AN ABSTRACT OF THE THESIS OF

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Title: Dietary Fish Meal and the Incidence of Sudden

Death Syndrome in Broiler Chickens

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Five experiments were conducted to investigate the

effect of dietary fish meal on the incidence of Sudden Death

Syndrome (SDS) in broiler chickens. Growth, feed

efficiency, total mortality and mortality due to SDS were

evaluated. Necropsy examination was performed on broilers

suspected of dying from SDS. Liver lipid and fatty acid

composition of liver lipid were analyzed to investigate the

possible biochemical changes occurred in SDS broilers.

Typical characteristics of SDS broilers were defined

(Exp. 1-5). The male broilers were more susceptible to SDS

than were females. Peak mortality was observed between 2-4

weeks of age. Body weight, feed conversion and total

mortality were not affected by dietary inclusion of varied

levels and types of fish meal (Exp. 1-5), except for Exp. 5

where broilers fed the diet containing 2.5% fish meal had

higher body weight compared to broilers fed corn-soy diet.

The effect of dietary treatments on mortality due to SDS was not consistent (Exp. 1-5). The incidence of SDS was similar for broilers fed diets containing 0 or 2.75% herring meal (Exp. 1). The amount of the dietary herring meal (0, 2.75 and 10%) did not affect mortality due to SDS. Different types of fish meal had different effects on the occurrence of SDS (Exp. 3). Variation in the incidence of SDS was not significant among broiler chicken strain crosses (Exp. 4). Additional biotin up to 500 ug/kg did not reduce the incidence due to SDS and dietary ASA at 0.2% increased mortality due to SDS (Exp. 5)

Liver lipid was similar between non-SDS and SDS broilers (Exp. 1-5). An elevated level of oleic acid and a decreased level of arachidonic acid were consistently observed in the liver lipids of SDS broilers (Exp. 1-5). Arachidonic acid is the direct precursor of prostaglandins which are involved in many physiological processes. Thus, a possible interrelationship among lipid metabolism, prostaglandins and SDS is suggested.

DIETARY FISH MEAL AND THE INCIDENCE OF SUDDEN
DEATH SYNDROME IN BROILER CHICKENS

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (CONTINUED)

DIETARY FISH MEAL AND THE INCIDENCE OF SUDDEN DEATH

SYNDROME IN BROILER CHICKENS

CHAPTER I

INTRODUCTION

A. What is Sudden Death Syndrome (SDS)?

Sudden Death Syndrome, abbreviated as SDS, is a

disorder mainly observed in broiler chickens, and

occasionally in broiler breeders (Brigden and Riddell, 1975;

Riddell and Brigden, 1976; Pass, 1983; Hopkinson et al.,

1983). It occurs in apparently healthy and well-nourished

birds. They show no sign of disease prior to the sudden

death. Affected birds unexpectedly jump in the air,

convulse, squawk, flip-over and die (Hopkinson et al., 1983;

Dendy, 1976). The majority of the SDS broilers are males

(Brigden and Riddell, 1975).

No specific signs/symptoms or lesions have been

identified on pathological and histological examination of

SDS birds, except for a full digestive tract and an empty

gall bladder (Cassidy et al., 1975; Ononiwu et al., 1979;

Hulan et al., 1980; Riddell and Orr, 1980). This suggests

that affected birds are able to consume feed normally until

the time of death (Ononiwu et al., 1979).

SDS occurs in broiler chickens of all ages. In broiler

breeders, it appears at the onset of sexual maturity.

Mortality due to SDS ranges from 0.5 to 9.62% in broiler
chickens (Jackson et al., 1972; Summers et al., 1987;
Gardiner et al., 1988), and 0.5 to 3% in broiler breeders
(Hopkinson et al., 1983).

B. Economic justifications

The incidence of SDS has continued to increase since it
was first recognized 20 to 25 years ago (Hemsley, 1965).
This increase seems to coincide with the rapid growth rate
in today's commercial broiler chickens (Eleazer, 1988). SDS
has been observed in many countries where other major
poultry diseases have been brought under control. In the
United States, SDS occurs in most broiler operations
(Buerostro and Kratzer, 1982) and is currently recognized as
the number one cause of broiler mortality (Haserbach &
Tagwerker, 1986). Lack of both the knowledge and a viable
control program has made SDS a major source of instant
profit loss. It causes tremendous economic burden for both
the commercial broiler grower and consumers.

In 1988, 5.24 billion broilers were produced in the
United States (Holleman and Majors, 1989). Based on this
figure, prevention of losses from SDS would result in an
estimated savings of $ 130-265 million dollars (based on
$ 0.85-1.90 production cost per broiler for bird 3-7 weeks
old) to the U.S. broiler industry. Therefore, investigation
to determine the cause of SDS is considered paramount
important if the problem is to be prevented.

C. Research objectives

Research on SDS is still at the exploratory stage. The involvements of hypothetical factors of nutritional, physiological and managerial nature have been implicated; however, findings are inconclusive. Limited information is available concerning the etiology of this syndrome, and the practical method of prevention is unknown at present.

A broiler chicken experiment (AGY 1707-83-4) conducted in the Department of Poultry Science at Oregon State University, showed that broiler chickens fed diet containing herring meal tended to have a higher mortality due to SDS (3.6%, 4/110) compared to those fed corn-soy basal diets (0%, 0/110). This observation motivated a more thorough investigation of this syndrome. The specific objectives of this dissertation are summarized as follows:

1. To define the characteristics of the SDS.

2. To examine the possible association between fish meal and the occurrence of SDS.

3. To evaluate the effects of the dietary fish meals at varying levels or from different sources on SDS.

4. To study the genetic implication (different strains) on the incidence of SDS.

5. To investigate the nutrient(s) that may be involved in the etiology of SDS.

6. To establish a mechanistic approach towards SDS research based on the knowledge obtained.

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CHAPTER II

REVIEW OF LITERATURE

Attempts have been carried out to unveil the mystery of Sudden Death Syndrome (SDS) in commercial broiler chickens. A review of the literature clearly indicates that the answer to this problem is complex and there may be more than one causative agent. Researchers in different disciplines have tried a wide range of approaches. None of the cited works in this review should be ignored simply because it did not define the etiology of SDS. It seems fair to state that etiology of SDS will only be solved through a multi-disciplinary approach and the integration of these findings.

This literature review is categorized into three major study areas: epizootiological, pathological and histological, and etiological.

Several different descriptive terms have been used to describe SDS: "Lung Edema", "Die in Good Condition", "Flip-Over", "Heart Attack" and "Acute Death Syndrome (ADS)". Such terms shall be considered synonymous since all of them appear to describe the same condition.

EPIZOOTIOLOGICAL STUDIES

SDS was first documented in England by Hemsley (1965).
However, Hemsley (1965) described the syndrome as "oedema in
the lungs", instead of SDS, because of the severe lung edema
observed in the affected broiler chickens. Mortality due to
this syndrome (0.46%) accounted for 23% of the total
mortality in Hemsley's survey which involved 100,000
broilers. The cause of mortality categorized as "oedema in
the lungs" included all deaths of unknown etiology where the
birds were in good condition but with lung edema. Male
broilers were more susceptible to this problem than were
females (74% vs. 26%). A marked difference in incidence was
observed among breed types. Progenies of less developed
breeds (Light Sussex crosses, "Chuncky") showed a lower
incidence than White Rock strains (Arbor Acres, Cobb and
Pilch).

In New South Wales, Australia, a similar condition was
found by Jackson et al., 1972. Their survey involved 69,068
chickens from day-old to processing. The term "die in good
condition", synonymous with "oedema in the lungs" (Hemsley,
1965), was used to describe those birds which had died
unexpectedly. It resulted in a flock mortality of 0.65%,
representing 15.6% of the total mortality. The affected
broilers showed one or more of the following three
conditions: 1) those that died from suffocation associated
with a panic; 2) those with similar necropsy findings but

without definite history of suffocation; and, 3) those with edema and/or congestion of the lung frequently accompanied by non-specific changes. Male broilers accounted for 72% of "die in good condition" birds.

Brigden and Riddell (1975) reported an excess acute mortality in 38,212 broiler chickens in Western Canada. Dead birds showed no specific cause of death on necropsy examination. Thus, the condition was named Acute Death Syndrome (ADS). Broilers classified as ADS included those which had died with good fleshing, full gastrointestinal tracts and no significant post-mortem lesion except for lung congestion or edema. ADS occurred as early as one week of age and continued throughout the growing period (8 weeks). Mortality increased rapidly once observed, peaked at the third week, and then declined by the eighth week. The average ADS mortality was 1.13%, representing 35% of the total mortality. More than 70% of the losses due to ADS occurred in male broilers.

In a survey of 51 broiler chicken flocks in Canada (Riddell & Springer, 1984), ADS and leg weakness were recognized as the two major causes of mortality. ADS birds were found dead on their backs, and the digestive tracts were full of feed. ADS was observed in broilers of all ages with mortality ranging from 0.71% to 4.07%. Peak ADS mortality occurred between 2-4 weeks of age in over 75% of the flocks. Male broilers accounted for 78% of ADS incidences.

Currently, SDS is considered an increasingly important problem for the poultry industry in many countries including the United States, the United Kingdom, Canada and Australia (Steele and Edgar, 1982). Thus, more research in the etiology of this syndrome is urgently needed.

PATHOLOGICAL AND HISTOLOGICAL STUDIES

A. Behavior of SDS Broiler Chickens Prior to Death

Observing the developing process can be a helpful tool in giving an insight into a disease problem. This is particularly true for SDS since no specific necropsy lesion has been associated with this problem. Moreover, SDS birds can not be identified before death occurs. Documenting the behavioral characteristics of affected chickens would greatly benefit the study of this syndrome by providing a mean to identify potential SDS birds.

By continuously video recording the behavior of chickens, researchers (Newberry et al., 1987) were able to capture the sequential activities of SDS broilers before, during and after the event took place. The video tapes containing the behavioral pattern of SDS chickens were analyzed to determine if SDS broilers showed any unusual behavioral traits during the final 12 hours preceding death.

The data collected from the video tapes indicated that all SDS chickens exhibited a sudden attack prior to death characterized by loss of balance, violent wing flapping and

strong muscular contractions. The average time from loss of balance to death was 53 ± 3.1 seconds. Affected birds fell forward or backward during the initial loss of balance and, most generally, turned over onto their backs or their sterna during the course of violent flapping.

No single behavior pattern or environmental event was associated with the onset of the sudden attack prior to death. No difference was observed between SDS chickens and their matched (healthy) controls in: behavioral patterns; proportion of time spent performing each behavior pattern and total percentage of time spent up on their feet (active) as opposed to laying down. The SDS chickens did not show signs of loss of appetite or lack of responsiveness to external stimuli indicative of ill-health. It was concluded that SDS chickens did not differ from healthy birds in gross observation. There was no consistent behavioral symptom which could be used to identify SDS chickens prior to death.

B. Symptoms

1) Broiler chickens

Since there is no specific symptoms available to diagnose the disease, SDS birds are generally characterized by the posture in which they are found along with the necropsy findings. The majority of SDS birds are found lying on their back with neck and feet extended. Generally, they are heavy, well-nourished, and apparently healthy. At the time of death, affected birds tend to jump in the air,

sometimes emit a loud squawk, give a few flaps and flip over
and die on their backs (Dendy, 1976; Buenrostro and Kratzer,
1982). The digestive tracts, particularly crop and gizzard,
in SDS broilers are full of feed, and the gall bladder is
usually empty (Steele and Edgar, 1982). This suggests that
the broilers dying of SDS eat normally right up to the time
of death, since the physiological function of the gall
bladder is to contract and supply bile rapidly to the
intestine during the process of digestion (Ononiwu et al.,
1979).

On necropsy examination, SDS broilers exhibit a pink
discoloration to the musculature and a pale abdominal
viscera. The contents of the intestine are milky and
suggestive of catarrhal enteritis (Brigden and Riddell,
1975). A blood structure is found in the heart chamber and
is of post-mortem origin as reported by Cassidy et al.
(1975):

> "The structures studied exhibited a smooth, shiny
> appearance and were molded to the shape of the wall of
> the chambers in which they were located... There was no
> evidence of attachment to the heart wall and the
> underlying endothelium showed no sign of damage.
> Microscopically, all specimens ... were composed of
> varying numbers of leucocytes and erythrocytes embedded
> in a matrix of serum and fibrin and no evidence of
> lysis was observed. The histological and histochemical
> examination of the clots failed to confirm them as
> thrombi."

Ononiwu et al. (1979) conducted a pathological study on
142 SDS broilers from six affected flocks. The lesions of
these birds at necropsy were generally similar. Severe
vascular congestion and edema were present in the lungs and

the frothy trachea contained an exudate. Hearts were somewhat enlarged. The intestines, particularly in the duodenum area, were markedly dilated, and the contents were pale and creamy. Livers were slightly enlarged, friable and pale. Gall bladders were discolored and empty. Kidneys were grayish and pale and thyroid gland, spleen and thymus were all congested.

Based on these histological findings, a hypothetical sequence of the pathological process in SDS was postulated (Ononiwu et al., 1979). When the course of a disease is acute, pathological lesions are generally associated with a vascular disturbance. Thus, it may be postulated that the development of SDS starts with circulatory lesions manifested by increased permeability of the peripheral circulatory system. Under the influence of certain physiological stress, a healthy normal capillary may become permeable, which is normally a reversible process. However, should the stimulus surpass the tolerance level, irreversible changes occurs resulting in damage. In the case of SDS, the cause may begin with the degenerative and inflammatory changes of the heart, which result in lung edema such that the chicken is unable to breathe. Sufficient fluid is lost from the circulatory system into the lung tissue spaces to result in the peripheral circulatory failure. Histological changes of intense congestion and edema of the lungs result in the tissue parenchyma becoming separated from fresh blood supply

leading to hypoxia. Much of the effective air space is lost because of engorgement of pulmonary capillaries. Lymphocytic infiltration, inflammation and edema fluid in lung tissue reduces gaseous exchange and enhances respiratory distress.

However, heart lesions and lung edema are not consistent features of SDS (Brigden and Riddell, 1975). Riddell and Orr (1980) conducted a series of histological studies to determine whether heart lesions were consistent symptoms of SDS. Their findings failed to agree with Ononiwu et al. (1979) in terms of the congestion and edema of the lungs. However, the lack of consistent histological lesions in SDS broilers did not rule out the possibility of ultrastructural or biochemical lesions in the heart that could result in the syndrome (Riddell and Orr, 1980).

Blood chemistry of SDS broilers was evaluated by Riddell and Orr (1980). No consistent differences were observed between the SDS and non-SDS birds. Blood lipid was similar for broilers dying from SDS and non-SDS broilers. Elevated levels of potassium, phosphorus, magnesium and glucose, and a decreased level of sodium were noted to occur in SDS birds. Similar changes were also found in non-SDS birds. These changes might be attributed to the movements of ions between the erythrocytes and sera (Coles, 1974).

Buenrostro and Kratzer (1982) compared the fatty acid profiles and biotin levels in livers between healthy broilers and broilers dying from SDS or Fatty Liver and

Kidney Syndrome (FLKS). Fatty acid analyses of liver lipids of FLKS birds showed an increase in palmitoleic (C16:1) and oleic (C18:1) acids, and a decrease in stearic (C18:0) and arachidonic (C20:4) acids. However, elevated palmitoleic acid level was not found in SDS birds, but a decrease in arachidonic acid was observed. A similar alteration has been observed by Rotter and Guenter (1985). Arachidonic acid level was lower in SDS birds. Liver biotin was lower in both SDS and FLKS birds when compared to normal birds. It was concluded that biotin deficiency was the nutritional factor implicated in SDS and FLKS.

2) Broiler breeders

Symptoms of SDS in broiler breeders are basically the same as those observed in broiler chickens. No overt signs of ill-health have been seen in breeder flocks prior to SDS outbreaks. Apparently normal breeders convulsed and died, particularly if startled. Most of SDS breeders died at feeding time (Hopkinson et al., 1983).

Post-mortem changes in broiler breeders were similar to that in broiler chickens. Hypertrophy of the heart and intense congestion of lungs, liver, spleen and ovaries were some of the changes observed. Histological changes present were minimal (Pass, 1983). Comparison of affected and unaffected flocks of the same age revealed significantly lower plasma potassium and phosphorus levels in the affected flock.

C. Mortality Distribution

SDS has been reported as a problem in many countries (Steele and Edgar, 1982). In the United States, this syndrome is present in most broiler operations (Buenrostro and Kratzer, 1982).

Buenrostro and Kratzer (1982) reported that the incidence due to SDS in a well managed broiler flock accounted for 50 to 75% of the total mortality, representing 2 to 3% of the flock mortality. The mortality is generally higher among males (over 70%) than females (Riddell and Brigden, 1976; Vock et al., 1974). A survey conducted in Nova Scotia, Canada, involving over 20,000 birds, recorded that mortality attributed to SDS averaged 3.22% in the male population and 1.11% in the females (Cassidy et al., 1975). SDS occurs at all ages, but the highest incidence appears to be when the birds are between 3 and 4 weeks of age (Buenrostro and Kratzer, 1982; Gardiner et al., 1988).

The incidence of SDS in broiler breeders (strain was not indicated in the literature cited) has been mainly found in Australia (Hopkinson et al., 1983 & 1984). Mortality generally occurs at the onset of sexual maturity. In some flocks, death due to SDS was observed before the first egg was laid. Peak mortality of up to 3% per week occurred at 20-30% rate of lay. Mortality then gradually declined to an acceptable level when the breeders reached 60% egg production. Flocks succumbing to SDS experienced mortality rates ranging from 0.5 to 3% per week throughout the laying

period. Subsequent egg production was always below the expected standard.

ETIOLOGICAL STUDIES

Although several factors have been implicated as the cause of SDS, the etiology of this syndrome can not be fully defined at this stage. However, investigations on SDS have provided helpful background information and have pointed to the need for further research.

A. <u>Growth Rate / Pelleting Factors</u>

Broiler chickens on a crumble-pellet dietary regimen will out perform birds on all-mash program (Calet, 1965). However, an increased incidence of SDS was observed in broilers fed crumble-pellet regimen compared with broilers fed the same diet in all-mash form (Proudfoot and Hulan, 1982). It was not clear from that initial experiment whether the growth rate itself or some pelleting factor(s) which enhanced growth rate was responsible for inducing an increased incidence of SDS. Thus, studies were conducted to investigate the involvement of growth rate on the incidence of SDS (Proudfoot <u>et</u> <u>al</u>., 1982). Different forms of feed (all-mash, ground crumble-pellet and crumble-pellet) were fed to broiler chickens, and the mortality rates were recorded.

Mortality due to SDS was significantly higher for broilers fed a crumble-pellet regimen in its original form or in a ground form when compared with birds fed all-mash diets. However, the growth rate of birds fed ground crumble-pellet diets was similar to that for birds fed all-mash regimen; both groups grew slower than birds on the crumble-pellet regimen. These results indicated that birds on a ground crumble-pellet regimen had growth rate similar to those on all-mash regimen (slower), but had SDS mortality similar to those on the crumble-pellet regimen (higher). Therefore, the authors concluded that the higher incidence of SDS was not caused by the stress of rapid growth, but rather, by some unidentified factor(s) resulting from the crumble-pelleting process.

Proudfoot et al. (1984) studied the effect of pelleted feed on the incidence of SDS by adding nutrients to the feed before or after the pelleting process. No difference in the occurrence of SDS was observed whether the micronutrients (a mixture of vitamins, d,l-methionine, minerals, ethoxyquin and amprolium) went through or by-passed the pelleting process. Similarily, by-passing the pelleting process with tallow had no effect on the incidence of SDS. The incidence of SDS was, however, numerically lower among broiler chickens fed a diet in which the soybean meal had been subject to the crumble-pelleting process. Although this difference was not statistically significant (P>.05), it warranted further investigation.

In a subsequent study, Proudfoot et al. (1984) demonstrated that the incidence of SDS in male broilers was reduced from 3.61 to 0.9% when broilers were fed a diet in which protein supplements (soybean meal, canola meal and fish meal) had by-passed the pelleting process. This suggested that some factor(s) derived from the pelletizing of the dietary protein components was involved in causing SDS. It was concluded that the pelleting process rather than the rapid growth rate resulting from the higher density of pelleted feeds was responsible for inducing SDS. However, the etiology of this observation, ie. the changes that resulted from pelleting the protein components which resulted in an increased incidence of SDS, was not defined.

B. Biotin and Other Vitamins

Proudfoot et al. (1976) suggested that vitamin K, which is an anti-hemorrhagic vitamin, might be involved in SDS since extensive hemorrhage of the kidney and blood clots were usually found in the SDS broilers. These investigators incorporated four different levels of vitamin K (1, 5, 10 and 20 mg/kg) into diets fed to broiler chickens and observed that overall mortality as well as mortality due to SDS was numerically lower among the broilers fed diets containing 10 mg/kg of vitamin K.

Hulan et al. (1980) fed broilers diets containing additional biotin, thiamine and pyridoxine and found that the incidence of SDS was reduced by the inclusion of

additional vitamins. Biotin supplementation at a level of

33 ug/kg in the diet reduced (P<.05) the incidence of SDS

compared to that for broilers fed the control diet (without

biotin supplementation). Steele et al. (1982) administered

20 and 100 ug/day biotin through the drinking water to

broiler chickens. Tissue concentrations of biotin were

measured to assess biotin status of affected broilers and

normal flock mates. They reported that additional biotin

supplementation did not affect either SDS mortality and

biotin levels in the liver of SDS or non-SDS broilers.

Whitehead and Randall (1982) also found that the occurrence

of SDS was unaffected by dietary biotin supplementation. No

explanation was offered in the latter two studies pertaining

to the differences of no effect of biotin on the incidence

of SDS as compared to the findings of Hulan et al. (1980).

The effects of other B-vitamins (choline, riboflavin

and pantothenic acid) and standard vitamin premix (Vitamin

Mix V-3) on the incidence of SDS have been investigated

(Whitehead and Randall, 1982). No dose-response relation-

ships between these vitamins and SDS were established.

C. Dietary Protein and Fat/Oil

Broiler chickens fed a 24% protein diet had a

significantly lower incidence of SDS than those fed a 19%

protein diet (Mollison et al., 1984). The reduced mortality

was suggested to be related to the fat content of the diet

or differences in the fat metabolism associated with a high

protein diet. Since broilers fed the higher protein rations tended to be leaner in terms of fat pad size, probably this reduction in fatness reduced the stress load on the heart and, consequently, reduced mortality due to SDS.

Riddell and Orr (1980) found elevated total serum lipids in some SDS individuals. This suggested a possible involvement of lipid metabolism in this syndrome. More research in this area is needed in order to draw a conclusion concerning the association between dietary fat (or lipid metabolism) and SDS.

Tissue fatty acid compositions are reflected by the dietary fat sources. The changes of fatty acid composition in SDS broilers suggested that fat metabolism and the dietary fat types might be involved in the etiology of SDS. Rotter and Guenter (1985) tested this hypothesis by incorporating tallow and/or sunflower oil (SFO) in broiler diets to make a comparison of fat sources from plant- or animal-origin. Their results indicated that dietary fat type did not have an effect (P>.05) on the total plasma lipid, triglycerides or cholesterol levels at 7 weeks of age. However, mortality due to SDS tended to be lower for broilers on the SFO diet than those fed a diet containing tallow. Fatty acid analyses of heart and liver of SDS and non-SDS birds revealed different fatty acid profiles. Palmitic (C16:0) and oleic (C18:1) acids were higher and linoleic (C18:2) and arachidonic (C20:4) acids were lower in SDS broilers compared to non-SDS broilers.

An explanation for the difference in fatty acid profile of SDS versus non-SDS broilers was offered by Rotter and Guenter (1985). They have suggested that the desaturation and elongation of the linoleic acid is competitively inhibited by oleic acid, resulting in reduced conversion of linoleic acid to arachidonic acid. Furthermore, this conversion is biotin dependent. Deficiency in biotin could lead to an inefficient production of arachidonic acid and result in lower tissue levels. This may be the reason biotin deficiency appeared to be associated with SDS in some cases (Hulan et al., 1980; Buenrostro and Kratzer, 1982) but not in others (Steele et al., 1982; Whitehead and Randall, 1982).

A major role of arachidonic acid is to serve as a direct precursor of prostaglandins which are essential for many physiological functions and have a variety of actions on cardiac tissue (Lands, 1982). As a conjesture, SDS observed in broilers may be the result of a sequential effect starting with a reduced conversion of linoleic acid to arachidonic acid, which would result in a reduced production of prostaglandins and lead to SDS (Rotter and Guenter, 1985).

Newberry et al. (1984) evaluated the possibility that the type of fat in the diet was related to SDS. The replacement of tallow with corn oil in the diets and fed to broilers did not reduce the incidence of SDS. Studies by Rotter et al. (1984) demonstrated that the incidence of SDS

was not affected by different dietary fat sources
(hydrogenated coconut oil, tallow, tallow+sunflower oil and
sunflower oil), nor did biotin supplementation reduce the
incidence of this syndrome.

D. Miscellaneous

    a. Stressors

Environmental factors such as sudden noise, ambient
temperature, social interaction, stocking density and
lighting programs have been suggested to increase the
incidence of SDS (Ononiwu et al., 1979; Hulan et al., 1980;
Cave, 1981; Hopkinson et al., 1983; Newberry et al., 1985 &
1986).

Apparently normal broiler breeders in a SDS susceptible
flock have been observed to convulse and die when startled
by sudden noise (Hopkinson et al., 1983). Steele and Edgar
(1982) reported that SDS birds were sometimes found in
groups near the feeding area. Possibly, the sudden noise
associated with the operation of the feeding equipment may
have startled the birds and caused some birds to succumb to
SDS. On the contrary, Newberry et al. (1985) observed that
SDS birds were scattered throughout the pens and that the
sudden on and off of the mechanical equipment (lights,
brooders and feeders) did not influence the incidence of
this syndrome.

Hopkinson et al. (1983) reported that SDS affected
flocks appeared to be more susceptible to heat stress than

other flocks. Mortality due to SDS was recorded to be higher on hot days.

Bolton et al. (1972) suggested that dense bird populations brought the risk of a higher incidence of the diseases. Ratcliff and Synder (1964) studied the incidence of myocardial infarction caused by social interaction in male and female chickens. Coronary arterial disease which results in infarction of the myocardium is a response to social interaction, especially the interaction associated with sexual behavior. The infarcts are attributed to limit blood flow to the heart muscle due to rapidly developing coronary arterial disease and also to the demand for high levels of myocardial activity. The majority of the broilers are raised without separating the sexes, and therefore, are subject to social interaction resulting stress.

Ononiwu et al. (1979) reported a higher incidence of SDS in broiler chickens reared under continuous light. Cave (1981) found no significant difference in a similar test. Hulan et al. (1980) suggested that high intensity light appeared to increase SDS, but Newberry et al. (1985 & 1986) concluded that light intensity had no effect on SDS.

The process by which light intensity may exert its effect on broiler chickens is unknown. Ononiwu et al. (1979) explained that if the intensity of illumination was above the optimum, it would induce cannibalism, excitement, fighting and piling. The effect of these vices would

probably adversely affect the more rapidly growing birds in a flock and result in SDS.

b. Minerals

Hopkinson et al. (1984) reported that SDS could be reproduced by manipulating the content of certain minerals (Na^+, K^+, Cl^-, Mg^{++}, I_2^{-2}, Mn^{++}, Zn^{++}, Cu^{++} and Mg^{++}) in the diets. When test diets containing lower levels of phosphorus, potassium, protein and energy than that in control diets were fed to broiler breeders, the incidence of SDS was successfully induced. At 24 and 28 weeks of age, plasma potassium and phosphorus levels from broiler breeders in test groups were lower ($P<.01$) than those for control birds. The lower than normal plasma values of these electrolytes had also been observed in field cases of SDS (Hopkinson et al., 1983).

Dietary fat can form insoluble calcium and magnesium soap in the digestive tract and decrease the retention of these minerals (Whitehead et al., 1971; Atteh et al., 1983; Atteh and Leeson, 1984). Julian (1986) theorized that SDS might be caused by acute hypomagnesemic tetany as the result of magnesium lost in the feces. However, when the test diets containing 0.2% calcium, 0.2% phosphorus and 0.2% magnesium were fed to broilers, the results indicated that the addition of these minerals had no significant effect on the incidence of SDS.

Hunt and Gardiner (1982) fed broiler chickens wheat-soy

diets supplemented with 0, 0.1, 0.2, and 0.3% potassium from K_2CO_3 to investigate the effect of additional potassium on SDS. Neither total mortality nor mortality due to SDS was influenced by the additional dietary potassium. Growth and feed consumption were significantly depressed by additional potassium. Mortality due to SDS as a percentage of total mortality was 55, 55, 48 and 57% with the increasing levels of dietary potassium, respectively.

c. Reserpine (3,4,5-trimethoxy-benzoyl methyl
   reserpate)

Reserpine is an anti-hypertensive and tranquilizing agent. This compound has been used as an anti-stress compound in chickens (Edens and Siegel, 1974) and has been reported to raise the level of circulating corticosterone in birds (Serbocan et al., 1972) and, enhance humoral response and the level of antibodies (Edens et al., 1975).

Gardiner and Hunt (1984) investigated the effect of low levels (0-3 mg/kg) of dietary reserpine on growth response and the incidence of SDS among broilers and roasters. Neither total mortality nor mortality due to SDS was affected by dietary reserpine. However, body weight was reduced (P<.05) in birds fed reserpine. It was concluded that this compound did have a physiological effect on the growth performance of chickens, although the effect might not be associated with SDS.

d. Aspirin, (Acetylsalicylic acid, ASA)

Aspirin has been reported to have beneficial effects on the cardiovascular system (Pick et al., 1979). Proudfoot and Hulan (1983) used ASA as a prophylactic drug and fed to broiler chickens at levels of 0.04, 0.08 and 0.16% of the diets to ascertain whether the drug would reduce the incidence of SDS. The results of this experiment showed that mortality due to SDS was not different (P>.05) for broilers fed diets containing ASA compared to birds fed the diets without ASA. They concluded that additional ASA (up to 0.16%) in broiler diets did not illicit a beneficial effect on the incidence of SDS.

e. Lactic Acid

SDS was speculated to be similar to acidosis in ruminants and laminitis in horses because of their resemblances in the following symptoms: lung edema, labored breathing, circulatory collapse, and acute death (Summers et al., 1987). It was hypothesized that a change in acid-base balance in the bird, resulting in increased plasma lactate, might be responsible for SDS. Thus, Summers et al. (1987) simulated a lactic acidosis condition in broilers in order to examine the possible involvement of lactic acid in SDS.

In the initial pilot study, 5 c.c. of a 20% lactic acid solution was pipetted into the crop of 2 weeks old male broilers. Within 30 seconds after administration, two birds squawked, jumped up and landed on their backs and died with

their feet up in a typical flip-over position. Similar
flip-over conditions were also observed in another group of
birds receiving lactic acid solution through wing vein
injections. However, such observations were not confirmed
in follow-up experiments where the lactic acid solution was
administered through either the feed or water. No
explanation was offered as to why broilers responded
differently to the lactic acid treatments. The authors
suggested that evidence had been accumulated to indicate
that SDS was a metabolic condition. They postulated that
alteration of acid-base balance rather than lactic acid per
se may be the factor triggering the SDS.

    f. Taurine

    SDS was proposed to be caused by heart damage (Ononiwu
et al ., 1979). Taurine is present in high concentration in
heart tissues. Therefore, the effects of dietary taurine
and guanidinoethyl sulfonate (GES, a taurine transport
inhibitor) on the incidence of SDS in broiler chickens were
studied (Blair et al., 1989; Jacob et al., 1989).

    The inclusions of up to 500 mg/kg taurine in broiler
diets did not reduce mortality due to SDS nor did
supplementations of GES at levels of 1.5% affect the taurine
levels in cardiac and brain tissues.

OTHER ASSOCIATED DISEASES

A. <u>Fatty Liver and Kidney Syndrome (FLKS)</u>

Fatty Liver and Kidney Syndrome (FLKS), is a disease
commonly observed in broiler chickens (Payne <u>et al</u>., 1974).
In general, affected birds are rapidly growing and are
heavier than their flock mates. The common characteristics
between FLKS and SDS birds are that both conditions are
usually associated with rapid growth. Both FLKS and SDS
birds show no indication of disease before death.
Apparently healthy birds may die of FLKS and SDS shortly
after imposition of stress (Steele <u>et al</u>., 1982; Hopkinson
<u>et al</u>., 1983). Finally, pinkish fat, enlarged and pale
liver and kidney are found in both FLKS and SDS birds.

However, FLKS differs from SDS in many ways (Steele and
Edgar, 1982; Hood, 1980; Payne <u>et al</u>., 1974; Wight and
Siller, 1975): (1) Stress is usually required to trigger
FLKS. This is not always true in the case of SDS. (2)
Hypoglycaemic coma precedes death in FLKS. Affected birds
become lethargic, they sit down and their heads slowly droop
to the floor, and they eventually die, whereas the SDS bird
remain alert and active until death. (3) Severe fatty
infiltration is always observed in the livers, kidneys and
hearts of FLKS birds, whereas such lesions are inconsistent
in SDS birds. And, (5) biotin deficiency is the main cause
of FLKS and the supplementation of biotin in the diets can

reduce FLKS, whereas effect of additional dietary biotin in reducing SDS has not been consistent.

B. Sudden Infant Death Syndrome (SIDS)

Sudden Infant Death Syndrome (SIDS), also referred to as "crib death", is defined as sudden and unanticipated death in an infant. With rare exception, the SIDS occurs in young infants between one to six months of age. As in the case of SDS in broiler chickens, the incidence of SIDS is higher for males than for females.

Several theories have been proposed as possible mechanisms of SIDS. Some of the hypotheses are: cardiovascular failure, respiratory system obstruction, imbalanced electrolytes (magnesium, potassium) and biotin deficiency (Guntheroth, 1982). These factors are similar to those that have been suggested as the causative agents of SDS. The levels of biotin were significantly lower in the livers of infants died of SIDS when compared to infants of similar age who died of known causes (Johnson et al., 1980). Since the course of SDS is similar to that of SIDS, knowledge on SDS may serve as helpful information for SIDS, and vice versa.

C. Ascites

Ascites, sometimes called "water belly", refers to the symptom of abnormal accumulation of fluid in the body cavity. Mortality from ascites runs from 5 to 15% (Dale,

1986). It occurs in chickens as young as 3 days old (Dale, 1986) and increases in severity over weeks and peaks at 7 weeks of age (Coello et al., 1987). Enlarged heart and liver are characteristic necropsy findings in birds with ascites. There has been much speculation as to the cause of ascites. Pelleted feed (as opposed to the mash feed), vitamin E and selenium deficiencies, and high fat/high density diet have been implicated as causes of ascites. Factors that promote growth rate also appear to increase the incidence of ascites (Dale, 1986).

Ascites and SDS are thought to be inversely related (Shane, 1986). It has been suggested that SDS is a precursor of ascites. SDS birds die acutely at an early stage (before 7 weeks of age) due to circulatory collapse. However, broilers that survive the initial growth period will later develop an accumulation of fluid in the body cavity (ascites) due to a heart defect.

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CHAPTER III

EXPERIMENTS 1 - 5:

EFFECTS OF DIETARY FISH MEAL AND STRAIN OF BROILER CHICKEN

ON THE INCIDENCE OF SUDDEN DEATH SYNDROME

Experiment 1: Dietary Herring Meal and the Incidence of
Sudden Death Syndrome in Broiler Chickens

Experiment 2: Effect of Dietary Levels of Herring Meal on
the Incidence of Sudden Death Syndrome in
Broiler Chickens

Experiment 3: Effect of Three Types of Fish Meal on the
Incidence of Sudden Death Syndrome in Broiler
Chickens

Experiment 4: Effect of Strain Crosses and Dietary Fish Meal
on the Incidence of Sudden Death Syndrome in
Broiler Chickens

Experiment 5: Effect of Dietary Biotin, Acetylsalicyclic
Acid and Fish Meal on the Incidence of Sudden
Death Syndrome in Broiler Chickens

EXPERIMENT 1

DIETARY HERRING MEAL AND THE INCIDENCE OF SUDDEN

DEATH SYNDROME IN BROILER CHICKENS

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ABSTRACT

An experiment was conducted to evaluate the effect of
herring meal on the incidence of Sudden Death Syndrome (SDS)
in broiler chicknes. A total of 800 broiler chicks was
randomly assigned to two dietary treatments: corn-soy (c-s)
and c-s+herring meal. Each treatment contained 8 replicates
with 50 broilers (25 males, 25 females) per replicate. Mean
body weight, feed conversion, total mortality and mortality
due to SDS were recorded. Creatine phosphokinase (CPK),
hematocrit and liver lipid were analyzed to investigate the
possible biochemical changes in the SDS birds.

Mean body weight and feed conversion were not different
(P>.05) between the two dietary treatments. Mortality due
to SDS was 2.75% in the group fed the c-s diet and 2.25% in
the c-s+herring group. Male broilers accounted for 80% of
the SDS deaths. Hematocrit readings and CPK values were not
different (P>.05) between the two dietary treatments. Liver
lipid was similar (P>.05) regardless of the dietary
treatments or causes of death. The fatty acid composition
of the liver were different for SDS and non-SDS broilers.
Palmitic (16:0) and oleic (18:1) acids were elevated, while
linoleic (18:2) and arachidonic (20:4) acids were lower for
SDS broilers compared to that of non-SDS broilers. This
difference was observed in all SDS broilers regardless of
the dietary treatments.

INTRODUCTION

The growth rate and feed efficiency of broiler chickens have been greatly improved during the past 30 years. Rapid growth rates in broiler chickens have resulted in a greater mortality due to Sudden Death Syndrome (SDS) (Martin, 1987; Bowes et al., 1988). SDS birds are apparently healthy and rapid growing (Bowes et al., 1988). Necropsy examinations do not identify any lesion specific to the syndrome. Factors suggested to be associated with SDS include: stress, the feed pelleting process and dietary vitamins and fat (Ononiwu et al., 1979; Hopkinson et al., 1984; Proudfoot et al., 1982; Whitehead and Randall, 1982; Rotter and Guenter, 1985). However, to date, there has been little reported information as to the possible cause of this syndrome. More research is needed to elucidate the etiology of SDS.

In a preliminary study in the Department of Poultry Science at Oregon State University, two groups of broilers (110 birds/group) were fed either a corn-soy (c-s) or c-s+herring meal diets. The results of the study showed that broilers fed the diet containing herring meal had a slightly higher incidence of SDS than broilers fed the c-s diet (3.6%, 4/110; 0%, 0/110). Therefore, this experiment was conducted to examine whether the higher incidence of SDS in the preliminary study was associated with dietary fish meal and to investigate the possible biochemical changes that might occur at the cellular level in the liver and blood.

MATERIALS AND METHODS

Eight hundred Hubbard broiler chicks were feather-sexed at the hatchery and randomly assigned to two dietary treatments. Each treatment consisted of 8 replicates with 50 chicks per replicate (25 males, 25 females). The chicks were fed either corn-soy (c-s) basal diet or c-s+2.75% herring meal diets (Table III.1.1.) from day-old to 7 weeks of age. Feed and water were provided _ad_ _libitum_ throughout the experiment.

The broilers were housed in an enclosed positive pressure mechanically ventilated house. Each pen (1.22 m x 2.44 m), or replicate, was equipped with an infra-red heat lamp, an automatic waterer (diameter 16.5 cm) and tube feeder (diameter 30.5 cm). The pens were covered with clean wood shaving (5 cm deep). The infra-red heat lamps were left on from day-old to approximately three weeks of age corresponding to the brooding period. Overhead continuous incandescent light (5.4 lux) was provided throughout the experiment.

Body weight and feed conversion were determined at 4 and 7 weeks of age. Mortality was recorded daily. Broilers suspected of dying from SDS were sent to the Diagnostic Laboratory at the College of Veterinary Medicine, Oregon State University, for necropsy examination. The livers were removed from broilers identified as dying from SDS and stored at $-17^{\circ}C$ for subsequent lipid and fatty acid

analyses. Non-SDS broilers from both dietary treatments were killed at the end of 7 weeks of age, and the livers were removed for total lipid and fatty acid analyses.

Total lipid content was determined by the methods described by Bligh and Dyer (1959) and Lowry (1977). Thawed and wet liver tissues (2 gm) were homogenized (using a modified Omni-Mixer) in 10 ml of chloroform and methanol (2:1). The homogenates were filtered, diluted with water (6.7 ml) and centrifuged for 10 min to separate the homogenate into two layers. The chloroform layer contained the lipids, and the methanol layer contained the non-lipids. The methanol layer was aspirated into a trap and discarded. The chloroform layer was taken to dryness using a $50^{\circ}C$ heating block. The residue was immediately redissolved in 5 ml of chloroform and weighed. A 100 ul of the chloroform was pipetted in an aluminum weighing pan. The chloroform was evaporated to dryness with the heating block. The lipid content was calculated and expressed as % of the sample weight.

Fatty acid analyses were performed by the method as outlined by Lowry (1977). The lipid extract (1 ml, from total lipid determination), ethyl ether (2 ml) and HCl-methanol (2 mls) were capped and heated for 90 min (using a heating block). At the end of the heating period, the samples were cooled and extracted with water (2 ml) and hexane (2 ml). The solution was centrifuged for 4 min to separate the two layers. The upper phase was pipetted into

a clean tube. The remaining was subjected to the second extraction procedure where 3 ml of hexane-ether (1:1) mixture was used. The upper phase was removed and combined with that from the first extraction and dried using heating block. The residue was immediately redissolved in hexane (1 ml). Five microliters of the hexane were injected into a gas liquid chromatography (Nupro Co., Cleveland, OH) to determine the fatty acid profile. A 61 m, 0.076-cm ID ethylene glycol succinate column was used with helium as a carrier gas at a flow rate of 3.4 ml/min. The injector temperature was maintained at $220^{O}C$. Total analysis time per sample was 60 min. The detector ports were maintained at $225^{O}C$. The fatty acid methyl esters were identified by comparison with Nu-Chek-Prep#15A standard mixture (Elysian, MN) and expressed as weight percentages.

The venipuncture method was used to obtain blood samples from the wing veins of 7 weeks old broilers from both dietary treatments (10 males and 10 females of each) for hematocrit determination. The blood samples were collected directly into capillary tubes, and the ends of the tubes were immediately sealed with a clay sealer (Seal-Ease, Clay Adams, Parsippany, NJ). The tubes were centrifuged for 5 min, and the hematocrit values were then read on the microhematocrit reader (Phillips-Drucker, OR) and reported as a percentage.

At the end of feeding period, 20 broilers (10 males and 10 females) from each dietary treatment were randomly

chosen, and blood samples were obtained for creatine phosphokinase (CPK) assays. The wing vein was punctured with a 21 G, 3.8 cm needle, and the blood was collected directly into heparinized tubes. The blood samples were then subject to 5 min centrifugation at 2,000 rpm, -8.8°C (using Beckman Model TJ-R Gefrigeration unit, Beckman Instruments, Inc., CA) to separate plasma and corpuscles (red blood cells). The plasma (upper layer) was then pipetted into a clean tube. Plasma specimen (.05 ml) was pipetted into vial containing 2.5 ml working solution (Cat. No. 126322, Single Vial CK-NAC kit, Boehringer Mannhein Diagnostics, Indianapolis, IN) and mixed well by gentle inversion. The mixture was quantitatively measured by the Bausch and Lomb spectrophotometer (SPECT 2000, Bausch & Lomb Co., Rochester, NY) using a wavelength of 340 nm and temperature of 30°C to determine the enzymatic activity. The values obtained were expressed as international enzyme unit.

The data were subject to one-way analysis of variance (Steel and Torrie, 1980). Arcsin square root transformations were made on percentage data (outside 25 to 75% range) prior to analysis. Significant difference between treatment means were separated by the least significant difference (LSD) test.

RESULTS

Mean body weight and feed conversion at 4 and 7 weeks of age and mortality to 7 weeks of age are presented in Table III.1.2. No difference (P>.05) was observed between the two dietary treatments for mean body weight or feed conversion. Total mortality and mortality due to SDS were similar for broilers fed a c-s or c-s+herring meal diets. The incidence of SDS accounted for 50% of the total mortality for the herring meal group and 46% for the c-s group. SDS occurred in chicks as young as 5 days of age and peaked when the broilers reached 2-3 weeks of age. Eighty percent of the SDS broilers were males.

The CPK, hematocrit and liver lipid data are presented in Table III.1.3. No differences (P>.05) were observed for CPK and hematocrit readings between c-s and the c-s+herring meal groups. Liver lipid content was similar (P>.05) for all broilers regardless of dietary treatments or the causes of death.

Liver lipids of SDS broilers tended to have higher (P>.05) levels of palmitic (16:0) and oleic (18:1) acids, and lower level of arachidonic acid (20:4) compared to that for non-SDS broilers regardless of dietary treatments (Table III.1.4.) A similar fatty acid pattern (higher 16:0 and 18:1, and lower 20:4) was observed from the liver lipids of non-SDS broilers which had beed fed a diet containing herring meal compared to that for broilers fed the c-s diet.

DISCUSSION AND CONCLUSION

SDS was not documented until modern meat type broilers were developed. The affected birds are normally heavier than their flock mates. The difference in fatty acid composition of liver lipid between SDS and non-SDS birds obtained in this study confirm that reported by others (Buerostro and Kratzer, 1982; Rotter and Guenter, 1985). These observations suggest that both genetic and nutritional factors may be associated with the occurrence of SDS.

Modern strains of broilers were developed for rapid growth. Furthermore, nutritionally well-balanced feeding programs have been developed to maximize this genetic potential. Synchronizing physiological supporting systems with this rapid rate of growth presents a major challenge. The rapid growth rate causes physiological disorders leading to SDS and leg weakness (Martin, 1987), which are the two major problems in the commercial production of broiler chickens today (Riddell and Springer, 1984).

The main function of red blood cell is to transport oxygen to the tissue. Abnormally low level of red blood cells will result in hypoxia (Brown, 1980). Ononiwu et al. (1979) suggested that SDS was, in part, due to lung hypoxia. The hematocrit reading of the experimental birds in this study was similar to the value (32%) reportd by Sturkie (1976). It was unlikely that the broilers in this study were subject to stresses which might alter normal blood

production. However, since the blood samples were collected only from normal broilers due to the fact that SDS birds could not be identified prior to the death, the blood chemistry of a bird before and after SDS occurs should be examined and compared when a method of identifying SDS birds prior to death has been developed.

CPK is an energy transfer enzyme, the activity of which increases during the myocardial infarction. Thus, CPK measurements are used to assess the extent of myocardial injury after infarction. CPK levels obtained from non-SDS broilers in this study were similar for the two dietary treatments. Thus, there was no indication that dietary treatments affected the biological status of the broilers rendering them more susceptible to myocardial damage. However, CPK levels were higher in male broilers than in female broilers, a heritable characteristic. This may partially explain why male broilers are more susceptible to SDS, if indeed, myocardial injury is involved in the etiology of this syndrome.

Liver lipid was similar for non-SDS and SDS broilers regardless of dietary treatments. The rates of synthesis and degradation of lipid in the liver were normal. This does not agree with the suggestion by Hood (1980) that SDS and Fatty Liver and Kidney Syndrome (FLKS), where fatty liver is a profound characteristic, are the same disorder.

Although the total lipid content of the livers was not affected by the dietary treatments or the causes of death,

fatty acid compositions of the liver lipid were different between non-SDS and SDS birds. Palmitic (16:0) and oleic (18:1) acids tended to be higher, and linoleic (18;2) and arachidonic (20:4) acids tended to be lower for SDS birds compared to that for non-SDS birds. A similar finding was reported by Rotter and Guenter (1985) that liver lipid of SDS birds showed increased levels of 18:1 and lower levels of 18:2 and 20:4 fatty acids. Because of these observations, lipid metabolism may be associated with SDS. Further investigation as to the involvement of lipid metabolism and SDS is indeed justified.

Table III. 1.1. Composition of corn-soy (c-s) and
c-s+herring meal diets (Exp. 1)

Ingredients	c-s	c-s+2.75% herring meal
	---------- (%) ----------	
Yellow corn	68.05	69.95
Soybean meal (47.5%CP)	21.45	17.20
Herring meal	-	2.75
Animal fat	0.90	0.50
Meat & bone meal	6.87	6.87
Alfalfa meal (17%CP)	2.00	2.00
Salt (iodized)	0.30	0.30
Trace mineral premix[1]	0.05	0.05
Vitamin premix[2]	0.20	0.20
d,l methionine (98%)	0.08	0.08
Baciferm[3]	0.05	0.05
Zoamix[4]	0.05	0.05
Calculated analyses:		
Crude protein, %	20.0	20.1
Metabolizable energy, kcal/kg	3068	3088

[1]Provided per kg of diet: Ca, 97.5 mg; Mn, 60 mg; Fe, 20 mg; Cu, 2 mg; Zn, 27.5 mg; Co, 2 mg.

[2]Provided per kg of diet: Vitamin A, 3300 IU; Vitamin D3, 1100 IU; riboflavin, 3.3 mg; d-pantothenic acid, 5.5 mg; niacin, 22 mg; choline, 190.9 mg; vitamin B12, 5.5 mcg; vitamin E, 1.1 IU; vitamin K, 55 mg; folic acid, 22 mg; ethoxyquin, 0.06 g.

[3]Gratuitously provided by International Mineral & Chemical Corp., Mundelein, IL 60060.

[4]Gratuitously provided by Salsbury Laboratories, Charles City, IA 50616-9989.

Table III.1.2. Effect of feeding broiler chickens a corn-soy (c-s) or c-s+ herring meal diets on mean body weight, feed conversion, total mortality and mortality due to Sudden Death Syndrome (SDS) to 7 weeks of age (Exp. 1)

Dietary treatments	Body weight[a]		Feed conversion[a]		Mortality (M) to 7 weeks[a]		
	4-week	7-week	4-week	7-week	Total (T)	SDS	SDS, % of TM
	(gm)				(%)	(%)	
c-s	794	1865	1.67	2.07	6.00(24)[1]	2.75(11)	46
c-s+herring	814	1910	1.79	2.05	4.50(18)	2.25(9)	50
Pooled SE[2]	18	23	.10	.02	1.82	.90	

[a]There was no significant difference (P>.05) between treatment means within the variables.

[1]( ) Number of birds.

[2]Standard error

Table III.1.3. Levels of plasma creatine phosphokinase (CPK), percent hematocrit and liver lipid of broiler chickens fed corn-soy (c-s) and c-s+ herring meal diets (Exp. 1)

Dietary treatments	CPK (mU)[a]		Hematocrit, %[a]		Liver lipid, %[a]	
	Male	Female	Male	Female	Non-SDS	SDS
c-s	1736	1227	32	32	4.9	5.3
c-s+herring	1754	1372	31	30	4.7	4.2
Pooled SE[1]	140	173	1.18	.87	.35	.39

[a]No significant difference (P>.05) between treatment means within variables.

[1]Standard error

Table III.1.4. Fatty acid composition of liver lipids of non-SDS (Sudden Death Syndrome) and SDS broiler chickens fed a corn-soy (c-s) or c-s+herring meal diet (Exp. 1)

Cause of death	Dietary treatments	Fatty acid composition[a], weight %						
		16:0	18:0	18:1	18:2	18:3	20:0	20:4
Non-SDS	c-s	20.90	23.25	14.95	17.18	.45	.40	17.32
	c-s+herring	24.02	21.00	20.90	15.15	.38	.60	11.01
Pooled SE[1]		1.44	.92	1.49	1.14	.27	.20	2.41
SDS	c-s	24.70	21.00	22.03	15.18	.75	.50	9.90
	c-s+herring	26.10	20.00	24.88	14.00	.19	.20	7.59
Pooled SE		3.11	4.98	8.53	4.30	.20	.19	1.31

[a]Within non-SDS or SDS birds, no significant difference (P>.05) between treatment means.

[1]Standard error

EXPERIMENT 2

EFFECT OF DIETARY LEVELS OF HERRING MEAL ON THE INCIDENCE OF SUDDEN DEATH SYNDROME IN BROILER CHICKENS

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ABSTRACT

An experiment was conducted to investigate the effect
of different levels of dietary herring meal on the incidence
of Sudden Death Syndrome (SDS) in broiler chickens. A total
of 830 broiler chicks was randomly assigned to 4 dietary
treatments: corn-soy (c-s), c-s+2.75%, +5% and +10% herring
meal. Each treatment contained 8 replicates with 26
broilers (13 males, 13 females) per replicate. Body weight,
feed conversion, total mortality, mortality due to SDS,
liver lipid and fatty acid composition of liver lipids were
measured.

No differences (P>.05) were observed among dietary
treatments for body weight, feed conversion, total mortality
or mortality due to SDS. The incidence of SDS did not
correlate with the increasing amount of herring meal in the
diets. Liver lipid was not affected (P>.05) by the dietary
treatments or the causes of death. For non-SDS broilers,
higher palmitic acid (16:0), and lower linoleic (18:2) and
arachidonic (20:4) acids were observed in the liver lipids
of broilers fed diets containing herring meal compared to
that of broilers fed c-s diet. The composition of the liver
lipids of SDS broilers showed a similar trend when compared
to that of non-SDS broilers regardless of the dietary
treatments.

INTRODUCTION

Sudden Death Syndrome (SDS) is a major problem in the commercial production of broiler chickens, and yet information concerning the biochemical changes of broilers succumbing to SDS is limited.

Herring oil contains an abundant quantity of oleic, 18:1 (24%), palmitic, 16:0 (14%) and arachidonic, 20:4 (9%) acids (Pryde, 1976). In Experiment 1, liver lipid of SDS broilers had an increase in oleic (18:1) and palmitic (16:0) acids and a decrease in linoleic (18:2) and arachidonic (20:4) acids when compared to that of non-SDS broilers. If this observation (alteration of fatty acid composition) is consistent in SDS birds, it will enhance SDS research by providing a diagnostic tool to identify potential subjects of this syndrome and should provide a lead for further research to uncover the etiology of SDS.

This study was designed: 1) to evaluate the relationship between the incidence of SDS and varying amounts of dietary herring meal, and 2) to examine the changes in the composition of liver fatty acids in response to the feeding herring meal.

MATERIALS AND METHODS

A completely randomized design was used involving 832
Hubbard broiler chickens. The day-old chicks were feather-
sexed and randomly assigned to four dietary treatments:
corn-soy (c-s), c-s+2.75%, c-s+5% and c-s+10% herring meal
(Table III.2.1.) Each treatment contained 8 replicates with
13 male and 13 female chicks per replicate. Each replicate
was housed in individual pen (1.22 m x 2.44 m) with 5 cm
deep wood shaving litter. An automatic waterer and a tube-
feeder were placed in each pen. Feed and water were given
ad libitum. Husbandry practices were as outlined previously
(Experiment 1).

Body weight and feed conversion were measured at 4 and
7 weeks of age. Mortality data were recorded daily.
Necropsy examinations were performed on broilers suspected
to have died from SDS (apparently healthy birds found on
their back). When no specific lesions were observed and the
cause of death was not determined, the dead birds were
categorized as SDS birds. The liver from SDS birds were
removed and stored at -17°C for subsequent total lipid and
fatty acid analyses. At the end of experiment, five male
broilers from each treatment were slaughtered, and the
livers were removed for lipid and fatty acid analyses
(representing the non-SDS birds).

Total lipid content of the liver was determined by the
method outlined by Bligh and Dyer (1959), and fatty acid

profile was analyzed by the method of Lowry (1977).

Detailed analytical procedures were as outlined earlier

(Experiment 1).

The data were analyzed using the one way analysis of

variance method (Steel and Torrie, 1980). When differences

were detected (P<.05), the least significant difference

(LSD) test was used to compare means. Percentage data were

subject to arcsin square root transformation before one way

ANOVA was performed.

RESULTS

Mean body weight, feed conversion and mortality data are presented in Table III.2.2. Supplementation of herring meal up to 10% of the diet did not affect (P>.05) mean body weight or feed conversion at 4 and 7 weeks of age. Total mortality and mortality due to SDS were similar (P>.05) among dietary treatments. Mortality due to SDS was .96, .96, 1.44 and .48% for broilers fed diets containing 0, 2.75, 5 or 10% herring meal, respectively. Male broilers accounted for 75% of SDS birds. SDS occurred most frequently between 2 to 5 weeks of age.

Total liver lipid was similar for non-SDS and SDS broilers (Table III.2.3.) Liver lipid tended to increase as the amount of dietary herring meal increased.

A summary of fatty acid compositions of the liver lipid is presented in Table III.2.4. The liver lipid of SDS birds showed increased levels of palmitic (16:0) and oleic (18:1) acids, and decreased levels of linoleic (18:2) and arachidonic (20:4) acids compared to that of non-SDS birds fed the same diets. For non-SDS broilers, higher (P<.05) levels of 16:0, and lower (P<.05) levels of 18:2 and 20:4 were observed in broilers fed diets containing herring meal compared to broilers fed the c-s diet.

DISCUSSION AND CONCLUSION

The inclusion of up to 10% of dietary herring meal did not affect the incidence of SDS. Interestingly, the percentage of total mortality due to SDS decreased linearly as the amount of herring meal increased in the diets (50, 25, 23 and 8% in c-s, c-s+2.75, +5 and +10% herring meal diets, respectively). A similar observation was noted by Hulan et al. (1989) where the incidence of SDS decreased as the level of red fish meal in the diet was increased.

The fatty acid composition of the livers from SDS broilers again showed the similar alterations to that observed in the previous experiment. Levels of palmitic and oleic acids were elevated in SDS birds compared to that for non-SDS birds fed the same diets, whereas the levels of linoleic and arachidonic acids were decreased. It is premature to conclude that lipid metabolism is involved in the development of this syndrome. Repeatedly, however, the concentration of arachidonic acid appeared to be much lower in SDS broilers, which provides a good rationale for further studies in the metabolism of arachidonic acid. More research with different types of fish meal may help to elucidate the association of dietary fat, and lipid metabolism in general and SDS.

Table III. 2.1. Composition of 0, 2.75, 5 and 10%
herring meal diets (Exp. 2)

| | Herring | meal (%) | | |
| | 0 (control) | 2.75 | 5.0 | 10.0 |
Ingredients				
		(%)		
Yellow corn	68.05	69.95	70.41	71.65
Soybean meal (47.5%CP)	21.45	17.20	14.49	3.61
Herring meal	-	2.75	5.00	10.00
Animal fat	0.90	0.50	-	-
Meat & bone meal	6.87	6.87	5.00	7.00
Alfalfa meal (17%CP)	2.00	2.00	4.36	7.00
Salt (iodized)	0.30	0.30	0.30	0.30
Trace mineral premix[1]	0.05	0.05	0.05	0.05
Vitamin premix[2]	0.20	0.20	0.20	0.20
d,l methionine (98)%	0.08	0.08	0.09	0.09
Baciferm[3]	0.05	0.05	0.05	0.05
Zoamix[4]	0.05	0.05	0.05	0.05
Calculated analyses:				
Crude protein, %	20.0	20.1	20.0	20.0
Metabolizable				
energy, kcal/kg	3068	3088	3063	3063

[1]Provided per kg of diet: Ca, 97.5 mg; Mn, 60 mg; Fe, 20 mg; Cu, 2 mg; Zn, 27.5 mg; Co, 2 mg.

[2]Provided per kg of diet: Vitamin A, 3300 IU; Vitamin D3, 1100 IU; riboflavin, 3.3 mg; d-pantothenic acid, 5.5 mg; niacin, 22 mg; choline, 190.9 mg; vitamin B12, 5.5 mcg; vitamin E, 1.1 IU; vitamin K, 55 mg; folic acid, 22 mg; ethoxyquin, 0.06 g.

[3]Gratuitously provided by International Mineral & Chemical Corp., Mundelein, IL 60060.

[4]Gratuitously provided by Salsbury Laboratories, Charles, City, IA 50616-9989.

Table III.2.2. Effect of feeding broiler chickens corn-soy (c-s), c-s+2.75%, +5% and +10% herring meal diets on mean body weight, feed conversion, total mortality and mortality due to Sudden Death Syndrome (SDS) to 7 weeks of age (Exp. 2)

Dietary treatments	Body weight[a] 4-week	Body weight[a] 7-week	Feed conversion[a] 4-week	Feed conversion[a] 7-week	Mortality (M) to 7 weeks[a] Total(T)[a] (%)	Mortality (M) to 7 weeks[a] SDS (%)	Mortality (M) to 7 weeks[a] SDS, as % of TM
	(gm)						
c-s	911	1856	1.69	2.24	1.92(4)[1]	0.96(2)	50
c-s+2.75% herring meal	953	1978	1.72	2.21	3.85(8)	0.96(2)	25
c-s+5% herring meal	928	2045	1.73	2.20	6.25(13)	1.44(3)	23
c-s+10% herring meal	980	1898	1.72	2.09	5.77(12)	0.48(1)	8
Pooled SE[2]	20	52	.04	.05	1.50	.80	

[a]No significant difference (P>.05) among treatment means within variables.

[1]( ) Number of birds.

[2]Standard error

Table III.2.3. Mean levels of liver lipid of Sudden Death
Syndrome (SDS) and non-SDS broiler chickens
fed corn-soy (c-s), c-s+2.75%, +5% and +10%
herring meal diets (Exp. 2)

Dietary treatments	Liver lipid, weight %[a]	
	Non-SDS birds	SDS birds
c-s	4.31	4.23
c-s+2.75% herring meal	5.23	5.71
c-s+5% herring meal	5.85	6.15
c-s+10% herring meal	7.53	7.20
Pooled SE[1]	.56	.39

[a]No significant difference (P>.05) among treatment means.

[1]Standard error

Table III.2.4. Fatty acid compositions of liver lipid for SDS (Sudden Death Syndrome) and non-SDS broiler chickens fed corn-soy (c-s), c-s+2.75%, +5% and +10% herring meal diets (Exp. 2)

Cause of death	Dietary treatments	Fatty acid composition[a], weight %						
		16:0	18:0	18:1	18:2	18:3	20:0	20:4
SDS	c-s	30.35	17.49	31.21	8.89	.34	.21	5.43
	+2.75% herring	32.24	17.39	32.71	6.44	.56	.39	2.24
	+5% herring	34.66	15.00	39.89	5.74	.23	.40	1.64
	+10% herring	37.67	15.04	35.63	5.24	.32	.41	1.20
Pooled SE[1]		1.56	.59	.59	.75	.05	.04	1.29
Non-SDS	c-s	22.12[b]	18.22[bc]	20.34	14.50[d]	.24	.34	13.01[d]
	+2.75% herring	25.71[c]	19.03[c]	20.13	12.83[c]	.29	.47	8.19[c]
	+5% herring	25.82[c]	15.64[b]	31.06	9.77[b]	.14	.28	4.51[b]
	+10% herring	31.13[d]	18.11[bc]	24.35	9.61[b]	.19	.46	3.22[b]
Pooled SE		.97	.50	2.85	.67	.04	.05	1.08

[a]For SDS birds, no significant difference (P>.05) among treatment means.

[bcd]For non-SDS birds, different superscripts denote P<.05.

[1]Standard error

EXPERIMENT 3

EFFECT OF THREE TYPES OF FISH MEAL ON THE INCIDENCE OF SUDDEN DEATH SYNDROME IN BROILER CHICKENS

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ABSTRACT

An experiment was conducted to study the relationship between three types of fish meal and the occurrence of Sudden Death Syndrome (SDS) in broiler chickens. A total of 832 Hubbard broiler chicks was randomly assigned to four dietary treatments: corn-soy (c-s), c-s+herring meal, c-s+anchovy-sardine (a-s) meal and c-s+halibut meal. Each treatment contained 8 replicates with 26 broilers (13 males, 13 females) per replicate. The three fish meals were added to each formulation to equate to 10% of the total protein of the diets.

Mean body weight of broilers fed the a-s meal diet were lower (P<.05) than that for broilers fed diets containing no fish meal, herring or halibut meal. Feed conversions were similar (P>.05) among dietary treatments. Overall mortality tended to be higher for broilers fed the diets containing fish meal. This increased mortality was attributed to the higher incidences of SDS, which accounted for 33%, 64% and 25% of the total mortality for the herring, a-s and halibut meal dietary groups, respectively. Histological examinations of the liver tissues did not identify lesions specific to SDS. There was a consistent rise in palmitic (16:0) and oleic (18:1) acids, and an equally consistent lowering of linoleic (18:2) and arachidonic (20:4) acids in SDS birds compared to non-SDS birds regardless of the dietary treatments.

INTRODUCTION

The low incidence of cardiovascular disease in
Greenland Eskimos has inspired interest in the biological
effects of fish oil (Dusheck, 1985; Carroll, 1986). This
low incidence of coronary heart disease has been attributed
to the polyunsaturated fatty acids (PUFAs) in marine oil,
particularly eicosapentaenoic acid (EPA, 20:5w3) and
decosahexaenoic acid (DHA, 22:6w3). These omega-3 PUFAs are
effective in lowering serum cholesterol and in reducing the
platelet aggregation, a major factor in heart attacks
(Kinsella, 1981; Carroll, 1986; Dusheck, 1985).

However, negative side effects of feeding fish oil have
been reported as well. The Eskimo population experience
higher death rates from cerebral hemorrhage and suffer from
a higher incidence of strokes than do Americans (Dusheck,
1985; Dyerberg, 1986). Small amounts of fish oil have been
reported to promote certain types of cancer (mammary tumor)
(Carroll, 1986). Experiments with mice showed that fish oil
reduced glomerulitis (one kind of kidney disease) but
aggravated necrotizing vasculitis (another kind of kidney
disease) (Dusheck, 1985).

Fish oil elicits diverse biological effects which
appear to come about through prostaglandins (PGs) and their
related compounds (Dusheck, 1985). PGs are a group of
chemically related compounds derived from PUFAs. PGs
originated from different fatty acids have different

biological activities (Dyerberg, 1986). Therefore, the effects of PUFAs are multifactorial through different mechanisms depending on the impact of the stimulus (Dyerberg, 1986).

In previous experiments, the level of arachidonic acid appeared to be consistently lower in Sudden Death Syndrome (SDS) broilers compared to that for non-SDS broilers. Evidence was inadequate to demonstrate the exact involvement of dietary herring meal in this finding. A comparison of the liver fatty acid composition of SDS birds fed different types of fish meal might provide a clue to the understanding of the relationship between fatty acid profile and SDS mortality. Thus, the effects of three types of fish meal on the incidence of SDS and liver fatty acid composition were studied. Histological examinations of the livers of SDS broilers were performed to ascertain whether specific lesions existed which were characteristic of SDS.

MATERIALS AND METHODS

A total of 832 Hubbard broiler chicks was randomly
assigned to four dietary treatments utilizing a completely
randomizd design. Each treatment contained 8 replicates
with 26 broilers (13 males and 13 females) per replicate.
Diets used were: corn-soy (c-s), c-s+herring meal, c-
s+anchovy-sardine meal (a-s, Wilbur-Ellis Co., Portland, OR)
and c-s+halibut meal (Icicle Seafood Co., Portland, OR).
The fish meals were formulated to replace 10% of the total
protein of the diets on an isonitrogenous and isocaloric
basis (Table III.3.1.). Feed and water were provided ad
libitum. Husbandry practices were as outlined previously
(Experiment 1).

Body weight and feed conversion were measured at 4 and
7 weeks of age. Mortality was recorded daily. Broilers
suspected of dying from SDS were sent to the Diagnostic
Laboratory at the College of Veterinary Medicine, Oregon
State University, for necropsy examination. Broilers were
categorized as SDS birds when no specific lesions were found
and the cause of death was not determined. Livers from SDS
birds were removed. Half of the livers was preserved in
formalin solution for subsequent histopathological
examinations (performed by Dr. E. Wallner-Pendleton, College
of Veterinary Medicine, Oregon State University). The other
half was stored at $-17^{\circ}C$ for subsequent total lipid and
fatty acid analyses. Similar procedures were performed on

the liver of non-SDS broilers killed at the end of the experiment. Analytical procedures for total lipid and fatty acid composition were as outlined previously (Experiment 1).

The data were subject to one way analysis of variance (Steel and Torrie, 1980). Percentage data outside the 25 to 75% range were subject to arcsin square root transformation before analysis. When statistical significance was detected (P<.05), the mean values were compared using the Least Significant Difference test (Steel and Torrie, 1980).

RESULTS

At 7 weeks of age, mean body weights for broilers fed c-s, c-s+herring, c-s+a-s or c-s+halibut meal were 1843, 1859, 1655 and 1816 gm, respectively. Broilers fed diet supplemented with a-s meal had lower (P<.05) mean body weights than the other three dietary treatments (Table III.3.2.) No differences (P>.05) among the treatments were detected for feed conversion at either 4 or 7 weeks of age.

Total mortality was similar (P>.05) among dietary treatments (Table III.3.2.) Numerically (P=.08), the incidence of SDS was higher for broilers fed a-s meal than the other three dietary treatments. Examination as to the causes of death revealed that 64%, 33%, 25% and 16% of the mortality was due to SDS for broilers fed a-s meal, herring meal, halibut meal and c-s diets, respectively. The incidence of SDS peaked at 2 weeks of age. Eighty-three percent of the SDS birds were male broilers.

For the non-SDS broilers killed at 7 weeks of age, liver lipid levels were higher (P<.05) for broilers fed the c-s and c-s+herring meal diets than broilers fed c-s+a-s and c-s+halibut meal diets (Table III.3.3.) For the SDS broilers, the highest (P<.05) total lipid level was found in the herring meal group; and the lowest (P<.05) in the c-s group.

Alterations were observed in the liver fatty acid composition between SDS and non-SDS birds (Table III.3.4.)

Generally, the changes were consistent with the previous findings (Experiments 1 and 2). A trend of elevated palmitic (16:0) and oleic (18:1) acids, and decreased linoleic (18:2) and arachidonic (20:4) acids was observed in SDS birds compared to the non-SDS birds regardless of the dietary treatments.

Necropsy examination revealed that all SDS birds had full gastrointestinal tract. Areas of breast muscle showed a pale pinkish color. Blood clot was found within the heart. Enlarged liver and/or kidney was/were observed in some of the SDS birds. Histological examinations of the liver of non-SDS birds revealed a large multifocal accumulations of lymphocytes. A similar observation was found in liver of SDS birds.

DISCUSSION AND CONCLUSION

Total mortality and mortality due to SDS were similar for broilers fed diets containing the different sources of fish meals. Inclusion of different fish meals to the diets affected the total lipid content in the livers. Alteration of the liver fatty acid composition observed in this experiment was similar to those obtained in the two previous studies reported here. Similar results had been found by others (Rotter et al., 1985; Buenrostro and Kratzer, 1982). No evidence has been shown to suggest that the changes in fatty acid profile is specifically associated with SDS. So far, however, this was the only consistent finding observed in all our experiments. Further research in this area is needed to elucidate the relationship between fatty acid metabolism and the incidence of SDS.

Histological examinations of the liver tissues of both non-SDS and SDS broilers failed to reveal changes specific to this syndrome. Multifocal lymphocytic inflammation is usually indicative of immunostimulation probably as a result of viral infection and not necessarily specific to SDS. All SDS birds had mild intracytoplasmic vacuolar change present within the hepatocytes. This condition is often seen in birds that are moderately obese. The typical physical description of SDS birds is that the birds are large, well-nourished and rapid-growing (Ononiwu et al., 1979; Haserbach and Tagwerker, 1986). Therefore, the mild vacuolar changes

seen in SDS birds should not be considered as a specific characteristic to this syndrome. A healthy appearance, full GI tract and sudden unexpected death remain the only typical characteristics which identify the SDS birds.

Of the dietary treatments tested, broilers fed a-s fish meal diet had the numerically (P=.08) highest incidence of mortality due to SDS. Because of this finding, a-s meal diet would be used in the subsequent studies.

Table III. 3.1. Composition of three types of fish meal
diets (Exp. 3)

Ingredients	Corn-soy (c-s)	c-s+ Herring	c-s+Anchovy- Sardine (a-s)	c-s+ Halibut
	------------		(%) --------------	
Yellow corn	65.90	70.10	68.47	68.50
Soybean meal (47.5%CP)	22.00	17.20	19.00	24.00
Herring meal	-	2.75	-	-
A-s meal	-	-	3.05	-
Halibut meal	-	-	-	3.60
Animal fat	1.35	0.50	0.50	0.50
Meat & bone meal	6.82	6.87	5.00	-
Alfalfa meal (17%CP)	2.00	2.00	3.30	2.72
Defluo. phos.	0.60	-	-	-
Limestone flour	0.55	-	-	-
Salt (iodized)	0.30	0.30	0.30	0.30
Trace mineral premix[1]	0.05	0.05	0.05	0.05
Vitamin premix[2]	0.20	0.20	0.20	0.20
d,l methionine (98%)	0.08	0.08	0.08	0.09
Baciferm[3]	0.05	0.05	0.05	0.05
Zoamix[4]	0.05	0.05	0.05	0.05
Calculated analyses:				
Crude protein, %	20.1	20.1	20.0	20.0
Metabolizable				
energy, kcal/kg	3039	3038	3038	3039

[1]Provided per kg of diet: Ca, 97.5 mg; Mn, 60 mg; Fe, 20 mg; Cu, 2 mg; Zn, 27.5 mg; Co, 2 mg.

[2]Provided per kg of diet: Vitamin A, 3300 IU; Vitamin D3, 1100 IU; riboflavin, 3.3 mg; d-pantothenic acid, 5.5 mg; niacin, 22 mg; choline, 190.9 mg; vitamin B12, 5.5 mcg; vitamin E, 1.1 IU; vitamin K, 55 mg; folic acid, 22 mg; ethoxyquin, 0.06 g.

[3]Gratuitously provided by International Mineral & Chemical Corp., Mundelein, IL 60060.

[4]Gratuitously provided by Salsbury Laboratories, Charles City, IA 50616-9989.

Table III.3.2. Effect of feeding broiler chickens corn-soy (c-s), c-s+herring, +anchovy-sardine(a-s), +halibut meal diets on mean body weight, feed conversion, total mortality and mortality due to Sudden Death Syndrome (SDS) to 7 weeks of age (Exp. 3)

Dietary treatments	Body weight (gm)		Feed conversion[c]		Mortality (M) to 7 weeks[c]			
	4-week	7-week	4-week	7-week	Total(T) (%)	SDS (%)	SDS,	as % of TM
c-s	934^a	1843^a	1.74	2.24	2.88(6)	.48(1)		16
c-s+herring	928^a	1859^a	1.74	2.15	4.32(9)	1.44(3)		33
c-s+a-s	843^b	1655^b	1.77	2.24	5.29(11)	3.37(7)		64
c-s+halibut	927^a	1816^a	1.76	2.24	5.77(12)	.48(3)		25
Pooled SE[1]	20	38	.02	.07	1.46	.91		

[ab]Different superscripts denote P<.05.

[c]No significant difference (P>.05) among treatment means within variables.

[1]Standard error

Table III.3.3. Mean levels of liver lipid of Sudden Death
Syndrome (SDS) and non-SDS broiler chickens
fed corn-soy (c-s), c-s+herring, +anchovy-
sardine (a-s) and + halibut meal diets
(Exp. 3)

Dietary treatments	Liver lipid, weight %	
	Non-SDS birds	SDS birds
c-s	3.55^a	1.06^a
c-s+herring meal	3.66^a	4.60^c
c-s+a-s fish meal	3.13^b	2.74^b
c-s+halibut meal	2.90^b	2.34^b
Pooled SE[1]	.08	.32

^{abc}Different superscripts denote P<.05

[1]Standard error

Table III.3.4. Fatty acid compositions of liver lipid for Sudden Death Syndrome (SDS) and non-SDS broiler chickens fed corn-soy (c-s), c-s+ herring, +anchovy-sardine (a-s) and +halibut meal diets (Exp. 3).

Cause of death	Dietary treatments	Liver fatty acid composition[a], weight %						
		16:0	18:0	18:1	18:2	18:3	20:0	20:4
SDS	c-s	38.3	–	34.7	5.3	0.6	0.4	3.0
	c-s+herring	32.1	12.4	33.5	6.0	0.6	0.8	2.4
	c-s+a-s	31.4	15.5	32.4	9.3	0.6	0.6	2.9
	c-s+halibut	32.3	11.4	28.1	9.3	1.1	1.0	2.5
Pooled SE[1]		2.1	1.8	2.5	.8	.1	.1	1.8
Non-SDS	c-s	21.5	19.2	20.7	15.1	0.3	0.6	12.9
	c-s+herring	23.3	18.5	25.3	13.9	0.4	0.3	12.4
	c-s+a-s	23.5	19.2	15.5	15.2	0.4	0.3	9.6
	c-s+halibut	24.3	22.5	11.1	17.3	0.5	0.6	12.0
Pooled SE		2.4	2.0	2.7	.9	.1	.1	2.0

[a]Within non-SDS or SDS birds, no significant difference (P>.05) among treatment means.

EXPERIMENT 4

EFFECT OF STRAIN CROSSES AND DIETARY FISH MEAL ON THE INCIDENCE OF SUDDEN DEATH SYNDROME IN BROILER CHICKENS

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ABSTRACT

A 4 x 2 factorial design experiment was conducted to evaluate the effects of four broiler chicken strain crosses and two dietary treatments (corn-soy, c-s; c-s+anchovy-sardine meal,a-s) on the incidence of Sudden Death Syndrome (SDS). The four broiler strain were: Hubbard x Hubbard (HxH), Ross x Arbor Acres (RxAA), Peterson x Arbor Acres (PxAA) and Vantress x Hubbard (VxH). A total of 768 broiler chicks was randomly assigned to eight treatments with 3 replicates per treatment. Each replicate consisted of 32 chicks (16 males, 16 females).

No interaction (P>.05) was observed between the strains and dietary treatments for growth or mortality. Diet alone did not (P>.05) affect mean body weight, feed conversion, total mortality or mortality due to SDS. The PxAA broilers had a lower (P<.05) mean body weight than those of the other three broiler strain crosses. Total mortality and mortality due to SDS were similar (P>.05) among the strain crosses. The levels of liver lipid were not different (P>.05) between the dietary treatments or among the strain crosses. An elevated level of palmitic acid (16:0) and a decreased level of arachidonic acid (20:4) were observed in the liver lipid of SDS broilers compared to non-SDS broilers regardless of the dietary treatments or strain crosses. A similar fatty acid pattern was found in the liver lipid of broilers fed c-s+a-s meal diet compared to that for broilers fed c-s diet.

INTRODUCTION

Hutt and Rasmusen (1982) have pointed out that differences among animals to tolerate diseases are clearly inherited. Examples in the fowl can be demonstrated for pullorum disease to which the Plymouth Rock breed is three to five times more susceptible than White Leghorns. Thus, in studying the Sudden Death Syndrome (SDS), the genetic background of the broiler chickens as a possible source of variability must be considered.

Intense broiler breeding started 25-30 years ago at which time SDS was first observed. The increased incidence of SDS appears to coincide with the increased rate of growth of the broiler chickens (Eleazer, 1988). Gardiner et al. (1988) reported that as the body weight of broilers increased, mortality due to SDS increased. Hemsley (1965) reported that mortallity due to SDS was lower (.03%) in the less developed breeds (Chuncky) compared to the progeny of more developed parent stocks (Arbor Acres, .6%; Cobbs, 1.0%; Pilch, .6%). However, the results of a more recent study by Riddell and Springer (1984) suggested that differences in the incidence of SDS between strain crosses were negligible.

The purpose of this experiment was to examine the relationship among four different broiler chicken strain crosses and two diets (corn-soy or corn-soy+anchovy-sardine meal) on the incidence of SDS.

MATERIALS AND METHODS

A 4 x 2 factorial experiment was designed to test the relationship among four broiler strain crosses and two dietary treatments. Each of the 8 treatment contained 3 replicates with 32 broilers (16 males and 16 females) per replicate. The four broiler strain crosses used were: Hubbard x Hubbard (HxH), Ross x Arbor Acres (RxAA), Peterson x Arbor Acres (PxAA) and Vantress x Hubbard (VxH). Hatching eggs were purchased from Keith Smith Company (Hot Spring, Arkansas) and incubated in the Department of Poultry Science incubator (model Jamesway 252). The chicks were feather-sexed at hatching and randomly distributed to each treatment. They were raised from day-old to 7 weeks of age on two different diets: the corn-soy (c-s) or c-s+anchovy-sardine (a-s) meal (Table III.4.1.) The broilers were housed in floor pens (1.22 m x 3.05 m) covered with 5 cm deep clean wood shavings litter. Each pen was equipped with an automatic waterer and trough feeder. Water and feed were provided ad libitum from day-old to 7 weeks of age. Overhead continuous lighting (5.4 lux) was provided throughout the experiment. Standard brooding and rearing methods were followed as outlined by North (1981).

Body weight and feed conversion were measured at 4 and 7 weeks of age. Mortality data were recorded daily. Broilers suspected of dying from SDS were sent to the Diagnostic Laboratory, the College of Veterinary Medicine,

Oregon State University, for necropsy examination. When no specific lesion was identified and the cause of death was not determined, the broiler was categorized as a SDS bird. Livers from SDS and 7-week-old non-SDS broilers were removed for total lipid and fatty acids analyses (Bligh et al., 1959; Lowry, 1977). Details of the analytical procedures have been outlined previously (Experiment 1).

The data were subjected to analyses of variance procedures appropriate for factorial designs (Steel and Torrie, 1980). Percentage data outside the 25 to 75% range were transformed to arsin angles for analysis. Means were separated by least significant difference (LSD) test, with significance level of P=.05.

RESULTS

No significant interactions (P>.05) between dietary treatments and broiler strain crosses were detected for mean body weight and feed conversion (Table III.4.2.) Supplementation of the corn-soy diet with a-s meal had no effect (P>.05) on the growth. However, there was a significant (P<.05) strain cross main effect for mean body weight. The PxAA strain cross had a lower (P<.05) mean body weight than the HxH, RxAA and VxH strain crosses at both 4 and 7 weeks of age.

Total mortality and mortality due to SDS were similar (P>.05) among the dietary treatments (Table III.4.3.) Among the strain crosses, PxAA broilers had highest mortality due to SDS. Interestingly, no incidence of SDS was observed in VxH broilers.

Dietary treatments and strain crosses did not affect the level of (P>.05) liver lipid of either the non-SDS or SDS birds (Table III.4.4.) For non-SDS birds, those fed diets containing a-s meal had more palmitic acid (16:0) and less arachidonic acid (20:4) compared to broilers fed the c-s diet (Table III.4.5.) A comparison between non-SDS and SDS broilers revealed increased levels of palmitic (16:0) and oleic (18:1) acids, and decreased levels of linoleic (18:2) and arachidonic (20:4) acids in the liver lipid of SDS birds compared to non-SDS birds regardless of the dietary treatments or strain crosses.

DISCUSSION AND CONCLUSION

The sudden death cases observed in this experiment follow the typical SDS patterns with male broilers being more susceptible than females, and the peak mortality appeared at about 3 weeks of age. Dietary treatments had no effect (P>.05) on the incidence of SDS. The PxAA broiler strain cross had a higher incidence of SDS, and no SDS was observed in VxH strain cross.

Riddell and Springer (1984) suggested that the differences in the incidences of SDS between the strain crosses were negligible. However, Newberry et al. (1985) observed strain differences in mortality due to SDS. The inconsistent results on the outbreak of SDS within and among strain crosses may be explained by the following. First, modern strains of broiler chickens have all originated from a similar gene pool. Although varied broiler breeding programs have been carried out over the years, the emphasis of these programs have always been focused on growth performance. Thus, it appears that similar breeding goals resulted in the selection of similar genetic components and similar strains of broilers. Second, the population size studied was too small to provide statistical significance, especially when one considers the circumstance stated in the first reason.

Brigden and Riddell (1975) reported mortality due to SDS ranging from 0.3% to 1.54% in 12 different broiler

flocks. However, the differences in incidences among flocks were not statistically significant. The authors suggested that the non-significant difference in mortality rate among flocks was due to the small number of chickens studied (24,452 broilers). Studies including larger populations and more strain cross comparisons are necessary in order to fully illustrate the impact of genetic variation on the occurrence of SDS.

The results of the lipid and fatty acid analyses in this study agreed with those in previous studies. Marked changes in fatty acid composition were consistently observed in SDS birds. The significance of these changes and how lipid metabolism might be associated with SDS will be discussed in the final chapter of this dissertation.

The results of the current study did not establish a strain cross-SDS relationship or a dietary treatment x strain interaction. However, this does not rule out the potential genetic resistance to SDS that may exist in broiler chickens.

Table III. 4.1. Composition of corn-soy (c-s) and c-
s+anchovy-sardine (a-s) meal diets
(Exp. 4)

Ingredients	corn-soy (c-s)	c-s+anchovy-sardine (a-s)
	-------- (%)	--------
Yellow corn	65.90	66.90
Soybean meal (47.5%CP)	22.00	19.00
Animal fat	1.35	1.00
Meat & bone meal	6.82	5.00
Alfalfa meal (17%CP)	2.00	3.30
A-s fish meal (65%CP)	-	3.05
Defluo. phos.	0.60	0.55
Limestone flour	0.55	0.55
Salt (iodized)	0.30	0.30
Trace mineral premix[1]	0.05	0.05
Vitamin premix[2]	0.20	0.20
d,l methionine (98)%	0.08	0.09
Baciferm[3]	0.05	0.05
Zoamix[4]	0.05	0.05
Calculated analyses:		
Crude protein, %	20.1	20.0
Metabolizable		
energy, kcal/kg	3039	3038

[1]Provided per kg of diet: Ca, 97.5 mg; Mn, 60 mg; Fe, 20 mg; Cu, 2 mg; Zn, 27.5 mg; Co, 2 mg.

[2]Provided per kg of diet: Vitamin A, 3300 IU; Vitamin D3, 1100 IU; riboflavin, 3.3 mg; d-pantothenic acid, 5.5 mg; niacin, 22 mg; cholin, 190.9 mg; vitamin B12, 5.5 mcg; vitamin E, 1.1 IU; vitamin K, 55 mg; folic acid, 22 mg; ethoxyquin, 0.06 g.

[3]Gratuitously provided by International Mineral & Chemical Corp., Mundelein, IL 60060.

[4]Gratuitously provided by Salsbury Laboratory, Charles City, IA 50616-9989.

Table III.4.2. Effect of feeding different broiler chicken strain crosses corn-soy (c-s) and c-s+anchovy-sardine (a-s) meal diets on mean body weight and feed conversion to 7 weeks of age (Exp. 4)

	Body weight (gm)		Feed conversion[a]	
	4-wk-age	7-wk-age	4-wk-age	7-wk-age
Main effects				
Diets	NS	NS	NS	NS
corn-soy	905	1975	1.70	2.19
c-s+a-s meal	906	2013	1.76	2.17
Pooled SE[2]	15	13	.02	.03
Strain crosses[1]	*	**	NS	NS
H x H	922	2025	1.77	2.20
R x AA	927	2044	1.74	2.14
P x AA	845	1893	1.81	2.23
V x H	926	2014	1.69	2.15
Pooled SE	21	19	.03	.04
Interaction				
Diet x Strain crosses	NS	NS	NS	NS

NS = No significant difference (P>.05).

* P<0.05

** P<0.01

[1]H x H = Hubbard x Hubbard
R x AA = Ross x Arbor Acres
P x AA = Peterson x Arbor Acres
V x H = Vantress x Hubbard

[2]Standard error.

Table III.4.3. Effect of feeding different broiler chicken strain crosses corn-soy (c-s) and c-s+anchovy-sardine (a-s) meal diets on total mortality and mortality due to Sudden Death Syndrome (SDS) (Exp. 4)

	Total mortality	SDS mortality %	SDS as % of total mortality
Main effects			
Diets	NS	NS	
corn-soy	2.60(10)[2]	0.78(3)	30
c-s+a-s meal	2.34(9)	0.78(3)	33
Pooled SE[3]	0.53	0.33	
Strain crosses[1]	NS	NS	
H x H	3.65(7)	1.04(2)	29
R x AA	1.56(3)	0.52(1)	33
P x AA	2.60(5)	1.56(3)	60
V x H	2.08(4)	0	0
Pooled SE	0.75	0.47	
Interaction			
Diet x Strain crosses	NS	NS	

NS = No significant difference (P>.05).

[1] H x H = Hubbard x Hubbard
  R x AA = Ross x Arbor Acres
  P x AA = Peterson x Arbor Acres
  V x H = Vantress x Hubbard

[2] ( ) Number of broilers

[3] Standard error.

Table III.4.4. Mean levels of liver lipid of non-Sudden Death Syndrome (SDS) and SDS broiler chickens of different strain crosses fed corn-soy (c-s) and c-s+anchovy-sardine (a-s) meal diets (Exp. 4)

Dietary treatments	Strain crosses[1]	Total lipid (%)	
		Non-SDS broilers[a]	SDS broilers
c-s	H x H	4.30	4.98
c-s	R x AA	4.08	-[2]
c-s	P x AA	4.46	5.25
c-s	V x H	4.28	-
c-s+a-s	H x H	4.38	4.82
c-s+a-s	R x AA	4.33	4.47
c-s+a-s	P x AA	4.63	5.15
c-s+a-s	V x H	4.50	-
Pooled SE[3]		0.35	0.95

[a]There was no significant difference (P>.05) among the treatment means.

[1]H x H = Hubbard x Hubbard
R x AA = Ross x Arbor Acres
P x AA = Peterson x Arbor Acres
V x H = Vantress x Hubbard

[2]No SDS bird

[3]Standard error

Table III.4.5. Fatty acid composition (weight %) of the
liver lipid for non-Sudden Death Syndrome
(SDS) and SDS broiler chickens of different
strain crosses fed corn-soy (c-s) and c-
s+anchovy-sardine (a-s) meal diets (Exp. 4)

Non-SDS broilers

| Diets | Strain cross[1] | Fatty acid composition[a], weight % | | | | | | |
		16:0	16:1	18:0	18:1	18:2	20:0	20:4
c-s	H x H	19.5	1.1	24.6	19.2	17.6	0.6	16.2
	R x AA	19.3	2.7	21.0	16.2	17.3	1.0	20.6
	P x AA	20.6	1.9	24.0	16.9	17.0	0.8	17.6
	V x H	21.4	1.4	21.0	18.8	15.9	0.6	17.1
Pooled SE[2]		1.1	.8	1.0	1.0	.1	.1	1.7
+a-s	H x H	25.4	0.9	26.8	15.1	17.7	0.1	12.8
	R x AA	25.3	1.8	23.4	20.1	14.1	0.1	9.5
	P x AA	23.3	1.1	24.5	14.3	18.8	0.1	12.7
	V x H	23.8	1.7	23.4	15.9	18.1	0.8	15.1
Pooled SE		1.1	.8	1.0	1.0	.1	.1	1.7

SDS broilers

Diets	Strain cross	16:0	16:1	18:0	18:1	18:2	20:0	20:4
c-s	H x H	29.4	6.6	12.8	40.2	5.5	–	1.4
	R x AA	--------		not	available[3]		-------	
	P x AA	30.2	1.6	15.8	39.6	4.5	0.8	1.9
	V x H	--------		not	available		--------	
Pooled SE		.7	1.4	1.9	1.0	.3	–	.3
+a-s	H x H	31.7	1.5	16.1	40.5	5.0	–	1.4
	R x AA	30.3	4.3	19.7	41.0	2.1	–	1.1
	P x AA	32.4	2.0	18.4	35.0	3.6	1.0	2.0
	V x H	--------		not	available		--------	
Pooled SE		.5	1.1	1.6	1.7	.3	–	.3

[a]Within non-SDS or SDS birds, no significant differences
(P>.05) among the treatment means.

[1]H x H = Hubbard x Hubbard, P x AA = Peterson x Arbor Acres
R x AA = Ross x Arbor Acres, V x H = Vantress x Hubbard

[2]Standard error

[3]No SDS bird.

EXPERIMENT 5

EFFECTS OF DIETARY BIOTIN, ACETYLSALICYLIC ACID AND FISH MEAL ON THE INCIDENCE OF SUDDEN DEATH SYNDROME IN BROILER CHICKENS

RH: Sudden Death Syndrome in broilers

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ABSTRACT

A 3 x 3 x 2 factorial experiment was conducted to evaluate the effects of biotin, acetylsalicylic acid (ASA) and anchovy-sardine (a-s) meal on the incidence of Sudden Death Syndrome (SDS) in broiler chickens. The basal corn-soy diet was supplemented with: 1) 0, 2.5 or 7.5% of a-s meal, 2) biotin at 0, 50 or 500 ug/kg of diet, and 3) ASA at 0 or .2% of diet. Each treatment contained 3 replicates with 40 male broilers per replicate.

At seven weeks of age, broilers fed the diet containing 2.5% a-s meal had higher (P<.05) body weight than the broilers fed no a-s meal. Dietary ASA at .2% had an adverse effect (P<.05) on the body weight, feed conversion, total mortality and mortality due to SDS.

The length of time (up to 4 hrs) between death and tissue removal had no effect (P>.05) on the level of liver or heart lipids. No differences (P>.05) were observed for the lipid content of the liver or heart among the treatments, and between non-SDS and SDS birds. Interestingly, broilers fed diets containing 0 or 500 ug biotin/kg had similar biotin level in the liver. Higher levels of omega-3 (w3) fatty acids and lower levels of omega-6 (w6) fatty acids were found in broilers fed 7.5% a-s meal when compared to broilers fed 0% a-s meal. The liver lipids of SDS birds contained more (P<.05) oleic acid (18:1w9) but less (P<.05) arachidonic acid (20:4w6) than

the liver lipids of non-SDS birds. This alteration of fatty acid composition suggested a possible association among lipid metabolism, prostaglandins and SDS.

INTRODUCTION

Lower levels of liver arachidonic acid have been consistently observed in broilers that have succumbed to Sudden Death Syndrome (SDS) (Wu and Nakaue, unpublished). Similar findings were reported by others (Buenrostro and Kratzer, 1982; Rotter and Guenter, 1985). Arachidonate is a direct precursor of prostaglandins (PGs) which are important for many biological functions (Gryzlewski et al., 1976; Lands, 1982). A possible relationship among arachidonic acid, PGs and SDS in broiler chickens may be suggested.

Under usual conditions, arachidonic acid (C20:4w6) is incorporated into phospholipids as an integral part of the cell membrane structure (Hadley, 1984). When cells are subjected to different types of extrinsic stimuli (pH changes, bacteria, cellular trauma and fatty acid intake), arachidonate is released and rapidly converted into PGs (Lands, 1982; Gerrard, 1985).

The PGs are a group of chemically related compounds with powerful local hormonal effects (Gerrard, 1985). Current research indicates that PGs exert many effects on biological systems which include inflammation, ion transport (Stryer, 1981), and a variety of actions on cardiac tissue (Lands, 1982). The amount and form of PGs can have a synergistic or antagonistic effect on the same physiological process (Lands, 1982; Hadley, 1984).

Ononiwu et al. (1979) proposed that the onset of SDS was characterized by heart inflammation which led to lung edema, hypoxia and an eventual heart attack in broilers. Watkins and Kratzer (1984) suggested that an underproduction of PGs from low level of arachidonic acid in the tissue might be associated with the cause of SDS in broiler chickens. In both hypotheses, PGs are implicated in the pathogenesis of SDS.

The rate of synthesis of PGs is affected by the availability of the substrate. The omega-3 fatty acids which are abundant in marine oils may interfere with the formations of PGs by acting as competitive inhibitors to omega-6 fatty aicds (Sanders, 1986). Biotin is an important cofactor involved in the carboxylation reactions. Biotin dependent carboxylase is involved in fatty acid metabolism. Biotin deficiency impairs the production of arachidonic acid which is the precursor for PGs synthesis (Watkins and Kratzer, 1987). Conversely, acetylsalicylic acid (aspirin, ASA) is capable of inhibiting the production of PGs from arachidonic acid (Watkins and Kratzer, 1987).

Therefore, in this experiment, varying combinations of fish meal, biotin and ASA were fed to broilers to evaluate the effects of these treatments on the incidence of SDS. The length of time between SDS death and tissue removal (for analyses) were varied since SDS is not identifiable before death. Thus, the effect of time intervals (after death) on liver and heart lipids was determined.

MATERIALS AND METHODS

A total of 2,160 male Hubbard broiler chicks was feather-sexed at day-old and randomly assigned to 18 dietary treatments. Each treatment contained 3 replicates with 40 male broiler chicks per replicate. The broilers were raised in floor pens (1.22 m x 3.05 m/pen) covered with 5 cm deep of clean woodshaving litter, and equipped with automatic waterers and trough feeders. Water and feed were provided ad libitum. Overhead continuous light (5.4 lux) was provided throughout the experiment. Standard brooding and rearing methods were practiced as described by North (1981).

A 3 x 3 x 2 factorial arrangement was used to create 18 different dietary treatments: corn-soy diets supplemented with 1) 0, 2.5 or 7.5% of anchovy-sardine (a-s) meal, 2) biotin at levels of 0, 50 or 500 ug/kg of feed, and 3) ASA at levels of 0 or .2% of the diet (Tables III.5.1.) The a-s meal contained 65% crude protein and was commercially distributed by Wilbur-Ellis Co., Portland, Oregon. The biotin premix (Rovimix H 100, 220.46 mg d-biotin/kg premix) was a product of Hoffmann-La Roche, Inc., Nutley, New Jersey. ASA (crystalline form with 98% purity) was purchased from Sigma Chemical Co., St. Louis, Missouri. All the diets were formulated on an isonitrogenous and isocaloric bases (Table III.5.2.)

Body weights and feed conversions were measured at the end of two, four and seven weeks of the experiment.

Mortality was recorded daily. Broilers dying from no obvious pathological reason were subjected to necropsy examination and were categorized as SDS birds when no specific lesions other than a full gastrointestinal tract and an empty gall bladder was found, and the cause of death was recorded as "undetermined". Liver and heart tissues were removed from SDS broilers for subsequent lipid analyses and biotin determinations. Also, at the end of week seven, nine broilers from each of the treatments one, two, five, six, 13, 14, 17 and 18 were killed, and the livers and hearts were removed for similar analyses. Another nine broilers from treatments five and 17 were killed, and the livers and hearts were collected at different time intervals (three of each organ at each time interval of one, two and four hr after death) to examine the effect of time on the levels of total lipid.

Tissue samples were placed in plastic bags and stored at $-17^{\circ}C$ for subsequent analyses. Total lipids in the tissues were extracted in chloroform/methanol (2:1, v/v) by the method of Bligh and Dyer (1959). Methyl esters were prepared after saponification using boron trifluoride (Metcalfe et al., 1966). The methyl esters were extracted in isooctane for chromatograph equipped with a flame ionization detector and a Nelson Analytical Data System (Hewlett Packard Co.) A DB225 (25% cyanoprophylphenyl) fused silica glass capillary column (30 m x 0.25 mm ID) purchased from J & W Scientific Co. (Rancho Cordova, CA) was

used with helium as a carrier gas at a flow rate of 3.4 ml/min. The oven temperature was maintained at 196°C for the first 10 min of the run and increased at a rate of .9°C per min to a final temperature of 212°C. Total analysis time per sample was 52 min. Both injector and detector ports were maintained at 250°C. Fatty acid standards for gas chromatography were purchased from Nu-Chek-Prep (Elysian, MN). Proportions of fatty acids present in each sample were determined as weight percentages.

The biotin contents of the liver tissues were determined by the method of Hood (1975 & 1977). The technique involved isotopic dilution of ^{14}C-biotin by unlabeled biotin and the use of avidin as the binding protein for biotin. Tissues were homogenized with sulfuric acid (2 N) for 45 seconds. The homogenate was autoclaved at 121°C for 1 hr to liberate biotin. Scintillation vials containing the d-^{14}C-biotin (Amersham, Arlington Heights, Inc.) from the biotin analyses and 3.5 ml cocktail (3a70B, Research Projects International Corp., T. Prospect, IL) were shaken vigorously. The radioactivity was counted using a Liquid Scintillation System (Searle Mark III, 6880 Liquid Scintillation System, Searle Analytic Inc.)

The data on body weights, feed conversions (feed consumed/weight gain) and mortalities were analyzed using a factorial design (Steel and Torrie, 1980). The main effects were A-S meal, biotin and ASA. The data on lipid contents, fatty acid composition and biotin level were subjected to

one way ANOVA analysis. Arcsin square root transformation was performed on the percentage data outside the 25 to 75% range. Analyses were performed using the analysis of variance procedure of the SAS statistical programs (Barr et al., 1980). When differences were detected (P<.05), treatment means were separated by Tukey's Studentized Range Test (Steel and Torrie, 1980).

RESULTS

No significant (P>.05) interactions between or among the a-s fish meal, biotin and ASA (2 ways or 3 way) were observed for growth response. However, a pronounced a-s meal x ASA interaction (P=.057) was observed for weight gain of 7-week-old broilers fed diets containing both a-s meal and ASA (Table III.5.3.)

When the a-s meal was evaluated as the main effect, broilers fed the a-s meal had similar mean body weight compared to those fed diet without a-s meal at two and four weeks of age (Table III.5.3.) At seven weeks of age, the mean body weight of broilers receiving 2.5% a-s meal was heavier (P<.01) than those fed no a-s meal. Feed conversion was not affected (P>.05) by the supplementation of a-s meal.

Dietary biotin level per se as the main effect did not affect (P>.05) growth performances (Table III.5.3.) The mean body weight and feed conversion of broilers receiving additional biotin (50 or 500 ug/kg) were similar to that of broilers on the treatment without supplemental biotin.

Dietary ASA (.2%) suppressed (P<.05) the growth of broilers at two, four and seven weeks of age (Table III.5.3.) and reduced the feed conversion of the birds at two and seven weeks of age when compared to the broilers fed no ASA.

Total mortality and mortality due to SDS (Table III.5.4.) were not affected (P>.05) by dietary a-s level (0,

2.5 or 7.5%) or by biotin supplementations (0, 50 or 500 ug/kg). The addition of ASA increased (P<.05) the total mortality from 2.78% to 6.68% (0 vs. .2% ASA), and mortality due to SDS from 1.76% to 3.06%. The incidence of SDS were more frequent between the second and fourth week, with the peak at four weeks of age.

Biotin supplementation did not (P>.05) affect the levels of biotin in the liver. Broilers fed diets without additional biotin had a liver biotin of 1775 ± 205 ug/kg, which was not different (P>.05) from 1404 ± 106 ug/kg in the livers of broilers fed biotin at 500 ug/kg.

Time lag (Table III.5.5.) between slaughter and sample collection did not (P>.05) affect the lipid content in the liver and heart tissues of broilers within the same treatments (Treatments five and 17) or between two different diets (Treatments five vs. 17).

Total lipid content of the liver and heart were not different (P>.05) among the non-SDS broilers from Treatments one, two, five, six, 13, 14, 17 and 18 (Table III.5.6.) In SDS broilers, more profound variations in the lipid content of liver and heart were observed among the different treatments; however, the majority of the values was similar to those found in non-SDS broilers.

Fatty acid composition of the liver lipid is summarized in Table III.5.7. Higher (P<.05) levels of 20:5w3, 22:5w3 and 22:6w3, and lower (P<.05) levels of 20:4w6 and 22:4w6 fatty acids were observed in non-SDS broilers fed 7.5% a-s

meal compared to broilers fed no a-s meal. An increase of 18:1w9 and 20:2w6, and a decrease of 18:0, 20:4w6 and 20:5w3 were observed in SDS birds compared to non-SDS birds.

DISCUSSION AND CONCLUSION

Because SDS can not be recognized before it occurs, tissue samples from SDS broilers are usually collected at different time intervals after the death. Thus, the reliability and accuracy of results from lipid determination can be questioned. Analyses of liver and heart tissue collected at different time periods (up to 4 hrs) after death indicated that total lipid in these samples were similar. It was concluded that the quality of these tissues, collected within 4 hrs after a broiler died, provided a physiologically valid source of information indicative of the status of the liver and heart lipid of SDS birds.

More omega-3 fatty acids and less omega-6 fatty acids were found in the liver lipids of broilers fed 7.5% a-s meal. This alteration of fatty acid concentrations was no doubt the result of feeding a-s meal. Marine oil is rich in w3 fatty acids which are capable of inhibiting the synthesis of w6 fatty acids (Lands, 1982). The presence of the w3 fatty acids in the a-s meal (Table III.5.8.) elevated the tissue w3 fatty acids and in the process reduced the levels of w6 fatty acid, especially arachidonic acid. This effect may lead to the reduced production of PGs which has been suggested to contribute to SDS mortality (Watkins and Kratzer, 1987).

May and McNaughton (1980) found that ASA feeding at a level of .3% in the diet did not affect the body weight of broilers. Proudfoot and Hulan (1983) observe that .16% of dietary ASA suppressed body weight of the broilers, but had no effect on the incidence of SDS. Inclusion of dietary ASA at .2% in the study reported here reduced (P<.05) the mean body weight and feed conversion of the broilers, and increased mortality due to SDS. ASA has been shown to inhibit PGs biosynthesis (Huang et al., 1986; Watkins and Kratzer, 1987). Thus, the higher incidence of SDS in broilers fed .2% of ASA in this study suggests the possible involvement of lower PGs in the etiology of SDS.

Buenrostro and Kratzer (1982) reported that biotin deficiency was the nutritional factor implicated in SDS. Whitehead and Randall (1982) found that the incidence of SDS was unaffected by dietary biotin concentrations up to 200 ug/kg. Hulan et al. (1980) observed that biotin at 300 ug/kg reduced SDS mortality. Haserbach and Tagwerker (1986) offered the conjecture that a rather high level (e.g. 500 ug/kg) of biotin might be required to achieve an effective prevention of SDS. In the study reported here, dietary supplementation of biotin (up to 500 ug/kg) did not affect either the total or SDS mortality. However, laboratory analyses revealed that liver biotin concentrations were similar among broilers fed diets with or without additional biotin. A deficiency of biotin in the chicken decreases the fatty acid precursors of PGs in the tissues (Kratzer et al.,

1984; Watkins and Kratzer, 1984 & 1987). Although this study did not establish a direct dose-effect relationship between the biotin status and SDS, more research should be conducted to investigate the possible association of biotin with SDS.

Lower level of arachidonic acid in the liver of SDS birds leads to the hypothesis that SDS is a disorder resulting from the competitive inhibition and interaction between w3 and w6 fatty acids which reduce the level of tissue arachidonic acid. Thus, synthesis of tissue PGs from arachidonic acid may be interfered, which could eventually upset heart function leading to SDS (Rotter et al., 1985; Watkins and Kratzer, 1987).

A higher incidence of SDS was observed in broilers fed ASA, a compound known to inhibit PGs synthesis (Clark et al., 1986). This supports the hypothesis that lower PGs may be associated with the occurrence of SDS. However, the biosynthesis of PGs from arachidonic acid is complex. More work is required to elucidate the relationship between lipid metabolism, PGs and SDS.

Table III.5.1. Factorial experimental design with
anchovy-sardine (a-s) meal, biotin
and acetylsalicylic acid (ASA)
(Exp. 5)

Treatment number	Anchovy-sardine (a-s) (%)	Biotin (ug/kg)	Acetylsalicylic acid (ASA) (%)
1	0	0	0
2	0	50	0.2
3	0	500	0
4	0	0	0.2
5	0	50	0
6	0	500	0.2
7	2.5	0	0
8	2.5	50	0.2
9	2.5	500	0
10	2.5	0	0.2
11	2.5	50	0
12	2.5	500	0.2
13	7.5	0	0
14	7.5	50	0.2
15	7.5	500	0
16	7.5	0	0.2
17	7.5	50	0
18	7.5	500	0.2

Table III.5.2. Composition of corn-soy basal diet
supplemented with 0, 2.5% and 7.5% of
anchovy-sardine (a-s) meal (Exp. 5)

| Ingredients | % Anchovy-sardine (a-s) meal | | |
	0	2.5	7.5
	-------	(%)	-------
Yellow corn	67.10	68.03	69.60
Soybean oil	.98	.70	.40
Soybean meal (47.5% CP)	22.00	20.20	14.50
A-s meal (65% CP)	0	2.50	7.50
Meat & bone meal	7.00	5.00	3.00
Alfalfa meal (17% CP)	1.17	1.79	3.50
Defluo. Phos. (32% Ca, 18% P)	.46	.50	.14
Limestone flour	.55	.60	.72
Salt	.25	.22	.22
Trace mineral premix[1]	.05	.05	.05
Vitamin premix[2]	.20	.20	.20
d,l methionine (98%)	.15	.12	.08
Baciferm[3]	.04	.04	.04
Zoamix premix (25%)[4]	.05	.05	.05
Calculated analyses:			
Crude protein, %	20	20	20
Metabolizable energy, kcal/kg	3066	3064	3066

[1]Provided per kg of diet: Ca, 97.5 mg; Mn, 60 mg; Fe,
20 mg; Cu, 2 mg; Zn, 27.5 mg; Co, 2 mg.

[2]Provided per kg of diet: Vitamin A, 3300 IU; vitamin D3,
1100 IU; riboflavin, 3.3 mg; d-pantothenic acid, 5.5 mg;
niacin, 22 mg; choline, 190.9 mg; vitamin B12, 5.5 mcg;
vitamin E, 1.1 IU; vitamin K, 55 mg; folic acid,
22 mg; ethoxyquin, 0.06mg.

[3]Gratuitously provided by International Mineral &
Chemical Corp., Mundelein, IL 60060.

[4]Gratuitously provided by Salsbury Laboratories, Charles
City, IA 50616-9989.

Table III.5.3. Effect of feeding broiler chickens diets
containing anchovy-sardine (a-s) meal,
biotin (B) and acetylsalicylic acid (ASA)
on mean body weight and feed conversion
at 2, 4 and 7 weeks of age (Exp. 5)

	Body weight (gm) Weeks of age			Feed conversion Weeks of age		
	2	4	7	2	4	7
Main effects						
a-s meal	NS	NS	**	NS	NS	NS
0	340	1017	2079^a	1.26	1.65	2.00
2.5%	345	1021	2173^b	1.25	1.66	2.00
7.5%	351	1034	2124^{ab}	1.34	1.68	2.02
Pool SD[1]	14	36	126	.30	.09	.17
Biotin, ug/kg	NS	NS	NS	NS	NS	NS
0	349	1043	2109	1.29	1.64	2.06
50	338	1018	2143	1.27	1.68	1.91
500	351	1041	2123	1.34	1.65	2.08
Pooled SD	32	48	131	.14	.05	.10
ASA, %	**	**	**	**	NS	*
0	360	1050	2213	1.23	1.68	1.93
0.2	332	998	2037	1.34	1.65	2.08
Pooled SD	24	34	76	.11	.06	.20
Interactions						
a-s x B	NS	NS	NS	NS	NS	NS
a-s x ASA	NS	NS	P=.057	NS	NS	NS
B x ASA	NS	NS	NS	NS	NS	NS
a-s x B x ASA	NS	NS	P=.048	NS	NS	NS

NS = No significant difference (P>.05)

* P<.05

** P<.01

[1]Standard deviation.

Table III.5.4. Total mortality and mortality due to Sudden Death Syndrome (SDS) for broiler chickens fed diets containing anchovy-sardine (a-s) meal, biotin (B) and acetylsalicylic acid (ASA) (Exp. 5)

	Total mortality (%)	SDS mortality (%)	SDS as % of tot. mortality
Main effects			
a-s meal	NS	NS	
0	4.87(35)[1]	3.47(25)[1]	71
2.5%	3.61(26)	1.39(10)	38
7.5%	5.70(41)	2.36(17)	41
Pooled SD[1]	3.44	2.57	
Biotin, ug/kg	NS	NS	
0	4.17(31)	2.22(16)	52
50	4.59(37)	1.81(13)	35
500	5.42(34)	3.19(23)	68
Pooled SD	3.61	2.68	
ASA, %	**	*	
0	2.78(30)	1.76(19)	63
0.2	6.68(72)	3.06(33)	46
Pooled SD	2.50	2.13	
Interactions			
a-s x B	NS	NS	
a-s x ASA	NS	NS	
B x ASA	NS	NS	
a-s x B x ASA	NS	NS	

[1]( ) = Number of broilers

NS = No significant difference (P>.05)

* P<.05

[1]Standard deviation.

Table III.5.5. Levels of total liver and heart
lipids for broiler chickens killed at
different time interval (Exp. 5)

Time between death and sample removal (hrs)	Liver[a]		Heart[b]	
	Trmt 5[1]	Trmt 17[1]	Trmt 5	Trmt 17
	-------	(weight %)		-------
1	3.84	4.46	3.36	3.18
2	4.28	4.76	3.25	3.17
4	4.32	4.32	3.25	3.18
Pooled SD[1]	.15	.37	.17	.17

[a,b]There was no difference (P>.05) on time effect or
betweem treatments.

[1]Treatment 5 = 0% a-s meal + 500 ug/kg biotin + 0% ASA
 Treatment 17 = 7.5% a-s meal + 500 ug/kg biotin + 0% ASA

[1]Standard deviation.

Table III.5.6. Levels of liver and heart lipids for non-Sudden Death Syndrome (SDS) and SDS broiler chickens fed diets containing anchovy-sardine (a-s) meal, biotin and acetylsalicylic acid (ASA) (Exp. 5)

Treatment number & description			Non-SDS broilers[a]		SDS broilers	
			Liver	Heart	Liver	Heart
a-s meal (%)	Biotin (ug/kg)	ASA (%)	-----	(weight	%)	------
1, 0	0	0	4.30	3.27	7.14	5.35
2, 0	0	0.2	4.36	3.36	4.11	3.22
5, 0	500	0	4.35	3.40	4.98	3.81
6, 0	500	0.2	4.42	3.26	3.13	2.99
13, 7.5	0	0	4.33	3.08	3.70	3.17
14, 7.5	0	0.2	5.07	3.18	4.97	3.34
17, 7.5	500	0	4.49	3.10	4.29	3.88
18, 7.5	500	0.2	4.60	2.90	4.29	3.88
Pooled SD[1]			.31	.19	.31	.19

[a]No significant differences (P>.05) among the treatments or between non-SDS and SDS birds within each variable

[1]Standard deviation.

Table III.5.7. Fatty acid composition (weight %) of the liver lipid of non-Sudden Death Syndrome (SDS) and SDS broiler chickens, and of non-SDS broiler chickens fed 0 and 7.5% anchovy-sardine meal diets (Exp. 5)

Fatty acid	Cause of death		Anchovy-sardine meal		Pooled SD[1]
	Non-SDS	SDS	0%	7.5%	
16:0	17.72	19.52	17.95	19.70	3.39
16:1w7	1.33^b	2.64^a	2.17	1.85	.66
18:0	16.63^a	14.73^b	15.62	15.60	2.36
18:1w9	14.25^b	20.81^a	17.72	17.81	7.06
18:2w6	10.58	10.03	10.64	9.80	1.95
18:3w3	.02	.11	.09	.04	.14
20:2w6	.37^b	.70^a	.63	.44	.31
20:3w6	.95	1.23	1.20	.96	.40
20:4w6	7.70^a	5.08^b	7.50^a	4.63^b	2.57
20:5w3	1.67^a	0.63^b	0.26^b	2.27^a	.67
22:4w6	0.46	0.23	0.54^a	0.06^b	.30
22:5w3	0.66	0.37	0.35^b	0.72^a	.39
22:5w6	.59	.28	.74	0	.39
22:6w3	4.98	2.66	1.39^b	6.92^a	1.41
n[2]	12	14	15	11	

[a,b]Mean values within each rows followed by different superscripts were significantly different (P<.05).

[1]Standard deviation.

[2]n = numbers of samples

Table III.5.8. Fatty acid composition of anchovy-sardine meal (Exp. 5)

Fatty acids	%
16:0	21.69
16:1w7	4.32
17:0	0.88
18:0	6.23
18:1w9	11.21
trans18:2w6	0.13
18:2w6	1.55
18:3w6	0.13
18:3w3	0.56
20:0	0.25
20:1w9	0.99
20:2w6	0.11
20:3w6	0.21
20:4w6	1.24
20:5w3	9.72
22:1w9	0.19
22:4w6	0.71
22:5w3	2.00
22:6w3	26.78
Others	11.10

CHAPTER IV

POSTULATION AND FUTURE RESEARCH

The objectives of this dissertation were to investigate the possible involvement of fish meal in the etiology of Sudden Death Syndrome (SDS) and hopefully, to develop a practical approach for the prevention/control of this disorder. Although the experimental results did not demonstrated a direct relationship between fish meal and SDS, it suggested that an imbalanced lipid metabolism might be involved.

In general, the characteristics of the SDS broilers observed in the experiments reported here agree with the observation of others (Brigden and Riddell, 1975; Ononiwu et al., 1979; Steele and Edgar, 1982). A decreased level of liver arachidonic acid (C20:4) was repeatedly detected in SDS broilers. This suggests the possible involvement of lipid and prostaglandins (PGs) metabolism in the development of SDS. Recommendations for the prevention/control of SDS based on the literatures available are discussed.

A. Characteristics of SDS Broilers

a. General appearances

In the experiments reported here, no clinical signs were noted in the broiler chickens prior to sudden death. All SDS birds appeared to be eating, moving and squatting

normally. There was no unusual behavior observed in the
flocks from which SDS birds were found. Newberry et al.
(1987) reported that no single pattern or environmental
event was associated with the onset of the sudden attack
prior to death. SDS chickens did not show any consistent
behavioral symptoms which could be used to identify the
problem before it occurred.

In the studies reported here, SDS birds were healthy
and of heavy body weight. The majority of them were found
lying on their backs, and some were found on their sterna.
The neck and leg of the dead birds were extended. These
observations agreed with the general descriptions of the SDS
birds found by others (Ononiwu et al., 1979; Hulan et al.,
1980; Steele and Edgar, 1982). The posture of SDS birds
suggested that immediately prior to the sudden death, they
probably suffered from convulsion or spasm. The final
position of some SDS birds lying on their side may have
resulted from intensive flapping just prior to death. An
imbalanced muscle contraction may be the result of an
abnormal/imbalance concentration of electrolyte, which have
been suggested to be involved in SDS (Hulan and Proudfoot,
1980). Loss of balance, violent flapping and strong muscle
contraction prior to sudden death have also been reported by
Newberry et al. (1987). In SDS birds, the time from loss of
balance to cessation of movement (death) varies from 37 to
69 seconds (Newberry et al., 1987).

Although lying on the back is a common posture of SDS birds, diagnosis of this syndrome should not be made solely based on the posture of the dead bird. Broilers observed to have had a sudden attack and die on their backs might not always show the typical necropsy lesions found in SDS birds (Newberry et al., 1987).

b. Findings from necropsy examination

A full gastrointestinal tract was found in all SDS broilers. Apparently, the affected birds consumed feed normally until the time of death. The contents of the intestines were pale and creamy suggesting a presence of catarrhal enteritis (Brigden and Riddell, 1975; Ononiwu et al., 1979).

Blood clot in the heart was found in most of the SDS broilers. However, Cassidy et al. (1975) suggested that these blood structure was post-mortem in nature.

A pink discoloration of musculature in SDS birds confirmed the observation of others (Brigden and Riddell, 1975; Buenrostro and Kratzer, 1982). Steele and Edgar (1982) suggested that the pink tinge muscle was due to vascular congestion.

Lesions including enlarged liver and kidney, pinkish carcass fat and congested lungs were observed in some of the SDS broilers. The color of the liver was pale or dark red rather than "usually pale" as described by Steele and Edgar (1982).

Fatty infiltrated and friable livers were observed in some, but not all, of the SDS broilers (histological examination of the liver in Experiment 3). Fat accumulation was found in the liver cells of both SDS and non-SDS broilers. However, this condition is common in moderately obese broilers and is not specific to SDS.

The accumulation of fluid in the body cavity was occasionally found in SDS broilers. Abnormal accumulation of fluid in the body is referred to as ascites complex or "water belly" (Dale, 1986). As in the case of SDS, male broilers are more susceptible to ascites than are females (Reed et al., 1987; Fitz-Coy and Harter-Dennis, 1988). It has been hypothesized that SDS is a precursor of ascites (Shane, 1986). However, the etiology of both disorders are not understood. More information is needed in order to elucidate the association between body fluid accumulation, ascites and SDS.

c. Mortality distribution

Distribution by time: Total mortality and mortality due to SDS in Experiments 1-5 reported here are summarized in Table IV.1. SDS accounted for 22-51% of the total mortality in these experiments. The distribution of SDS by time is presented in Table IV.2. SDS first appeared at one week of age, and continued for the entire life span of the birds, i.e. 7 weeks. Sixty-five percent of the SDS broilers were found during the period from the 2-4 weeks.

A mortality survey done by Brigden and Riddell (1975) revealed that mortality due to SDS in different broiler flocks varied form 0.45 to 2.13% with the peak mortality at the third week of age. Riddell and Springer (1984) observed that SDS mortality was 0.71-4.0% with the peak at 2-4 weeks of age. Gardiner et al. (1988) found that SDS death ranged from 1.31 to 9.62% among 23 broiler flocks, and mortality due to SDS reached a maximum when birds were 3-4 weeks of age.

Sex: SDS was found in both male and female broilers. However, over 75% of SDS birds were males (Table IV.3.) This confirmed the observation of Brigden and Riddell (1975) who reported that more than 70% of the SDS birds in all flocks studied were males. In the other survey, 78% of SDS broilers were found to be males (Riddell and Springer, 1984). It appears that female broilers are not as susceptible to this syndrome as male broilers.

Season of the year: Relationship between season of the year and the incidence of SDS has been discussed by Gardiner et al. (1988). They reported that death rate from SDS was affected by the starting month of the experiments, with the highest mortality occurring in January and the lowest in July.

The effect of season was not studied here. SDS mortality for experiments conducted during the cold season (October-December) ranged from 0.63 to 1.44% and during the warm season (April-September) from 2.41 to 2.5% (Table

IV.4.). However, the size and the number of the flocks were too small to reach a conclusive statement.

d. Laboratory analyses

In an attempt to find physiological indicators specific to SDS, hematocrit levels, creatine phosphokinase (CPK) activity (Experiment 1), histological examinations of the liver tissues (Experiment 3) and liver lipid levels (Experiments 1-5) were determined. No differences were observed in the hematocrit readings or CPK activity among broilers fed different dietary treatments. The livers of SDS birds did not exhibit lesions specific to this disorder. Thus far, SDS can still only be determined by the appearance (posture) of the dead bird and the findings in the necropsy examination.

Diagnosis of SDS is limited to the gross examination. No laboratory test is available to identify this disorder either prior to or after death. However, the liver lipid of SDS birds does show a consistent change in the fatty acid composition. The association of this change with the occurrence of SDS is unknown and, therefore, it can not be used as a diagnostic tool. However, this observation suggests a promising lead for further research.

B. Recommendations

    a. Prevention and control

There is no therapeutic program or treatment for SDS. However, several environmental and nutritional factors are thought to be associated with SDS. Based on the review of the literature, certain recommendations can be made which may help to reduce the incidence of SDS.

1) All forms of stress should be minimized. Particularly, crowding, sudden disturbances, loud noise, and high light intensity should be avoided, since these factors have been suggested to induce SDS (Ononiwu et al., 1979; Steele and Edgar, 1982; Hopkinson et al., 1983; Smith, 1984; Eleazer, 1988).

2) SDS affected flocks are reported to be more sensitive to temperature changes, in particular, extreme heat (Hopkinson et al., 1983). Good rearing practices should be followed utilizing proper ventilation and cooling systems, which will improve the living environment for the birds.

3) The incidence of SDS has been suggested to be related to growth rate (Bowes et al., 1988). Diets with higher nutrient densities and increased fat levels can enhance growth but may also increase the incidence of SDS (Martin, 1987). Feeding mash rations, restricting feed intake by 15-20%, or feeding lower carbohydrate rations will help in reducing the incidence of SDS (Eleazer,

1988). However, this is may prove to be more costly than the economic losses associated with SDS.

4) SDS is thought to be a metabolic condition. Vitamins and minerals (electrolytes) are essential in metabolic processes where they serve as cofactors. Thus, it is important to ensure that broiler breeder diets are well fortified with these nutrients to guarantee healthy progenies. Starting broiler chicks on a well-balanced rations supplemented with B complex vitamins are also recommended (Smith, 1984).

b. Future research

Time and fundings are often two major barriers in conducting research. The efficient growth rate of broilers (5.5-7 weeks to marketable age) reduces the time required when conducting the studies with meat-type chickens. However, several other unique situations are present in dealing with SDS: 1) the onset of SDS is not recognizable, therefore, it is impossible to observe the development of this disorder; 2) no specific or unique lesion has been found to be associated with SDS which could provide an indication as to the possible etiology of the problem; and, 3) the incidence of SDS within a given population of birds is relatively low, therefore, large numbers must be used in studying this syndrome.

To fully understand SDS requires the combined efforts of researchers from various disciplines. The lack of

clinical signs suggest that more complete pathological,
histological and chemical studies of the SDS broilers are
needed for a full characterization of the syndrome.
Investigating background information such as the source
(breed origin), age, nutritional status and physiological
conditions of the parent stocks from which SDS flocks are
derived may help to elucidate the genetic link of the
disorder. The possible involvement of nutrients (fat,
carbohydrates, protein, minerals and vitamins) in SDS should
be tested to evaluate the impact of nutrition on the
disorder.

C.   The Possible Involvement of Lipid Metabolism in SDS

The total lipid and fatty acid composition of the liver
were determined in the studies reported here. Accumulation
of fat in the livers was observed in a few, but not all, SDS
broilers. Despite the dietary diversity, SDS broilers
consistently exhibited an altered fatty acid composition of
the liver. Elevated levels of oleic (C18:1) and decreased
levels of linoleic (C18:2) and arachidonic (C20:4) acids
were found. Similar results have been reported by other
researchers (Buenrostro and Kratzer, 1982; Rotter et al.,
1985). To date, this is the only consistent and specific
finding associated with SDS.

Arachidonic acid (C20:4w6), a polyunsaturated fatty
acid, serves as the substrate for the biosynthesis of
prostaglandins (PGs) and their related compounds (Lands,

1982). Given the fact that the concentration of arachidonic acid decreases in SDS broilers, and its metabolites (PGs) possess a wide range of effects on physiological processes, one might postulate that changes in fatty acid metabolism and possibly altered PGs biosynthesis may be involved in the etiology of SDS.

Arachidonic and linoleic acids are considered essential fatty acids (EFA) for poultry (Balnave, 1970), and their major functions are to act as precursors of PGs (Sturkie. 1976; Moncada and Vane, 1978).

PGs are a family of lipid-like compounds derived from polyunsaturated fatty acids. They are synthesized in cell membranes in response to several different types of extrinsic stimuli, such as change in pH, bacteria, cellular trauma and fatty acid intake (Lands, 1982 and 1986; Hadley, 1984; Martin, 1985; Gerrard, 1985). These compounds were discovered in 1930s but have only recently become prominent. The molecular basis of the major actions of PGs is not yet fully understood.

PGs are involved in a wide range of physiological processes. They mediate inflammation, regulate blood flow, control vascular smooth muscle activity, and promote or inhibit aggregation of platelet, etc. (Gryzlewski et al., 1976; Lands, 1982). Arachidonic acid may be utilized in one or more different pathways to produced a different series of PGs depending on the tissue concerned and the particualr nature of the extrinsic stimulus. They often

alter the activities of the cells in which they are
synthesized. The nature of these effects may vary from one
type of cell to another. The overall effects of these
chemicals in a given situation, therefore, depends
critically on the balance of production and the subsequent
effects of individual PGs. Due to this complexity, two
hypothetical theories are proposed to link PGs with the
development of SDS.

Hypothesis 1

The over conversion of arachidonic acid into PGs
results in a lower level of arachidonic acid in the
liver of SDS birds. PGs are capable of stimulating
inflammatory effects (Stryer, 1981) as well as
controlling smooth muscle contractions (Dusheck, 1985).
Ononiwu et al. (1979) postulated that SDS in broilers
began with degenerative and inflammatory changes in the
heart, which resulted in lung edema, reduced gaseous
exchange and respiration. Thus, higher level of PGs
may be involved in the development of SDS by inducing a
sequential pathological process which causes hypoxia,
enhances respiratory distress and leads to SDS.

Hypothesis 2

The pathway by which arachidonic acid is synthesized
(from linoleic acid) is sensitive to competitive
inhibition of other fatty acids, especially of the

oleic acid family (Cunnane, 1982). The increased oleic acid and decreased linoleic acid in SDS broilers suggest the following synario: desaturation and chain elongation of linoleate are competitively inhibited by oleate, thereby, reducing conversion of arachidonic acid from linoleic acid. Consequently, lower levels of arachidonic acid reduces the amount of PGs being synthesized, which eventually interferes with heart function, leading to fibrillation or arrhythmia (Rotter et al., 1985).

At this stage it is difficult to determine which of the proposed hypotheses may better explain the mechanism causing SDS. The biosynthesis and mode of action of PGs are complex. The amount and types of PGs can have either a complementary or antagonistic effects on the same physiological process (Lands, 1982; Hadley, 1984). Studies including mechanistic approaches, should be designed in an attempt to elucidate the interrelationship among lipid metabolism, PGs synthesis and SDS.

Table IV.1. Total mortality and mortality due to Sudden
Death Syndrome (SDS) of broiler chicken
flocks studied in Experiments 1-5.

Exp. No.	Total no. of broilers	Total mort. (%)	SDS mort. (%)	SDS as % of tot. mortality
1	800	5.25	2.50	48
2	832	4.33	0.96	22
3	832	4.54	1.44	32
4	768	1.98	0.63	22
5	2160	4.72	2.41	51

Table IV.2. Weekly distribution of mortality due to
Sudden Death Syndrome (SDS) of broiler
chicken flocks studied in Experiments 1-5.

Weeks of age	Experiments					Total
	1	2	3	4	5	
	----	No. of SDS birds			----	
1	3	0	3	0	4	10
2	5	2	7	1	9	24
3	4	2	0	4	9	19
4	3	2	1	0	15	21
5	0	2	1	1	6	10
6	3	0	0	0	8	11
7	2	0	0	0	1	3

Table IV.3. Distribution of mortality due to Sudden Death
Syndrome (SDS) by sex of broiler chicken flocks
studied in Expriments 1-5.

Exp. No.	SDS mortality	Mortality distribution by sex	
		Males (%)	Females
1	2.50	80	20
2	0.96	75	25
3	1.44	83	17
4	0.63	100	0
5*	2.41	100	0

*Only male broilers were used.

Table IV.4. Mortality due to Sudden Death Syndrome
(SDS) in broiler chicken flocks and season
of the year when Experiments 1-5 were
conducted.

Exp. No.	Experimental period (months)	SDS mortality (%)
1	April 17 - June 5	2.50
2	Octo. 30 - Dec. 18	0.96
3	Octo. 15 - Nov. 16	1.44
4	Octo. 21 - Dec. 9	0.63
5	June 29 - Sept. 8	2.41

CHAPTER V

SUMMARY AND CONCLUSION

Five experiments were conducted to investigate the cause of Sudden Death Syndrome (SDS) in broiler chickens. The nature of the SDS and the association of dietary fish meal with the occurrence of SDS were evaluated. Under the experimental conditions employed, the following observations were made.

1) Affected broilers appear to be well-nourished and healthy. Necropsy examination revealed no specific lesion except for a full gastrointestinal tract and usually an empty gall bladder. The majority of SDS birds were males (Table V.1.)

2) SDS and Fatty Liver and Kidney Syndrome (FLKS) are two different problems. SDS birds remain alert and active until death. The levels of liver lipid in non-SDS and SDS birds were similar (Experiments 1-5). Biotin supplementation (500 ug/kg) did not reduce the incidence of SDS (Experiment 5). With FLKS, on the other hand, hypoglycaemic coma is common, fatty liver is a typical characteristic, and additional biotin is an effective methods of prevention (Payne et al., 1974; Wight and Siller, 1975; Whitehead and Blair, 1976; Hood, 1980).

3) The response of broilers to dietary fish meal with regard to the occurrence of SDS was not consistent (Experiments 1-5). No dose-response relationship between fish meal and SDS could be established.

4) Genetic variation with regard to the resistance to SDS was not observed. However, the number of the broiler chickens used and the number of strain tested in this study were limited (Experiment 4).

5) Evidence has been accumulated to suggest that SDS is a metabolic condition and that its nature is complex (Experiments 1-5). It appears to be a result of a series of contributing factors involving nutrition, genetic and environment. A joint effort of scientists from various disciplines will be required if the etiology of the syndrome is to be elucidated.

6) The relationship between dietary biotin, lipid metabolism and SDS should be investigated further. Fatty acid composition is an indirect indication of the biotin status of the chicken. An elevated level of oleic acid and a decreased level of arachidonic acid, which have been consistently observed in SDS broilers (Experiments 1-5), are typical characteristics of a biotin deficiency (Buenrostro and Kratzer, 1983). Lipid metabolism is a biotin dependent process (Stryer, 1981).

These observations indicate a possible association between biotin, fatty acid metabolism and the occurrence of SDS.

7) There is also evidence to link prostaglandins (PGs) to SDS through an imbalanced lipid metabolism. PGs are a group of lipid-like compounds derived from arachidonic acid, the latter of which was observed to be consistently low in the liver lipid of SDS broilers regardless of the dietary treatments (Experiments 1-5). PGs and their related compounds are powerful local hormones with a wide range of effects on physiological processes. This suggests a good starting point for further research. Investigation regarding the interrelationship among lipid metabolism, PGs and SDS may help to elucidate the etiology of SDS.

The need for research on SDS is urgent because the syndrome has continued to increase since it was first recognized 20 to 25 years ago (Hemsley, 1965; Eleazer,1988). SDS results in enormous economic losses which, in the end, are compensated for through increased cost of the product to the consumer. Thus, a solution to this problem will prove to benefit both the poultry industry and the consumer.

Heart disease is a major known health problem. Pathological observations of SDS indicate the similarity to

heart disease in man with the failure of the cardiovascular system and imbalanced lipid metabolism (Cassidy et al., 1975; Hulan et al., 1980; Rotter et al., 1985). The domestic chicken provides a suitable animal model for the experimental induction and manipulation of atherosclerosis (Sturkie, 1976). Therefore, information as to the cause and prevention of SDS will help to provide a better understanding of human heart diseases.

Table V.1. Symptomatology of Sudden Death Syndrome (SDS) in broilers

DEFINITION	SDS is a non-infectious disease characterized by an unexpected death of well-fleshed broilers with healthy appearance.
OCCURRENCE	SDS affects broilers of all ages but more frequently during 2-4 weeks of age. Over 75% of SDS birds are males. It has been reported in commercial and experimental flocks in Canada, the United States, the United Kingdom and Australia.
CLINICAL SIGNS	SDS occurs without any prior indication. The affected broilers appear to be alert and active only to die moments later. They jump in the air, squawk, flip over and die. The majority of the SDS birds exhibit the posture of lying on their backs with their neck and leg extended. SDS mortality ranges 0.5% - 9.6%.
LESIONS	There are few characteristic signs. A full GI tract and,usually an empty gall bladder. The intestine contents are pale and creamy suggestive of a catarrhal enteritis. The liver is swollen, pale to dark red. The kidney frequently is enlarged. No specific histological lesions are found on examination of spleen, liver, heart, lung, kidney, bone marrow and bursa. Blood chemistry revealed no specific finding.
DIAGNOSIS	Sudden death of well-nourished individual broilers showing the classical 'flip-over' posture and full GI tract. The majority of the SDS birds are males, but the mortality due to SDS within a given population is relatively low.
CONTROL	No prevention/treatment program has been developed for SDS.

Sources: Cassidy et al., 1975; Ononiwu et al., 1879; Riddell and Orr, 1980; Hsaerbach and Tagwerker, 1986; Newberry et al., 1987; Experiments 1-5 (Wu and Nakaue, 1989).

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APPENDIX

Table A.1. Mean 4 and 7 week body weights of male and
female broiler chickens fed corn-soy (c-s)
and c-s+herring meal diets (Experiment 1)

Dietary treatment	Mean body weight			
	4 weeks of age		7 weeks of age	
	Male[a]	Female[a]	Male[a]	Female[a]
	------	(gm)	------	
c-s	833	755	2010	1718
c-s+herring meal	883	742	2075	1743
Pooled SE	19	18	36	15

[a]No significant difference (P>.05)

Table A.2. Mean 4 and 7 week body weights of male and
female broiler chcikens fed corn-soy (c-s),
c-s+2.75%, +5% and +10% herring meal diets
(Experiment 2)

Dietary treatment	Mean body weight			
	4 weeks of age		7 weeks of age	
	Male[a]	Female[a]	Male[a]	Female[a]
	------	(gm)	------	
c-s	945	871	1962	1747
c-s+2.75% herring meal	1011	893	2127	1826
c-s+5% herring meal	991	863	2230	1858
c-s+10% herring meal	1068	890	2052	1758
Pooled SE	42	20	74	50

[a]No significant difference (P>.05)

Table A.3. Mean 4 and 7 week body weights of male and female broiler chickens fed corn-soy (c-s), c-s+herring, +anchovy-sardine (a-s) and +halibut meal diets (Experiment 3)

Dietary treatment	Mean body weight			
	4 weeks of age		7 weeks of age	
	Male	Female	Male	Female
	------	(gm)	------	
c-s	984^a	893	2015^a	1670^a
c-s+herring meal	967^a	889	2000^a	1716^a
c-s+a-s meal	898^b	786	1788^b	1522^b
c-s+halibut meal	968^a	885	1909^a	1789^a
Pooled SE	15	30	47	46

[a,b]Different superscripts denote P<.05.

Table A.4. Mean 4 and 7 week body weights of male and female broiler chickens fed corn-soy (c-s) and c-s+anchovy-sardine (a-s) meal diets (Experiment 4)

Dietary Treatment	Mean	body	weight	
	4 weeks of age		7 weeks of age	
	Male	Female	Male	Female
	-------		(gm)	-------
Main effects				
Diets	NS	NS	NS	NS
c-s	963	844	2147	1800
c-s+a-s meal	947	855	2230	1843
Pooled SE	17	16	31	22
Strain crosses	*	NS	*	*
H x H	972	866	2290	1838
R x AA	987	858	2204	1883
P x AA	880	809	2089	1691
V x H	982	864	2170	1854
Pooled SE	23	22	44	31
Interaction				
Diet x Strain	NS	NS	NS	NS

NS = No significant difference (P>.05).

* P<.05