as for Experiment 1 (Table 5). Two thousand one hundred one day old broilers were randomly distributed among 28 pens, at 70 or 80 birds per pen. Seven pens of each density were randomly assigned to each of the two dietary formulations. The starting and finishing periods were from 0-28 and 28-49 days of age, respectively. Weight gains and feed/gain ratios were calculated for 0-2, 2-4 and 4-7 weeks. At the end of the experiment 15 birds of each diet (1 bird/pen plus 1) were sacrificed for biochemical analysis of heart and liver tissues. Mortalities were recorded to 49 days of age as they occurred. From the 4th week to the end of the experiment all hearts and livers of SDS and culled

(leg problems) birds were removed during the post mortem examination. For the analysis, samples from five (5) birds of each diet, for the SDS and culled groups were used (10 birds per group). The samples were immediately frozen, stored at -20°C and finally lyophylized for approximately 30 hours in a freeze-dryer (Lab Conco "Vac-stop" Tray dryer Model 75159 and Lab Conco Freeze dryer 18 litre with shelf freezer Model 75015). The lyophylized samples were finely ground (Janke and Kunkel A10-5 stainless steel grinder) and placed into polyethylene screw top vials. The methyl esters of heart and liver tissue fatty acids were prepared by a modification of the procedure described by Metcalfe and Schmitz (1961). The fatty acid composition of the tissues was determined using a Varian Vista 6000 GC equipped with a 8' x 1/4" x 2 mm glass column packed with 3% SP-2310 and 2% SP-2300 on 100/120 Chromosorb WAW, and a Vista 4042 data system.

In the preparation for mineral analysis [calcium (Ca), copper (Cu) and zinc (Zn)] subsamples of lyophilized liver and heart tissue were wet ashed according to the method of Thompson and Blanchflower (1971). The determinations were done using an Instrumentation Laboratory Model 551 atomic absorption spectrophotometer.

The performance data were analyzed as a 2x2 factorial of a completely randomized design (Snedecor and Cochran, 1980). Mortality data are presented according to dietary treatment because stocking density did not significantly affect mortality. Comparisons of means were made using the SNK-Test. One-way anova was used to compare the mineral contents of the individual hearts and livers between diets and between SDS and culled

birds. For the comparison of fatty acid composition in hearts and livers of the two diets, as well as for SDS and culled birds, a simple T-test was used.

RESULTS AND DISCUSSION

Means of the 7 week body weight, average weight gain and feed to gain ratio are summarized in Table 6 for Experiment 1 and in Table 7 for Experiment 2. The average weight gain over the period from 0-7 weeks was significantly ($P<0.05$) improved when the diets contained SFO as a source of dietary fat. In Experiment 1 an improvement in the feed:gain ratio with increased amounts of SFO in the diet was observed. No significant differences, ($P>0.05$) among treatments, however, were observed for the feed to gain ratios in Experiment 2.

The fatty acid analysis of both fat sources and composition of the diets indicate a wide range of linoleic acid content (Table 5). This would account for more efficient utilization of the diets supplemented with SFO (Renner and Hill, 1960). Leeson and Summers (1976) postulated that when components of fatty acids of tallow, lard and soybean oil are fed to the chicken as three separate mixtures the absorbability of palmitic and stearic acids increases as the proportion of unsaturated fatty acids increases. Therefore the balance of saturated to unsaturated fatty acids in a fat or oil can have a marked influence on the over-all digestibility of the fat. It is also worthwhile to consider the fatty acid synergism in terms of "extra caloric" properties attributed to the fat and its influence on carcass composition (Griffiths et al., 1977).

Tables 8 and 9 illustrate the total and SDS mortality trends for Experiments 1 and 2. Although overall mortality was unaffected, SDS mortality was significantly ($P<0.05$) reduced for birds fed exclusively the SFO diet. There were also more cases of dehydration and nonvisual

Table 6. Effects of different fat sources on performance of male broilers - Experiment 1

Parameters	Dietary treatment			
	Tallow	Tallow/SFO[1]	SFO	±SE
7 Weeks body weight (g)	2218[a]	2260[a,b]	2271[b]	±5.0
Average weight gain (g) (0-7 weeks)	2174[a]	2221[b]	2227[b]	±5.0
Feed/gain (0-7 weeks)	1.89[a]	1.84[b]	1.81[c]	±0.001

[a,b,c]Means within a row having different superscripts are significantly different ($P<0.05$).

[1]SFO = Sunflower oil.

Table 7. Effects of different fat sources and densities on performance of broilers - Experiment 2

	Dietary treatment				
	Tallow		SFO[1]		
Density[2]	70	80	70	80	±SE
Parameters					
7 weeks body weight (g)	2322[a]	2319[a]	2395[b]	2384[b]	±5.8
Average weight gain (g) (0-7 weeks)	2280[a]	2276[a]	2352[b]	2342[b]	±7.0
Feed/gain (0-7 weeks)	1.89[a]	1.88[a]	1.88[a]	1.86[a]	±0.02

[a,b]Means within a row having different superscripts are significantly different ($P<0.05$).

[1]SFO = Sunflower oil.

[2]0.09 m^2 per bird = 70 birds per pen.
0.08 m^2 per bird = 80 birds per pen.

Table 8. SDS mortality as a percent of total number of birds per treatment - Experiment 1

Parameters	Periods (weeks)	Dietary treatment		
		Tallow	Tallow/SFO[1]	SFO
Total no. of SDS mortalities (%)	0-4	4(0.71)[a][2]	5(0.89)[a]	2(0.36)[a]
	4-7	8(1.43)[a]	11(1.97)[a]	4(0.71)[b]
	0-7	13(2.32)[a]	16(2.86)[a]	6(1.07)[b]
Total no. of mortalities (%)[3]	0-7	30(5.36)[a]	32(5.71)[a]	27(4.82)[a]

[a,b]Means within a row having different superscripts are significantly different ($P<0.05$).

[1]SFO = Sunflower oil.

[2]Number outside of parenthesis indicates the actual number of birds died per dietary treatment and the number within the parenthesis percent mortality.

[3]Includes culled birds.

Table 9. SDS mortality as a percent of total number of birds per treatment - Experiment 2

Parameters	Periods (weeks)	Dietary treatment: Tallow	Dietary treatment: SFO[3]
Total no. of SDS mortalities (%)	0-4	14(1.33)[a][1]	7(0.67)[a]
	4-7	25(2.41)[a]	18(1.75)[a]
	0-7	39(3.71)[a]	25(2.38)[b]
Total no. of mortalities (%)[2]	0-7	77(7.33)[a]	58(5.52)[b]

Source	d.f.	M.S.
Diet	1	83.421*
Density	1	.214
Diet x density	1	19.773
Error	24	15.517

[a,b]Means within a row having different superscripts are significantly different ($P<0.05$).

[1]Number outside of parenthesis indicates the actual number of birds died per treatment and number within parenthesis percent mortality.

[2]Includes culled birds.

*Significant ($P<0.05$).

[3]SFO = Sunflower oil.

lesions on the SFO diet than on the tallow and tallow/SFO diets (Experiment 1). In Experiment 2 the SDS mortality tended to be lower on SFO diet as well. Because under our experimental conditions stocking rate did not have a significant ($P>0.05$) effect on the number of SDS mortalities, as indicated by analysis of variance, the mortality data were summarized according to dietary treatment only. Nevertheless, high stocking density (as a form of stress) should be further investigated, since stress can contribute to the syndrome (Buenrostro and Kratzer, 1982).

As indicated in Table 8, supplementation with a 50:50 tallow SFO mixture (Diet 2) did not reduce SDS mortality. If the level of linoleic acid in the diet was actually a significant contributing factor in reducing SDS on diet 3, then presumably the oleic and linoleic acid families competitively inhibited each other.

Mean total plasma lipids, triglycerides and cholesterol values are presented in Table 10. Total lipids were only significantly ($P<0.05$) affected at 4 weeks of age. Otherwise dietary fat type did not have any significant ($P>0.05$) effect on any other blood parameters. Values presented in Table 10 were similar to those reported in the literature (Marion et al., 1961; Rudas et al., 1972; Nir et al., 1973; Leclerq et al., 1974).

The fatty acid analysis was performed on heart and liver tissue for both dietary treatments (tallow and SFO) and for the SDS and culled birds. The fatty acid composition of heart tissue is presented in Table 11. The analysis at 7 weeks of age showed significant ($P<0.05$) effects of diet on oleic acid (18:1), linoleic (18:2) and arachidonic acid (20:4). The

Table 10. Mean total plasma lipids, triglycerides and cholesterol values of male broilers - Experiment 1

Parameters	Dietary treatment			
	Tallow	Tallow/SFO[1]	SFO	±SE
	Week 4			
Total lipids (mg/dl)	441[a,b]	469[a]	413[b]	±3.7
Triglycerides (mg/dl)	71[a]	66[a]	59[a]	±0.9
Cholesterol (mg/dl)	110[a]	113[a]	111[a]	±0.8
Weight (g)	839[a]	849[a]	826[a]	±4.3
	Week 7			
Total lipids (mg/dl)	430[a]	422[a]	391[a]	±3.8
Triglycerides (mg/dl)	101[a]	90[a]	89[a]	±1.4
Cholesterol (mg/dl)	138[a]	135[a]	133[a]	±1.2
Weight (g)	2209[a]	2292[a]	2293[a]	±9.6

[a,b]Means within a row having different superscripts are significantly different ($P<0.05$).

[1]SFO = Sunflower oil.

Table 11. Heart fatty acid pattern in response to dietary fat and comparison with the heart fatty acid pattern found in SDS birds and culled birds - Experiment 2

Dietary treatment	(%) 14:0	14:1	16:0	16:1	17:0	18:0	18:1	18:2	18:3	20:4
Tallow	0.36±0.03[a]	0.10±0.01[a]	20.8±0.4[a]	2.9±0.1[a]	0.34±0.02[a]	18.4±0.4[a]	21.6±0.2[a]	22.4±0.5[a]	0.25±0.01[a]	12.1±0.3[a]
SFO[1]	0.34±0.05[a]	0.08±0.01[a]	20.7±0.4[a]	2.4±0.1[a]	0.35±0.02[a]	17.2±0.2[a]	16.4±0.5[b]	26.4±0.4[b]	0.27±0.01[a]	14.9±0.5[b]
	Deaths due to[2]									
SDS	0.73±0.04[a]	0.16±0.02[a]	26.4±0.5[a]	4.1±0.4[a]	0.40±0.04[a]	17.5±0.4[a]	25.2±0.9[a]	18.3±0.5[a]	0.13±0.01[a]	6.2±0.2[a]
Culled	0.41±0.05[a]	0.09±0.01[a]	20.4±0.5[b]	2.6±0.3[a]	0.35±0.02[a]	15.1±0.7[a]	16.9±0.7[b]	31.3±0.3[b]	0.28±0.01[a]	11.7±0.7[b]

[a,b]Means (± standard error) within a column (tallow vs SFO and SDS vs culled) having different superscripts are significantly different ($P<0.05$).

[1]SFO = Sunflower oil.

[2]Values given for SDS and culled birds consist of pooled samples from both diets between 4-7 weeks.

SDS birds had greater tissue levels of palmitic (16:0), palmitoleic (16:1), stearic (18:0) and oleic (18:1) acids and decreased levels of linoleic (18:2) and arachidonic (20:4) acids as compared with the birds culled within the same period of the experiment (4-7 weeks).

A similar fatty acid analysis was performed on the liver tissue (Table 12). The SDS birds tended to have lower levels of 18:0, 18:2 and 20:4 and higher levels of 18:1 as compared with culled birds. Buenrostro and Kratzer (1982), who reported similar values for SDS birds (increased level of 18:1) and decreased levels of 20:4, indicated that SDS birds were in the biotin-deficient state. In both tissues the level of arachidonic acid in SDS birds was lower than in the culled birds although theoretically a sufficient amount of linoleic acid was supplied by the diet to serve as a source of arachidonic acid. The linoleic acid is desaturated and elongated through a series of steps which are sensitive to competitive inhibition from other fatty acids especially those from the oleic acid family (competition for Δ6-desaturase) (Cunnane, 1982). The elevated level of oleic acid (especially in the heart) would suggest that the desaturation and elongation of the linoleic acid was competively inhibited by the oleic acid, thereby reducing conversion of linoleic to arachidonic.

An important part of the conversion of linoleate to arachidonate is the microsomal chain elongation system, where the 2-carbon units are added to γ-linoleate via malonyl-CoA to give dihomo γ-linoleate (Roland and Edwards, 1971). Because biotin is necessary for the synthesis of malonyl-coenzyme A (Lehninger, 1982), this would indicate that biotin

Table 12. Liver fatty acid pattern in response to dietary fat and comparison with the liver fatty acid pattern found in SDS birds and culled birds - Experiment 2

Dietary treatment	(%) 14:0	14:1	16:0	16:1	17:0	18:0	18:1	18:2	18:3	20:4
Tallow	0.41±0.05[a]	0.11±0.01[a]	26.5±0.4[a]	3.3±0.2[a]	0.26±0.05[a]	22.0±0.6[a]	26.5±0.6[a]	13.5±0.3[a]	0.18±0.01[a]	6.5±0.5[a]
SFO[1]	0.50±0.03[a]	0.12±0.01[a]	27.3±0.6[a]	2.9±0.3[a]	0.30±0.04[a]	19.9±0.6[a]	18.8±0.5[b]	21.5±0.3[b]	0.21±0.01[a]	7.7±0.5[a]
	Deaths due to[2]									
SDS	0.75±0.04[a]	0.19±0.01[a]	30.3±0.5[a]	4.5±0.3[a]	0.31±0.04[a]	18.4±0.7[a]	27.3±0.6[a]	14.2±0.6[a]	0.17±0.01[a]	3.1±0.2[a]
Culled	0.59±0.08[a]	0.14±0.01[a]	29.2±0.8[a]	3.3±0.5[a]	0.20±0.06[a]	20.2±0.4[a]	23.2±0.9[b]	16.4±0.8[a]	0.15±0.02[a]	5.7±0.7[b]

[a,b]Means (± standard error) within a column (tallow vs SFO and SDS vs culled) having different superscripts are significantly different ($P<0.05$).

[1]SFO = Sunflower oil.

[2]Values given for SDS and culled birds consist of pooled samples from both diets between 4-7 weeks.

is directly involved in the conversion of linoleic to arachidonic acid. Therefore, a biotin-deficiency at the enzyme level could also result in reduced conversion rate, and a lower level of arachidonic acid in the tissue. Arachidonic acid, however, serves as a precursor of the dienoic prostaglandins (PGs) (Karmazyn and Dhalla, 1983), which have been found to have a wide range of biochemical effects. Some established physiological roles include a variety of actions on cardiac tissue (effect on the myocardial contractile force, heart rate and cardiac rhythm), modulating and regulating its function (Karmazyn and Dhalla, 1983). Hwang et al. (1975) demonstrated that biosynthesis of PGs in tissues of animals deficient in essential fatty acid is dependent on the availability of their precursors. Also, the ability of PGs to modulate cardiac rhythm may be a concentration-dependent phenomenon (Karmazyn and Dhalla, 1983).

Overall, there is a possibility of combined effect of competitive inhibition and biotin-deficiency at the enzyme level, reducing the conversion rate of linoleic to arachidonic acids. Lower arachidonic acid levels would reduce the amount of PGs being synthesized, which can finally upset heart function, leading to fibrillation or arrythmia.

Because the biotin analysis on the liver tissue was not performed, the biotin status of those birds is not known. Steele et al. (1982) demonstrated that SDS birds had adequate biotin concentrations in the liver and supplementation of biotin via drinking water did not reduce SDS mortality. In contrast, Hulan et al. (1980) reported that addition of biotin significantly reduced SDS mortality. In view of these reports

the issue remains controversial.

Copper, Zn and Ca values of heart tissue in response to dietary treatments (tallow and SFO) and for SDS and culled birds are given in Table 13. Calcium levels were found to be higher in SDS birds and significantly ($P<0.05$) different from culled birds. Mollison (1983) observed much higher Ca values for SDS and culled birds (400 µg/g vs 441 µg/g) but the difference was not significant. Table 14 presents Cu and Zn values of the liver tissue. The SDS birds had significantly ($P<0.05$) lower Cu and Zn concentration in the liver compared to the culled birds. It is difficult to speculate if the lower Cu and Zn concentrations are correlated with the increased incidence of the syndrome. Both elements have been shown to be integral components in fatty acid metabolism, particularly at the level of linoleic and stearic acid desaturation (Cunnane, 1982). With Cu stimulating the metabolism of the oleic acid family and Zn stimulating the metabolism of linoleic acid family, a substantial interaction exists between those trace elements. Further research is required to investigate whether Cu and Zn are involved in the development of SDS.

ACKNOWLEDGMENTS

The authors appreciate the assistance of G. Crow in the data analysis. They also wish to thank H. Muc, the University of Manitoba Poultry Barn Staff and numerous graduate students for their technical assistance. The research was supported in part by NSERC grant No. A0457.

Table 13. Copper, zinc and calcium content of heart tissue in response to the diet and for SDS and culled birds - Experiment 2

	Dietary treatment[1]		Cause of mortality[1]	
Parameters	Tallow	SFO[2]	SDS birds	Culled birds
Copper (µg/g)	18.3±1.7[a]	18.4±1.1[a]	16.3±2.6[a]	14.8±1.8[a]
Zinc (µg/g)	124±23[a]	125±13[a]	106±25[a]	108±8[a]
Calcium (µg/g)	220±26[a]	210±15[a]	321±82[a]	258±25[b]

[a,b]Means within each row (tallow vs SFO and SDS vs culled) having different superscripts are significantly different ($P<0.05$).

[1]All values (mean ± standard deviation) expressed on dry matter basis.

[2]SFO = Sunflower oil.

Table 14. Copper and zinc content of liver tissue in response to the diet and for SDS and culled birds - Experiment 2

	Dietary treatment[1]		Cause of mortality[1]	
Parameters	Tallow	SFO[2]	SDS birds	Culled birds
Copper (µg/g)	14.5±2.1[a]	14.2±2.1[a]	13.1±1.8[a]	15.3±3.3[b]
Zinc (µg/g)	89±11[a]	91±14[a]	87±17[a]	104±17[b]

[a,b]Means within a row (tallow vs SFO and SDS vs culled) having different superscripts are significantly different ($P<0.05$).

[1]All values (mean ± standard deviation) expressed on dry matter basis.

[2]SFO = Sunflower oil.

Manuscript II: Contribution of Dietary Tallow to the Incidence of Sudden Death Syndrome in Broiler Chicks[1]

[1]Submitted to Poultry Science

ABSTRACT The effects of different dietary tallow concentrations versus cornstarch on the general performance of broiler chicks and the incidence of sudden death syndrome (SDS) were observed. Wheat-soy diets supplemented with increased amounts of tallow were found to improve performance characteristics significantly ($P<0.05$) between 0-4 weeks of age. At 7 weeks of age a significant ($P<0.05$) reduction in liver weight was observed, however liver lipid and abdominal fat pad were not affected ($P>0.05$). Overall mortality was not significantly ($P>0.05$) different. There was a trend for the SDS mortality to be lowest when the highest concentration of tallow was fed. It appears that the early growth phase (0-4 weeks) is more sensitive ($P = 0.08$) to dietary manipulation than the grower period (4-7 weeks). Heart weight/body weight (g/kg) ratios and copper, zinc and calcium content of heart tissues from the SDS mortalities and culled (leg problems) birds were not significantly ($P>0.05$) different.

Key words: sudden death syndrome, broilers, dietary fat, mineral composition.

INTRODUCTION

Sudden death syndrome (SDS) was found to be significantly ($P<0.05$) reduced when a wheat-soybean diet was supplemented with unsaturated fat as opposed to saturated fat (Rotter et al., 1985a). Two experiments conducted at the University of Manitoba showed a beneficial effect when sunflower oil was incorporated in the diet, reducing the number of SDS mortalities up to 7 weeks. Also Mollison (1983) reported that wheat-soy diets fed ad libitum or restricted resulted in higher (1.9%) incidence of SDS than corn-soy diets (0.95%). These findings suggested that a linoleic acid deficiency might be involved in sudden death syndrome.

Wheat-soy-cornstarch diets are deficient in linoleic acid (0.8%) based on the recommendation of 1.2% suggested by Scott et al. (1982) or 1% (NRC, 1977) for young chicks. Tallow, although a poor source of linoleic acid when added to wheat-soy diets replacing the cornstarch should result in a reduced mortality due to SDS if linoleic acid deficiency is a factor. The objective of the present experiment was to examine the effect of different dietary amounts of tallow (ranging from 5 to 54 g/kg of diet) vs cornstarch on general performance and SDS mortality of young broilers.

MATERIALS AND METHODS

The experiment employed sixteen hundred and eighty (1680) one-day old male, vaccinated (Marek's) broiler chicks of commercial (Cobb x Cobb) parentage. All birds were randomly allocated to 24 floor pens (1.54 x 4.31 m), at a stocking density of 11 birds/m^2 (70 birds/pen), in an environmentally controlled house under continuous low intensity lighting. Each diet in mash form was fed to 560 birds reared in 8 randomly assigned pens. Feed and water were available ad libitum. The dietary formulations for starter (0-4 weeks) and finisher (4-7 weeks) are presented in Table 15.

The wheat-soy diets were supplemented with different amounts of tallow. The cornstarch was used to adjust the energy content of diets low in tallow to maintain equal calorie to protein ratios in the diets. Weight gains and feed conversion were calculated for the experimental periods of 0-2, 2-4 and 4-7 weeks. Mortalities were recorded to 49 days of age and each mortality was submitted for post-mortem necropsy. Birds were diagnosed as SDS based on criteria applied in previous experiments (Rotter et al., 1985a). From week 4 to the end of the experiment the hearts of twenty-one (21) SDS and sixteen (16) age-matched culled (leg problems) birds were removed during the post-mortem examination and weighed. At the end of the experiment 16 birds of each diet were sacrificed, their hearts, livers and abdominal fat pad removed and relative organ weights determined.

All samples were immediately frozen, stored at -20°C and finally lyophilized for approximately 30 hours in a freeze-dryer (Lab Conco "Vac stop" tray dryer Model 75159 and Lab Conco freeze-dryer 18 litre with shelf freezer Model 75015). The lyophilized samples were ground

Table 15. Composition and calculated analysis for starter and finisher diets

Ingredients	Starter			Finisher		
	Cornstarch	Cornstarch/ Tallow	Tallow	Cornstarch	Cornstarch Tallow	Tallow
	g/kg					
Wheat	651.0	598.1	587.7	742.6	722.4	704.7
Soyabean meal (47.5%)	254.5	283.5	283.5	173.6	193.6	189.6
Fish meal (65%)	22.5	21.5	32.0	19.0	11.0	25.0
Tallow	5.0	30.0	54.0	5.0	21.0	40.0
Cornstarch	24.0	24.0	-	23.0	8.5	-
Limestone	12.5	12.5	12.5	6.0	11.5	11.0
Dicalcium-phosphate	14.0	14.0	14.0	15.0	16.0	14.0
Vitamin-mix (standard)[1]	10.0	10.0	10.0	10.0	10.0	10.0
Mineral-mix (standard)[2]	5.0	5.0	5.0	5.0	5.0	5.0
DL-methionine	1.5	1.4	1.3	0.8	1.0	0.7
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Calculated analysis: (determined)						
Metabolizable energy (ME), kcal/kg	2860	2938	3016	2936	2959	3035
Crude protein (P), %	22.0	22.6	23.2	19.1	19.3	19.8
ME/P ratio	130	130	130	153	153	153
Ether extract, %	2.13	4.57	6.84	2.20	3.75	5.68
Linoleic acid, %	0.84 (0.86)	0.93 (1.02)	1.04 (1.22)	0.91 (1.03)	.98 (1.10)	1.06 (1.21)

[1]Supplies the following per kg of complete feed: vitamin A, 8250 I.U.; vitamin D_3, 870 I.C.U.; vitamin E, 5.5 I.U.; riboflavin, 5.5 mg; Ca pantothenate, 11 mg; niacin, 16.5 mg; choline chloride, 275 mg; menadione, 1.1 mg; santoquin, 250 mg.

[2]Supplies the following in mg per kg of complete feed: manganese oxide, 166; zinc oxide, 14.5, ferrous sulfate, $7H_2O$, 31; copper sulfate, $5H_2O$, 25.5; iodized salt, 4760.

(Janke and Kunkel A10-5 stainless steel grinder) and put into polyethylene screw top vials. The subsamples of lyophilized heart tissue were used for mineral analysis [calcium (Ca), copper (Cu) and zinc (Zn)]. Analytical procedures followed the modified wet-ashing method of Thompson and Blanchflower (1971) with subsequent mineral determination using an Instrumentation Laboratory model 551 atomic absorption spectrophotometer. The lipid content of the livers (16/diet) was determined by the method of Folch et al. (1957). The linoleic acid content of the feed samples was determined using the fat extraction method by Bligh and Dyer (1959) and methylation procedure by Metcalfe et al. (1966).

Data collected (performance and mortality data - on a pen basis) were statistically analyzed as a completely randomized design using one way analysis of variance procedures of the Statistical Analysis System (SAS Institute, Inc., 1982). Balanced data were subjected to ANOVA, while unbalanced data were analyzed using General Linear Models (GLM). Mortality data were converted by arc sine transformation before statistical analysis was done, however, for interpretation the results are presented as percents. Where significant differences were observed, means were further subjected to Student-Neuman-Keuls test (Snedecor and Cochran, 1980).

Growth curves were calculated using average pen weights at 0, 2, 4 and 7 weeks. The weights of birds dying from SDS are indicated on the graph on the day of their death.

RESULTS AND DISCUSSION

The performance characteristics of broiler chickens were significantly ($P<0.05$) improved when the amount of dietary fat was increased (Table 16). Birds fed the diet containing tallow had the best weight gain and feed to gain ratio, but this effect was primarily observed between 0-4 weeks of age. The effect of fat on growth rate may be due to the presence of a required nutrient and also the higher energy content of diets with added fat. Hopkins and Nesheim (1967) demonstrated that addition of graded levels of linoleic acid to a diet containing hydrogenated fat (isocaloric substitution for sucrose) resulted in improved weight gain. They suggested that a level of 1.4% of linoleic acid is required for maximum growth. This value could be slightly overestimated however, because the diets contained high proportions of saturated fatty acids, which are known to enhance the metabolic requirement of unsaturated fat (Renner and Hill, 1961b). Menge (1970) recommended a level of 1.2% dietary linoleic acid, but these studies were performed with Leghorn chicks, which are characterised by slower growth rate than broilers and therefore may differ in dietary requirements. More recent studies indicated adequate growth rates using 1.0 (NRC, 1977) and 1.2% of linoleic acid (Scott et al., 1982) in the diet. The linoleic acid values used in our study were slightly below or borderline to these recommendations. The reduced feed consumption of birds fed the tallow diet between 0 to 4 weeks of age indicates that they were able to utilize the diet more efficiently than those on the cornstarch diet. This is demonstrated by higher weight gains (Table 16).

Table 16. Effect of different levels of tallow on performance of male broilers

Parameters	Dietary treatment			
	Cornstarch	Cornstarch/ Tallow	Tallow	±SE
7 Weeks body weight (g)	2020[a]	2068[a,b],	2102[b]	12.3
Average weight gain (g)				
0-4 weeks	814[a]	829[b]	848[c]	2.5
4-7 weeks	1161[a]	1194[a]	1209[a]	12.2
0-7 weeks	1975[a]	2023[a,b]	2057[b]	12.3
Feed/gain:				
0-4 weeks	1.79[a]	1.76[b]	1.69[c]	0.005
4-7 weeks	2.36[a]	2.40[a]	2.33[a]	0.015
0-7 weeks	2.12[a]	2.13[a]	2.06[b]	0.008

[a,b]Means within a row having different superscripts are significantly different ($P<0.05$).

The observed performance differences can be attributed to a number of reasons, the major being extra caloric effect of supplemental fat. This effect can be explained by two main factors: 1) synergism between saturated and unsaturated fats in the diet and 2) enhanced nutrient absorption by slowing down the rate of passage of food (Summers, 1984). The other factors could be: differences in dietary energy and growth stimulating effect of fat.

The effects of dietary treatments on various body parameters are illustrated in Table 17. A significant ($P<0.05$) reduction in liver weight was observed with increased tallow content in the diet whereas liver lipid content had a tendency to decrease ($P = 0.08$). Haghighi-Rad and Polin (1982) postulated that lipid in the diet (in proper proportions) may act through a feed-back mechanism to prevent excessive accumulation of liver lipid but it accumulates when starch is present. Further, it has been observed that, when carbohydrate intake is held constant, the addition of fat to the diet (with a simultaneous increase in energy intake) has no effect on in vivo hepatic lipogenesis (Hillard et al., 1980). However, when dietary fat increases at the expense of carbohydrate, but with a constant calorie to protein ratio (ME/P), a reduction in hepatic fatty acid synthesis and the activity of associated lipogenic enzymes, malic and citrate cleavage enzymes is noted (Leveille et al., 1975). Although there were no significant differences ($P>0.05$) between treatments, there was a tendency for abdominal fat pad to increase with added dietary fat (Table 17). As reported by Kubena et al., (1974) and Deaton et al. (1981), increasing the energy content of broiler rations while maintaining a constant ME/P ratio could result in an increased abdominal fat deposition.

Table 17. Effects of dietary treatment on various body parameters at 7 weeks of age

	Dietary treatment		
Parameters	Cornstarch	Cornstarch/Tallow	Tallow
Body weight (g)	2190±33[a,1]	2138±27[a]	2218±40[a]
Liver weight (g)	45.09±1.77[a]	41.19±1.27[b]	37.00±.74[c]
Fat pad (g)	38.31±2.78[a]	40.62±3.55[a]	43.41±2.79[a]
Liver weight (g/100 g body weight)	2.06±.08[a]	1.93±.06[a]	1.67±.05[b]
Fat pad (g/100 g body weight)	1.75±.14[a]	1.90±.16[a]	1.96±.11[a]
Liver lipid, % of dry weight	17.48±.60[a]	16.29±.69[a]	15.70±.31[a]

[a,b,c]Means ±SE within a row having different superscripts are significantly different ($P<0.05$).

[1]16 observations per mean.

Figures 1, 2 and 3 represent growth curves for birds fed the cornstarch, cornstarch/tallow and tallow diets, respectively, and the weight of SDS mortalities. The majority of SDS birds were lighter than their respective time-matched live birds. In contrast, previous studies reported that weights of SDS birds were higher than their flock mates (Ononiwu et al., 1979a; Steele and Edgar, 1982), while Brigden and Riddell (1975), indicated that they were of average weight. Between 0-4 weeks of age a trend towards reduced SDS mortality was observed on the tallow diet ($P = 0.08$). The total mortality was not significantly ($P>0.05$) affected (Table 18). Overall, the mortality response to different treatments was more prevalent between 0-4 weeks of age and this early phase appears to be more sensitive to dietary manipulation than the grower period (4-7 weeks).

It can be speculated that selection for rapid growth in meat-type birds may have resulted in increased requirements for specific nutrients (e.g. linoleic acid, 18:2). Although SDS may be a result of a moderate deficiency of essential fatty acids (EFAs) or their metabolites, the dietary level is sufficient for maintaining a normal growth rate. The classical ways of assessing EFA deficiency include measurement of linoleic acid intake, blood and tissue levels of linoleic acid and triene:tetraene (20:3/20:4) ratio in the tissue (Horrobin and Cunnane, 1981). The biological activity of linoleic acid largely depends on its conversion to γ-linolenic acid by the Δ-6-desaturase enzyme. Since the desaturation pathway is shared with oleic acid under normal conditions, defects in desaturation due to lack of enzymes or their cofactors can also result in functional EFA deficiency. This will not be manifested by any abnormalities detectable by the 3

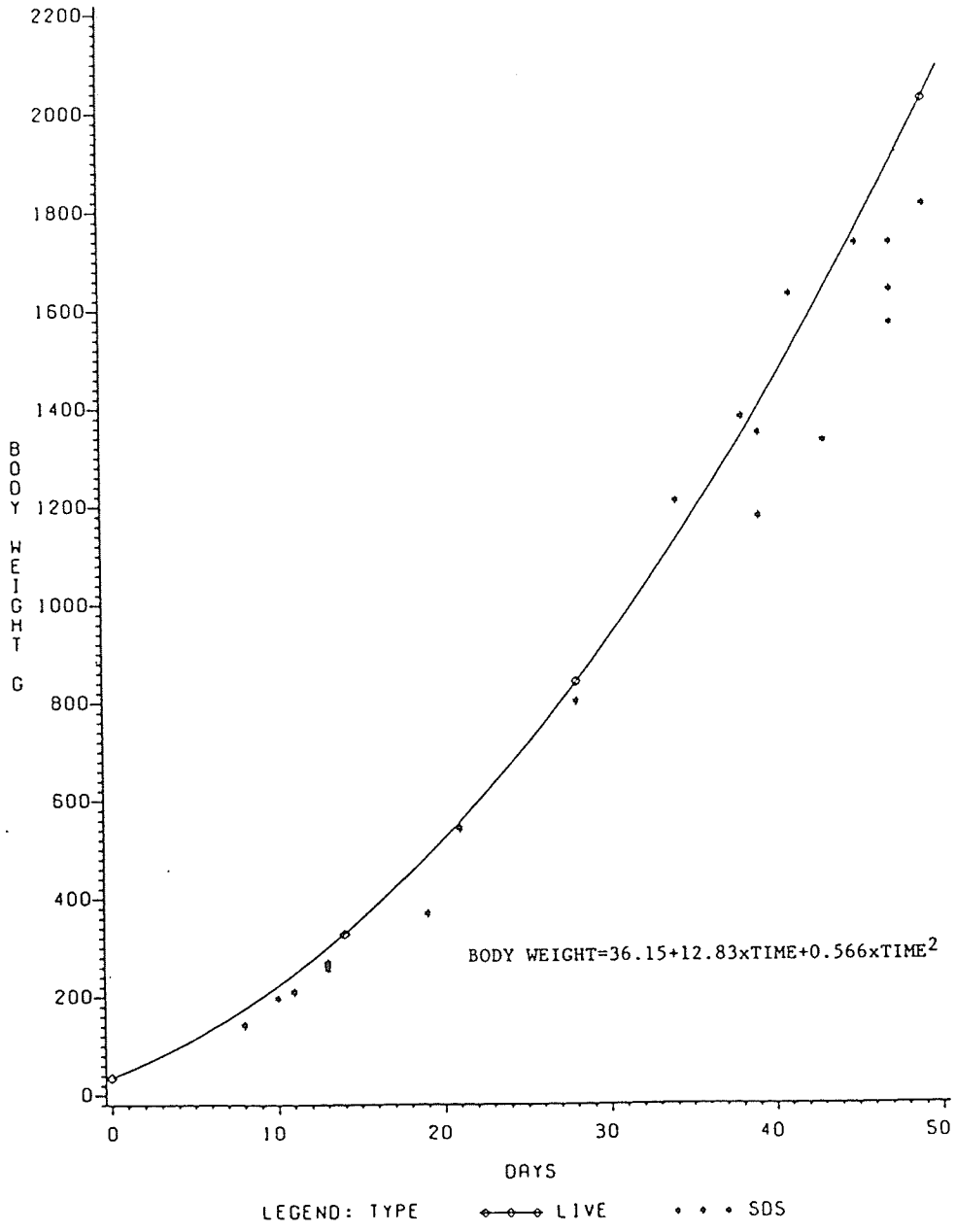

Figure 1. Growth curve of birds fed the cornstarch diet and observations of SDS birds on the same diet.

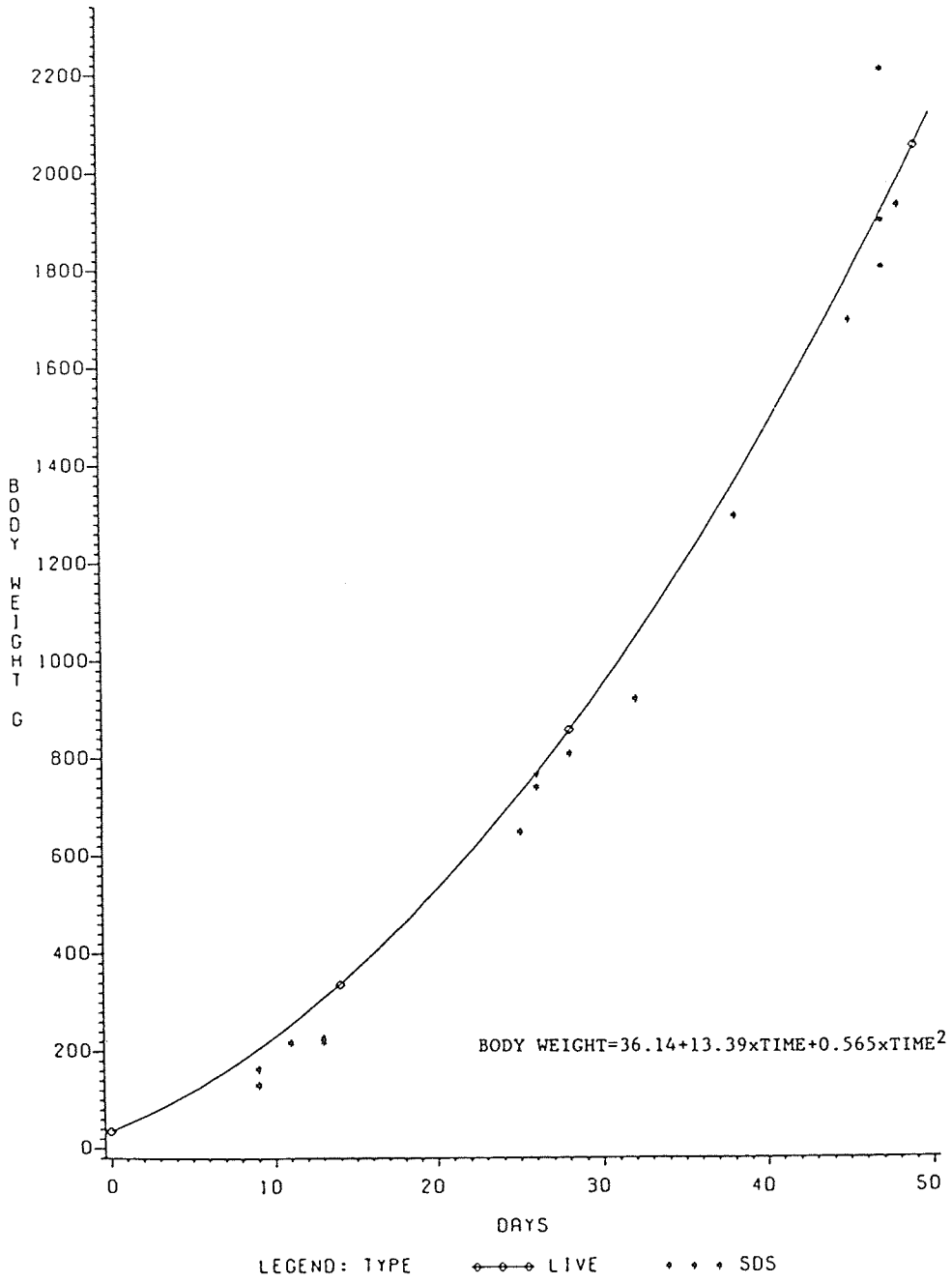

Figure 2. Growth curve of birds fed the cornstarch/tallow diet and observations of SDS birds on the same diet.

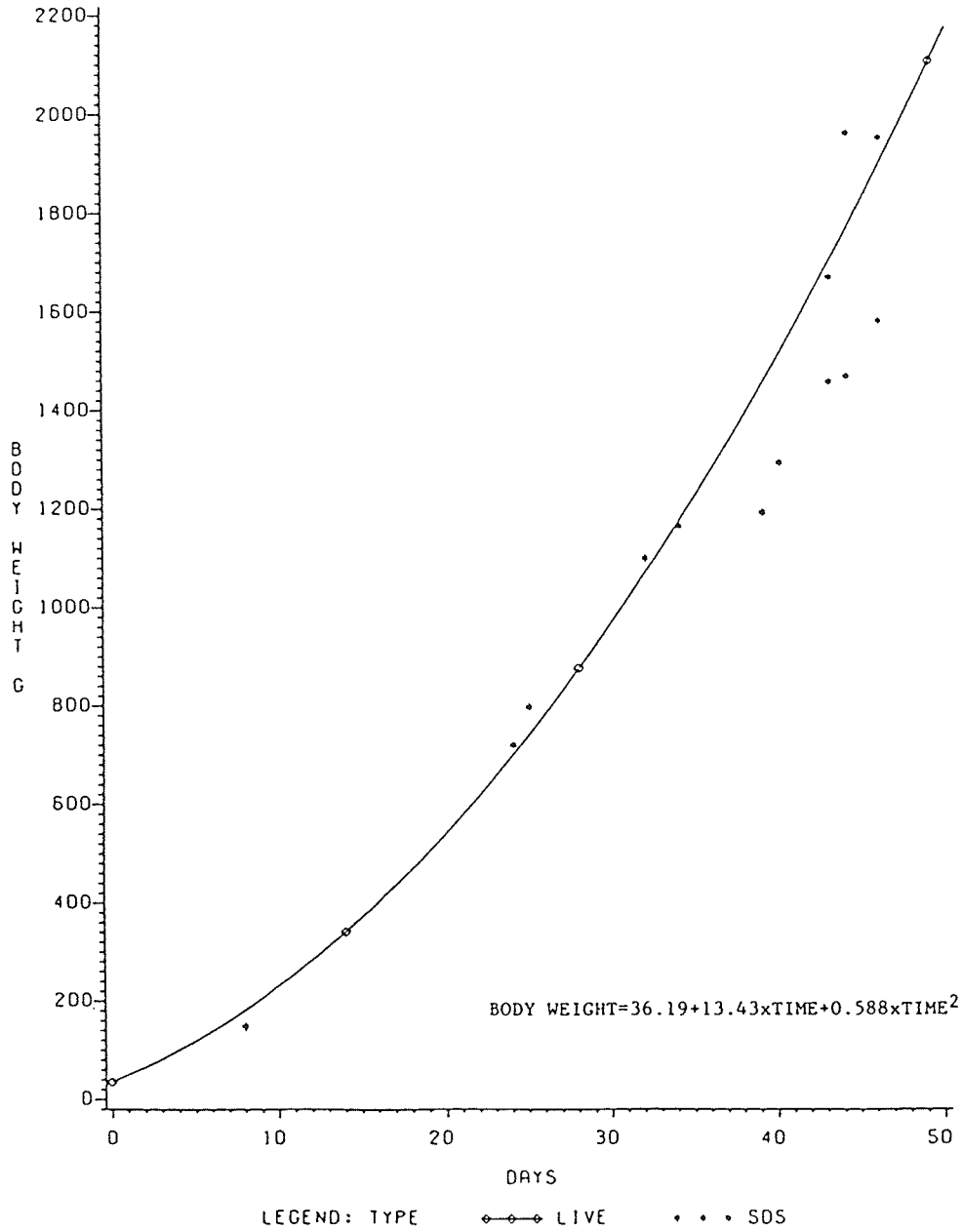

Figure 3. Growth curve of birds fed the tallow diet and observations of SDS birds on the same diet.

Table 18. SDS mortality as a percent of total number of birds per treatment

Parameters	Periods (weeks)	Dietary treatment[1] Cornstarch	Cornstarch/ Tallow	Tallow
Total number of SDS mortalities (%)	0-4	9(1.61)[2]	9(1.61)	3(0.53)
	4-7	11(2.03)	7(1.28)	10(1.82)
	0-7	20(3.57)	16(2.86)	13(2.32)
Total number of mortalities (%)[3]	0-7	33(5.89)	29(5.18)	27(4.82)

[1]There were no significant differences among treatments (P>0.05).

[2]Number outside of parenthesis indicates the actual number of birds died per dietary treatment and the number within parenthesis percent mortality.

[3]Includes culled birds.

classical ways of assessing EFA status. In addition, Holman (1971) reported that a deficiency of EFA can be more severe when saturated fatty acids were simultaneously fed to the animal. They will compete with the EFA and supress their activity (Crawford et al., 1981). Diet 3 supplied an adequate amount of linoleic acid, so the saturated fatty acids were likely of less concern. If linoleic acid is involved, this would also explain the trend towards reduced SDS mortality on this diet.

Heart weight/body weight (g/kg) (H wt/B wt) ratios for dietary treatments and mortalities were not significantly ($P>0.05$) different although the ratio for SDS birds was slightly higher (Table 19). Pass (1983) reported that broiler-breeder hens and egg-laying strains had a significant increase in H wt/B wt ratio compared to non-SDS matched birds. The SDS birds were divided in two groups, with elevated and normal heart weights. In our study, all hearts fell into the same category and some hearts which appeared "visually" large had normal weights, indicating that the enlargement was probably due to dilation. Transverse sections of the organs to examine for possible hypertrophy were not performed.

Copper, Zn and Ca content of the heart tissue for dietary treatments and mortalities are illustrated in Table 20. Diet had no effect on mineral content and no significant ($P>0.05$) differences were found between SDS and culled birds. Previously, Rotter et al. (1985a) found the Ca concentration to be significantly higher in SDS birds, while Mollison (1983) reported no differences in its content.

Table 19. Heart weight/body weight (g/kg) ratios

Dietary treatment[1]			Cause of mortality[3]	
Cornstarch[2]	Cornstarch/Tallow	Tallow	SDS[4]	Culled birds[5]
4.00±0.09	3.79±0.07	3.86±0.09	4.92±0.19	4.68±0.22

[1]Means ± SE within the row were not significantly different (P>0.05).

[2]16 observations per mean.

[3]Least square means ±SE within the row were not significantly different (P>0.05).

[4]21 observations per mean.

[5]16 observations per mean.

Table 20. Copper, zinc and calcium content of heart tissue in response to the diet and for SDS and culled birds

	Dietary treatment[1,2]			Cause of mortality[1,4]	
Parameters	Cornstarch[3]	Cornstarch/Tallow	Tallow	SDS birds[5]	Culled birds[6]
Copper (µg/g)	15.7±.4	15.7±.4	15.7±.4	15.2±0.3	15.2±0.3
Zinc (µg/g)	111±1.5	114±1.8	116±2.1	106±3.4	110±3.1
Calcium (µg/g)	229±3	230±3	236±3	338±20	330±18

[1]All values are expressed on dry matter basis.

[2]Means ±SE within the row were not significantly different (P>0.05).

[3]16 observations per mean.

[4]Least square means ±SE within the row were not significantly different (P>0.05).

[5]21 observations per mean.

[6]16 observations per mean.

In summary, further research is required to investigate how dietary manipulation during the growing phase (0-4 weeks) can effectively reduce the SDS mortality in young broilers.

ACKNOWLEDGEMENTS

The authors wish to express appreciation to Harry Muc, the University of Manitoba poultry barn staff and several graduate students for their technical assistance. The study was supported, in part, by NSERC Grant No. A0456.

Manuscript III: Sudden Death Syndrome in Broilers: Effects of Different Dietary Fats on the Incidence and Tissue Composition

ABSTRACT: The effects of different dietary fat sources on the general performance of broiler chicks and the incidence of sudden death syndrome (SDS) were studied. Birds fed hydrogenated coconut oil (HCO) gained significantly ($P<0.05$) less and had a poorer feed conversion when compared with other dietary treatments (tallow, tallow/sunflower oil (SFO) and SFO) between 0 to 4 weeks of age. No treatment effect was observed on liver weight and abdominal fat pad. Birds fed the HCO diet had a very low and significantly ($P<0.05$) different SDS incidence from the SFO treatment, while total mortality was not affected. Serum samples collected at 4 and 7 weeks of age indicated a significant ($P<0.05$) decrease in albumin concentration with increased amount of unsaturated fat. The total serum protein also had a tendency to decrease. At 7 weeks of age serum calcium was reduced, while potassium increased with increased dietary unsaturated fat. Mineral composition of the heart muscle revealed differences in sodium and potassium concentration between SDS and control birds (matching penmates), but there was no difference in copper, zinc, calcium and magnesium. The liver tissue of SDS and control birds did not vary significantly ($P>0.05$) in the protein, lipid, glycogen and biotin content. The biotin concentration was found to be adequate for the SDS and control birds. Analysis of the heart tissue for fatty acid composition indicated differences in palmitoleic and arachidonic acids, while no differences were found in the liver tissue. The monoiene:diene (18:1/18:2) ratios were calculated for heart and liver tissues. The ratios in both tissues remained the same at 2% and 3.8% of dietary linoleate, indicating an adequacy at 2% of the diet.

Key words: sudden death syndrome, broilers, dietary fat, tissue composition.

INTRODUCTION

Sudden death syndrome (SDS) is a widely recognized cause of mortality in broiler chickens throughout North America. The reported incidence in Canada varies between 1.0 and 2.16% (Brigden and Riddell, 1975; Cassidy et al., 1975; Ononiwu et al., 1979a). Mortality due to SDS can occur from the first week up to marketing at 7 weeks of age. A recently conducted epizootiological study and strain comparison in Saskatchewan, Canada, indicated that variation in the incidence may be due more to nutritional and environmental factors than genotype (Riddell and Springer, 1985). Nutrition has been implicated in experimental broiler feeding trials. Although Hulan et al. (1980) and Buenrostro and Kratzer (1982) suggested that biotin deficiency may be a cause for SDS, Steele et al. (1982) found that high levels of biotin supplementation neither prevented SDS or reduced total mortalities in the flock. Therefore, the biotin involvement remained unsolved. Other nutritional factors investigated in regards to SDS suggested a decrease in incidence when corn-soy diets were fed instead of wheat-soy diets (Mollison et al., 1984). In a similar study, a reduced mortality was observed when birds were fed diets supplemented with sunflower oil compared with tallow (Rotter et al., 1985a). Another experiment, utilizing cornstarch vs tallow showed a trend toward reduced SDS incidence between 0 and 4 weeks of age on the latter ($P=0.08$) (Rotter et al., 1985b). These observations indicated that: 1) birds seem to respond to dietary manipulation, which can result in reduced mortality due to SDS and 2) essential fatty acids or their metabolites may be involved in development of the syndrome.

The objective of the present study was to investigate the effects of various fat sources on the performance parameters, total mortality and mortality due to SDS. The study also evaluates the results of dietary treatments on blood and tissue composition and their possible interactions with SDS.

MATERIALS AND METHODS

The experiment used nineteen hundred and sixty (1960) one-day old male, vaccinated (Marek's) broiler chicks of commercial (Cobb x Cobb) parentage. Birds were distributed randomly among 28 floor pens (1.54 x 4.31 m each), at a stocking rate of 11 birds/m^2 (70 birds/pen), located in an environmentally controlled house with continuous lighting at low intensity. All diets were fed in mash form and water supplied ad libitum, each to 490 birds. The wheat-soy diets were supplemented either with hydrogenated coconut oil (HCO), tallow, tallow/sunflower oil (SFO) or SFO. Starter (0-4 weeks) and finisher (4-7 weeks) dietary formulations are presented in Table 21. Performance parameters (weight gain and feed conversion) were calculated for the experimental periods 0 to 2, 2 to 4 and 4 to 7 weeks of age.

At 4 and 7 weeks of age, one bird from each pen (7 birds/treatment) was selected at random and blood was collected from the wing vein. The samples were placed into 5-cc vacutainers (Becton Dickinson, USA) and put on ice until centrifuged. Serum samples were analyzed to establish a biochemical profile, using a Discrete Analyzer with Complete Optical Scanning (Coulter Electronics, Inc., Texas Instrument). The electrolytes (Na, K, Ca, P, Mg) were analyzed using a Klina Flame (Beckman, USA) and chloride was determined using a Chloride Meter 920M (Corning, USA).

Mortalities were recorded to 49 days of age and submitted for post-mortem examination. Birds identified as SDS were diagnosed according to the previously applied criteria (Rotter et al., 1985a). Between 4 and 7 weeks of age, 18 birds were killed by cervical dislocation (control birds) to match the time of death, pen, weight and

Table 21. Composition and calculated analysis of starter and finisher diets

Ingredients	Starter				Finisher			
	Diet 1 HCO[3]	Diet 2 Tallow	Diet 3 Tallow/SFO[4]	Diet 4 SFO	Diet 1 HCO	Diet 2 Tallow	Diet 3 Tallow/SFO	Diet 4 SFO
	g/kg							
Wheat	598.5	598.5	601.5	599.5	716.4	708.4	715.4	717.4
Soybean meal (47.5% protein)	279.0	274.0	274.0	280.0	179.6	179.6	179.6	179.6
Fish meal (65%)	30.0	30.0	30.0	30.0	25.0	25.0	25.0	25.0
Fat source	52.0	57.0	35.4/18.6	50.0	38.0	46.0	10.0/29.0	37.0
Limestone	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0
Dicalcium-phosphate	13.0	13.0	13.0	13.0	14.3	14.3	14.3	14.3
Vitamin mix (standard)[1]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Mineral mix (standard)[2]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
DL-methionine	1.5	1.5	1.5	1.5	0.7	0.7	0.7	0.7
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Analysis: Calculated (determined)								
ME[5] (kcal/kg)	3062	3041	3060	3098	3069	3065	3090	3096
Crude protein (%)	23.5	23.3	23.4	23.6	20.1	20.1	20.2	20.2
Linoleic acid (%)	.77 (.85)	1.06 (1.39)	2.08 (2.01)	3.81 (3.81)	0.86 (1.09)	1.09 (1.30)	2.68 (2.92)	3.11 (3.28)
Total biotin (mg/kg)	(0.09)	(0.11)	(0.11)	(0.12)	(0.11)	(0.12)	(0.12)	(0.13)

Continued .....

Table 21 (Continued)

[1]Supplies the following per kg of complete feed: vitamin A, 8250 IU; vitamin D_3, 870 ICU; vitamin E, 5.5 IU; riboflavin, 5.5 mg; Ca pantothenate, 11 mg; niacin, 16.5 mg; choline chloride, 275 mg; menadione, 1.1 mg; santoquin, 250 mg.

[2]Supplies the following in mg per kg of complete feed: manganese oxide, 92.5, zinc oxide, 56.2; ferrous sulfate$\cdot H_2O$, 259; copper sulfate$\cdot 5H_2O$, 16.2; selenium selenite, 0.5; iodized salt, 4080.

[3]HCO = Hydrogenated coconut oil.

[4]SFO = Sunflower oil.

[5]Metabolizable energy.

diet of SDS birds. Dead birds were immediately removed from the barn and stored in a cooler. At the end of the experiment 28 birds (7 birds/treatment) were killed and their abdominal fat pad and liver weights determined. During post-mortem necropsy hearts and livers of SDS and killed birds were taken out and later used for analysis. Obtained samples were immediately frozen, stored at -20°C and lyophilized for approximately 30 hours in a freeze-dryer (Lab Conco "Vac-stop" tray dryer Model 75159 and Lab Conco freeze-dryer 18 litre with shelf freezer Model 75015). After lyophilization samples were finely ground (Janke and Kunkel A10-5 stainless steel grinder) and put into polyethylene screw top vials.

Protein content of the liver tissue was determined according to the method of the Association of Official Analytical Chemists (1984). Glycogen analysis was carried out using the method of Seifer et al. (1950). Liver tissue was analyzed for biotin content using an isotope dilution technique described by Bhullar et al. (1985). The total lipid content was determined by the method of Folch et al. (1957). The methyl esters of heart and liver tissue were prepared using a modified procedure by Metcalfe and Schmitz (1961) and subsequent fatty acid composition of the tissues was determined using a Varian Vista 6000 gas chromatograph equipped with with 8' x 1/4" x 2-mm glass columns packed with 3% SP-2310 and 2% SP-2300 on 100/120 Chromosorb WAW and a Vista 4042 data system. Identifications of individual fatty acids were made by comparison with authentic standards (Nu Chek Prep. Inc., Elysian, Minn.). The linoleic acid content of the feed samples was determined using the fat extraction method of Bligh and Dyer (1959) and the

methylation procedure of Metcalfe et al. (1966).

In the preparation for mineral analysis [calcium (Ca), copper (Cu), zinc (Zn), magnesium (Mg), sodium (Na), potassium (K)], subsamples of lyophilized liver and heart tissue were wet ashed following the modified procedure of Thompson and Blanchflower (1971). The determinations were performed using an Instrumentation Laboratory Model 551 atomic absorption spectrophotometer.

All performance parameters, blood samples and mortality data (on a pen basis) were analyzed as completely randomized designs, using a one-way analysis of variance procedure. Mortality data were converted by arc sine transformation prior to statistical analysis; however, for interpretation the results are expressed as percentages. Differences between means were determined using the Student-Newman-Keuls (SNK) test (Snedecor and Cochran, 1980).

Data collected for SDS and control birds were analyzed as a split plot design using 18 pair of birds (HCO = 4, Tallow = 6, Tallow/SFO = 3, SFO = 5).

RESULTS AND DISCUSSION

The effects of different dietary fat treatments on performance parameters are illustrated in Table 22. Birds fed HCO gained significantly ($P<0.05$) less when compared with other treatments between 0 to 4 weeks of age, however feed conversion was only different from the SFO fed birds. The difference observed between HCO and SFO for average weight gain and feed/gain ratio can be mainly attributed to the difference in metabolizable energy (ME) and linoleic acid content, as well as to the physical properties of the fat. In a very young chick (<1 week old), the ability to assimilate dietary fat is impaired by a deficiency of bile production (Freeman, 1984). During this period of growth, fats which emulsify readily (e.g. corn oil) are digested to a greater extent than fats with higher melting point (e.g. tallow) (Carew et al., 1972). The same could be true for SFO and HCO. As indicated in Table 21, HCO and tallow/SFO diet had the same ME value, but there was an improved weight gain on the latter. There are several factors responsible for the observed effect, including composition and absorbability of the fats. HCO is composed of mainly medium-chain (C_{12}-lauric, C_{14}-mystiric) saturated fatty acids with absorbability of 65% (C_{12}) and 25% (C_{14}) respectively (Renner and Hill, 1961a). Tallow contains palmitic and stearic acids (saturated fatty acids) and oleic acid (unsaturated fatty acid). Renner and Hill (1961b) found that the absorbability of saturated fatty acids varies directly with the level of unsaturated fatty acids in the mixture. Therefore, combining SFO and tallow should additively improve the absorbability of the two fats. Further, it was demonstrated that fatty-acid binding protein (FABP), which influences transport of fatty acid through cytosol of the ab-

Table 22. Effects of different fat sources on performance of male broilers

Parameters	Dietary treatment				
	HCO[1]	Tallow	Tallow/SFO[2]	SFO	±SE
7 weeks body weight (g)	2170[a]	2213[b]	2230[b]	2286[c]	±7.1
Average weight gain (g)					
0-4 weeks	822[a]	843[b]	858[c]	904[d]	±2.6
4-7 weeks	1304[a]	1326[a]	1328[a]	1338[a]	±6.5
0-7 weeks	2126[a]	2169[b]	2186[b]	2242[c]	±7.2
Feed/gain					
0-4 weeks	1.61[ab]	1.63[a]	1.59[b]	1.55[c]	.004
4-7 weeks	2.18[a]	2.18[a]	2.18[a]	2.21[a]	.007
0-7 weeks	1.96[a]	1.96[a]	1.94[a]	1.94[a]	.004

[a,b,c]Mean within a row having different superscripts are significantly different ($P<0.05$).

[1]HCO = Hydrogenated coconut oil.

[2]SFO = Sunflower oil.

sorptive cell has greater affinity for unsaturated than saturated fatty acid. Virtually no affinity was observed for medium or short chain fatty acids (Ockner and Manning, 1972, 1974).

No significant ($P>0.05$) differences were observed among dietary treatments on liver weight and abdominal fat pad (Table 23). Kubena et al. (1972, 1974) found that increasing the energy content of broiler rations, while maintaining constant ME:protein (P) ratio could increase abdominal fat deposition. The ME value for their diets ranged from 3141 to 3306 for starter and from 3042 to 3372 for finisher, while in this study the ME values were very similar. Bartov (1979) indicated however that ME:P ratio is of greater importance in regards to fat deposition than is energy per se. The overall correlation coefficient between body weight and abdominal fat pad was $r = 0.61$, which is slightly higher than reported by Becker et al. (1981) $r = 0.49$. Birds fed the tallow diet appeared to deposit more fat than the birds on the other diets.

Dietary treatments did not affect overall mortality with the exception of SDS mortality between 0 to 4 weeks of age (Table 24). HCO diet had a very low and significantly ($P<0.05$) different mortality from the SFO treatment. This observation is difficult to explain in view of previous findings, where SDS mortality was found to be significantly reduced on SFO diets (Rotter et al., 1985a).

Serum samples collected at 4 and 7 weeks of age indicated differences in total serum protein and the albumin fraction among treatments (Tables 25 and 26). At 4 weeks of age there were no significant ($P>0.05$) differences in the albumin content between the tallow, tallow/SFO or

Table 23. The effects of dietary treatments on various body parameters of 7 week old broilers

Parameters	Dietary treatment				Significance
	HCO[1]	Tallow	Tallow/SFO[2]	SFO	
Body weight (g)	2218±49[a]	2373±36[b]	2283±19[ab]	2360±39[b]	*
Fat pad (g)	39.10±3.84	54.46±5.73	43.29±4.74	44.79±5.36	NS[3]
Liver weight (g)	34.43±0.76	38.66±1.46	35.14±1.64	37.21±2.09	NS
Liver weight (g/100 g body weight)	1.56±0.05	1.63±0.05	1.54±0.06	1.57±0.07	NS
Fat pad (g/100 g body weight)	1.75±0.15	2.28±0.22	1.89±0.21	1.88±0.21	NS

[a,b]Means ± SE within the row having different superscripts are significantly different (*P<0.05).

[1]HCO = Hydrogenated coconut oil.

[2]SFO = Sunflower oil.

[3]NS = No significant difference (P>0.05).

Table 24. SDS mortality as a percent of total number of birds per treatment

Parameters	Periods	Dietary treatment			
		HCO[3]	Tallow	Tallow/SFO[4]	SFO
Total no. of SDS mortalities (%)	0-4	3(.61)^{a}[1]	8(1.63)^{ab}	9(1.84)^{ab}	12(2.45)^{b}
	4-7	8(1.68)^{a}	10(2.12)^{a}	16(3.37)^{a}	10(2.14)^{a}
	0-7	11(2.24)^{a}	18(3.67)^{a}	25(5.10)^{a}	22(4.49)^{a}
Total no. of mortalities (%)[2]	0-7	27(5.51)^{a}	37(7.55)^{a}	39(7.96)^{a}	44(8.98)^{a}
No. of culled birds	0-7	5	10	6	14

^{a,b}Means within a row having different superscripts are significantly different ($P<0.05$).

[1]Number outside of parenthesis indicates the actual number of birds died per dietary treatment and the number within the parenthesis percent mortality.

[2]Includes culled birds

[3]HCO = Hydrogenated coconut oil.

[4]SFO = Sunflower oil.

Table 25. Average body weight and serum parameters of 4 week old male broilers fed different dietary fats

Parameters	Dietary treatment[1]				Significance
	HCO[2]	Tallow	Tallow/SFO[3]	SFO	
Weight (g)	903±26[a]	984±20[b]	977±26[b]	1011±9[b]	*
Total protein (g/ℓ)	32.8±0.9[a]	30.7±1.0[ab]	29.1±0.6[b]	30.3±1.0[ab]	*
Albumin (g/ℓ)	13.3±0.3[a]	12.4±0.5[ab]	11.7±0.3[b]	11.6±0.2[b]	*
Calcium (mmol/ℓ)	2.59±0.03	2.51±0.05	2.44±0.06	2.38±0.09	NS[4]
Phosphorus (mmol/ℓ)	2.20±0.04	2.21±0.06	2.24±0.04	2.25±0.04	NS
Magnesium (mmol/ℓ)	0.97±0.02	0.94±0.02	0.99±0.03	1.02±0.05	NS
Sodium (mmol/ℓ)	155±1	153±1	153±1	153±1	NS
Potassium (mmol/ℓ)	5.8±0.2	5.5±0.2	5.5±0.1	5.2±0.2	NS
Chloride (mmol/ℓ)	114±0.7[a]	111±1[ab]	111±0.8[ab]	108±1.5[b]	*

[a,b]Means ± SE within a row having different superscripts are significantly different (*P<0.05).

[1]Seven observations per mean.

[2]HCO = Hydrogenated coconut oil.

[3]SFO = Sunflower oil.

[4]NS = No significant difference (P>0.05).

Table 26. Average body weight and serum parameters of 7 week old male broilers fed different dietary fats

Parameters	Dietary treatment[1]				Significance
	HCO[2]	Tallow	Tallow/SFO[3]	SFO	
Weight (g)	2202±55^{a}	2383±47^{b}	2242±30^{ab}	2331±33^{ab}	*
Total protein (g/ℓ)	43.0±2.2	40.7±1.4	39.8±0.9	38.7±2.2	NS[4]
Albumin (g/ℓ)	13.3±0.4^{a}	13.0±0.2^{a}	12.1±0.3^{ab}	11.7±0.4^{b}	*
Calcium (mmol/ℓ)	2.71±0.08^{a}	2.75±0.03^{a}	2.61±0.05^{ab}	2.45±0.11^{b}	*
Phosphorus (mmol/ℓ)	2.16±0.05	2.09±0.04	2.16±0.08	2.04±0.04	NS
Magnesium (mmol/ℓ)	0.90±0.06^{a}	1.92±0.34^{b}	1.19±0.05^{a}	1.13±0.08^{a}	*
Sodium (mmol/ℓ)	148±0.7	148±1.4	149±0.6	150±0.9	NS
Potassium (mmol/ℓ)	4.2±0.3^{a}	4.6±2.2^{a}	5.1±0.1^{ab}	5.8±0.3^{b}	*
Chloride (mmol/ℓ)	113±0.9	112±0.9	116±1.7	116±0.8	NS

^{a,b}Means ± SE within a row having different superscripts are significantly different (*P<0.05).

[1]Seven observations per mean.

[2]HCO = Hydrogenated coconut oil.

[3]SFO = Sunflower oil.

[4]NS = No significant difference (P>0.05).

SFO diets, however the difference was observed between HCO and tallow/SFO, as well as between HCO and SFO diets. The increased albumin concentration on HCO diet could be due to the presence of medium-chain fatty acids, as the albumin would be required to improve their transport. The total protein was significantly ($P<0.05$) different between HCO and tallow/SFO diets at 4 weeks of age, however there were no differences among treatments at 7 weeks. Previously, Leveille et al. (1960) observed that total serum protein was significantly depressed on low protein diets, but not influenced by dietary fat (HCO or corn oil).

To our best knowledge, an effect similar to that observed in this study, has not been reported previously. Albumin is known to act as a carrier of many nutrients, including fatty acids (Sturkie, 1976). Because of better absorbability of the fat in the SFO diet more albumin may have been involved in the transport of fatty acids evidenced by a lower level of albumin in the serum. The values for individual electrolytes were comparable to those reported by Vo et al. (1978) (Tables 25 and 26). A significant ($P<0.05$) reduction in chloride concentration was observed with increased amount of unsaturated dietary fat, but this effect was not seen at 7 weeks of age. Other significant changes at 7 weeks involved Ca and K. Calcium was reduced, while potassium increased with increased unsaturated dietary fat. Julian (personal communication, 1984) observed that serum K levels were higher in the high nutrient group (protein 28%, ME 3100 kcal/kg) as well as in the high protein (31%) fed group.

Mineral content of the heart muscle was studied due to its involvement in blood pressure and heart muscle function. Calcium, Cu, Zn and Mg concentrations did not reveal differences, but the Na and K levels

were significantly ($P<0.05$) different (Table 27). Previously, Mollison (1983) had only reported differences between Cu levels of the hearts for SDS and culled birds. It has been observed in human studies that cardiac diseases have an effect on electrolyte concentration in serum and muscle tissue. Speich et al. (1980) reported changes in heart electrolytes (Ca, Mg, Na, K) due to myocardial infarction, indicating increases in Ca and Na and reduction in K and Mg. Our findings also indicated increase in Na (7%) and reduction in K (6%) in SDS birds compared with controls. How either dietary intake or metabolism of Na and K may be linked to the etiology of sudden coronary death, ventricular fibrillation or myocardial infarction remains controversial (Blaustein, 1977; Folkow, 1982; Guyton et al., 1980).

Although both elements play important roles in governing the electrical activity of the heart, it is not clear if cardiac muscle cells or the coronary vascular smooth muscle cells are altered (Altura and Altura, 1982). Whether they influence the structural design of vascular walls by effecting an osmotic movement of H_2O, Na-Ca exchange mechanisms or electrogenic transport of cations is still speculation. Ononiwu et al. (1979b) suggested that in case of SDS it could be a result of circulatory lesions, increasing the permeability of the circulatory system, but caused by increases in blood pressure. If blood pressure is related to SDS, sex differences could be somewhat explained as males have been found to have higher blood pressure than females (Sturkie, 1976).

No significant ($P>0.05$) differences were observed in the mineral composition of the liver tissue (Table 28). Copper and Zn contents

Table 27. Mineral composition of the heart tissue for SDS and control birds

Parameters	Type of bird[1]	Heart tissue	Significance
Ca (µg/g)	SDS	289±10.4	NS[2]
	Control	277±8.7	
Cu (µg/g)	SDS	16.6±0.31	NS
	Control	16.3±0.28	
Zn (µg/g)	SDS	110±1.9	NS
	Control	112±1.7	
Na (%)	SDS	0.71±0.02[a]	*
	Control	0.66±0.02[b]	
K (%)	SDS	1.38±0.02[a]	*
	Control	1.47±0.02[b]	
Mg (%)	SDS	0.098±0.001	NS
	Control	0.104±0.004	

[a,b]Means ± SE for individual parameters were significant at *P<0.05.

[1]All values expressed on dry matter basis.
18 observations per mean.

[2]NS = No significant difference (P>0.05).

Table 28. Comparison of liver tissue parameters for SDS and control birds

Parameters	Type of bird[1]	Liver tissue
Protein (%)	SDS	66.19±1.37
	Control	64.14±0.91
Glycogen (mg/g)	SDS	29.91±5.2
	Control	32.91±2.5
Biotin (µg/g)	SDS	7.69±1.17
	Control	6.30±0.91
Cu (µg/g)	SDS	11.3±0.3
	Control	12.3±0.3
Zn (µg/g)	SDS	79±2.1
	Control	83±1.8

[1]Means ± SE for individual parameters were not significantly different (P>0.05).

All values expressed on dry weight basis.

18 observations per mean.

were reduced in SDS birds as reported previously (Rotter et al., 1985a), but were not significantly different from the control birds. The protein and glycogen content in the liver did not differ significantly ($P>0.05$) (Table 28). Glycogen values of livers from SDS birds were highly variable ranging from 4.61 to 91.90 mg/g, while the control birds ranged from 9.35 to 47.74 mg/g. Since glycogen status in the liver is regulated by hormones such as catecholamines and corticosteroids, it can be argued that stressful situation such as sudden death will promote release of catecholamines from the adrenal medulla (Wells and Wight, 1971). This in turn can cause depletion of glycogen stores in the liver and introduce a large variation due to different time duration of the stress. Cervical dislocation if considered as another type of stress would take place over a shorter time period. This would stop the circulation of hormones to the liver, which may influence glycogen stability.

Biotin concentration in the liver was found not to be significantly ($P>0.05$) different between SDS and control birds (Table 28). Frigg and Brubacher (1976) observed that liver biotin content above 1.5 µg/g (wet basis) indicates sufficient dietary biotin. This would suggest that SDS birds in our study had an adequate amount (7.69 µg/g dry basis) of biotin in the liver. Similar findings were reported earlier by Steele et al. (1982). Furthermore, the syndrome and total mortality were found unaffected by dietary biotin concentration (Steele et al., 1982; Whitehead and Randall, 1982; Mollison, 1983). In contrast, Hulan et al. (1980) showed that supplemental biotin in the ration significantly reduced SDS and total mortality, while Buenrostro and Kratzer (1982) observed that chicks dying from SDS had significantly lower biotin levels in the liver compared to other dead birds. Buenrostro

and Kratzer (1982) and Whitehead and Randall (1982) suggested a relationship between fatty liver kidney syndrome (FLKS) and SDS, and the occurrence was related to the biotin status of chicks. In our study, no cases of FLKS based on the visual necropsies (Wight and Siller, 1975) were observed. FLKS is known to be caused by biotin deficiency and associated with increased palmitoleic and decreased stearic acid in the liver (Roland and Edwards, 1971; Payne et al., 1974). Previously reported findings (Rotter et al., 1985a) indicated an increase in oleic and decrease in arachidonic acids in heart and liver tissues, which according to Buenrostro and Kratzer (1982) could also be a sign of biotin deficiency. In the present study, a significant ($P<0.05$) reduction in arachidonic acid and an increase in palmitoleic acid were observed in the heart tissue, while no differences were found in the liver tissue (Table 29). Several factors could be responsible for some of the differences, including the improved method of sample collection (killing of matching control at the time of SDS occurrence) and in turn reduction in the degree of post-mortem changes in the necropsy materials.

The monoiene:diene (18:1/18:2) ratios for heart and liver tissues were calculated to estimate the dietary linoleic acid adequacy (Table 30). Menge (1970) reported that when dietary linoleate was present at 1.2%, Leghorn chicks attained adequate growth and the 18:1/18:2 ratios for the heart and liver were 1.00 and 0.62, respectively. In the present study a ratio of 0.89 (SDS) and 0.92 (controls) was found in the heart tissue, but it corresponded with 1.4% dietary linoleate. At 2.0% and 3.8% of linoleic acid, ratios in both tissues remained the same, indicating adequacy at 2% of the diet. Some discrepancy between the values can be attributed to strain differences (Leghorn vs broiler)

Table 29. Heart and liver fatty acid composition for the SDS and control birds[1]

Type of bird	% of total methyl esters										
	14:0	14:1	16:0	16:1	17:0	18:0	18:1	18:2	18:3	20:4	Fat content (%)[2]
	Liver										
SDS	1.18±0.26	0.27±0.07	28.3±0.8	4.2±0.5	0.22±0.02	19.4±0.7	22.1±1.2	17.9±1.1	0.23±0.01	5.9±0.6	11.16±0.58
Control	1.18±0.26	0.28±0.06	28.1±0.9	4.6±0.4	0.22±0.04	18.6±0.6	23.3±1.2	17.3±1.2	0.31±0.06	5.8±0.5	11.49±0.47
	Heart										
SDS	0.81±0.19	0.17±0.03	22.3±0.3	3.2±0.2[a]	0.27±0.02	17.8±0.3	18.8±0.8	26.7±0.7	0.21±0.01	9.0±0.6[a]	7.83±0.47
Control	0.86±0.22	0.14±0.02	22.3±0.4	2.5±0.1[b]	0.26±0.02	18.1±0.3	18.4±0.8	26.3±0.6	0.19±0.01	10.1±0.6[b]	8.03±0.66

[1]Means ± SE within a column (SDS vs control) with different superscripts are significantly different ($P<0.05$).

[2]Dry weight basis.

Table 30. Effect of different dietary treatments on the monoiene:diene (18:1/18:2) ratios of the heart and liver tissues of SDS and control birds

Cause of mortality	Diet	Heart tissue	Liver tissue	Dietary linoleate, % Starter	Finisher
SDS	HCO[1]	0.78	1.91	.85	1.09
Control		0.75	1.61		
SDS	Tallow	0.89	1.71	1.39	1.30
Control		0.92	2.36		
SDS	Tallow/SFO[2]	0.72	0.90	2.01	2.92
Control		0.62	1.00		
SDS	SFO	0.72	0.92	3.81	3.28
Control		0.62	0.98		

[1]HCO = Hydrogenated coconut oil.

[2]SFO = Sunflower oil.

and therefore different dietary requirements. The 18:1/18:2 ratio was suggested as more accurate than triene:tetraene (20:3/20:4) (Reid et al., 1964; Menge et al., 1968). However, it does not provide the information about the interconversion products, which are either arachidonic or eicosatrienoic acids.

ACKNOWLEDGEMENTS

The authors wish to express appreciation to G. Crow for cooperation and advice in the data analysis. The excellent assistance of H. Muc, the University of Manitoba poultry barn staff and numerous graduate students in conducting the study is gratefully acknowledged. The research was supported, in part, by NSERC Grant No. A0457.

Manuscript IV: Effect of Dietary Fat Source and Biotin Supplementation on the Incidence of Sudden Death Syndrome in Broiler Chicks

ABSTRACT: The effects of supplemental biotin added to the diet containing tallow or sunflower oil (SFO) on the general performance of broiler chickens and the incidence of sudden death syndrome (SDS) were investigated. The weight gain and feed/gain ratio were not different among dietary treatments to 7 weeks of age ($P>0.05$). Overall mortality was not affected, but SDS mortality was significantly ($P<0.05$) lower when birds were fed the SFO diet alone (without added biotin) between 0 to 4 weeks of age. The results would suggest that supplemental SFO could be recommended as a means of reducing SDS mortality, but addition of biotin does not seem to be a further benefit. The histopathological examination of the heart and liver tissues did not reveal any differences in lesions between SDS and control birds (matching penmates).

INTRODUCTION

In recent years broiler growers have become increasingly concerned with mortality problems caused by sudden death syndrome (SDS). Its widespread prevalence in broiler chickens, high incidence in males and lack of significant macroscopic pathology suggest that SDS is a metabolic condition (Riddell and Springer, 1985). An epizootiological and strain comparison field study recently conducted in Saskatchewan indicated a lot of variation in the incidence due to different nutritional and environmental factors (Riddell and Springer, 1985). Experimental broiler feeding trials have identified some of the nutritional aspects related to SDS. The syndrome was found to be reduced when a corn-soy diet replaced a wheat-soy diet (Mollison et al., 1984) and sunflower oil (SFO) was substituted for tallow (Rotter et al., 1985a). Hulan et al. (1980) reported that the amount of biotin in the ration may affect the incidence of SDS. Further, Buenrostro and Kratzer (1982) demonstrated that chicks dying from SDS had significantly lower biotin levels in the liver when compared with other dead birds. In contrast, Steele et al. (1982) showed that supplementation of biotin via drinking water did not reduce SDS or total mortality. The SDS-affected birds also had adequate amounts of biotin in the liver compared to clinically normal birds. A further study by Rotter et al. (1985c) supported these findings.

An interrelationship has been shown between biotin and unsaturated fatty acids, especially oleic acid in many microorganisms (Scott et al., 1982). Oleic acid can spare the requirement for biotin and vice versa in the growth process. However, it has never been demonstrated

that oleic acid has any effect on sparing the biotin requirement of animals.

This study was designed to examine if supplemental biotin added to the diet containing tallow or SFO would further reduce SDS mortality. In addition, histological evaluations of the heart and liver tissue in SDS and control birds were carried out.

MATERIALS AND METHODS

A total of 1890 male day-old, vaccinated (Marek's), broiler chicks of commercial (Cobb x Cobb) parentage were randomized in 27 pens (70 birds/pen) with a floor area of 6.64 m^2 each. Due to a mistake in the distribution of birds, one pen (diet tallow plus biotin) had 50 birds instead of 70. The pens were located in an environmentally controlled house with continuous lighting at low intensity. Each diet was fed to 630 birds (610 birds for the tallow plus biotin diet) in mash form and water was supplied ad libitum. The wheat-soy diets were supplemented either with tallow or sunflower oil (SFO) as a dietary fat source. In one of each of the diets biotin was added at 0.15 mg/kg. Starter (0 to 4 weeks) and finisher (4 to 7 weeks) dietary formulations are presented in Table 31. Performance parameters (weight gain and feed conversion) were calculated for the experimental periods 0 to 2, 2 to 4 and 4 to 7 weeks of age. Mortalities were recorded to 49 days of age and submitted for post-mortem necropsy as they occurred.

Diagnosis of SDS birds was based on the criteria described in previous experiments (Rotter et al., 1985a). Between 4 and 7 weeks of age 8 birds were killed by cervical dislocation and used as matching controls for SDS birds (time of death, pen, weight and diet). All birds were immediately removed from the barn and stored in a cooler. During post-mortem necropsy hearts and livers of SDS and control birds were removed. The tissues were fixed in ten percent-buffered formalin at room temperature before trimming. The heart was divided into three parts: top, middle and apex and sections were taken from each of these areas. Following trimming, they were stored in formalin solution before embedding in paraffin using Autotechnicon Mono (Technicon

Table 31. Composition and calculated analysis of starter and finisher diets

Ingredients	Starter	Finisher
	g/kg	
Wheat	598.5	690.0
Soybean meal[1]	274.0	198.0
Fish meal (65%)	30.0	25.0
Fat source[2]	57.0	46.0
Limestone	11.0	11.0
Dicalcium-phosphate	13.0	14.3
Vitamin mix - standard[3]	10.0	10.0
Mineral mix - standard[4]	5.0	5.0
DL-methionine	1.5	0.7
Total	1000.0	1000.0
Calculated analysis: (chemical analysis)		
Metabolizable energy (ME) (kcal/kg)	3043-3098	3011-3041
Crude protein (P) (%)	23.3-23.6	19.9-20.1
Linoleic acid (%)	1.06-3.32 (0.96-3.58)	1.08-2.73 (1.02-2.90)
ME:P	131	151

[1]Protein content of the soybean meal was 47.5% in the starter diet and 44% in the finisher diet.

[2]Supplies tallow (Diet 1) or sunflower oil (Diets 2 and 3).

[3]Supplies the following per kg of complete feed: vitamin A, 8250 I.U.; vitamin D_3, 870 I.C.U.; vitamin E, 5.5 I.U.; riboflavin, 5.5 mg; Ca pantothenate, 11 mg; niacin, 16.5 mg; choline chloride, 275 mg; menadione, 1.1 mg; santoquin, 250 mg. Total biotin determined (mg/kg): tallow + biotin - 0.23; SFO - 0.11; SFO + biotin - 0.24.

[4]Supplies the following in mg per kg of complete feed: manganese oxide, 92.5; zinc oxide, 56.2; ferrous sulfate$\cdot H_2O$, 259; copper sulfate$\cdot 5H_2O$, 16.2; selenium selenite, 0.5; iodized salt, 4080.

Corporation, U.S.A.). Sections were stained with hematoxylin and eosin (Galigher and Kozloff, 1971). The evaluation of the sections followed criteria used by Ononiwu et al. (1979b) to identify the histopathological changes in both tissues. The examination was performed by a Poultry Pathologist from the Manitoba Department of Agriculture.

The linoleic acid content of the feed samples was determined using the fat extraction method by Bligh and Dyer (1959), followed by methylation procedure according to Metcalfe et al. (1966).

All performance and mortality data (on a pen basis) were analyzed as completely randomized designs, using a one-way analysis of variance procedure. Mortality data were converted by arc sine transformation, but for interpretation are reported as percents. Differences between means were determined using the Student-Newman-Keuls (SNK) test (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

There were no significant ($P>0.05$) differences among dietary treatments in weight gain and feed/gain ratios to 7 weeks of age (Table 32). Similarly, Stallings et al. (1985) reported that wheat-soy diets with or without biotin supplementation were essentially equal in performance as measured by body weight and feed utilization. In this study, an improved feed efficiency and weight gain between 0 to 4 weeks of age mainly due to the SFO was assumed to be due to its better absorption over tallow. The digestibility of animal fat, tallow in particular, is poor initially in the very young bird, but it increases gradually to 8 weeks of age (Freeman, 1976). The effects of age on digestibility of vegetable oils are not as pronounced, and impaired digestibility occurs only during the first one or two weeks. Whitehead and Fisher (1975) found that corn oil and lard were digested with equal efficiency (97 and 91%) at 2, 4 and 8 weeks, whereas the true digestibility of tallow over the same time increased from 57 to 70 and 74%, respectively. In the very young chick, the ability to assimilate dietary fat is impaired by a deficiency in bile salt production and possibly other mechanisms necessary for efficient digestion and absorption of saturated fatty acids (Krogdahl, 1985). Between 4 and 7 weeks of age, birds fed tallow plus biotin showed improved feed conversion when compared with SFO diet alone, which could be due to a compensatory ability to utilize the fat more efficiently at the older age.

The overall mortality was not significantly ($P>0.05$) affected by any of the dietary treatments, but SDS mortality was significantly ($P<0.05$) reduced on the SFO diet alone between 0 to 4 weeks of age (Table 33). Since Hulan et al. (1980) reported a significant reduction

Table 32. Effects of different fat sources and biotin on performance of male broilers

Parameters	Dietary treatment			
	Tallow + biotin	SFO[1]	SFO + biotin	±SE
7 weeks body weight (g)	2442[a]	2475[a]	2482[a]	±11.1
Average weight gain (g)				
0-4 weeks	1002[a]	1047[b]	1016[a]	± 7.4
4-7 weeks	1399[a]	1387[a]	1426[a]	±11.4
0-7 weeks	2401[a]	2435[a]	2442[a]	±11.1
Feed/gain				
0-4 weeks	1.64[a]	1.49[b]	1.54[b]	± 0.02
4-7 weeks	2.42[a]	2.50[b]	2.46[a,b]	± 0.02
0-7 weeks	2.09[a]	2.05[a]	2.07[a]	± 0.01

[a,b]Means within a row having different superscripts are significantly ($P<0.05$) different.

[1]SFO = Sunflower oil.

Table 33. SDS mortality as a percent of total number of birds per treatment

Parameters	Periods	Dietary treatment		
		Tallow + biotin	SFO[1]	SFO + biotin
Total no. of SDS mortalities (%)	0-4	30 (4.92)[a2]	11 (1.75)[b]	18 (2.86)[a,b]
	4-7	11 (1.98)[a]	14 (2.34)[a]	16 (2.69)[a]
	0-7	41 (6.72)[a]	25 (3.97)[a]	34 (5.40)[a]
Total no. of mortalities (%)[3]	0-7	71 (11.64)[a]	54 (8.57)[a]	58 (9.21)[a]
No. of culled birds	0-7	18	11	11

[a,b]Means within a row having different superscripts are significantly different ($P<0.05$).

[1]SFO = Sunflower oil.

[2]Number outside of parenthesis indicates the actual number of birds died by dietary treatment and the number within the parenthesis percent mortality.

[3]Includes culled birds.

in SDS on diets supplemented with biotin it was expected to achieve a further reduction by combining SFO and biotin. This combination did not result in significant reduction when compared to tallow plus biotin, however, numerically a trend towards reduced SDS mortality (significant at P = 0.1) was indicated. Therefore, the results would suggest that supplementation with additional biotin does not seem to further alleviate the condition. Our observations agree with the study reported by Steele et al. (1982), demonstrating that dietary addition of biotin did not reduce total or SDS mortality.

The second part of this study consisted of microscopic examinations of heart and liver tissues of SDS and control birds. The histopathological findings are summarized in Table 34. Previously, Ononiwu et al. (1979b) reported lesions in both tissues for SDS birds. In contrast, Riddell and Orr (1980) did not find significant differences between SDS and control birds in their study. Similarly to their findings, the results of our experiment indicated that there were no differences between SDS and control birds. The lack of significant lesions in SDS birds does not rule out the possibility of ultrastructural lesions in atrium, ventricular septum, sinus valve and coronary arteries. Further research in this area is warranted.

ACKNOWLEDGEMENTS

The authors wish to thank Harry Muc, the University of Manitoba Poultry Barn Staff and numerous graduate students for their valuable help.

Table 34. Detailed histopathological lesions of hearts and livers of SDS and control birds

	SDS									Controls								
Lesions	1	2	3	4	5	6	7	8	$\bar{x}$	1a	2a	3a	4a	5a	6a	7a	8a	$\bar{x}$
Heart:																		
Vascular congestion	1	1	1	1	1	2	2	1	1.20	1	1	1	1	1	1	1	1	1.00
Myocardial degeneration	0	0	1	1	1	1	1	1	0.75	0	0	1	0	1	0	1	0	.37
Mononuclear cell aggregates	0	1	1	0	0	0	1	0	0.37	1	0	1	1	0	1	1	1	.75
Heterophilic infiltration	0	1	1	0	0	0	0	0	0.25	1	0	0	0	0	1	0	0	.25
Myocardial separation	1	1	1	1	1	1	2	2	1.20	0	1	1	0	1	0	1	1	.80
Liver:																		
Sinusoidal congestion	1	2	1	2	1	2	2	2	1.62	0	2	1	1	2	2	1	2	1.37
Bile duct hyperplasia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mononuclear cell aggregates	1	1	1	1	1	2	2	1	1.25	1	1	1	1	3	2	3	1	1.62
Heterophilic infiltration	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	1	0	.50
Constricted bile ducts	1	0	0	1	0	1	1	0	.50	0	0	0	1	0	1	0	1	.37
Periportal hepatitis	0	1	0	0	0	0	2	0	.37	0	0	1	2	0	1	2	0	.75

Indicates: (0) none, (1) slight, (2) moderate or (3) severe.

GENERAL DISCUSSION

Sudden death syndrome (SDS) can cause a significant amount of mortality in rapidly growing broiler chickens, but its exact etiology and pathology has not been well established. The immediate cause of death however, seems to be a cardiac failure due to ventricular fibrillation (Julian, personal communication, 1984). Literature regarding possible causes or prevention of SDS is limited, as well as the chemical characteristics of SDS birds. Several factors have been linked to SDS including sex, growth rate, vitamin supplementation and feed pelleting process (Brigden and Riddell, 1975; Mollison et al., 1984; Hulan et al., 1980; Proudfoot et al., 1984).

The approach of the current research was based on findings reported by Mollison (1983) and was expanded using biochemical observations. A series of experiments was carried out to examine the possibility of linoleic acid and/or its metabolites being linked to the syndrome.

It is currently thought, that any mechanism responsible for the utilization of dietary fat is mediated through the prostaglandin system (Puska and Iacono, 1985). Further it is known that dietary linoleic acid is converted to arachidonic acid and then to prostaglandins (PGs) (Fig. 4). They in turn are known for their physiological effects on the cardiac system (including myocardial contractile force, heart rate and blood pressure) (Karmazyn and Dhalla, 1983). Thus, diets high in polyunsaturated fatty acids (linoleic acid) could possibly lead to higher levels of PGs in different tissues.

When wheat-soy diets were supplemented with sunflower oil (SFO),

Figure 4. Outline of the step-wise parallel metabolism of LA and stearic acid via the common enzymes Δ^6 and Δ^5 desaturase. The conversion of D-γ-LA and AA to their respective series of PGs occurs via the intermediate cyclic endoperoxides and the enzymes endoperoxide synthetase and cyclooxygenase. The w3 family which is derived from α-LA (9,12,15-18:3w3) and the w7 family which is derived from palmitic acid (16:0) also compete for the Δ^9, Δ^6 and Δ^5 desaturase enzymes but are not shown in this Figure.

Modified from Cunnane (1982).

a significantly ($P<0.05$) lower SDS mortality was observed as opposed to tallow diets. Simultaneously, birds on the former diet exhibited increased body weight and improved feed utilization possibly due to a better digestibility of the fat. In turkeys, Whitehead and Fisher (1975) noted that corn oil and lard were digested with equal efficiency (≈97 and 91%) at 2, 4 and 8 weeks, whereas the true digestibility of tallow over the same time increased from 57 to 70 and 74%, respectively. Also, with increased concentration of tallow in the diet (i.e. increasing level of linoleic acid) a trend towards reduced SDS incidence between 0 to 4 weeks of age was observed ($P = 0.08$). However, the results with hydrogenated coconut oil (HCO) were inconsistent in that a lower SDS mortality was noted on this diet compared with the SFO treatment between 0 to 4 weeks of age. Based on these findings, SFO supplementation as a means of reducing SDS incidence can only be conditionally recommended, considering the inconsistency in the results and cost of the oil.

Hulan et al. (1980) reported that biotin supplementation resulted in reduced SDS mortality. Other studies, however, have not confirmed this finding (Steele et al., 1982). In the current study, SFO alone was more effective in reducing the SDS incidence between 0 to 4 weeks of age than tallow plus biotin, however there was no difference ($P>0.05$) between the tallow plus biotin and SFO plus biotin diets. Therefore, supplementation with biotin does not seem to reduce the incidence of SDS.

The addition of SFO to the diet as compared with tallow resulted in an increase in the concentration of arachidonic acid in the heart tissue. Further, SDS birds had a higher concentration of oleic and

decreased levels of linoleic and arachidonic acid in the heart tissue when compared with the birds culled within the same period of the experiment (4 to 7 weeks). A similar pattern was observed in the liver tissue. These findings suggested a possibility of a competitive inhibition between the oleic and linoleic acid families and/or reduced conversion rate from linoleic to arachidonic acid (Fig. 4). In regards to SDS, it can be hypothesized that some individuals are more susceptible to a malfunction in the conversion pathway, and so a good source of linoleic acid (SFO) could alleviate some of problems. As a result a lower incidence of SDS would be observed. Also, Buenrostro and Kratzer (1982) reported similar findings in the liver when they compared SDS birds with other mortalities. However, when a different method of tissue collection (killing a matching control at the time of an SDS occurrence) was introduced, a significant ($P<0.05$) reduction in arachidonic acid was only observed in the heart tissue. The linoleic acid concentration in SDS and control birds was not different ($P>0.05$). This in turn would suggest an inadequate conversion from linoleic to arachidonic acid in the heart tissue possibly due to enzyme deficiency in the pathway.

Biotin is involved in the conversion of γ-linolenic acid to dihomo-γ-linolenic acid by being a cofactor in the synthesis of malonyl-coenzyme A (Fig. 4). Biotin analysis indicated that SDS birds had an adequate amount of the vitamin in the liver when compared with control birds. Similar findings were reported by Steele *et al.* (1982), but Buenrostro and Kratzer (1982) indicated that SDS birds were in a biotin

deficient state. It is possible however, that in the current study although adequate levels of biotin were in the tissue, differences existed in biotin availability for the optimum enzyme activity in the heart tissue between these two groups of birds.

The mineral content of the heart muscle tissue indicated an increase in sodium and reduction in potassium concentrations in SDS birds compared with controls. Similar observations have been made in human studies and implied in cardiac diseases (Speich et al., 1980). However, how these minerals may be linked to the etiology of sudden coronary death or ventricular fibrillation remains controversial (Blaustein, 1977; Folkov, 1982; Guyton et al., 1984).

Overall, there is the possibility of an interaction among several factors, which could lead to SDS:reduced linoleic acid content of the diet and enzyme deficiencies could result in an inadequate conversion rate from linoleic to arachidonic acid in SDS affected individuals. Lower arachidonic acid would reduce the amount of PGs being synthesized or result in an imbalance among major PGs having opposite effects. This could possibly upset heart function, leading to fibrillation or arrythmia.

SUMMARY AND CONCLUSIONS

Five experiments were carried out to investigate the possible involvement of several nutritional factors in the development of sudden death syndrome (SDS). The condition was found to begin as early as 5 to 10 days of age and continued until market age at 7 weeks. It may affect up to 6.7% of the population and account for approximately 58% of total mortality. In three out of four experiments, SDS mortality was significantly reduced by 40% to 50% when birds were fed diets supplemented with sunflower oil (SFO) as opposed to tallow. However, when hydrogenated coconut oil (HCO) was included in the diet, a significantly ($P<0.05$) lower SDS mortality occurred between 0 to 4 weeks of age when compared to a SFO diet.

The histological and biochemical findings on the heart and liver tissues were:

a) Histologically, there were no major differences between SDS and control birds.

b) Fatty acid analysis of the heart tissue showed reduction in arachidonic and an increase in palmitoleic acid concentration.

c) Mineral analysis of the heart tissue revealed that SDS birds had increased sodium and reduced potassium concentrations when compared with control birds.

The incidence of SDS is irregular and variable, and chemical and morphological conditions of necropsy materials can be complicated by post-mortem changes. It is therefore essential to identify a SDS-affected bird at the time of death. A matching control bird should be used for comparison.

Examination of collected data leads to the following conclusions:

1. The use of SFO as a dietary source of linoleic acid and energy seems to be beneficial in reducing SDS incidence when compared with tallow and could be conditionally recommended. However, as the cost of SFO is twice as high as tallow, diets would be uneconomical, unless the reduction in mortality is sufficient to compensate for the extra cost of the diet.
2. Addition of biotin to a SFO diet did not further reduce the incidence of SDS whereas SFO in the diet alone was found to be more effective between 0 to 4 weeks of age than tallow plus biotin diet.
3. Under our experimental conditions, stocking rate did not have a significant effect on the number of SDS mortalities.
4. Factors such as nutritional (low linoleic acid of the diet and arachidonic acid in the heart tissue) and/or physiological (an electrolyte imbalance in the heart tissue) status of the bird could contribute to the initiation of the syndrome.

Considering the present investigations, the following recommendations for future studies can be made:

A. Methodology and economics.

1. In order to minimize the post-mortem changes in the blood and tissues, it is necessary to take the samples at the "dying moment", and preserve appropriately depending on the type of analysis to be done.

2. A maximum number of male birds should be used in order to detect treatment differences. The number of birds per treatment should be between 1500 to 2000 birds according to calculations based on the current results and a formula from Snedecor and Cochran (1980).
3. The cost of possible preventive measures has to be taken into account, so the reduction in income due to SDS are not offset by increases in feed cost.

B. New objectives

1. The heart tissue should be examined for ultrastructural lesions in myocardium, as well as associated coronary arteries. This may be feasible by electron microscopy.
2. Some of the blood parameters could be used as a criteria for cardiovascular disorders - plasma glucose, lactate, free fatty acids and catecholamines.
3. The content of glycogen should be determined since it is used as energy source by the heart.
4. The examination of heart electrolytes in reference to their role in maintaining heart function warrants further studies, since they have been linked to the etiology of cardiac disorders.
5. Since, there is an indication of inadequate conversion of linoleic to arachidonic acid, member of both families - linoleic and oleic acid should be determined quantitatively.

Determination of the enzyme activities could be also beneficial (malonyl-coenzyme A). The reduced tissue content of arachidonic acid is of interest, because of its further conversion to the "2nd series" of prostaglandins.

6. The requirement for linoleic acid should be determined under different vitamin E and Ca regimes, because of its involvement in membrane structure and function (permeability).

LITERATURE CITED

Association of Official Analytical Chemists, 1984. Official Methods of Analysis. 14th ed. Assoc. Off. Anal. Chem., Washington, D.C.

Altura, B.M. and B.T. Altura, 1982. Mg, Na and K interactions and coronary heart diseases. Magnesium Reprint, Vol. 1, No. 3-6 (pp. 241-265), Karger Medical and Scientific Publishers.

Bartov, I., 1979. Nutritional factors affecting quantity and quality of carcass fat in chickens. Fed. Proc. 38:2627-2630.

Becker, W.A., J.V. Spencer, L.W. Mirosh and J.A. Verstrate, 1981. Abdominal and carcass fat in five broiler strains. Poultry Sci. 60:693-697.

Bhullar, R.P., S.H. Lie and K. Dakshinamurti, 1985. Isotope dilution assay for biotin. Ann. NY Acad. Sci. 447:122-127.

Blaustein, M.P., 1977. Sodium ions, calcium ions, blood pressure regulation and hypertension: a reassessment and a hypothesis. Am. J. Physiol. C232:165-173.

Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.

Brigden, J.L. and C. Riddell, 1975. A survey of mortality in four broiler flocks in Western Canada. Can. Vet. J. 16:194-200.

Buenrostro, L.J. and F.H. Kratzer, 1982. A nutritional approach to the "flip-over" syndrome. Pages 76-79 in Western Poult. Dis. Conf. Proc.

Carew, L.B., Jr., R.H. Machemer, Jr., R.W. Sharp and D.C. Foss, 1972. Fat absorption by the very young chick. Poultry Sci. 51:738-742.

Cassidy, D.H., H.A. Gibson and F.G. Proudfoot, 1975. The histology of cardiac blood clots in chicks exhibiting the "flip-over" syndrome. Poultry Sci. 54:1882-1886.

Classen, H.L., G.L. Campbell and R.K. McMillan, 1982. The influence of low nutrient density diets on chicken broiler productivity. Poultry Sci. 61:1438-1439, (Abstr.).

Crawford, M.A., P. Stevens, G. Williams and R.W.D. Turner, 1981. Dietary fats and heart disease in Progress in Lipid Research 20:589-593, ed. T. Holman, Pergamon Press Ltd.

Cunnane, C.S., 1982. Differential regulation of essential fatty acid metabolism to the prostaglandins: Possible basis for the interaction of zinc and copper in biological systems. Prog. Lip. Res. 21:73-90.

Deaton, J.W., J.L. McNaughton, F.N. Reece and B.D. Lott, 1981. Abdominal fat of broilers as influenced by dietary level of animal fat. Poultry Sci. 60:1250-1253.

Folch, J., N. Less and C.H. Sloane-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Folkov, B., 1982. Physiological aspects of primary hypertension. Physiol. Rev. 62:347-504.

Freeman, C.P., 1976. Digestion and absorption of fat. Pages 117-142 in Digestion in the Fowl, ed. by K.N. Boorman and B.M. Freeman, British Poultry Science Ltd., Edinburgh, Scotland.

Freeman, C.P., 1984. The digestion, absorption and transport of fats-non ruminants. Pages 105-122 in Fats in Animal Nutrition, ed. by J. Wiseman, Butterworths, England.

Frigg, M. and G. Brubacher, 1976. Biotin deficiency in chicks fed a wheat-based diet. Inter. J. Vit. Nutr. Res. 46:314-321.

Frings, C.S. and R.T. Dunn, 1970. A colorimetric method for determination of total serum lipids based on sulphosphospho-vanillin reaction. Am. J. Clin. Path. 53:89-91.

Galigher, A.E. and E.N. Kozloff, 1971. Essentials of Practical Microtechnique. Lea and Febiger, Philadelphia.

Gardiner, E.E. and J.R. Hunt, 1984. Effect of dietary reserpine on the incidence of sudden death syndrome in chickens. Can. J. Anim. Sci. 64:1015-1018.

Gasperdone, H., 1981. Feeding schedules and "flippers". Canada Poultryman 68(12):34-36.

Griffiths, L., S. Leeson and J.D. Summers, 1977. Influence of energy system and level of various fat sources on performance and carcass composition of broilers. Poultry Sci. 56:1018-1026.

Guyton, A.C., T.G. Coleman, D.B. Young, T.E. Lohmeier and J.W. De Clue, 1980. Salt balance and long-term blood pressure control. A. Rev. Med. 31:15-27.

Haghighi-Rad, F. and D. Polin, 1982. Lipid: the unidentified factor for alleviating fatty liver syndrome. Poultry Sci. 61:2075-2082.

Hemsley, L.A., 1965. "The Fatty Liver and Kidney Syndrome" of young chickens. Vet. Rec. 77:467-472.

Hillard, B.L., P. Lundin and S.D. Clarke, 1980. Essentiality of dietary carbohydrate for maintenance of liver lipogenesis in the chick. J. Nutr. 110:1533-1542.

Holman, R.T., 1971. Biological activities of and requirements for polyunsaturated acids. Progr. Chem. Fats Other Lipids 9:607-682.

Hood, R.L., 1980. Fatty Liver and Kidney Syndrome in broiler chickens: An Australian Experience. Feedstuffs 52(53):13-15.

Hopkins, D.T. and M.C. Nesheim, 1967. The linoleic acid requirement of chicks. Poultry Sci. 46:872-881.

Horrobin, D.F. and S.C. Cunnane, 1981. Is the triene/tetraene ratio always a valid indicator of functional essential fatty acid deficiency in Progress in Lipid Research 20:831-833, ed. T. Holman, Pergamon Press Ltd.

Howell, J., 1972. Flip-over disease. Report of the 25th Meeting of the Canadian Poultry Disease Conference held at the Animal Diseases Research Institute, Hull, Quebec.

Hulan, W.H., F.G. Proudfoot and K.B. McRae, 1980. Effect of vitamins on the incidence of mortality and acute death syndrome ("flip-over") in broiler chicks. Poultry Sci. 59:927-931.

Hunt, J.R. and E.E. Gardiner, 1982. Effect of various diets on the incidence of acute death syndrome ("Flip-Over") of chickens. Poultry Sci. 61:1481, (Abstr.).

Hwang, D.H., M.M. Mathias, J. Dupont and D.L. Meyer, 1975. Linoleate enrichment of diet and prostaglandin metabolism in rats. J. Nutr. 105:995-1002.

Jackson, C.A.W., D.J. Kingston and L.A. Hemsley, 1972. A total mortality survey of nine hatches of broiler chickens. Aust. Vet. J. 48:481-487.

Karmazyn, M. and N.S. Dhalla, 1983. Physiological and pathophysiological aspects of cardiac prostaglandins. Can. J. Physiol. Pharmacol. 61:1207-1225.

Krogdahl, A. 1985. Digestion and absorption of lipids in poultry. J. Nutr. 115:675-685.

Kubena, L.F., B.D. Lott, J.W. Deaton, F.W. Reece and J.D. May, 1972. Body composition of chicks as influenced by environmental temperature and selected dietary factors. Poultry Sci. 51:517-522.

Kubena, L.F., T.C. Chen, J.W. Deaton and F.N. Reece, 1974. Factors influencing the quantity of abdominal fat in broilers. Poultry Sci. 53:974-978.

Leclerq, B., I. Hassan and J.C. Blum, 1974. The influence of force-feeding on the transport of plasma lipids in the chicken (Gallus gallus L.). Comp. Biochem. Physiol. 47:289.

Leeson, S. and J.D. Summers, 1976. Fat ME values: The effect of fatty acid saturation. Feedstuffs 48(46):26-28.

Lehninger, L.A., 1982. Principles of Biochemistry. Worth Publishers, Inc., New York.

Leveille, G.A., A.S. Feigenbaum and H. Fisher, 1960. The effect of dietary protein, fat and cholesterol on plasma cholesterol and serum protein components of the growing chisk. Arch. of Biochem. and Bioph. 86:67-70.

Leveille, G.A., D.R. Romsos, Yu-Yan Yeh and E.K. O'Hara, 1975. Lipid biosynthesis in the chick. A consideration of site of synthesis, influence of diet and possible regulatory mechanisms. Poultry Sci. 54:1075-1093.

Marion, J.E., H.M. Edwards, Jr. and J.C. Driggers, 1961. Influence of diet on serum cholesterol in the chick. J. Nutr. 74:171-175.

Menge, H., 1968. Linoleic acid requirement of the hen for reproduction. J. Nutr. 95:578-582.

Menge, H., 1970. Comparative requirements of linoleic acid for male and female chicks. Poultry Sci. 49:178-183.

Metcalfe, L.D. and A.A. Schmitz, 1961. The rapid preparation of fatty acid esters for gas chromatography analysis. Anal. Chem. 33:363-364.

Metcalfe, L.D., A.A. Schmitz and J.R. Pelka. 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal. Chem. 38:514-515.

Mollison, B.J., 1983. Studies of some possible nutritional aspects of sudden death syndrome and abdominal fat pad deposition in the broiler chicken. Thesis, Univ. of Manitoba.

Mollison, B., W. Guenter and B.R. Boycott, 1984. Abdominal fat deposition and sudden death syndrome (SDS) in broilers: The effects of restricted intake, early life caloric (fat) restriction and calorie: protein ratio. Poultry Sci. 63:1190-1200.

Moses, G., E. Ollvero and T.F. Draisey, 1975. Simultaneous determination of serum cholesterol and triglyceride after preliminary column chromatography. Clin. Chem. 21:428-431.

National Research Council, 1977. Nutrient Requirements of Poultry, 7th revised edition. National Academy of Science, Washington, D.C.

Newberry, R.C., E.E. Gardiner and J.R. Hunt, 1985. The behaviour of chickens dying from sudden death syndrome. Page 51 in Can. Soc. Anim. Sci. Proc., 35th Annual Conf. (Abstr.).

Nir, I., V. Levy and M. Perek, 1973. Response of plasma glucose, free fatty acids and triglycerides to starving and re-feeding in cockerels and geese. Br. Poultry Sci. 14:263-268.

Ockner, R.K. and J.A. Manning, 1972. A binding protein for fatty acids in cytosol of intestinal mucosa, liver, myocardium and other tissues. Science 77:56-58.

Ockner, R.K. and J.A. Manning, 1974. Fatty acid-binding protein in small intestine. Identification, isolation and evidence for its role in cellular fatty acid transport. J. Clin. Invest. 54:326-338.

Ononiwu, J.C., R.G. Thompson, H.C. Carlson and R.J. Julian, 1979a. Studies on effect of lighting on "Sudden Death Syndrome" in broiler chickens. Can. Vet. J. 20:74-77.

Ononiwu, J.C., R.G. Thompson, H.C. Carlson and R.J. Julian, 1979b. Pathological studies of "Sudden Death Syndrome" in broiler chickens. Can. Vet. J. 20:70-73.

Pass, D.A., 1983. A cardiomyopathy ("Sudden Death Syndrome") of adult hens. Avian Pathology 12:363-369.

Payne, C.G., P. Gilchrist, J.A. Pearson and L.A. Hemsley, 1974. Involvement of biotin in the fatty liver and kidney syndrome of broilers. Br. Poult. Sci. 15:489-498.

Proudfoot, F.G. and H.W. Hulan, 1982. Effects of reduced feeding time using all mash or crumble-pellet dietary regimes on chicken broiler performance, including the incidence of acute death syndrome. Poultry Sci. 61:750-754.

Proudfoot, F.G., H.W. Hulan and K.B. McRae, 1982. The effect of crumbled and pelleted feed on the incidence of sudden death syndrome among male chicken broilers. Poultry Sci. 61:1766-1768.

Proudfoot, F.G. and H.W. Hulan, 1983. Effects of dietary aspirin (acetylsalicylic acid) on the incidence of sudden death syndrome and the general performance of broiler chickens. Can. J. Anim. Sci. 63:469-471.

Proudfoot, H.W. Hulan and K.B. McRae, 1984. The effects of dietary micronutrient, fat and protein components in pelleted feeds on the incidence of sudden death syndrome and other traits among male broiler chickens. Can. J. Animal Sci. 64:159-164.

Puska, P. and J.M. Iacono, 1985. Blood pressure and dietary fat. Pages 230-248 in Handbook of Hypertention, Vol. 6: Epidemiology of Hypertension. Elsevier Science Publishers B.V.

Reid, M.E., J.G. Bien, P.A. Mack and E.L. Andrews, 1964. Nutritional studies with the guinea pig. X. Determination of the linoleic acid requirement. J. Nutr. 82:401-408.

Renner, R. and F.W. Hill, 1960. The utilization of corn oil, lard and tallow by chickens of various ages. Poultry Sci. 39:849-854.

Renner, R. and F.W. Hill, 1961a. Utilization of fatty acids by the chicken. J. Nutr. 74:259-264.

Renner, R. and F.W. Hill, 1961b. Factors affecting the absorbability of saturated fatty acids in the chick. J. Nutr. 74:254-258.

Riddell, C. and J.P. Orr, 1980. Chemical studies of the blood and histological studies of the heart of broiler chickens dying from acute death syndrome. Avian Dis. 24:751-757.

Riddell, C. and R. Springer, 1985. An epizootiological study of acute death syndrome and leg weakness in broiler chickens in western Canada. Avian Dis. 29:90-102.

Roland, D.A. and H.M. Edwards, Jr., 1971. Effect of essential fatty acid deficiency and type of dietary fat supplementation on biotin-deficient chicks. J. Nutr. 101:811-818.

Rotter, B., W. Guenter and B.R. Boycott, 1985a. Sudden death syndrome in broilers: dietary fat supplementation and its effect on tissue composition. Poultry Sci. 64:1128-1136.

Rotter, B., W. Guenter and B.R. Boycott, 1985b. Contribution of dietary tallow to the incidence of sudden death syndrome in broiler chicks. Poultry Sci. (unpublished).

Rotter, B., W. Guenter and B.R. Boycott, 1985c. Sudden death syndrome in broilers: effects of different dietary fats on the incidence and tissue composition. Poultry Sci. (unpublished).

Rudas, B., G. Wick and R.K. Cole, 1972. Serum lipid pattern in chicks of the obese strain. J. Endocrinol. 55:609-615.

SAS Institute Inc., 1982. SAS User's Guide: Basic edition. Cary, NC: SAS Institute Inc. 923 p.

Scott, M.L., 1981. Importance of biotin for chickens and turkeys. Feedstuffs 53(8):59-67.

Scott, M.L., M.C. Nesheim and R.J. Young, 1982. Nutrition of the Chicken. M.L. Scott and Associates, Ithaca, NY.

Seifer, S., S. Dayton, B. Novic and E. Muntwyler, 1950. The estimation of glycogen with anthrone reagent. Arch. of Biochem. 25:191-200.

Snedecor, G.W. and W.G. Cochran, 1980. Statistical methods. 7th edition. Iowa State Univ. Press, Ames, IA.

Speich, M., B. Bousguet and G. Nicolas, 1980. Concentrations of magnesium, calcium, potassium and sodium in human heart muscle after acute myocardial infarction. Clin. Chem. 26:1662-1665.

Stallings, K.K., B.C. Dilworth, S. Omar and E.J. Day, 1985. Biotin supplementation of wheat diets fed to broilers. Poultry Sci. 64:42 (Abstr.).

Steele, P. and J. Edgar, 1982. Importance of acute death syndrome in mortalities in broiler chicken flocks. Austr. Vet. J. 58:63-66.

Steele, P., J. Edgar and G. Doncon, 1982. Effect of biotin supplementation on incidence of acute death syndrome in broiler chickens. Poultry Sci. 61:909-913.

Sturkie, P.D., 1976. Heart and circulation. Pages 76-101 in Avian Physiology, 3rd ed. Springer-Verlag, New York, Heidelberg, Berlin.

Summers, J.D., 1984. The extra caloric value of fats in poultry diets. Pages 265-276 in Fats in Animal Nutrition, ed. by J. Wiseman, Butterworths, England.

Thompson, H.R. and J. Blanchflower, 1971. Wet-ashing apparatus to prepare biological materials for atomic absorption spectrophotometry. Lab. Prac. 20(II):859-861.

Vo, K.V., Boone, M.A. and Torrence, A.K., 1978. Electrolyte content of blood and bone in chickens subjected to heat stress. Poultry Sci. 57:542-544.

Volk, M., M. Herceg, B. Marzan, M. Kralj, S. Meknic and V. Tadic, 1974. Fatal syncope in broilers: occurrence, clinical symptoms: lesions and pathogenesis. Vet. Arch. 44:14-23.

Wells, J.W. and P.A.L. Wight, 1971. The adrenal glands. Pages 489-516 in Physiology and Biochemistry of the Domestic Fowl, ed. D.J. Bell and B.M. Freeman, Academic Press Inc., London and New York.

Whitehead, C.C. and C. Fisher, 1975. The utilization of various fats by turkeys of different ages. Br. Poultry Sci. 16:481-485.

Whitehead, C.C. and C.J. Randall, 1982. Interrelationship between biotin, choline and other B-vitamins and the occurrence of FLKS and SDS in broiler chickens. Br. J. Nutr. 48:177-183.

Wight, P.A.L. and W.G. Siller, 1975. The histopathology of fatty liver and kidney syndrome in chicks. Res. Vet. Sci. 19:173-184.