Pancreatic lipase activity and utilization of dietary fat in young turkeys as affected by 17[beta]-estradiol dipropionate

Fernando Escribano
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Pancreatic lipase activity and utilization of dietary fat in young turkeys as affected by 17β-estradiol dipropionate

Escribano, Fernando, Ph.D.
Iowa State University, 1989
Pancreatic lipase activity and utilization of dietary fat in young turkeys as affected by 17B-estradiol dipropionate

by

Fernando Escribano

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

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For the Major Department

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INTRODUCTION AND LITERATURE REVIEW

General

The following review of the literature includes background information concerning: (1) fat supplementation in diets for young turkeys; (2) retention of dietary fat by young turkeys; (3) determination of pancreatic lipase (EC 1.3.3.1) activity, assay systems, and the lipolytic reaction; and (4) the exocrine pancreas and the influence of sex steroids on its secretory function.

In many instances, no report was found in the literature referring to the above subjects and turkeys. Thus, information from studies with other species have been included. For example, the glycerol trioleate method for determination of lipase activity was originally developed for milk lipases in rats, swine, cats, dogs, and humans. Furthermore, when referring to the effect of sex steroids on the exocrine pancreas, most of the research was done with rats and baboons.

Fat Supplementation in Diets of Young Turkeys

Improved growth and feed efficiency by supplementing turkey diets with fat have been reported by many researchers. The first report of beneficial effects of supplemented fat in turkey diets was that of Biely and March (1954). Since then, numerous studies (for reviews see, Kagan, 1981; Sell, 1985) have documented the effect of fat on turkey performance. Besides their contribution to the energy concentration of the diet, supplemental fats
improve feed efficiency by exerting what is termed as an "extracaloric effect" (Touchburn and Naber, 1966; Vermeersch and Vanschoubroek, 1968). The improvement in performance that is usually observed with the addition of supplemental fat to diets of young turkeys seems to be derived mostly from the increased energy concentration of the diet. For example, Whitehead and Fisher (1975) found that 2-week-old poult's grew at the same rate when fed diets that were isocaloric with or without fat. Furthermore, Hurwitz et al. (1988) found that the performance of young turkeys improved with increasing dietary energy concentration by adding fat, but did not change when fat was added isocalorically into diets of 1- to 3-week-old poult's. It appears that the "extracaloric effect" of fats is not of great significance for turkeys during the first weeks after hatching.

Turkeys have a limited ability to consume food during the first weeks after hatching. For example, poult's at hatch are heavier than chicks. However, feed consumption of poult's during the first two weeks after hatching is less than that of chicks (National Research Council, 1984). This lower feed consumption limits their energy intake and, consequently, their rate of growth during this period. Increasing the energy concentration of the starter diet by supplementing it with fat should, therefore, increase the energy intake of young poult's. As mentioned earlier, however, young poult's do not seem to be able to fully utilize the beneficial effects of supplemental fat. Sell et al. (1986) found that fat retention from diets with different amounts of added fat improved as poult's aged. The authors concluded that young turkeys had a limited ability to utilize dietary fat during early life. The
reasons for the poor ability of young turkeys to utilize dietary fat are not clear, but adequate fat digestion and absorption requires the presence, among other factors, of enough pancreatic lipase, colipase, bile salts, and fatty-acid binding protein. One or more of these factors may not be fully developed in the young turkey, thus limiting the utilization of dietary fat. Several research reports can be found in the scientific literature trying to establish which is the first limiting factor in utilization of fat from the diet. For example, Polin et al. (1980) reported improvements in the utilization of dietary fat when bile acids and/or lipase were added to the diet of broiler-chicks. Also, Sell et al. (1986) reported that improvements in utilization of dietary fat with age may be the result of increased bile secretion and/or increased pancreatic lipase activity. Moreover, Krøgdahl and Sell (1984) found that pancreatic lipase activity increased with age, after a lag-period of about 14 days and attained maximum levels by 42 to 56 days after hatching. In an experiment done concomitantly with this, Sell et al. (1986) found that utilization of dietary fat also approached a maximum between 42 and 56 days of age. Thus, the authors suggested that pancreatic lipase could be involved in the improved utilization of dietary fat with age. These research reports by Krøgdahl and Sell (1984) and Sell et al. (1986) are the only works found in the literature in which retention of fat from the diet was determined simultaneously with pancreatic lipase activity in the young turkey.
Retention of Dietary Fat by Young Turkeys

A substantial amount of research has been done on retention of dietary fat by broiler chickens, laying hens, and growing turkeys (Joshi and Sell, 1964; Touchburn and Naber, 1966; Jensen et al., 1970; Waibel et al., 1977; Sell and Owings, 1981). For the growing turkey, when fats were used to increase the energy concentration of the diet, improvements in feed efficiency were frequently observed. These improvements seem to be firstly determined by the age of the turkeys and secondly by the type of dietary fat used (for a review see, Sell, 1985). In general, retention of dietary fat by turkeys improved with age and the improvement was greater for saturated than for unsaturated fats. These observations are supported, as well, by the work of Whitehead and Fisher (1975) and Salmon (1977) who studied the retention of dietary fat (absorbability) as affected by age and the source of fat. These authors concluded that fat absorbability from vegetable oils was high at 1 week and remained high as poults aged until 6 weeks. Fat retention from animal tallow, however, decreased or remained relatively constant between 1 and 2 weeks and then increased as poults aged until 6 weeks, with most of the improvement occurring around 3 weeks of age. Whitehead and Fisher (1975) and Salmon (1977) concluded that differences in absorbability between soy oil and tallow were due to differences in the rate of absorption of their constituent fatty acids. Because absorbability from vegetable oils was already high by 1 week of age, it could be concluded, as well, that hydrolysis was not limiting its utilization. So, 1-week-old poults appeared to have enough pancreatic lipase, colipase, and bile salts to utilize fat from vegetable oils. It
cannot be concluded however that fat utilization from tallow was limited only by its rate of absorption. A greater amount of lipase could be required for hydrolyzing tallow than soy oil. In fact, Desnuelle (1972) demonstrated that lipase activity was affected by the physical and chemical characteristics of the substrate. Substrates with longer and more saturated fatty-acid chains were hydrolyzed slower than shorter and more unsaturated ones. Thus, it would be of interest to simultaneously determine the retention of saturated and unsaturated fat and the activity of pancreatic lipase by young turkeys.

Determination of Lipase Activity. Assay Systems. The Lipolytic Reaction

General considerations

Ingested lipids undergo emulsification, hydrolysis, micellar solubilization, cell membrane permeation, intracellular esterification and incorporation into lipoproteins before release to the interstitial fluid and transport to the liver through the portal blood system. Lipid hydrolysis in poultry does not begin until dietary lipids reach the upper small intestine. No lipolytic activity has been found in the saliva, proventriculus, and gizzard in poultry. Emulsification, as a first step in lipid digestion starts, however, in the gizzard and is greatly enhanced as the chyme enters the small intestine and is mixed with biliary and pancreatic secretions. Once the emulsion is formed by cooperation of bile salts, phospholipids, proteins and fats, pancreatic lipase, in conjunction with colipase, hydrolyzes the triglyceride at positions 1 and 3 of the glycerol moiety.
Until an enzyme has been obtained in a pure state, it cannot be directly characterized. This is obvious from a quantitative point of view. An impure enzyme cannot be weighed and we are obligated, therefore, to take the magnitude of its catalytic activity as a measure of quantity. In other words, enzymatic units are determined and if done under appropriate conditions, they are proportional to the amount of enzyme present (Desnuelle and Savary, 1963). When homogenates from whole pancreatic tissue are used for determination of lipase activity, one should be particularly careful with the fact that several different enzymes can catalyze the same reaction, even though at different rates. That is one reason why the substrate specificity concept is so important in enzymology studies, and in particular in studies of pancreatic lipase activity. In the case of pancreatic lipase, substrate specificity is understood as the influence exerted by the physical state and chemical nature of the substrate on lipase activity. Physical state requirements for a substrate to be a lipase substrate are to be liquid at the temperature of the assay and to be insoluble in water. Chemical requirements are those related to the chemical nature of the fatty acyl moiety and the alcohol moiety of the triacylglycerol molecule.

Pancreatic lipase activity can be measured \textit{in vitro} by using triacylglycerol substrates. The rate of hydrolysis is greatly affected, however, by the length of the fatty acid chain. Longer chains are hydrolyzed more slowly than shorter ones (Desnuelle, 1972). Because the main biological role of pancreatic lipase is to catalyze the hydrolysis of dietary triacylglycerols, the preferred way to determine lipase activity \textit{in vitro} is to
use a water insoluble, long-chain triacylglycerol in an emulsified state. However, emulsion systems containing short-chain fatty acid triacylglycerols (i.e., tributyrin) have been proven useful and, at present, are frequently utilized in lipase studies.

**Glycerol tributyrate assay system**

With the glycerol tributyrate (GTB) assay system, the rate of hydrolysis of emulsified GTB is measured titrmetrically in the presence of sodium taurodeoxycholate and an excess of pancreatic colipase. Because this procedure utilizes a partially soluble substrate that contains secondary as well as primary ester groups, it does not discriminate against other lipolytic and esterolytic enzymes and cannot, therefore, be used reliably to measure only triacylglyceride lipase in all tissue preparations. For pancreatic extracts, this assay system is most reliable if treatment of the extract with diisopropylfluorophosphate (DFP) is done. DFP inhibits other pancreatic enzymes that hydrolyze tributyrin without affecting pancreatic lipase (Brockman, 1981).

**Glycerol trioleate assay system**

The glycerol trioleate (GTO) assay system was originally developed by Hernell and Olivecrona (1974) for determination of human milk lipase activity and is currently in use for determination of gastric lipases activity in several animal species (Liao et al., 1983; Freed et al., 1986). In this assay system, the rate of hydrolysis of a sonified emulsion of $^3$H-GTO is measured in the presence of sodium taurodeoxycholate and an excess of pancreatic colipase, as required for pancreatic lipase. The $^3$H-oleic acid released is
separated from the partial glycerides and triacylglycerol by liquid-liquid partitioning (Belfrage and Vaughan, 1969) and measured by liquid scintillation. The use of radioactive triacylglycerols greatly increases the sensitivity of the assay. Moreover, the physical state and chemical nature of the substrate fulfill the requirements recommended by Desnuelle (1972) for being a specific pancreatic lipase substrate. On the other hand, it has been shown that certain pancreatic enzymes (i.e., carboxyl ester lipase) can hydrolyze long-chain fatty acid triglyceride substrates as well. Thus, as mentioned earlier for the GTB assay system the GTO assay system is most reliable when using pancreatic extracts if treatment of the extract with DFP is done.

Although the selection of the proper substrate is of great importance, several other components of an assay system and their functions in determining lipase activity deserve mention. The lipase enzyme itself will be described first.

Pancreatic lipase

Pancreatic lipase is the best known and most often investigated of all lipolytic enzymes. It specifically hydrolyzes esters of primary alcohols and is essential for the utilization of dietary fat by vertebrate animals. Dietary triglycerides must be partially hydrolyzed before they can be absorbed in the intestinal tract, as first noted by Claude Bernard (1849). In contrast with other pancreatic enzymes, lipase has a strong specificity for its substrates, triacylglycerols. Therefore, lipase exists in its final active form in the pancreatic gland (Sardá et al., 1964). Chicken pancreatic lipase has a
molecular weight of 48,000 (Bosc-Bieme et al., 1984), which is in the range of equivalent proteins from pig, cow, horse, and man. No report has been found in the scientific literature referring to the molecular weight of turkey's pancreatic lipase.

**Colipase**

Colipase is a peptide cofactor for pancreatic lipase which is necessary to maintain lipase activity in physiological conditions (Borgström and Erlanson, 1973). Colipase is produced by the pancreas in a "pro" form. Procolipase cannot assist lipase while in the "pro" form, but must be activated by trypsin. Chicken colipase has a molecular weight of 11,000 (Bosc-Bieme et al., 1984).

**Bile salts**

Bile salts, more in particular sodium taurodeoxycholate, can be used as an emulsifying agent. Emulsifying agents are needed in the reaction medium to reduce surface tension, maximize surface area and stabilize the emulsion. Bile salts, in combination with colipase, protect lipase from interfacial denaturation (Brockerhoff, 1971; Borgström and Erlanson, 1973; Momsen and Brockman, 1976; Rietsch et al., 1977). Furthermore, bile salts may play an important role in the solubilization of the products of hydrolysis pushing the equilibrium towards the formation of product and thereby favoring the reaction.

**Sodium chloride**

Sodium chloride is necessary to increase the ionic strength of the reaction medium. Increasing the ionic strength has been shown to increase
pancreatic lipase activity when lipase is in solution (Entressangles and Desnuelle, 1968). In other words, sodium chloride improves the lipase-colipase-bile salts interaction by affecting the "interfacial quality".

**Calcium chloride**

Calcium chloride ions are needed for pancreatic lipase to act on long-chain fatty acid triacylglycerol emulsions in the presence of bile salts (Benzonana, 1968). It has been proposed that calcium ions may compensate for the electrostatic repulsion between enzyme and bile salts at the interface. Also, Benzonana and Desnuelle (1968) suggested that calcium ions may provide electrostatic force whereby the divalent ions prevent inhibition of lipase by the fatty acids released during hydrolysis of triacylglycerols.

The most crucial components of a pancreatic lipase assay system have just been described. Several other factors could be added but it is still not clear whether they are necessary in all cases or are effective only in particular conditions. Thus, no further consideration will be given to them in the present review. For additional information, the reader is referred to the book on lipases published by Borgström and Brockman (1984).

**The lipolytic reaction**

Lipolytic enzymes have been defined as "long-chain fatty acid ester hydrolases (Brockerhoff and Jensen, 1974). This definition, though useful in practical terms, indirectly calls for a more fundamental distinction between lipases and esterases. This distinction is based on the involvement of a lipid-water interface in the lipolytic process (Benzonana and Desnuelle, 1965; Brockman, 1984). For many years enzymologists have tried to apply methods
and concepts of enzymology in a homogeneous milieu to what is by definition a heterogeneous reaction system. The environment in which lipolysis occurs contains a minimum of two and frequently four or more different phases. By virtue of their physical properties, substrate, products, and enzyme will partition themselves among these bulk and surface phases. Furthermore, the milieu changes as lipolysis proceeds (Patton and Carey, 1979). Hydrolysis of triglycerides may happen in solution, but efficient hydrolysis requires a lipid-water interface (Brockman et al., 1973). It is generally accepted that lipolysis occurs at the lipid-water interface. However, in an absolute sense, it is difficult to define where the interface is located because of the continuous transition of physical and chemical parameters from one phase to another.

To surmount these difficulties, Gibbs (1870) introduced the thermodynamical concept of surface phase as a two-dimensional surface separating two bulk phases. Because of the interfacial nature of lipolysis, the velocity of a lipolytic reaction should be related more directly to the surface excess of enzyme and substrate than to their overall concentrations. The applicability of the concept of interfacial concentration was demonstrated by Benzonana and Desnuelle (1965), and it has been the conceptual foundation for the lipase research in general and its explosive development in recent years, as described by Borgström and Brockman (1984).

To the complex nature of interfacial lipolysis, Brockman (1984) added that different molecular states can exist in different phases and within a single phase. If now we include the heterogeneity of the biological systems, it becomes almost impossible to predict accurately the behavior of pure lipids
and lipid mixtures at interfaces. This is why studies on regulation of lipolysis on a molecular level have been confined to model systems. Two of these model systems were described previously in this section and have been utilized in the research work presented herein to determine the activity of pancreatic lipase in young turkeys.

The Exocrine Pancreas. Influence of Sex Steroids on its Secretory Functions

Before reviewing the influence of sex steroids on the exocrine pancreas, a brief update of the main theories of the process of pancreatic exocrine secretion is presented.

The pancreatic acinar cell is the predominant secretory cell of the pancreas. Its prime functions are to synthesize and secrete hydrolases for the digestion of dietary macromolecules. Contained within the acinar cells are specialized structures called zymogen granules, that store these hydrolases (Grossman, 1984). When acinar cells are stimulated physiologically, either by acetylcholine elicited from parasympathetic nerve endings or by the gut hormone cholecystokinin (CCK), these granules emit their contents extracellularly via a series of reactions culminating in fusion of the secretory granule with the plasma membrane (Palade, 1975; Williams, 1980; Schulz, 1980), a process called exocytosis. About 20 different proteins are contained within the secretory products (Scheele, 1975). This theory of pancreatic exocrine secretion was called the "cisternal packaging-exocytosis theory" and Palade (1975) was awarded the Nobel Prize for his work on this pancreatic secretory mechanism. Rothman (1975), however, reported that contrary to
the "cisternal packaging-exocytosis theory", digestive enzyme secretion by
the pancreas does not appear to be a simple zymogen packaging (mass
transport) and exocytosis process. Furthermore, digestive enzymes were
neither completely nor continuously isolated from the cytoplasm of the cell.
Rothman (1975) concluded that another secretory process must occur in
which the cytoplasm of the cell plays an important role during transport and
secretion. This process was named the "equilibrium theory of protein
secretion" and emphasized the transport of pancreatic digestive enzymes
through the membranes of the acinar cells in contrast to the segregation of
digestive enzymes in zymogen granules, followed by exocytosis (Rothman,
1980). The "equilibrium theory of protein secretion" has been very helpful to
explain the non-parallel changes in enzyme composition of the pancreatic
exocrine secretion under the influence of physiological (acetylcholine, and
CCK) and exogenous secretagogues (i.e., carbachol, secretin).

The secretory function of the exocrine pancreas has been shown to be
affected by glucocorticoids and/or estrogenic steroids (Beaudoin et al., 1986)
in rats. Sex steroids are commonly regarded as modulators of physiological
activities of the reproductive systems of male and female animals. Evidence
for the ability of sex steroids to influence these organ systems came from the
demonstration of the presence of specific cellular receptors and the change
in the physiological response in the presence or absence of the hormone
(Winborn et al., 1987). Evidence for the influence of sex steroids on the
exocrine pancreas was demonstrated on this basis as well. Several reports
have appeared in the scientific literature referring to the presence of
estrogen receptors in pancreatic tissue (Ullberg and Bengtson, 1963; Sandberg et al., 1973; Sandberg and Rosenthal, 1974). Also, Grossman et al. (1969) reported that pancreatic integrity depended on adrenal and ovarian secretions. When ovaries and adrenal glands were removed, the protein content of the pancreas markedly decreased and zymogen granule depletion occurred. Similar results were obtained by Beaudoin et al. (1986) who found that depletion of zymogen granules in acinar cells from castrated male rats was totally reversed by estradiol treatment. Estradiol treated rats had an even greater number of zymogen granules than control, non-castrated rats. The authors noted that a significant drop in plasma estradiol levels was observed in all rats after both adrenals and testes were removed. Beaudoin et al. (1986) concluded that estradiol was inhibiting the secretion of zymogen granules into the acinar lumen, thus allowing them to accumulate in the cytosol of the acinar cells. This effect of estradiol on the exocrine pancreas was thought to be a direct effect of the hormone on the gland. To support this direct effect Beaudoin et al. (1986) reported the presence of an estradiol-binding protein in rat acinar cells which required a coligand to bind estradiol. Grossman et al. (1984) presented evidence that somatostatin (SRIF\textsuperscript{14}) was the primary coligand required for specific binding of estradiol in rat pancreatic tissue. Somatostatin has been shown to inhibit secretion in a number of tissues: growth hormone release from the pituitary (Brazeau et al., 1973); acetylcholine from the myenteric plexus (Guillemin, 1976); CCK from the gut (Schlegel et al., 1977); salivary secretion (Girod et al., 1980); gastric secretion (Thomas, 1980); insulin and glucagon from the endocrine pancreas (Koerker
et al., 1974; Guillemin, 1976; Honey et al., 1980; Mandarino et al., 1981) and pancreatic exocrine secretion (Susini et al., 1978). It seems reasonable to consider that the inhibitory effects of somatostatin and that of estradiol are causally related. However, it should be noted that estradiol also may be exerting its influence on the acinar cells by increasing the rate of synthesis of certain proteins. This could result in an increased number of zymogen granules in the cytoplasm unless the rate of secretion was also increased. For example in the case of the chicken oviduct, Munro and Kosin (1940) and Brant and Nalbandov (1956) reported a dramatic increase in weight of the oviduct after treatment with 17β-estradiol dipropionate. Furthermore, Schrader and O'Malley (1980) demonstrated a rise in both the rate of ovalbumin gene transcription and in the accumulation of protein in the cytosol. Schrader and O'Malley proposed that estrogens act by directly regulating gene expression. At present, the mechanism by which estradiol has its effect is not well established, and it may be possible that it is different in different body tissues.

Because pancreatic lipase is a component of the zymogen granule and 17β-estradiol is a naturally occurring hormone in turkeys (Bacon et al., 1978), it is of interest to determine whether treatment with 17β-estradiol dipropionate would increase lipase activity in the pancreas of young turkeys. Furthermore, a potential increase in fat retention from the diet could result as well if pancreatic lipase would be inadequate for optimum fat digestion.
Explanation of Dissertation Format

The three sections that constitute this dissertation represent three complete manuscripts which are or will be submitted for publication in the Poultry Science Association Journal. The first manuscript (Section I) will be published under the authorship of Fernando Escribano, Donald C. Beitz, and Jerry L. Sell. The second and third manuscripts (Section II and Section III) will be published under the authorship of Fernando Escribano and Jerry L. Sell. Fernando Escribano is the senior author of all these manuscripts.
SECTION I. GLYCEROL TRI [9,10 (n)-\(^3\)H] OLEATE ASSAY OF TURKEY PANCREATIC LIPASE
Glycerol tri [9, 10 (n)-³H] oleate assay of turkey pancreatic lipase

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ABSTRACT

An assay developed by Hernell and Olivecrona (Biochim. Biophys. Acta 369:234-244, 1974) for human milk lipases was modified and used to measure pancreatic lipase activity (LA) in young turkeys. LA (μmoles of oleic acid (OA) released/min) was determined in pancreatic homogenates. Glycerol trioleate (GTO) was emulsified by sonication in a MES buffer solution with 2% gum arabic. The final incubation system (200 μl) containing the pancreatic homogenate and an excess of turkey colipase was: 3 x 10^5 dpm ^3H-GTO, 5.16 mM GTO, 2mM Mes buffer pH 6.5, 6 mM sodium taurodeoxycholate, .15 M sodium chloride, 1 mM calcium chloride, .3 mM sodium azide, 2 mM benzamidine hydrochloride, 2 mM hydrocinnamic acid, and .5 mM N^benzoyl-D,L-arginine. The latter three constituents were added as proteinase inhibitors. Diisopropylfluorophosphate (1 mM) was added as inhibitor of ester hydrolases other than lipase. Incubations were done at 40° C for 10 min in a water bath after 5 min preincubation time. The released OA was separated by liquid-liquid partitioning and centrifugation. The upper phase contained 89% of the OA but trace amounts of partial glycerides. LA was linear with both the amount of pancreas dry weight added into the medium (P<.0001, R^2=.98) and the time of incubation (P<.0001, R^2=.96) up to 20 min. LA was maximal at .5 μmoles GTO and above. This assay allowed accurate and repeatable determinations of LA with only 2 μg of pancreas dry weight.

Key words: Lipase activity, glycerol trioleate, turkeys
INTRODUCTION

Pancreatic lipase is responsible for catalyzing the hydrolysis of dietary triacylglycerols as they reach the small intestine. Dietary triacylglycerols are mainly formed of long-chain fatty acids; the biological role of pancreatic lipase being thus to hydrolyze long-chain fatty acid triacylglycerol molecules into species that can be readily absorbed through the intestinal wall (i.e., monoglycerides and fatty acids).

Several methods have been proposed for determining lipase activity with triacylglycerol emulsions. Desnuelle (1972), in a review on lipolytic enzymes, pointed out that any method using a triacylglycerol emulsion and giving a true expression of initial velocity could be used. Lipolytic enzymes act at the interface between the lipid substrate and the water phase (Brockman, 1984). Thus, the physical and chemical characteristics of the interface play an important role in the ability of lipase to hydrolyze its substrate. Consequently, the use of long-chain or short-chain fatty acid triacylglycerol substrates will result in different values for lipase activity.

Of all the methods, titrametric methods using either short- or long-chain fatty acid triacylglycerol substrates are the most widely used for determining lipase activity. Continuous titration to a constant pH, as introduced by Marchis-Mouren (1959), is currently a common practice, and has recently been used for determination of pancreatic lipase activity in turkeys (Krogdahl and Sell, 1984; Escribano et al., 1988). Important advantages of this method are that hydrolysis and titration are occurring
simultaneously, which simplifies the procedure, and that a graphic printout of the kinetics of the reaction is obtained while hydrolysis is taking place. Disadvantages of this method, however, are that long-chain fatty acid triacylglycerol substrates require the presence of emulsifiers in the assay medium (Erlanson and Borgström, 1970). Another disadvantage is that the pH of the reaction medium has to be 8 or greater. If the pH is lower than 8, the liberated fatty acids would not be dissociated (protonated), the proton will not be released, and, therefore, lipase activity would be underestimated. This is not a problem when short-chain fatty acid triacylglycerol substrates are used (i.e., glycerol tributyrate). At the pH of the small intestine (pH 6.5) short-chain fatty acids will be completely dissociated. Dietary triacylglycerols are mainly long-chain fatty acid triacylglycerols; so it seems reasonable that pancreatic lipase activity should be determined with such substrates. Furthermore, pancreatic lipase activity should be determined at pH 6.5, and not greater, to more closely simulate the intestinal conditions under which this enzyme acts in the turkey. To solve the dilemma of using long-chain fatty acid triacylglycerols and running the assay at physiological pH, an assay procedure developed by Hernell and Olivecrona (1974) for milk lipases and currently in use for determination of gastric lipases (Freed et al., 1986) was modified and adapted to the particular case of turkey pancreatic lipase. By using this method, a ³H-labelled glycerol trioleate (GTO) emulsion can be incubated at pH 6.5 in a strongly buffered medium. When incubation is terminated the released oleic acid (OA) is separated from the acyl glycerols by liquid-liquid partitioning (Belfrage and Vaughan, 1969) and counted by liquid
scintillation. With this procedure, incubation and partitioning are separated in two steps that occur sequentially instead of simultaneously. This separation allows the use of the most optimal conditions for each step.

The objectives of the research reported herein were: to define the conditions of the GTO assay system for determination of pancreatic lipase activity of turkeys, to validate the assay system, and to compare the values of pancreatic lipase activity obtained with the GTO assay with those determined by automatic titration by using a glycerol tributyrate emulsion substrate.
MATERIALS AND METHODS

Lipase Source, Colipase Source, Homogenizing Buffer, Substrate Emulsion, and Partitioning Solution

Lipase source

Pancreases collected from 14-day-old poult's were used as lipase source. Pancreatic homogenates were prepared in a ground glass tissue homogenizer by dispensing 10 mg of finely pulverized, freeze-dried pancreatic tissue in 10 ml of homogenizing buffer, pH 6.5. Homogenates were then diluted 20-fold in homogenizing buffer and frozen in 5 ml aliquots at -65°C. These diluted homogenates, containing .05 mg of dry pancreas per ml, were used for the lipase assay as described later in this section. Protein concentration in the homogenates was determined by using the bicinchoninic acid method (Smith et al., 1985).

Colipase source

Colipase, free of lipase activity, was partially purified from adult turkey pancreases. Fresh pancreases were obtained immediately after slaughter from a commercial slaughterhouse, freeze-dried, and delipidated by successive washings with chloroform, butanol, and diethyl ether, as indicated by Brockman (1981). About 20 g of dried delipidated pancreas powder were obtained from every 100 g of fresh pancreas weight and were kept at -65°C until used. Colipase was solvent-extracted following a procedure adapted from that described by Canioni et al. (1977) for the preparation of homogeneous colipase from porcine pancreas. Specific colipase activity (in lipase units)
averaged 30-50 μmoles butyric acid hydrolyzed /min x mg protein as determined at the Hormel Institute (MN, USA) by courtesy of H. L. Brockman and W. E. Momsen.

**Homogenizing buffer**

All reagents were bought from Sigma Chemical Co. (St. Louis, MO, USA), unless specified otherwise. The homogenizing buffer contained 2 mM MES (2-(N-morpholino)ethanesulfonic acid, sodium salt, Calbiochem, CA, USA) buffer, 6 mM sodium taurodeoxycholate (Calbiochem, CA, USA), .15 M sodium chloride, 1 mM calcium chloride, .3 mM sodium azide, 2 mM benzamidine hydrochloride, 2 mM hydrocinnamic acid, and .5 mM N\textsuperscript{α} benzoyl-D,L-arginine. The Mes buffer was used because it has a pKa of 6.15, which is slightly lower than the pH of the reaction medium. This allowed for an increased buffering capacity as hydrolysis occurred, thus minimizing inhibition of lipase by the released fatty acids from the triacylglycerol molecule. Entressangles and Desnuelle (1968) found that fatty acids have a strong impact on the characteristics of the interface on which the lipolytic reaction takes place. Sodium taurodeoxycholate was used as emulsifying agent and to interact with colipase in protecting lipase against interfacial denaturation (Brokerhoff, 1971; Borgström and Erlanson, 1973; Momsen and Brockman, 1976; Rietsch et al., 1977). Furthermore, bile salts may play an important role in the solubilization of the products of hydrolysis, pushing the equilibrium towards the formation of product and thereby favoring the reaction. Sodium chloride was added to increase the ionic strength of the medium. Increasing the ionic strength has been shown to increase pancreatic lipase activity when lipase is in solution.
Entressangles and Desnuelle, 1968). In other words, sodium chloride improves the lipase-colipase-bile salts interaction by affecting the "interfacial quality". Calcium chloride was added because calcium ions have been found to be necessary for porcine pancreatic lipase to act on long-chain fatty acid triacylglyceride emulsions in the presence of bile salts (Benzonana, 1968). The role of calcium ions is not yet clear, but it has been postulated that they may compensate the electrostatic repulsion between enzyme and bile salt at the interface, or they may provide electrostatic force whereby the divalent ion prevents inhibition by the fatty acids released in the lipolysis of trioleylglycerols (Benzonana and Desnuelle, 1968). Sodium azide was added to avoid bacterial growth. Benzamidine hydrochloride, hydrocinnamic acid, and N\textsuperscript{\textbeta} benzoyl-D,L-arginine were added as proteinase inhibitors to avoid lipase degradation during homogenization, storage, and incubation (Rudd et al., 1987).

**Substrate emulsion**

The substrate emulsion contained: 7.5 \( \times \) 10\(^6\) dpm of \(^3\)H-TOG (Amersham, IL, USA), and 50 \( \mu \)l unlabeled TOG in 5 ml of homogenizing buffer pH 6.5 with 2 \% gum arabin. Gum arabin (an exudate of high molecular weight of Acacia senegal) was used for its amphipatic properties, i.e., as a surface component needed for the formation of a surface monolayer on the dispersed apolar lipid, producing a stable homogeneous emulsion. The substrate emulsion was prepared by sonification (Branson sonifier 350 with double step microtip) to make the emulsion uniform in size. Emulsions were sonified two times for 1 min at continuous setting and output control 1.5. By this procedure, a particle
diameter of less than .5 μm is obtained, providing maximal interfacial area and number of available substrate molecules for a given amount of substrate. Also $^{14}$C-palmitate (sodium salt, Amersham, IL, USA) was used to determine the influence of the pH of the potassium carbonate buffer on the partitioning efficiency.

**Partitioning solution**

The partitioning solution was prepared by mixing methanol (Fisher Co., MO, USA), chloroform, and heptane (1.41:2.25:1.0, by vol.) as indicated by Belfrage and Vaughan (1969). Also, OA and GTO (carriers) were added at a ratio of 100 times the concentration of GTO in the reaction medium to minimize problems derived from the "mass effect" during centrifugation. Fatty-acid salts can be trapped by compounds of greater mass (molecular weight, i.e., gum arabic), physically forcing them down into the organic phase during centrifugation. A factorial experiment was done with 5 different amounts of pancreas dry weight added into the reaction medium and two partitioning solutions (with and without carriers added). Two replicates were used of each treatment combination. All assays were conducted simultaneously and a pancreatic homogenate from one 14-day-old poult was used. Results of LA obtained when carriers were added were used. Another experiment was conducted to determine the effect of the pH of the potassium carbonate buffer (Fisher Co. MO, USA) on the efficiency of separation between of OA from the GTO and partial glycerides. Three pH treatments (10, 12, and 13) were used with two replications of each treatment. One pancreatic homogenate from a 14-day-old poult was used in this experiment as well. Reagents, solutions, and
other materials used for determining lipase activity by using a glycerol tributyrmate substrate and automatic titration procedures were presented elsewhere (Escribano et al., 1988).

Assay Principle, Procedure, and Validation

Assay principle

The principle of the assay described herein was to quantitate the amount of $^3$H-OA released after incubating pancreas homogenates with a $^3$H-GTO emulsion in the presence of bile salts and an excess of pancreatic colipase. The products of hydrolysis were separated by liquid-liquid partitioning and counted by liquid scintillation.

Assay procedure

Diisopropylfluorophosphate (DFP, 1 mM) was added into each of the pancreas homogenates just prior to starting the assay. The DFP was added as a serine-esterase inhibitor that has been shown to have no effect on porcine pancreatic lipase while inhibiting other pancreatic lipases and esterases (Maylé et al., 1972; Momsen and Brockman, 1977). The GTO emulsion (100 µl) was placed in 16 X 100 disposable glass culture tubes (Fisher Co., MO, USA). Then 40 µl of homogenizing buffer and 20 µl of colipase preparation were added. The tubes were then placed in a 40°C shaking (100 oscillations/min) water bath for 5 min of preincubation. When preincubation was finished, 40 µl of pancreatic homogenate were added into each tube. Tubes were vortexed and incubated, shaking at the same temperature for 10 min. After the incubation period was finished, the reaction was stopped by putting the tubes
for 1 min and adding 3.25 ml of partitioning solution and 1.05 ml of potassium carbonate buffer pH 12. Tubes were then vortexed carefully to mix well and centrifuged at 2,000 X g. From the aqueous fraction (top), .5 ml aliquots were pipetted into scintillation vials, and 7.5 ml scintillation cocktail (Scintisol, Isolab, OH, USA) were added. Radioactivity was measured in a scintillation counter (Beckman LS 750, Beckman, CA, USA). Also .2 ml aliquots of the organic fraction (bottom) were pipetted and counted to check the percent recovery and percent hydrolysis to be sure that the reaction was occurring at initial velocity. The objective was to obtain between 5-10% hydrolysis of the GTO substrate as recommended by Hernell and Olivecrona (1974). Lipase activity was defined as the μmoles of OA released per min of incubation. The μmoles of OA released were calculated as the quotient of the dpm in the aqueous phase (OA) by the specific radioactivity of the GTO substrate (GTO specific radioactivity = 1 Ci/mmol) and multiplied by 3. This multiplication by 3 is based on the assumption that lipase in vitro hydrolyzes the three OAs from the GTO molecule. Specific lipase activity was defined as the lipase activity per mg of pancreas protein.

**Assay validation**

Linearity of lipase activity with the amount of pancreas homogenate added into the assay medium was used as the first criterion. An experiment was done with five different amounts of pancreas dry weight and two replicates were used of each amount. The assay was conducted simultaneously for all the treatments to minimize variation among treatments. The amount of tissue to be selected would be that giving between 5 and 10% substrate
hydrolysis. The second validation criterion used was that lipase activity should not be affected by the amount of substrate emulsion added into the assay medium, (substrate saturation). An experiment was done with increasing amounts of substrate emulsion added to determine the substrate concentration at which lipase activity was not limited by the availability of substrate. Each substrate concentration was replicated two times. A ratio of enzyme to substrate giving between 5 and 10% hydrolysis was selected. The third validation criterion used was that lipase activity should be linear with the incubation time. An experiment was done with different incubation times between 0 and 30 min. For each incubation time the assay was replicated three times. An incubation time resulting in a 5 to 10% substrate hydrolysis was selected. Once these three conditions were fulfilled, the assay procedure was considered satisfactory for in vitro determination of turkey pancreatic lipase.

Statistical analysis was done by linear regression and analysis of variance by using the Statistical Analysis System (SAS) developed by the SAS Institute (1982). Also, orthogonal comparisons were done to determine the plateau during the substrate saturation experiment.
RESULTS AND DISCUSSION

Effect of the pH of the Potassium Carbonate Buffer

Results comparing the partitioning efficiency of $^{14}$C-palmitate (sodium salt) with potassium carbonate buffers of different pHs are shown in Table 1.

Table 1. Effect of the pH of the potassium carbonate buffer on the recovery rate of $^{14}$C-Na palmitate into the aqueous phase

<table>
<thead>
<tr>
<th>pH</th>
<th>$^{14}$C recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>80.5 ± .72&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>89.1 ± .40</td>
</tr>
<tr>
<td>13</td>
<td>78.9 ± 3.10</td>
</tr>
</tbody>
</table>

<sup>a</sup>Standard deviation, based on two replicates.

The amount of $^{14}$C-palmitate recovered in the aqueous phase (methanol:water) after centrifugation was greater ($P<.001$) at pH 12 than at pH 10 or 13. The lower recovery rate of $^{14}$C palmitate at pH 10 was probably due to its lower solubility in water. For long-chain fatty acids to be soluble in water, they have to be dissociated. The dissociation constant (pKa) of long-chain fatty acids can be greater than 10. This means that $^{14}$C-palmitate will be more dissociated at pH 12 than 10. Thus, more $^{14}$C-palmitate will separate into the aqueous phase. Hernell and Olivecrona (1974), reported similar recovery rates of fatty acid soaps separated into the aqueous phase (80%) when using a pH of 10. In the research reported herein, an increase in the pH of the
potassium carbonate buffer to 12 resulted in an even better recovery rate (89%). At pH 13, the partitioning solution became totally cloudy and did not lose its cloudiness after centrifugation, and the partitioning properties of the solution were reduced resulting in a lower recovery rate than at pH 12.

Effect of Adding Carriers into the Partitioning Solution

Results comparing lipase activity (LA, nmoles/min) as affected by the addition of carriers (glycerol trioleate, GTO; oleic acid, OA) into the partitioning solution are shown in Table 2. Carriers were added at 100 times their maximal concentration in the reaction medium (i.e., 5.13 mM for GTO). This addition of carriers resulted in a greater amount of the $^3$H-OA ($P<.001$) in the aqueous phase after centrifugation, thereby giving a more accurate assessment of lipase activity. This effect of the carriers on the partitioning of $^3$H-OA was called the "mass effect" (Brockman, The Hormel Institute, University of Minnesota); it was named after the effect that high mass (high molecular weight, i.e., gum arabic) compounds have on smaller ones ($^3$H-OA) during centrifugation. Gum arabic can entrap $^3$H-OA and physically force it down into the organic phase during centrifugation. The probability for $^3$H-OA being forced down into the organic phase was reduced by increasing the concentration of non-labelled OA by 100 times. Reducing the probability for the $^3$H-OA to be entrapped with addition of carriers thus resulted in a reduced probability for $^3$H-OA to be pushed down into the organic phase. This effect of the carriers was more pronounced when the amount of pancreas dry weight in the reaction medium increased (Table 2).
Table 2. Effect of adding glycerol trioleate and oleic acid (carriers) on lipase activity

<table>
<thead>
<tr>
<th>µgDW&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Carriers</th>
<th>LA, nmoles/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>no</td>
<td>0.98 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>yes</td>
<td>1.32 ± 0.3</td>
</tr>
<tr>
<td>0.5</td>
<td>no</td>
<td>15.37 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>yes</td>
<td>16.64 ± 1.18</td>
</tr>
<tr>
<td>1.0</td>
<td>no</td>
<td>22.85 ± 0.55</td>
</tr>
<tr>
<td></td>
<td>yes</td>
<td>29.67 ± 5.05</td>
</tr>
<tr>
<td>2.0</td>
<td>no</td>
<td>40.86 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>yes</td>
<td>44.65 ± 0.4</td>
</tr>
<tr>
<td>4.0</td>
<td>no</td>
<td>56.39 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>yes</td>
<td>71.37 ± 2.06</td>
</tr>
</tbody>
</table>

<sup>a</sup>In 200 µl of reaction medium.

<sup>b</sup>Standard deviation, based on two replicates.
Determination of the Amount of Enzyme Source Needed

Results for linearity of LA with increasing amounts of dry pancreas added into the reaction medium are presented in Table 3 and Figure 1.

Table 3. Effect of the amount of pancreas dry weight (DW) on lipase activity (LA)

<table>
<thead>
<tr>
<th>DW(^{a, \mu g})</th>
<th>LA, nmoles/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>.5</td>
<td>15.95</td>
</tr>
<tr>
<td>1.0</td>
<td>31.2</td>
</tr>
<tr>
<td>2.0</td>
<td>49.6</td>
</tr>
<tr>
<td>4.0</td>
<td>84.1</td>
</tr>
<tr>
<td>SEM(^b)</td>
<td>3.4</td>
</tr>
</tbody>
</table>

\(^{a}\)In 200 \(\mu l\) of reaction medium.

\(^{b}\)Standard error of a treatment mean.

Lipase activity increased linearly \((P<.0001)\) between 0 and 85 nmoles OA/min as the pancreas dry weight (DW) increased between 0 and 4 \(\mu g\).

Once linearity of LA with DW was obtained, the amount of pancreas DW that resulted in a 5 to 10% conversion of substrate into product was determined. A pancreas DW of 2 \(\mu g\) was selected as the most adequate to fulfill this requirement, thus ascertaining the conditions for the reaction to occur at initial velocity.
LA = 5.7 + 20.3 DW, \( R^2 = 0.98, \ P < 0.0001 \)

Figure 1. Linearity of lipase activity on pancreas dry weight (DW)
Determination of the Amount of Substrate Needed

Results of LA as affected by the substrate (GTO) concentration of the reaction medium are presented in Table 4 and Figure 2.

Table 4. Effect of the concentration of glycerol trioleate (GTO) emulsion substrate in the reaction medium on lipase activity

<table>
<thead>
<tr>
<th>GTO, µl</th>
<th>GTO, µmoles</th>
<th>GTO, mM</th>
<th>LA, nmoles/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>.21</td>
<td>1.032</td>
<td>21.7</td>
</tr>
<tr>
<td>10</td>
<td>.42</td>
<td>2.064</td>
<td>28.0</td>
</tr>
<tr>
<td>20</td>
<td>.84</td>
<td>4.128</td>
<td>24.7</td>
</tr>
<tr>
<td>40</td>
<td>1.68</td>
<td>8.256</td>
<td>28.3</td>
</tr>
<tr>
<td>60</td>
<td>2.52</td>
<td>12.384</td>
<td>29.0</td>
</tr>
</tbody>
</table>

SEM\(^b\) 1.32

\(^a\) In 200 µl of reaction medium.
\(^b\) Standard error of a treatment mean.

A sharp increase in LA occurred between 0 and .42 µmoles. Then, a slow, not significant (P>.10) increase in LA occurred with increasing concentrations of GTO between .42 and 2.52 µmoles. A horizontal line, thus, was fitted through these points to indicate that no further increases in LA were obtained for substrate concentrations above .42 µmoles of GTO/200 µl of reaction medium. Once the substrate was found not to be limiting the rate of
Figure 2. Effect of the amount of glycerol trioleate in 200 μl of reaction medium on lipase activity (LA). Differences in LA for substrate concentrations above .5 μmoles were not significant (P>.05), but their average differed significantly (P<.05) from the LA at .25 μmoles. Thus, a break-point has been approximated at about .5 μmoles of GTO.
the reaction, the concentration of substrate that would give initial velocity conditions was selected. Consequently, a substrate concentration between 1 and 1.5 moles/200 μl of reaction medium was selected. The lower LA obtained at 0.84 moles could not be explained and was thought to be due to an inadequate emulsion preparation.

It should be noted, however, that the lipolytic reaction occurs at the interface between the lipid substrate and the water medium. Thus, the reaction rate is more dependent on the amount of surface area available for the lipase enzyme to attach than on the concentration of substrate itself in the reaction medium. Because of the difficulties in determining the interfacial area, however, substrate saturation can be a helpful criterion for evaluating the conditions of the assay system.

Determination of the Time of Incubation

Results for LA as influenced by incubation time between 0 and 30 min are shown in Table 5 and Figure 3. Once the amount of enzyme and substrate to be used were defined, it was necessary to determine if the reaction proceeded linearly with the incubation time. To be most suitable the reaction should not have shown a sudden increase at start, and it should not decrease during incubation. No sudden increase was observed at the start of the reaction, and no decrease in the reaction rate occurred during the 30 min of incubation tested.
Table 5. Effect of incubation time on lipase activity

<table>
<thead>
<tr>
<th>Time, min</th>
<th>LA, nmoles OA/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.5</td>
</tr>
<tr>
<td>5</td>
<td>16.1</td>
</tr>
<tr>
<td>10</td>
<td>25.4</td>
</tr>
<tr>
<td>20</td>
<td>38.3</td>
</tr>
<tr>
<td>30</td>
<td>46.7</td>
</tr>
<tr>
<td>SEM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.25</td>
</tr>
</tbody>
</table>

<sup>a</sup>Standard error of a treatment mean.

Lipase activity increased linearly ($R^2 = .96, P<.0001$) with incubation time between 0 and 30 min. Similar results were reported by Hernell and Olivecrona (1974) for bile salt-stimulated lipase, who found that lipase activity developed linearly until about 100 nmoles of OA were released into the reaction medium. From the results obtained in the research reported herein, an incubation time of 10 min was selected as most adequate. The reaction occurred in a linear manner for at least 20 min, and in 10 min of incubation time the conversion of substrate into product was between 5 and 10%.
Figure 3. Effect of the incubation time on lipase activity
Comparison with the Glycerol Tributyrate Assay

Results for specific lipase activity (SLA, μmoles/min x mg protein) in six pancreases collected from 14-day-old turkeys are presented in Table 6.

Table 6. Specific lipase activity (SLA) as determined by using glycerol tributyrate (GTB) or glycerol trioleate (GTO) emulsion substrate

<table>
<thead>
<tr>
<th>Sample</th>
<th>SLA, μmoles/min x mgPW</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.5</td>
</tr>
<tr>
<td>2</td>
<td>16.7</td>
</tr>
<tr>
<td>3</td>
<td>19.3</td>
</tr>
<tr>
<td>4</td>
<td>21.0</td>
</tr>
<tr>
<td>5</td>
<td>29.5</td>
</tr>
<tr>
<td>6</td>
<td>31.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>GTB</th>
<th>GTO</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEMa</td>
<td>1.86</td>
<td>.79</td>
</tr>
</tbody>
</table>

aStandard error of a treatment mean.

Determinations of LA were done by using the GTO assay system presented herein and by using the glycerol tributyrate (GTB) emulsion substrate and the automatic titration assay described by Escribano et al. (1988). Although no reports have been found in the scientific literature making quantitative comparisons of pancreatic lipase activity by using both methods, comparative data obtained in the current research were used as an additional criterion to validate the GTO assay. On the average, SLA was significantly greater (P<.001) when determined by using GTB procedure as compared with
GTO. However, both methods give results that followed similar trends and were highly correlated. The correlation coefficient between SLA determined with the two methods was $R^2 = .80$ (P<.001). Therefore, this relationship could be described by the following equation:

$$SLA_{GTB} = -6.11 + 2.11 \times SLA_{GTO} \quad R^2 = .80, \quad P<.001$$

Several factors may be involved in the difference in LAs obtained from each of these methods. Besides the differences due to the method itself, it is also reasonable to think that LA determined by using GTB will be greater. GTB can be hydrolyzed by most lipases known. Therefore, other pancreatic enzymes than lipase (i.e., carboxyl ester lipase, EC 3.1.1.13, Rudd and Brockman, 1984) could be contributing to the greater activity of pancreatic lipase using GTB than using GTO. It should also be considered that the GTO assay system reported herein contains diisopropylfluorophosphate which inhibits pancreatic esterases activity without affecting pancreatic lipase.

In summary, the activity of pancreatic lipase, or amount of active enzyme in the pancreatic gland will vary with the assay system used. The selection of one system or another should be made by the investigator according to the objectives of the research. The assay system presented herein, however, is recommended for determination of lipase activity in pancreas homogenates of turkeys. Because of its high sensitivity, it is particularly recommended when the amount of pancreatic tissue available is very small. Furthermore, because dietary lipids are mostly composed of long-chain fatty acid triacylglycerols, the use of a GTO substrate emulsion should give better estimates of the digestive capacity of pancreatic lipase. Moreover,
when lipase is not isolated from other pancreatic enzymes; and when only small portions of the pancreatic gland or pancreatic juice can be collected, the use of a radiolabelled substrate greatly increases the sensitivity of the assay.
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SECTION II. EFFECT OF 17β-ESTRADIOL DIPROPIONATE ON THE DEVELOPMENT OF PANCREATIC LIPASE ACTIVITY IN YOUNG TURKEYS
Effect of 17β-estradiol dipropionate on the development of pancreatic lipase activity in young turkeys

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Jerry L. Sell, B.S., M.S., Ph.D.

From the Department of Animal Science, Iowa State University, Ames, IA 50011
Two experiments were conducted to evaluate the effect of 17β-estradiol dipropionate \((E_2)\) on pancreatic lipase activity during the first few weeks after hatching in turkeys. In Experiment 1, pancreatic lipase activity of control (non-injected) male poults was compared with that of male poults injected with 5.5 mg \(E_2/\text{kg}\) of body weight (BW) on days 1 and 3 of age. The total amount of \(E_2\) given was 8.0 µg. Lipase activity was determined in pancreases from 1-, 4-, 7-, 10-, and 13-day-old poults by measuring the release of butyric acid (BA) hydrolyzed from a glycerol tributyrate (GTB) substrate in an automatic titrator. In Experiment 2, male and female poults were injected with 0, 0.02, 0.2, and 2 mg of 17β-estradiol dipropionate \((E_2)/\text{kgBW}\) on alternate days from 2 to 8 days post-hatch. The total amounts of \(E_2\) injected were 0, 8.8, 88, and 880 µg per poult. Non-injected poults were used as negative control. All injections were given into the breast muscle. In Experiment 2, pancreases from one poult from each replicate were collected at 9, 14, and 21 days post-hatch. Lipase activity was determined by measuring the release of oleic acid (OA) from a glycerol tri [9, 10 (n)-\(^3\)H] oleate substrate.

In Experiment 1, specific lipase activity \((\text{SLA, } \mu \text{moles } \text{BA/min x mg protein})\) was greater \((P<.05)\) at 1 day than at 4, 7, 10, and 13 days of age. Poults injected with \(E_2\) had greater \((P<.05)\) SLA, total lipase activity \((\text{TLA, } \mu \text{moles BA/min})\), and pancreas dry weight \((\text{DW})\) at 4 days of age than non-injected poults. Poults treated with 5.5 mg \(E_2/\text{kgBW}\) presented a high incidence of rectal prolapses, hyperirritability, cannibalism, and mortality. In
Experiment 2. SLA (μmoles OA/min x mg protein) was greater at 9 than 14 or 21 days of age regardless of E2 dosage (19.0 vs 14.0 and 15.0, respectively). Sex did not influence SLA (P>.5). SLA was numerically greater with increasing doses of E2 at all ages. Pancreas DW and TLA per pancreas increased with increasing doses of E2. TLA averaged 1,700 at 9 days and increased (P<.01) to 2,400 and 4,300 μmoles OA/min at 14 days and 21 days of age, respectively. Poult injected with 2 and .2 but not with .02 mg of E2/kgBW developed rectal prolapse, but no mortality occurred during Experiment 2. Treatment with .02 mg of E2/kgBW increased TLA and thereby might improve utilization of dietary lipids by young turkeys.
INTRODUCTION

Sex steroids are commonly regarded as modulators of physiological activities of the reproductive systems of male and female animals. Evidence for the ability of sex steroids to influence these organ systems comes from the demonstration of the presence of specific cellular receptors and the change in the physiological response under the presence or absence of the hormone. Estrogen receptors have been found in a variety of tissues other than the reproductive tract, including the pituitary gland (Everett, 1964; Flerkó and Szentagothai, 1957), the cardiovascular system (McGill et al., 1980), the liver (Viladiu et al., 1975; Porter et al., 1983), and the pancreas (Sandberg et al., 1973; Tesone et al., 1979; Grossman et al., 1985; Winborn et al., 1987) of various animal species. The importance of sex hormones in regulating the growth, as well as the synthesis and secretion of specific albumen proteins by the chicken oviduct was shown by Brant and Nalbandov (1956) and by O'Malley et al. (1969). In more recent studies (Grossman et al., 1983; Boctor et al., 1983; Grossman et al., 1985; Beaudoin et al., 1986), it was found that sex steroids can also play an important role in modulating physiological activities of the endocrine and exocrine pancreas. For example, a marked depletion of zymogen granules in acinar cells of the pancreas was observed after adrenalectomy and/or ovariectomy in female rats. Treatment with 17ß-estradiol (E2) resulted in a complete restoration of these secretory vesicles, suggesting that this hormone exerts its action directly on the pancreatic
gland. Also, it has been shown that E2 is a naturally occurring hormone in chickens (O'Grady, 1966) and in turkeys (Bacon et al., 1978).

Pancreatic lipase activity in turkeys has been found to be relatively low during first weeks after hatching (Escribano et al., 1988). Krogdahl and Sell (1984) and Sell et al. (1986) suggested that increments in pancreatic lipase activity with age could be involved in the improved utilization of dietary fat as the young poult aged. Also, supplementation of chicken starter diets with exogenous lipase resulted in an increased utilization of dietary fat (Polin et al., 1980).

The objectives of the research reported herein were to see whether treatment with 17β-estradiol dipropionate will increase lipase activity in the pancreatic gland and to determine a dose of E2 that increases lipase activity but does not cause any adverse side-effects in the young poult.
MATERIALS AND METHODS

Experimental Procedure

Experiment 1

Experiment 1 was designed as a preliminary experiment to determine if 17β-estradiol dipropionate (E2) has any influence on pancreatic lipase activity in young turkeys.

Ninety 1-day-old male poults were obtained from a commercial hatchery. At reception and before any food or E2 was given, 18 poults were killed and pancreases were collected to obtain baseline data on pancreatic lipase activity. From these 18 poults, six samples were prepared by pooling the pancreases collected from every three poults to make one sample. Pooling was necessary to obtain enough tissue for lipase activity determinations. The remaining 72 poults were placed at random in 12 (100 X 35 X 30 cm, length X width X height) pens in a battery brooder at a number of six poults per pen. Electrical heaters were preset at 35°C and 32°C for the first and second week, respectively. Six pens were randomly assigned to two treatments (control and 5.5 mg E2/kgBW). The 5.5 mg E2/kgBW dose was selected on the basis of research done by Akiba et al. (1982) who used a similar dose of E2 with chicks. Even though these researchers did not study lipase activity, their research was the only reference found using E2 in young poultry and thus was used as a starting point for our study. Injections of E2 dissolved in .2 ml of soybean oil, were given into the breast muscle on days 1 and 3 after hatching. Expected body weights at 1 and 3 days of age were 65 and 80 g, respectively;
therefore, poults under E2 treatment received 3.6 and 4.4 μg on days 1 and 3. On days 4, 7, 10, and 13 of age, poults were weighed and killed by cervical dislocation after halothane anesthesia. Immediately after poults were killed, pancreases were excised, weighed, frozen in liquid nitrogen, freeze-dried, and stored at -65°C until used. Lipase activity was determined in homogenates of dry pancreas powder in pH 6.5 Tris-HCl buffer. The procedure for preparation of the pancreas homogenates, the composition of the homogenizing buffer and the composition of the reaction medium were those given by Escribano et al. (1988). Briefly, lipase activity was determined by using a glycerol tributyrate emulsion substrate. The released butyric acid was measured in an automatic titrator (pH-Stat, Sargent-Welch Scientific Company, Skokie, IL). One unit of lipase activity was defined as the amount of enzyme that catalyzed the production of one micromole of butyric acid (BA) per min at 40°C. Specific lipase activity (SLA, μmoles BA/min x mg of protein) was defined as the lipase activity per unit of pancreatic protein. Pancreatic protein was determined by the bicinchoninic acid method (Smith et al., 1985). Feed intake was determined during the 4, 7, 10, and 13 days of age periods. Food and water were offered ad libitum throughout the experiment. The composition of the experimental diet is given in Table 1.
Table 1. Composition of the experimental diet. Experiments 1 and 2

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean meal (44% protein)</td>
<td>55.91</td>
</tr>
<tr>
<td>Corn</td>
<td>36.03</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>3.61</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>2.23</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.48</td>
</tr>
<tr>
<td>Poult vitamin premixa^</td>
<td>.30</td>
</tr>
<tr>
<td>Poult mineral premix^</td>
<td>.40</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>.04</td>
</tr>
<tr>
<td>Calculated analysis</td>
<td></td>
</tr>
<tr>
<td>Dry matter, %</td>
<td>89.71</td>
</tr>
<tr>
<td>Metabolizable energy, kcal/kg</td>
<td>2800</td>
</tr>
<tr>
<td>Crude protein, %</td>
<td>27.50</td>
</tr>
<tr>
<td>Ether extract, %</td>
<td>5.42</td>
</tr>
<tr>
<td>Crude fiber, %</td>
<td>4.87</td>
</tr>
<tr>
<td>Total sulfur amino acids, %</td>
<td>.90</td>
</tr>
<tr>
<td>Methionine, %</td>
<td>.46</td>
</tr>
<tr>
<td>Lysine, %</td>
<td>1.72</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>1.20</td>
</tr>
<tr>
<td>Available phosphorus, %</td>
<td>.60</td>
</tr>
</tbody>
</table>

^aThe vitamin premix supplied the following per kg of diet: vitamin A, 5,000 IU; vitamin D₃, 1,500 IU; vitamin E, 12 IU; vitamin B₁₂, 11 µg; menadione, 1.8 mg; riboflavin, 2.7 mg; pantothenic acid, 7.2 mg; niacin, 75 mg; choline, 520 mg; folic acid, 55 mg; biotin, 75 µg.

^bThe mineral premix supplied the following per kg of diet: manganese, 89 mg; zinc, 47 mg; iron, 44 mg; copper, 6.7 mg; sodium chloride, 3.1 g.
Experiment 2

Experiment 2 was designed to determine the dosage of E2 that would not have undesirable side-effects on the poults, but would still increase lipase activity in the pancreas. The weight of the left oviduct was used as criterion to ascertain that E2 was received by the poults and to evaluate side-effects. In the following section, only those aspects of the experimental procedure in which experiment 2 differed from experiment 1 will be described.

A 2 x 5 factorial experiment was designed with two sexes and five E2 doses (control, 0.0, 0.02, 0.2, and 2.0 mg E2/kg BW). One hundred and fifty (75 males and 75 females), 1-day-old poults with body weights between 55 and 65 g, were randomly distributed within each sex group to 30 pens (five poults/pen), and three pens were randomly assigned to each treatment combination. All poults were debeaked and their toe nails cut prior to distribution into the pens. This was necessary to avoid problems of cannibalism due to hyperirritability of E2-treated poults as observed during Experiment 1. Also, a creosote ointment was used to spread onto the vent to decrease further problems in birds showing incidence of rectal prolapse. Electrical heaters were preset at 35, 32, and 29°C for the first, second, and third week, respectively. Ingredient composition and calculated analysis of the experimental diet were the same as in experiment 1 (Table 1). A stock solution of E2 (Sigma Chemical Company, ST. Louis, MO) was prepared with 1.6 mg E2 per ml of corn oil. Working solutions of the desired concentrations for each E2 dosage (0, .02, .2, and 2 mg/kg BW) were prepared by serial dilutions of the stock solution in corn oil. Poults were injected intramuscularly with each
dose of E2 dissolved in .2 ml of corn oil on days 2, 4, 6, and 8 post-hatching, and non-injected poults were used as negative control. Because expected body weights at 2, 4, 6, and 8 days of age were 60, 90, 130, and 160 g, respectively, poults in each of the treatment groups received a total of 0.0, 8.8, 88.0, or 880.0 µg E2 by Day 8. Body weight and feed intake were recorded during each period. Pancreases from one poult randomly selected from each pen were collected at 9, 14, and 21 days after hatching, weighed, and stored as described previously in Experiment 1. Also, the left oviduct was carefully collected and weighed. Because the oviduct is known to be the primary target tissue for estrogen hormones, its weight was the criterion used for evaluating overdosage and undesirable side-effects.

Pancreatic homogenates were prepared in a ground glass tissue homogenizer with 10 mg of finely ground freeze-dried pancreatic powder in 10 ml of homogenizing buffer, pH 6.5. The composition of the homogenizing buffer was given by Escribano et al. (1989). Homogenates were diluted 20-fold and frozen in 5-ml aliquots at -65°C. These diluted homogenates (0.05 mg pancreas dry weight (DW)/ml) were used for the lipase assay. The original homogenates were diluted 2-fold for determination of protein concentration. Protein was determined by the bicinchoninic acid method (Smith et al., 1985). Lipase activity was determined using a glycerol trioleate (GTO) instead of the glycerol tributyrate (GTB) substrate that was used in Experiment 1. The GTO assay system used herein was described by Escribano et al. (1989). Briefly, the assay involves the determination of 3H-oleic acid (3H-OA) released from the glycerol tri [9, 10 (n)-3H] oleate substrate after incubation with an aliquot of
the pancreatic homogenate at 40°C for 10 min in the presence of bile salts and an excess of turkey pancreatic colipase. The released $^3$H-OA was then separated from the partial glycerides and triglycerides by liquid-liquid partitioning and counted by liquid scintillation. One lipase unit was defined as 1 µmole OA released from the glycerol trioleate molecule per min at 40°C. Specific lipase activity (SLA, µmoles OA/min x mg of protein) was calculated as the lipase activity per unit of pancreas protein. Total lipase activity (TLA, µmoles OA/min) per pancreas was calculated as the product of SLA times the protein content of the pancreas. The reason for using a GTO assay system was based on the greater specificity that the GTO substrate has for determining lipase activity in whole pancreas homogenates. Moreover, when samples of pancreas are collected from very young poults differences in lipase activity as determined by the two assay systems can be of considerable importance (Escribano et al., 1989).

Statistical Analysis

Results of Experiment 1 were analyzed by analysis of variance to determine whether the effect of 5.5 mg E2/kgBW was significant and for how long the effect lasted after the last dose was given. Regression analysis was used, however, to study the changes in lipase activity with age. Results from Experiment 2 were analyzed statistically by analysis of variance, and orthogonal comparisons were made to determine the effect of the injection itself by using the non-injected poults as negative control. The effect of E2 was analyzed by regression of each of the studied criteria on the logarithm of
the dose of E₂. Statistical calculations were done by using the system developed by the SAS Institute (1982).
Experiment 1

Injection of 5.5 mg E2/kgBW did not affect (P>.10) body weight of poult's during the experiment. Body weight of poult's averaged 64, 92, 146, 203, 281 g by 1, 4, 7, 10, and 13 days of age, respectively. Feed intake was consistently greater for E2-treated poult's (10.5 vs 8.5 g/poult per day) from day 1 to 4 after hatching, but differences were not significant (P>.10). This trend for a greater feed intake disappeared by day 7, 3 days after the last E2 dose was given.

Results for SLA, TLA, and pancreas dry weight (DW) are presented in Table 2 and Figures 1 and 2. SLA in control poult's was greater at 1 day than at 4, 7, 10, and 13 days of age. For poult's treated with E2, SLA remained high until day 4, but by day 7, SLA was reduced to that of control poult's. Thus, SLA was greater (P<.05) for E2-treated than for control poult's (57 vs. 36 μmoles BA/min x mg protein) at 4 days of age. No differences (P>.05) between treatments were detected when poult's were 7-day-old or older. TLA increased from 1,050 to 1,500, 4,200, 6,050, and 8,450 μmoles BA/min between 1 and 4, 7, 10, and 13 days of age, respectively, for control poult's. However, TLA increased more drastically from 1,050 to 3,900 μmoles BA/min between 1 and 4 days of age, when poult's were treated with E2. This dramatic increase in TLA per pancreas was due to an increase in SLA together with an increase in the weight of the pancreas (total protein) itself. Pancreas DW at 4 days of age was greater (P<.05, 65 vs. 95 g) for E2-treated poult's than for control poult's.
Table 2. Effect of 17β-estradiol dipropionate (E2) on specific lipase activity (SLA), total lipase activity (TLA), and pancreas dry weight (DW) of poults during the first two weeks after hatching. Experiment 1

<table>
<thead>
<tr>
<th>Age, d</th>
<th>SLA, μmoles BA/ min x mg protein</th>
<th>TLA, μmoles BA/min</th>
<th>DW, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>E2</td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>54</td>
<td>-</td>
<td>1050</td>
</tr>
<tr>
<td>4</td>
<td>36</td>
<td>57*</td>
<td>1500</td>
</tr>
<tr>
<td>7</td>
<td>44</td>
<td>43</td>
<td>4200</td>
</tr>
<tr>
<td>10</td>
<td>42</td>
<td>42</td>
<td>6050</td>
</tr>
<tr>
<td>13</td>
<td>46</td>
<td>54</td>
<td>8450</td>
</tr>
</tbody>
</table>

SEM<sup>a</sup>  5.0  545  10.3

<sup>a</sup>Standard error of a treatment mean.
<sup>*P<.05.</sup>
Figure 1. Specific lipase activity in pancreases from control (○—○—○) and 17β-estradiol dipropionate treated (●—●—●) pouls. Experiment 1
Figure 2. Total lipase activity per pancreas in control (○○○○○) and 17β-estradiol dipropionate treated (●●●●●) poults. Experiment 1
In this preliminary trial, about 80% of the E2-treated poults developed rectal prolapse, and about 30% of the treated poults died. Mortality was mainly due to hyperirritability and cannibalism among the poults and occurred mostly during 4 days after the last dose of E2 was given. Spreading a creosote ointment on their vents totally eliminated mortality by day 10.

Experiment 2

Growth

Results for body weight gain, feed intake, and feed efficiency (gain:feed) of males and females are shown in Table 3. Males grew faster and ate more food than females (P<.05). Males were more efficient than females throughout the experiment, but differences were statistically significant (P<.05) only during the 1- to 14-day period. Effects of E2 injection on the performance criteria measured are shown in Table 4. The injection itself had a negative effect on weight gain and feed intake. Poults injected with .2 ml of corn oil containing no E2 had lower (P<.10) body weight gain and feed intake than did negative control (non-injected) poults during the 1- to 9-day period. The negative effect of the injection was not significant (P>.10) after 9 days and totally disappeared by 21 days of age. No influence (P>.10) of the injection on feed efficiency was observed.
Table 3. Body weight gain, feed intake, and feed efficiency of male and female turkeys during the first 3 weeks after hatching.

Experiment 2

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Period, days</th>
<th>Females</th>
<th>Males</th>
<th>SEM^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain, g/poult per day</td>
<td>1-9</td>
<td>12.4</td>
<td>13.2</td>
<td>.30</td>
</tr>
<tr>
<td></td>
<td>1-14</td>
<td>16.2</td>
<td>18.0*</td>
<td>.42</td>
</tr>
<tr>
<td></td>
<td>1-21</td>
<td>20.0</td>
<td>22.3**</td>
<td>.62</td>
</tr>
<tr>
<td>Feed intake, g/poult per day</td>
<td>1-9</td>
<td>14.5</td>
<td>15.3*</td>
<td>.44</td>
</tr>
<tr>
<td></td>
<td>1-14</td>
<td>22.2</td>
<td>24.0**</td>
<td>.41</td>
</tr>
<tr>
<td></td>
<td>1-21</td>
<td>29.3</td>
<td>33.1**</td>
<td>1.00</td>
</tr>
<tr>
<td>Gain/feed, g/g</td>
<td>1-9</td>
<td>.85</td>
<td>.87</td>
<td>.028</td>
</tr>
<tr>
<td></td>
<td>1-14</td>
<td>.73</td>
<td>.75*</td>
<td>.009</td>
</tr>
<tr>
<td></td>
<td>1-21</td>
<td>.68</td>
<td>.67</td>
<td>.016</td>
</tr>
</tbody>
</table>

*P<.05. Contrast between males and females.
**P<.01. Contrast between males and females.

^aStandard error of a treatment mean.
Table 4. Cumulative body weight gain, feed intake, and feed efficiency of turkeys during the 9, 14, and 21 days after hatching injected with increasing doses of 17β-estradiol dipropionate (E₂). Experiment 2

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Period, Negative dosage, ug</th>
<th>Linear trend</th>
<th>0</th>
<th>8.8</th>
<th>88</th>
<th>880</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>days</td>
<td>control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight gain, g/poult per day 1-9</td>
<td>12.8</td>
<td>12.1</td>
<td>12.9</td>
<td>12.7</td>
<td>13.5</td>
<td>P&lt;.10</td>
</tr>
<tr>
<td></td>
<td>1-14</td>
<td>16.9</td>
<td>16.5</td>
<td>17.2</td>
<td>17.4</td>
<td>17.4</td>
</tr>
<tr>
<td></td>
<td>1-21</td>
<td>21.5</td>
<td>21.5</td>
<td>20.3</td>
<td>21.7</td>
<td>20.5</td>
</tr>
<tr>
<td>Feed intake, g/poult per day 1-9</td>
<td>14.9</td>
<td>14.1</td>
<td>14.6</td>
<td>14.9</td>
<td>16.1</td>
<td>P&lt;.05</td>
</tr>
<tr>
<td></td>
<td>1-14</td>
<td>22.6</td>
<td>22.2</td>
<td>22.6</td>
<td>23.6</td>
<td>24.5</td>
</tr>
<tr>
<td></td>
<td>1-21</td>
<td>30.7</td>
<td>31.1</td>
<td>31.8</td>
<td>31.3</td>
<td>31.0</td>
</tr>
<tr>
<td>Gain/feed, g/g</td>
<td>1-9</td>
<td>.86</td>
<td>.86</td>
<td>.92</td>
<td>.85</td>
<td>.84</td>
</tr>
<tr>
<td></td>
<td>1-14</td>
<td>.75</td>
<td>.75</td>
<td>.76</td>
<td>.74</td>
<td>.71</td>
</tr>
<tr>
<td></td>
<td>1-21</td>
<td>.70</td>
<td>.69</td>
<td>.65</td>
<td>.69</td>
<td>.66</td>
</tr>
</tbody>
</table>

aInjections given on days 2, 4, 6, and 8 after hatching.
bSignificance level for a straight line with the natural logarithm of E₂ dose. Negative control is not included.
cNot significant.
Body weight gain during the 1- to 9-day period increased linearly (P<.10) with the logarithm of the dose of E2 injected. The effect of E2 on body weight decreased throughout the experiment, and by 14 days it was not significant (P>.10). Feed intake during the 1- to 14-day period increased linearly (P<.05) with the logarithm of the dose of E2 injected, but, as for body weight, linearity disappeared thereafter. Feed efficiency was not altered by E2 injection (P>.10) except from the 1- to 14-day period during which it was impaired (P<.10).

Rectal prolapses developed in 0, 10, 75, 100% of the poult injected with 0, 8.8, 88, and 880 μg of E2, respectively. No mortality, however, occurred throughout the experiment.

**Oviduct**

Data showing the effect of E2 treatment on the weight of the left oviduct collected at 9, 14, and 21 days of age are presented in Table 5. No difference in oviduct weight was detected between negative control poult (non-injected) and poult injected with no E2 (corn oil carrier only) or poult injected with .02 mg E2/kgBW (8.8 μg E2 total). Injection with .2 mg E2/kgBW (88 μg in total) increased oviduct weight, but it was not detected until day 14. In contrast, injection with 2.2 mg E2/kgBW (880 μg in total) produced a dramatic increase in oviduct weight by day 9. Furthermore, poult given the higher doses of E2 had a high incidence of rectal prolapse, cannibalism, and hyperirritability, as mentioned before.
Table 5. Weight of the left oviduct of pouls injected with increasing doses of 17β-estradiol dipropionate (E2). Experiment 2

<table>
<thead>
<tr>
<th>Age, days</th>
<th>Negative control</th>
<th>E2, µg/poult</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>.015 (.001)a</td>
<td>.015 (.009)</td>
</tr>
<tr>
<td>14</td>
<td>.030 (.015)</td>
<td>.030 (.010)</td>
</tr>
<tr>
<td>21</td>
<td>.070 (.008)</td>
<td>.070 (.024)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses are standard deviations of each mean based on three values.*
Pancreas

Results for SLA and TLA averaged across sex and E2 treatment are presented graphically in Figure 3. SLA in the pancreas averaged 18.8 at 9 days of age and decreased (P<.001) to 14.1 and 15.6 μmoles OA/min x mg protein at 14 and 21 days of age, respectively. TLA per pancreas increased from 1,760, to 2,420, and 4,280 μmoles OA/min. This increase in TLA was mostly due to increased pancreas weight. Pancreas DW averaged 142 mg at 9 days of age and increased to 252 and 407 mg at 14 and 21 days of age.

Figure 3. Specific lipase activity (SLA, •—• ) and total lipase activity (TLA, O—O ) of 9, 14, and 21-day-old turkeys
The SLA, TLA, and pancreas DW data of female and male poults at 9 days of age are presented in Table 6. No significant (P>.10) differences between females and males were detected in SLA or TLA, irrespective of E2 treatment. Males had a slightly heavier (P=.10) pancreas than did females. There seemed to be a linear increase in SLA of pancreases with the logarithm of the dose of E2 used; however, the relationship was not significant (P>.10). TLA per pancreas and pancreas DW increased linearly (P<.001) with increasing doses of E2 injected. TLA increased from 1,430 μmoles OA/min for the no-E2 treated group, to 1,720, 1,930, and 2,170 μmoles OA/min for the groups treated with .02, .2, and 2 mg E2/kg BW. At 14 days of age, the same trend in SLA with the logarithm of the E2 dose observed at 9 days was obtained. Also, for TLA and pancreas DW, results at 14 days of age paralleled those at 9 days. By 21 days of age, the linear increase in SLA with the logarithm of the dose of E2 injected became significant, the effect of E2 on pancreas DW disappeared (P>.10), and only a trend toward greater TLA was observed, but the relationship was not significant (P>.10).

No significant (P>.10) interaction effects of age by dose or age by dose by sex were detected for body weight, feed intake, SLA, TLA, and pancreas DW.
Table 6. Specific lipase activity (SLA), total lipase activity (TLA), and pancreas dry weight (DW) of 9-day-old turkeys injected with increasing doses of 17β-estradiol dipropionate (E2)

<table>
<thead>
<tr>
<th>Negative control</th>
<th>E2 dose, µg</th>
<th>SEMa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>8.8</td>
</tr>
</tbody>
</table>

**SLA, µmoles OA/min per mg protein**
- **Females**
  - 17.5
  - 17.2
  - 18.7
  - 21.4
  - 19.6
- **Males**
  - 18.2
  - 18.9
  - 18.8
  - 19.6
  - 20.5
- **Mean**
  - 17.8
  - 18.1
  - 18.8
  - 20.5
  - 20.1
  - 1.7

**TLA, µmoles/min**
- **Females**
  - 1370
  - 1391
  - 1384
  - 2208
  - 1892
- **Males**
  - 1950
  - 1468
  - 2057
  - 1660
  - 2441
- **Mean**
  - 1660
  - 1430
  - 1720
  - 1930
  - 2170
  - 321

**DW, mg**
- **Females**
  - 128
  - 127
  - 110
  - 154
  - 143
- **Males**
  - 162
  - 124
  - 160
  - 131
  - 179
- **Mean**
  - 145
  - 126
  - 135
  - 143
  - 161
  - 18

aStandard error of a treatment mean.
DISCUSSION

Body weight gain and feed intake increased with increasing doses of E2 administered to female and male poults until they were 9 days old. The magnitude of the influence of E2 on growth decreased throughout the experiment, even though its effect on feed intake lasted until day 14. It should be remembered that the last E2 injection was given at 8 days of age. These observations disagree with those reported by Munro and Kosin (1940) for baby chicks. These researchers found that daily intramuscular injections of 100 µg of E2 for 10 days to 6-day-old female chicks did not affect growth. However, a growth depression was observed when injections were given to male chicks. The authors suggested that the E2 was having its effect through an inhibiting action upon the pituitary. No mention of the effect of E2 on feed intake was reported. This is the only report that was found in the literature describing the influence of E2 on the performance of young poultry. However, with other estrogenic compounds (i.e., diethylstilbestrol, a synthetic estrogen), weight gain and feed intake were improved in young chickens, with no change in feed efficiency (Hill et al., 1958; Sell and Balloun, 1961). The authors suggested that the estrogen effect on growth was a result of increased feed intake.

Administration of E2 to female turkeys during the first 8 days after hatching caused a dramatic growth of the oviduct. This response was used as a criterion to ascertain that E2 was received by the poults without having to determine concentrations of estrogen in blood. The oviduct is a very
responsive organ to estrogenic hormones. Similar responses of the oviduct to estrogen administration were observed previously by many investigators in the case of the chicken (for a review, see O'Malley et al., 1969; Schrader and O'Malley, 1980). In the research reported herein, the stimulatory effect of E2 continued for at least 6 days after treatment, after which, the oviduct started to regress to its normal size. Hawkins et al. (1969), found that in the domestic fowl about 60 or 90% of the intravenously injected E2 disappeared from the blood in 20 or 120 min, respectively, accumulating primarily in the bile. Even though in our experiment E2 was given intramuscularly, thus entering the circulation at a slower rate, it appears that E2 was still circulating through the blood several days after administration. It seems reasonable, therefore, to postulate that the clearance mechanisms for E2 were not fully developed in the young turkey.

The SLA determined in the preliminary experiment (Experiment 1) was consistently greater than that determined in Experiment 2 (45 μmoles BA/min x mg protein vs. 16 μmoles OA/min x mg protein). Similar differences were found by Escribano et al. (1989), who reported that SLA determined by using the glycerol trioleate substrate (GTO) assay was about 50% of that determined by using the glycerol tributyrate assay. These authors suggested that these differences were due to differences in the specificity of pancreatic lipase for the two substrates, differences in the rate with which lipase hydrolyzes each substrate, and differences inherent in the assay procedures themselves. Notwithstanding the absolute difference in SLA as determined by using the
two assays, the changes in TLA with E2 treatment or age paralleled one another in Experiment 2.

The pattern of change in SLA with age observed in Experiment 1 differed from that of Experiment 2. In Experiment 1, SLA remained relatively constant or increased slightly between 7 and 13 days of age, whereas in Experiment 2, SLA decreased between 9 and 14 days of age. No explanation could be found for this decline in SLA observed in Experiment 2. Regardless of the increase or decrease in SLA per unit of pancreas protein, TLA per pancreas and pancreas DW increased as poults aged in both experiments. Similar increases in TLA were reported by Krogdahl and Sell (1984) and Escribano et al. (1988).

In Experiment 1, SLA and TLA in pancreases from 4-day-old poults were increased drastically by the E2 treatment. However, more than 50% of the poults developed hyperirritability, rectal prolapse, and cannibalism. In Experiment 2, SLA and TLA consistently tended to increase with increasing doses of E2 injected, but doses were not high enough to give a response as strong as that obtained in Experiment 1. Treatment of poults with E2 given on alternate days between 2 and 8 days after hatching, somehow increased SLA and TLA in the pancreas without having negative effects on performance. No report was found in the scientific literature on the influence of estrogenic hormones directly on pancreatic lipase activity. Several reports, however, were found on the influence of estrogenic hormones on the acinar cells of the exocrine pancreas. Grossman et al. (1983) demonstrated that the zymogen granules in pancreatic acinar cells disappear almost completely after
adrenalectomy of male rats. The zymogen granules reappeared in normal number and size after administration of E2 to the rats. Thus, the authors concluded that E2 played an essential role in the normal functioning of the exocrine pancreas. In the research reported herein, a similar effect to that observed by Grossman et al. (1983) may have occurred. Furthermore, because lipase is a component of the zymogen granule, it seems reasonable to conclude that E2 will affect the activity of lipase in the pancreatic gland. Doctor et al. (1983), suggested that E2 would be inhibiting the secretion of the zymogen granule into the lumen of the acinar cells. This inhibition, thus, would result in an accumulation of zymogen granules in the acinar cell's cytoplasm, which would bring with it an increase in the concentration of lipase in the pancreas. Whether this happened or not in our experiments can not be concluded. On the one hand, if the zymogen granules accumulate in the pancreatic gland, less digestive enzymes would be secreted into the intestine, and therefore an impairment of digestion should have taken place. On the other hand, no reduction in the performance was observed in either Experiments 1 or 2. Thus, if E2 altered the secretion process of the zymogen granule, it did not have a discernible impact for the performance of the young turkeys. Another possible way for E2 to increase lipase activity could be by directly increasing the rate of synthesis of the lipase protein by the acinar cell. For example, Brant and Nalbandov (1956) and O'Malley et al. (1969) demonstrated that sex hormones regulated the synthesis of specific albumen proteins in the chicken oviduct. From the research presented here,
no explanation on the way E2 produced its effect on pancreatic lipase activity of young turkeys can be given.

In summary, E2 increases pancreatic lipase activity in turkeys during the first weeks after hatching. No undesirable side-effects occurred when E2 was administered at .02 mg/kgBW. It would be of interest to further investigate whether the effect of E2 on lipase activity could result in an improved utilization of dietary fat by the young turkey.
REFERENCES


Munro, S. S., and I. L. Kosin, 1940. Relative potency of several estrogenic compounds tested on baby chicks of both sexes. Endocrinology 27:687-692.


SECTION III. PANCREATIC LIPASE ACTIVITY AND UTILIZATION OF DIETARY FAT BY YOUNG TURKEYS AS AFFECTED BY AGE, 17β-ESTRADIOL DIPROPIONATE AND THE TYPE OF FAT
Pancreatic lipase activity and utilization of dietary fat by young turkeys as affected by age, 17β-estradiol dipropionate and the type of fat

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Jerry L. Sell, B.S., M.S., Ph.D.

From the Department of Animal Science, Iowa State University, Ames, IA 50011
ABSTRACT

An experiment was conducted to determine pancreatic lipase activity and the utilization of dietary fat as affected by age, 17β-estradiol dipropionate (E2), and the type of dietary fat in turkeys during the first weeks after hatching. One hundred-forty-four Nicholas White male poults were randomly assigned to 24 pens. A factorial arrangement of two diets (tallow or soybean oil) and two doses of E2 (0 or .02 mg/kgBW) was used. E2 injections were given intramuscularly on days 2, 4, 6, and 8 after hatching. At 5, 9, and 21 days of age, pancreatic lipase activity, fat retention, and nitrogen-corrected metabolizable energy (MEn) were determined.

Apparent fat retention averaged 85.3, 84.6, and 93.5% at 5, 9, and 21 days of age, respectively. E2 treatment did not affect (P>.10) retention of dietary fat. Retention of fat from tallow was greater (P<.10) than from soybean oil at 5 days of age. By day 9 there were no differences (P>.10) and by day 21 retention from tallow was lower (P<.10). Even though apparent fat retention was relatively high at 5 days of age, the greatest increase in fat retention occurred between 9 and 21 days of age. MEn of the diets was not affected by E2 treatment. The soybean oil diet had greater (P<.01) MEn than the tallow diet at all ages.

Specific lipase activity of the pancreas (SLA) averaged 6, 14, 16, and 20 μmoles of oleic acid (OA) hydrolyzed/min x mg protein at 1, 5, 9, and 21 days of age, respectively. Treatment with E2 increased SLA at all ages, but the E2 effects were not significant (P>.10). Poults fed the tallow diet had greater
(P<.10) SLA than poults fed the soybean oil diet at 5 days of age. By day 9 there were no differences (P>.10) and by day 21 poults fed the tallow diet had lower (P<.10) SLA. A positive relationship between fat retention and SLA was observed ($r^2 = .92$). Total lipase activity per pancreas (TLA) increased exponentially with age and averaged 100, 700, 1,400, 8,000 μmoles OA/min at 1, 5, 9 and 21 days of age, respectively. Poults fed the tallow diet had greater (P<.10) TLA than poults fed the soybean oil diet. Electron microscopic view of the midportion section of pancreases showed more zymogen granules of greater diameter in acinar cells from poults injected with E2, but differences were not statistically significant (P>.10).
Pancreatic lipase catalyzes the hydrolysis of dietary triglycerides. Dietary fat is mainly composed of triglycerides and their hydrolysis is an essential step in utilization. Incomplete digestion derived from inadequate activity of pancreatic lipase could result in reduced utilization of dietary fat. Escribano et al. (1988) found that lipase activity in the pancreas of turkeys was relatively low during the first 2 weeks after hatching, but fat utilization was not determined simultaneously. Polin et al. (1980) found that utilization of dietary fat increased when chickens were fed starter diets supplemented with bile salts and/or pancreatic lipase, but lipase activity in the pancreas was not determined simultaneously. Krogdahl and Sell (1984) found that pancreatic lipase activity increased with age after a lag-period of about 14 days, and attained maximum levels by 42 to 56 days after hatching. In an experiment done concomitantly, Sell et al. (1986) reported that utilization of dietary fat also approached a maximum by 42 to 56 days after hatching. Sell et al. (1986) suggested that pancreatic lipase could be involved in the improved utilization of dietary fat with age. No reports have been found in the scientific literature referring to the utilization of dietary fat by poultry younger than 1 week of age. Similar effects of age to those mentioned above on fat utilization by turkey poults were found by Whitehead and Fisher (1975) and Salmon (1977). Moreover, these researchers found that fat utilization (absorbability) was not only affected by age but also by the type of fat. Absorbability of corn oil and rapeseed oil was high and that of tallow was low.
for 1- and 2-week-old poults. Absorbability remained high for oils, but increased for tallow as poults aged. Whitehead and Fisher (1975) and Salmon (1977) concluded that differences in utilization between the vegetable oils and tallow were related to different rates of absorption of their constituent fatty acids after hydrolysis. However, no determinations of lipase activity were done in any of these studies. Because absorbability of vegetable oils was already high by 1 week of age, it seems reasonable to conclude that pancreatic lipase was not limiting their utilization. On the other hand, vegetable oils and tallow differ markedly in physical and chemical characteristics and this, in turn, may result in a greater need of lipase for digesting tallow than corn oil. No reports have been found relating the activity of pancreatic lipase with the utilization of vegetable oils and animal fats by the young turkey.

The development of the digestive functions of the gastrointestinal tract and its accessory glands have been shown to be modulated by several hormones, including steroid hormones (Winborn et al., 1987). Furthermore, estrogen receptors have been found in pancreatic acinar cells (Beaudoin et al., 1986). These researchers showed, by using electron microscopic and autoradiographic techniques, that 17β-estradiol dipropionate (E2) and glucocorticoids played a key role during the formation and secretion of zymogen granules in the rat pancreas. Zymogen granules contain lipase together with other digestive enzymes responsible for the digestion of dietary components. Therefore, as a means to increase lipase activity in the pancreatic gland of the young turkey, Escribano and Sell (1989) treated poults with E2, and found that lipase activity increased by increasing doses of E2.
given intramuscularly. A dose of .02 mg E₂/kg of body weight (BW) was observed to be effective for increasing pancreatic lipase activity without having undesirable side-effects. No determinations of fat utilization, however, were done simultaneously.

The research reported herein was designed to determine pancreatic lipase activity and utilization of dietary fat in young turkeys as affected by age, E₂, and the type of fat.
MATERIALS AND METHODS

Nicholas White male poults were obtained from a commercial hatchery at 1 day of age. At reception, poults were weighed and those at the upper and lower extremes were discarded. Six poults were placed randomly in each of twenty four, 100 X 35 X 30 cm (length X width X height), pens located in an electrically heated battery. Electric heaters were preset at 35°C for the first week, and reduced to 32°C during the second and to 30°C during the third week. Poults received 24 hours of light per day. Batteries were equipped to facilitate periodic collection of excreta. A factorial arrangement of two diets differing in fat source (tallow or soybean oil) and two doses of 17β-estradiol dipropionate (E2, 0 or .02 mg/kgBW) was used. The composition of the diets is shown in Table 1 and the fatty acid composition of the fats is shown in Table 2. Diets were formulated to satisfy minimum nutrient requirements recommended by the National Research Council (NRC, 1984) except for metabolizable energy (ME).
Table 1. Composition of the experimental diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean meal (44% protein)</td>
<td>58.04</td>
</tr>
<tr>
<td>Oats</td>
<td>10.00</td>
</tr>
<tr>
<td>Fat(^a)</td>
<td>10.00</td>
</tr>
<tr>
<td>Corn</td>
<td>7.18</td>
</tr>
<tr>
<td>Oat hulls</td>
<td>5.00</td>
</tr>
<tr>
<td>Alfalfa meal (17% protein)</td>
<td>5.00</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>2.26</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.23</td>
</tr>
<tr>
<td>Vitamin premix(^b)</td>
<td>.40</td>
</tr>
<tr>
<td>Mineral premix(^c)</td>
<td>.40</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>.19</td>
</tr>
<tr>
<td>Chromic oxide</td>
<td>.30</td>
</tr>
</tbody>
</table>

Chemical analysis

<table>
<thead>
<tr>
<th></th>
<th>Calculated</th>
<th>Tallow diet</th>
<th>Soybean oil diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter, %</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Metabolizable energy, kcal/kg</td>
<td>3040.00</td>
<td>2990.00(^d)</td>
<td>3170.00(^d)</td>
</tr>
<tr>
<td>Crude protein, %</td>
<td>31.30</td>
<td>29.20</td>
<td>29.50</td>
</tr>
<tr>
<td>Ether extract, %</td>
<td>12.30</td>
<td>11.60</td>
<td>12.00</td>
</tr>
<tr>
<td>Others, %(^e)</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

\(^a\)Tallow or soybean oil.

\(^b\)The vitamin premix supplied the following per kg of diet: vitamin A, 6,666 IU; vitamin D3, 2,000 IU; vitamin B12, 14.7 \(\mu\)g; menadione, 2.4 mg; riboflavin, 3.6 mg; pantothenic acid, 9.6 mg; niacin, 100 mg; choline, 693 mg; folic acid, .73 mg; biotin, 100 \(\mu\)g.

\(^c\)The mineral premix supplied the following per kg of diet: manganese, 89 mg; zinc, 47 mg; iron, 44 mg; copper, 6.7 mg; sodium chloride, 3.10 g.

\(^d\)Nitrogen-corrected metabolizable energy averaged across ages.

\(^e\)Calculated composition: methionine, .67; TSAA, 1.16; lysine, 2.00; crude fiber, 8.73; calcium, 1.33; available phosphorus, .66; total phosphorus, .95.
Table 2. Determined gross energy, metabolizable energy and composition of tallow and soybean oil

<table>
<thead>
<tr>
<th></th>
<th>Tallow</th>
<th>Soybean oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross energy, kcal/kg</td>
<td>9586 ± 18^a</td>
<td>9592 ± 22</td>
</tr>
<tr>
<td>Fatty acids, %b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:0</td>
<td>26.0 ± 2.2</td>
<td>11.8 ± .1</td>
</tr>
<tr>
<td>C18:0</td>
<td>15.1 ± .7</td>
<td>3.33 ± .02</td>
</tr>
<tr>
<td>C18:1</td>
<td>47.4 ± .9</td>
<td>21.30 ± .07</td>
</tr>
<tr>
<td>C18:2</td>
<td>5.7 ± .6</td>
<td>54.10 ± .07</td>
</tr>
<tr>
<td>C18:3</td>
<td>.4 ± .1</td>
<td>9.15 ± .07</td>
</tr>
<tr>
<td>Others</td>
<td>5.4</td>
<td>.32</td>
</tr>
</tbody>
</table>

^aStandard deviation.
^bPercent of methyl esters.

Dietary ME was slightly lower than NRC (1984) (2750 kcal ME/kg) to stimulate intake of the dietary fat, and thus, exacerbate potential differences derived from having two different fat sources. Chromic oxide was included as a non-absorbable index substance. Feed and water were offered ad libitum. Each diet was fed to six control and six E2 pens. Injections of E2 in .1 ml of corn oil were given intramuscularly into the breast muscle on alternate days between 2 and 8 days of age. Because expected body weights at 2, 4, 6, and 8 days of age were, 60, 90, 130, and 160 g, respectively, poults under E2 treatment received a total of 2.8 μg and 8.8 μg by days 4 and 8, respectively. On day 1, before poults had access to food or water, 12 poults were killed and pancreases from each of two poults were pooled to give six samples. All poults were killed by cervical dislocation after halothane anesthesia. At 5, 9, and 21 days of age,
poults were fasted for 1.5 h, then one poult from each pen, close in body weight to the pen average, was killed and the pancreas excised, weighed, frozen in liquid nitrogen, freeze-dried and stored at -65°C for future determination of lipase activity and protein concentration. The assay system used to determine lipase activity was that described by Escribano et al. (1989). The procedure involved the determination of \(^3\)H-oleic acid (OA) released from the glycerol tri [9, 10 (n)-\(^3\)H] oleate substrate after incubation of pancreatic homogenates in the presence of bile salts and excess of colipase. Pancreatic homogenates were prepared in a ground glass tissue homogenizer with 10 mg of finely pulverized freeze-dried pancreatic tissue in 10 ml of buffer, pH 6.5. Homogenates were then diluted 20-fold and frozen in 5 ml aliquots at -65°C. These diluted homogenates, containing .05 mg of dry pancreas per ml, were used for the lipase assay. Specific lipase activity (SLA, \(\mu\)moles OA/min x mg protein) was defined as the lipase activity per mg of pancreas protein, and total lipase activity (TLA, \(\mu\)moles OA/min) per pancreas was calculated as the product of SLA times protein content of the pancreas. Protein concentration was determined by the bicinchoninic acid method (Smith et al., 1985). The composition of the homogenizing buffer was given by Escribano et al. (1989). Also at 5, 9, and 21 days of age, body weight and feed intake data were recorded and samples of excreta from each pen were collected. The samples of excreta were freeze-dried for 3 days and left for 2 days to equilibrate. Feed and freeze-dried excreta samples were ground and analyzed for dry matter, gross energy, nitrogen, fat, and chromium content. Duplicates of 2 g samples were dried at 70°C for 48 h in a force draft oven to determine dry matter.
adiabatic bomb calorimeter was used to determine gross energy content. Nitrogen content was determined in a micro-kjeldahl unit (protein was calculated as nitrogen X 6.25). Fat was determined gravimetrically after extraction with ethyl ether in a Goldfisch extraction unit. The ether extracts were dried, and the material that dissolved in chloroform was considered to be fat. Chromic oxide was determined by atomic absorption spectrometry using the method of Williams et al. (1962). These data were used to determine apparent fat retention from and nitrogen-corrected metabolizable energy (MEn) of each diet. The ME of tallow and soybean oil were determined by multiplying their gross energy by the apparent fat retention from each diet.

At 9 days of age, one extra poult was killed from each of the pens fed the soybean oil diet to determine the effect of E2 on the number and size of the zymogen granules in pancreatic acinar cells. A 3 mm section of the midportion of the pancreatic gland was cut into 1 to 2 mm cubes and immediately fixed in glutaraldehyde overnight at 4°C and postfixed in osmium tetroxide. Then the fixed tissues were dehydrated with ethanol, embedded into epoxy resin in gelatin capsules, and blocks were polymerized in a 60 to 90°C oven. From these blocks, thick sections (1-3μm) were mounted on glass slides, stained with toluidine blue, and checked under the light microscope for selection of areas for ultrathin sectioning (600-900 Å). Ultrathin sections were made, collected on copper grids and immersed in solutions of uranyl acetate and lead citrate. These thin sections were examined with a Hitachi HS-9 electron microscope at 75,000 V of accelerating voltage. Five pictures from different portions of each section and two thin sections from each pancreas
were photographed at 2,500 X true magnification. A total of 10 photographs from each pancreas were taken. The number and size of the mature zymogen granules were counted with a Zeiss SEM-IPS (IBAS; 8 bit) image analysis system, interfaced to a Panasonic WV cd50 CCD camera, as previously used by Sowter et al. (1987) for bladder tissue. Also the total cytoplasmic surface area occupied by mature zymogen granules was determined for pancreatic tissue of both control and E2-treated poults.

The statistical analysis was done by analysis of variance and linear regression by using the Statistical Analysis System (SAS) developed by the SAS Institute (1982). Because only three ages were tested, in the case that the lack of fit to a straight regression line was significant, orthogonal comparisons were done to compare the results of 5- vs 9- and 5- and 9- vs 21-day-old poults.
RESULTS

Results for body weight gain and feed conversion during the 1 to 5, 5 to 9, and 9 to 21 days of age periods are presented in Table 3. Poults injected with E2 had a slightly greater body weight gain and improved feed conversion (feed:gain) (P<.10) during the 1 to 5 day period than non-injected control poults. This E2 effect, however, disappeared as the experiment progressed. No differences (P>.10) in body weight gain were obtained between poults fed diets supplemented with tallow or soybean oil. Feed needed per unit of gain (feed conversion) was lower (P<.10) at 9 and 21 days of age for poults fed the soybean oil diet; however, no differences were observed when poults were 5-day-old. None of the interaction effects between E2, diet, and age on body weight gain or feed conversion were significant (P>.10) during the experiment. Results for feed intake, fat intake and MEn intake are summarized in Table 4. Feed intake was greater (P<.05) for poults fed the tallow diet than for poults fed the soybean oil diet at 9 and 21 days of age. Because feed intake was greater, tallow-fed poults had consistently greater fat and nitrogen intakes than poults fed the soybean oil diet (Table 4). Even though feed intake was greater, MEn intake was not different (P>.10) between treatment groups at any age.
Table 3. Effect of 17β-estradiol dipropionate (E2) on body weight gain and feed conversion of pouls fed tallow or soybean oil supplemented diets from 1-5, 5-9, and 9-21 days of age

<table>
<thead>
<tr>
<th>E2 dose</th>
<th>Diet</th>
<th>Weight gain</th>
<th>Feed conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/kgBW</td>
<td></td>
<td>1-5a 5-9 9-21</td>
<td>1-5 5-9 9-21</td>
</tr>
<tr>
<td>0</td>
<td>Tallow</td>
<td>6.6 13.3 27.3</td>
<td>1.10 1.25 1.57</td>
</tr>
<tr>
<td></td>
<td>Soy oil</td>
<td>6.7 11.6 27.5</td>
<td>1.09 1.30 1.48</td>
</tr>
<tr>
<td>.02</td>
<td>Tallow</td>
<td>6.7 12.9 27.0</td>
<td>1.06 1.38 1.59</td>
</tr>
<tr>
<td></td>
<td>Soy oil</td>
<td>7.4 12.1 27.5</td>
<td>1.04 1.29 1.49</td>
</tr>
<tr>
<td>SEMb</td>
<td>.38</td>
<td>.80 .70</td>
<td>.025 .043 .026</td>
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Source of variation

<table>
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<tbody>
<tr>
<td>E2</td>
</tr>
<tr>
<td>Diet</td>
</tr>
<tr>
<td>E2 X Diet</td>
</tr>
</tbody>
</table>

aDays of age.
bStandard error of a treatment mean.
cNot significant, P>.20.
Table 4. Effect of 17B-estradiol dipropionate (E2) on feed intake, fat intake, and nitrogen-corrected metabolizable (MEn) energy intake of 5-, 9-, and 21-day-old pouls fed tallow or soybean oil supplemented diets

<table>
<thead>
<tr>
<th>E2 dose</th>
<th>Diet</th>
<th>Feed intake</th>
<th></th>
<th>Fat intake</th>
<th></th>
<th>MEn intake</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kgBW</td>
<td></td>
<td>5a 9 21</td>
<td>5 9 21</td>
<td>5 9 21</td>
<td>5 9 21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------</td>
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<td>-----</td>
<td>------------</td>
<td>-----</td>
<td>------------</td>
<td>-----</td>
</tr>
<tr>
<td>0</td>
<td>Tallow</td>
<td>7.5 16.9 42.8</td>
<td>.87</td>
<td>1.96</td>
<td>4.97</td>
<td>22.0</td>
<td>51.5</td>
</tr>
<tr>
<td></td>
<td>Soy oil</td>
<td>7.2 15.4 40.8</td>
<td>.86</td>
<td>1.84</td>
<td>4.90</td>
<td>22.5</td>
<td>50.0</td>
</tr>
<tr>
<td>.02</td>
<td>Tallow</td>
<td>7.2 17.4 43.0</td>
<td>.82</td>
<td>2.00</td>
<td>5.00</td>
<td>21.0</td>
<td>52.5</td>
</tr>
<tr>
<td></td>
<td>Soy oil</td>
<td>7.7 15.4 41.2</td>
<td>.93</td>
<td>1.85</td>
<td>4.95</td>
<td>24.0</td>
<td>46.0</td>
</tr>
<tr>
<td>SEMb</td>
<td>.34</td>
<td>.53</td>
<td>.92</td>
<td>.04</td>
<td>.06</td>
<td>.11</td>
<td>1.09</td>
</tr>
</tbody>
</table>

Source of variation:

| Probabilities |
| E2          | NSc NS NS NS NS NS NS NS |
| Diet        | NS .01 .05 NS .10 NS NS NS |
| E2 X Diet   | NS NS NS NS NS NS NS NS |

---

aDays of age.
bStandard error of a treatment mean.
cNot significant, P>.20.
Results for apparent fat retention, ME\textsubscript{n} of the diets and ME of the fats at 5, 9, and 21 days of age are presented in Table 5. The apparent retentions of dietary fat by pouls fed the soybean oil diet were 83.6, 84.6 and 95.0% at 5, 9 and 21 days of age, respectively. Retentions of dietary fat by pouls fed the tallow diet were 87 and 85% at 5 and 9 days of age, respectively, and then increased significantly (P<.0001) to 92% by 21 days of age. Fat retention by pouls injected with E\textsubscript{2} was similar (P>.10) to that of control pouls. Treatment of pouls with E\textsubscript{2} had no effect on ME\textsubscript{n} of the diet or ME of the fat at any age. However, pouls fed tallow retained more fat (P<.05) at 5 days of age than did pouls fed soybean oil (87 vs 84%). Subsequently, a transition in fat utilization occurred whereby fat retention at 9 days was the same for pouls fed tallow or soybean oil, but, by 21 days, fat retention of pouls fed soybean oil exceeded (P<.01) that of pouls fed tallow. Because the ME of each fat was calculated by multiplying fat retention by the GE of the fat, differences between ME\textsubscript{s} for tallow and soybean oil at each age paralleled those of fat retention. No interaction (P>.10) occurred between E\textsubscript{2} and type of fat added to the diet at any age. Regardless of the type of diet, the ME\textsubscript{n} of the diet increased slightly between 5 and 9 days and then increased significantly (P<.0001) between 9 and 21 days of age. Treatment with E\textsubscript{2} did not alter (P>.10) the change in ME\textsubscript{n} of the diet with age. Despite some inconsistency in fat retention with age, the ME\textsubscript{n} of the soybean oil diet was greater (P<.01) than that for the tallow diet at all ages. This difference was not affected by the E\textsubscript{2} treatment, and no interaction between E\textsubscript{2} and diet was obtained.
Table 5. Effect of 17β-estradiol dipropionate (E2) on the apparent fat retention\(^a\), nitrogen-corrected metabolizable energy of the diet (MEn)\(^b\), and metabolizable energy of the fat (ME)\(^c\) for 5-, 9-, and 21-day-old pouls fed tallow- or soybean oil-supplemented diets

<table>
<thead>
<tr>
<th>E2 dose</th>
<th>Diet</th>
<th>Fat retention</th>
<th>MEn of diet</th>
<th>ME of fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diet</td>
<td>5(^d)</td>
<td>9</td>
<td>21</td>
</tr>
<tr>
<td>mg/kgBW</td>
<td></td>
<td>5(^d)</td>
<td>9</td>
<td>21</td>
</tr>
<tr>
<td>0</td>
<td>Tallow</td>
<td>86.6</td>
<td>85.0</td>
<td>90.7</td>
</tr>
<tr>
<td></td>
<td>Soy oil</td>
<td>86.2</td>
<td>85.0</td>
<td>94.9</td>
</tr>
<tr>
<td>.02</td>
<td>Tallow</td>
<td>87.2</td>
<td>84.2</td>
<td>93.3</td>
</tr>
<tr>
<td></td>
<td>Soy oil</td>
<td>81.0</td>
<td>84.2</td>
<td>95.0</td>
</tr>
<tr>
<td></td>
<td>SEM(^e)</td>
<td>2.5</td>
<td>3.4</td>
<td>.9</td>
</tr>
</tbody>
</table>

Source of variation

- E2: NS\(^f\), NS, .15
- Diet: NS, NS, .01
- E2 X Diet: NS, NS, .15

Source of variation

- E2: NS, NS, .15
- Diet: NS, NS, .01
- E2 X Diet: NS, NS, .01

\[^a\]Fat retention = \[\frac{[\%fat \text{ diet} - \%fat \text{ feces} (\text{Cr}_2\text{O}_3 \text{ diet}/\text{Cr}_2\text{O}_3 \text{ feces})]}{\%fat \text{ diet}}\] X100.

\[^b\]MEn = ME - 8.22 X NRet; ME = GE diet - GE feces (Cr2O3 diet/Cr2O3 feces); NRet = nitrogen retention; GE = gross energy; GE of tallow = 9592 kcal/kg; GE of soy oil = 9586 kcal/kg.

\[^c\]ME fat = \[\frac{\text{GE fat} \times \text{fat retention}}{100}\].

\[^d\]Days of age.

\[^e\]Standard error of a treatment mean.

\[^f\]Not significant, P > .20.
Results for specific lipase activity (SLA, μmoles OA/mg protein/min), total lipase activity (TLA, μmoles OA/min) per pancreas, and pancreas dry weight (PDW, mg) as influenced by E2 treatment and the type of supplemented fat are presented in Table 6. SLA averaged 6, 14, 16 and 20 μmoles OA/mg protein/min at 1, 5, 9 and 21 days after hatching, respectively. A much greater increase in SLA occurred between day 1, before poults ate any food, and day 5 than between days 5 and 21. Poults treated with E2 had consistently greater SLA than control poults at all ages, but differences were not significant (P>.10). At 5 days of age, poults fed the tallow diet had significantly greater (P<.10) SLA than soybean oil-fed poults. However, by 9 days, SLA did not differ (P>.10) between tallow- or soybean oil-fed poults, and by day 21 tallow fed-poults had lower SLAs. Therefore, an interaction (P<.05) between type of fat and age occurred for SLA. A positive relationship between SLA and apparent fat retention was found. This relationship was described by a second degree polynomial (Fat retention= 110 - 4.3 SLA + .17 SLA^2, R^2=.92). Poults with greater SLA had greater fat retention, but fat retention increased slowly until SLA was 16 μmoles/mg protein/min, and then increased more rapidly.

Pancreas size, as represented by its dry weight (PDW), increased exponentially (R^2=.92, P<.0001) from 25 to 70, 130, and 600 mg from 1 to 5, 9, and 21 days of age, respectively. The rate of increase of PDW with age was greater than for SLA per mg of pancreas protein, but the protein concentration in the pancreatic gland did not change (P>.10) as poults aged between 5 and 21 days.
Table 6. Effect of 17β-estradiol dipropionate on the specific lipase activity (SLA), total lipase activity (TLA) and pancreas dry weight (PDW) of 5-, 9-, and 21-day old poult's fed tallow or soybean oil supplemented diets

<table>
<thead>
<tr>
<th>E2 dose</th>
<th>Diet</th>
<th>SLA</th>
<th>TLA</th>
<th>PDW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1^a</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>mg/kgBW</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Tallow</td>
<td>5.6</td>
<td>15.3</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td>Soy oil</td>
<td>5.6</td>
<td>11.6</td>
<td>15.6</td>
</tr>
<tr>
<td>.02</td>
<td>Tallow</td>
<td>5.6</td>
<td>16.0</td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td>Soy oil</td>
<td>5.6</td>
<td>12.9</td>
<td>16.6</td>
</tr>
<tr>
<td>SEM^b</td>
<td></td>
<td>1.0</td>
<td>1.8</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Source of variation

<table>
<thead>
<tr>
<th>E2</th>
<th>Probabilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>---</td>
<td>NS^c NS NS NS</td>
</tr>
<tr>
<td>Diet</td>
<td>.10 .20 .10 .10</td>
</tr>
<tr>
<td>E2 X Diet</td>
<td>NS NS NS NS</td>
</tr>
</tbody>
</table>

^a Days of age.
^b Standard error of a treatment mean.
^c Not significant.
A linear relationship was found between PDW and the total protein content per pancreas (PDW = 16 + 1.36 mg protein, $R^2 = .96$, $P < .0001$). Also, PDW was significantly correlated ($R^2 = .54$, $P < .0001$) with body weight. Treatment with E2 did not affect ($P > .10$) PDW, but a trend toward larger pancreases for E2-treated poults was observed at every age. No interaction effects between E2 and age or dietary fat on PDW were obtained. On the average, tallow-fed poults had larger pancreases ($P < .10$) than soybean oil fed poults and differences became more prominent ($P < .10$) as poults aged.

Total lipase activity (TLA, μmoles OA/min) per pancreas increased exponentially ($R^2 = .85$, $P < .0001$), averaging 100, 700, 1,400, and 8,000 μmoles OA/min at 1, 5, 9, and 21 days of age, respectively. A trend towards greater TLA with E2 treatment was obtained at all ages, but differences were not significant ($P > .10$). No interaction between E2 and age or diet occurred for TLA. Poults fed the tallow diet had greater TLA ($P < .10$) than poults fed the soybean oil diet at 5 and 9 days of age. At 21 days of age TLA was considerably greater as well, but for unknown reasons the experimental error increased greatly and the level of significance ($P < .10$) was not reached. The opposite trend observed for SLA indicated that TLA increased mainly as a result of increased pancreas weight. No significant interaction ($P > .10$) effect occurred between diet and age for TLA.

Pictures of the electron microscopic view of a midportion section of the pancreas from control and E2 treated 9-day-old poults are presented in Figures 1 and 2. The dark spherical structures represent the zymogen granules that contain pancreatic lipase as well as other digestive enzymes.
The number of zymogen granules per unit of surface area was about 20% greater for E2-treated poults than for non-treated poults, but the difference was not significant (P > .10) (Table 7). Also, the average diameter of each granule and the total cytoplasmic surface area occupied by zymogen granules were greater for E2-treated poults but differences were not statistically significant.

Table 7. Effect of 17β-estradiol dipropionate (E2) on the number, size and total area occupied by the zymogen granules in pancreatic acinar cells of 9-day-old turkeys

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of granules a</th>
<th>Granule diameter, μm</th>
<th>Total area, % b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>57 (20-141) c</td>
<td>2.10 (1.4-2.5)</td>
<td>4.0 (1.1-11.0)</td>
</tr>
<tr>
<td>E2</td>
<td>72 (14-248)</td>
<td>2.14 (1.5-2.8)</td>
<td>5.0 (.8-14.1)</td>
</tr>
<tr>
<td>SEM d</td>
<td>40</td>
<td>1.7</td>
<td>2.5</td>
</tr>
</tbody>
</table>

a Number of mature zymogen granules in the pancreas section area of an electron micrograph.

b Percent of the cytoplasmic area occupied by zymogen granules.

c Number in parenthesis represents the minimum and maximum for each determination.

d Standard error of a treatment mean.
Figure 1. Electron micrograph of the midportion section of the pancreas of a 9-day-old poult from the control treatment group. The dark spherical structures represent the zymogen granules that contain pancreatic lipase and other digestive enzymes. 2,500 X magnification
Figure 2. Electron micrograph of the midportion section of the pancreas of a 9-day-old poult from the 17ß-estradiol dipropionate treatment group. The dark spherical structures represent the zymogen granules that contain pancreatic lipase and other digestive enzymes. 2,500 X magnification
DISCUSSION

Body weight gain and feed conversion were not affected by E₂ treatment. This result is in agreement with those reported by Akiba et al. (1982). These researchers found that injections of 5.5 mg E₂/kgBW in chickens did not affect feed intake. On the other hand, Escribano and Sell (1989) observed that body weight and feed intake increased with increasing doses of E₂ given intramuscularly to 1- to 9-day-old turkeys. This difference in the effect of E₂ on feed intake in turkeys may be due to the different dose of E₂, or to the different class of poultry (chicken vs. turkey) used. The .02 mg E₂/kgBW dose used in the current experiment may not have been high enough to have a detectable effect on performance.

Feed conversion was not affected by the type of dietary fat at 5 days of age. By day 9, poults fed the soybean oil diet were more efficient, and by day 21, differences were more prominent. The differences in feed conversion were due to a different feed intake rather than to a different body weight gain. Tallow-fed poults ate more than soybean oil-fed poults. Both tallow- and soybean oil-fed poults grew at the same rate throughout the experiment. Furthermore, the MEn intake and thus the MEn intake per unit of weight gain were the same for poults fed either diet. These results agree with the hypothesis that poults were eating primarily to satisfy their energy requirements. Except when poults were younger than 5 days, feed intake reflected differences in MEn of the diets; poults adjusted their intake to the MEn concentration of the diet. Similar results were found by Joshi and Sell.
who reported a reduction in feed consumption and an improvement in feed conversion when turkeys were fed diets containing 10% supplemental soybean oil during the first 6 weeks of life. In the current research, only when poults were younger than 5 days of age, feed intake did not reflect differences in MEn of the diets.

Apparent fat retention was somewhat greater at 5 than at 9 days of age, but by 21 days of age fat retention was maximum. Similar results in fat retention were reported by Salmon (1977), who studied fat retention (absorbability) in poults from 1 to 6 weeks of age. Salmon (1977) reported that fat absorbability from rapeseed oil or tallow was reduced from 87.6 to 86.5% by 1 and 2 weeks of age, respectively, and then increased between 2 and 6 weeks. Most of the increase occurred by 3 weeks of age. No report on fat retention by poults younger than 1-week-old has been found in the scientific literature. Research done with rats by Berendsen and Blanchette-Mackie (1979), suggested that pinocytosis of lipid droplets may be an important pathway for lipid absorption in the newborn. In this process, intact globules of fat could enter the enterocyte, form lipoproteins, and pass into the portal system. This pathway could be of importance also for the turkey until the adult mode of micellar absorption appears. Moreover, Romanoff (1960) reported that during the retraction process of the yolk membrane, some yolk is propelled into the small intestine. This process could be of considerable importance for the development of other digestive functions. Whether this concept holds true for the young turkey is not known, but it seems reasonable to think that
the enterocyte's membrane is partly permeable to yolk and to some dietary fat during the early post-hatching life.

By day 9, the enterocyte's membrane may not be permeable any longer. The utilization of dietary fat would then become dependent on the regular digestive and absorptive processes and the factors involved in them. Fat retention at 5 days was greater for poults fed the tallow than the soybean oil diet. By 9 days no difference in fat retention was found, and by 21 days of age fat retention was greater for soybean oil- than for tallow-fed poults. Most of the research reviewed comparing utilization of saturated and unsaturated fats indicates that saturated fats are utilized less efficiently than unsaturated fats, with this difference being most pronounced in young animals (Renner and Hill, 1960; Sibbald et al., 1961; Whitehead and Fisher, 1975; Salmon, 1977; Hamosh, 1979; and Sell et al., 1986). Unless the pinocytosis pathway mentioned earlier in this section is more prominent for tallow, no other explanation can be given at present for the greater fat retention from tallow than from soybean oil at 5 days as observed in this research.

Results for MEn did not parallel those for fat retention. The greater nitrogen retention from the soybean oil diet may partially explain this difference. The soybean oil diet had a greater MEn than the tallow diet at all ages. These results support the generally accepted idea that unsaturated fats, when added to the diet, increase the MEn more than an equivalent amount of saturated fat.

Treatment with E2 resulted in a numerical increase in SLA at all ages. A similar numerical increase was reported by Escribano and Sell (1989). SLA
was very low at hatch and increased thereafter through day 21 post-hatch. This increase in SLA with age is in conflict with the reduction in SLA during a similar age period as reported by Escribano and Sell (1989). A possible explanation for this contrasting change in SLA with age is related to the different composition of the diets used. The diets used in the current research had 10% supplemented fat whereas less than 4% fat was added to the diets tested by Escribano and Sell (1989). Influence of the composition of the diet on pancreatic lipase activity has been reported previously for poultry (Hulan and Bird, 1972; and Dror et al., 1976) and for mammals (Corring, 1980). However, Krogdahl and Sell (1984) found that pancreatic lipase was not very responsive to the concentration of dietary fat until poults were 14 days old or older. Differences like these could be explained on the basis of: first, a different method of collection of the pancreas (fasted vs. non-fasted poults before killing), second, the different assay used for lipase (tributyrin vs. triolein substrate), and third, differences inherent in the turkeys themselves.

Furthermore, in the experiment reported herein, the type of fat also affected the change in SLA with age. Poults fed the tallow diet as compared with those fed the soybean oil diet had greater SLA at 5 days of age. By day 9 there was no difference in SLA, but by day 21 poults fed the soybean oil diet had greater SLA than poults fed the tallow diet. It should be noted that the type of dietary fat affected SLA and fat retention in a parallel manner within each age. No cause-effect relationship, however, can be concluded from these results, but it may be indicative of the importance of pancreatic lipase for the utilization of dietary fat by the young turkey. Krogdahl and Sell (1984) found
that changes in fat retention between 4 and 6 weeks of age corresponded with changes in intestinal lipase activity. These authors suggested that intestinal lipase activity could be a limiting factor for lipid digestion during the early post-hatch period of turkeys.

Whether the type of fat is having its effect on SLA directly or through feed intake, cannot be concluded from this experiment. As mentioned before in this section, tallow-fed poults had greater feed consumption than soybean oil-fed poults. This greater feed consumption would increase the demand for pancreatic digestive enzymes and, with it, an increase in the weight of the pancreas itself. But this increase in weight would not necessarily indicate an increased production of pancreatic lipase. In fact, the greater PDW for turkeys fed the tallow diet was accompanied by a lower SLA. As a result, TLA did not differ between tallow- and soybean oil-fed poults at any age.

Both PDW and TLA increased exponentially during the first 3 weeks post-hatch. The rate of increase in PDW was greater than that of TLA. This difference indicates that the pancreas was growing faster than the increase in synthesis of pancreatic lipase during these first weeks after hatching.

The high correlation between PDW and total pancreas protein found in this research indicates that units of lipase activity could legitimately be expressed per unit of PDW or per mg of pancreas protein during these early stages of development. This could be of importance when very small amounts of pancreatic tissue are available as usually happens when working with very young turkeys. In this instance, PDW could be used as a basis for expressing SLA and, concurrently, there would not be a concern that
components of the lipase buffer may interfere with the protein
determination.

The number of zymogen granules per unit of surface area of pancreas
and the percent of cytoplasmic area occupied by them were numerically
greater for E2-treated than for control poulets. These observations are in
general agreement with those of Grossman et al. (1985) and Beaudoin et al.
(1986). They reported a marked depletion in zymogen granules in acinar cells
of the pancreas after adrenalectomy and/or ovariectomy of female rats.
Treatment with E2, resulted in a complete restoration of these secretory
vesicles. Even though a numerical increase in zymogen granules was found
after E2 treatment of turkeys in the current research, no increase in lipase
activity nor in fat retention was obtained. Therefore, regardless of the effect
of E2 on pancreatic lipase activity, this hormone did not perceptibly improve
utilization of dietary fat by the young turkey.
REFERENCES


SUMMARY AND GENERAL DISCUSSION

General

The objectives of the research described in this dissertation were: (1) to develop a method for determining lipase activity in pancreatic homogenates by using a long-chain fatty acid substrate and to compare it with a method that utilized a short-chain fatty acid substrate, (2) to determine pancreatic lipase activity in young turkeys during the first weeks after hatching, (3) to evaluate the effect of 17β-estradiol dipropionate on pancreatic lipase activity, and (4) to determine the retention of fat from the diet as affected by 17β-estradiol dipropionate and the type of dietary fat.

The main results corresponding to each of the previously mentioned objectives will be summarized and discussed. The aim of this section will be to synthesize the concepts and results presented previously in sections I, II, and III, to establish the areas in which further research is needed, and to give recommendations when possible. For details and further discussion, the reader is referred to the particular areas of each section.

Glycerol Trioleate Assay of Pancreatic Lipase Activity

A glycerol trioleate (GTO) assay method originally developed for milk lipases and currently in use for gastric lipases has been adapted to the particular case of the turkey pancreatic lipase. In this assay system, the rate of hydrolysis of emulsified $^3$H-GTO is measured in the presence of sodium taurodeoxycholate and an excess of pancreatic colipase. The released $^3$H-oleic acid (OA) is separated from the partial glycerides by liquid-liquid partitioning.
and measured by liquid scintillation. Modifications of the original procedure were done to adapt it to the physiological conditions of the small intestine of turkeys and simultaneously improve the partitioning efficiency. Specifically, the assay was conducted at pH 6.5, non-labelled GTO and OA were added into the partitioning solution, and the pH of the potassium carbonate buffer used during partitioning was increased from 10 to 12. The effect of adding carriers (non-labelled GTO and OA) plus the increase in pH of the buffer resulted in a 10% improvement (from 80 to 90%) in partitioning efficiency. Lipase activity increased linearly with the amount of pancreas dry weight added into the reaction medium, substrate concentration did not affect the rate of the reaction, and lipase activity increased linearly with the time of incubation. Based on the fulfillment of these three conditions, the GTO assay method presented herein was considered valid for determination of lipase activity in pancreatic homogenates.

Pancreatic lipase activity has been determined previously in young turkeys by using a glycerol tributyrate (GTB) substrate. It was of interest, therefore, to determine how results obtained with the two methods compared. Lipase activity determined with the GTB method was consistently greater than that measured with the GTO method. This difference was expected because the rate of hydrolysis is known to be influenced by the type of substrate used. For example, the reaction rate is slower with increasing length of the fatty acid chain. Even though lipase activity was greater when the GTB substrate was used, both assay methods gave results that were very highly correlated ($R^2 = .80$). One exception should be mentioned though, the correlation between
results obtained by using the two methods was lower ($R^2 = .52$) when lipase activity was determined in pancreases from 1-day-old poults. The lower correlation between the two methods at one day of age suggested that more than one enzyme was hydrolyzing the substrate. For example, carboxyl ester lipase has been shown to be present in the pancreas during early life. In the recently hatched turkey, carboxyl ester lipase could be playing an important role in the utilization of cholesterol esters coming from the remaining yolk. By 7 to 10 days of age, very little yolk remains inside of the body cavity and thus, less carboxyl ester lipase would be needed. This would help explain why lipase activity obtained with the two methods correlated better for 14-day-old poults than for 1-day-old poults.

In summary, lipase activity in the pancreas varied with the assay method used. It is recommended that the GTO assay method be used in nutritional studies because dietary fat occurs mostly as long-chain fatty acid triacylglycerols. In particular, the GTO assay method is specially recommended if very small amounts of pancreatic tissue are available, such as may be the case when pancreatic lipase activity is determined during embryonic life or during the first days post-hatch. If the GTB assay were used, overestimation of pancreatic lipase activity should be expected, irrespective of the age of the turkeys.
Development of Pancreatic Lipase Activity During the First Weeks After Hatching. Effect of the Type of Dietary Fat

In this section, only results for lipase activity determined by using the GTO assay method will be considered. Specific lipase activity per mg of pancreas protein (SLA, μmoles OA/min x mg protein) averaged 6 μmoles OA/min x mg protein at one day of age. SLA slowly increased to 14, 16, and 14 μmoles OA/min x mg protein by days 5, 9, and 14 days, respectively, and then increased to 20 μmoles OA/min x mg protein by day 21 of age. It seems that pancreatic lipase activity increased rapidly when poults started eating, remained relatively constant until about 14 days of age, and then increased again by 21 days of age. These changes in SLA with age were similar to changes obtained in previous research (Escribano et al., 1988). It should be noted, however, that for 1-day-old poults SLA was much lower in the research reported herein than that reported by Escribano et al. (1988). No conclusion can be stated regarding these differences, but differences could just be due to the different assay methods used for determination of lipase activity. Total lipase activity (TLA, μmoles OA/min) per pancreas, calculated as the product of SLA per mg of pancreas protein times the total mg of pancreas protein, increased exponentially with age, averaging 100, 700, 1,400, 2,500, and 8,000 μmoles OA/min at 1, 5, 9, 14, and 21 days of age, respectively.

Poults fed the diet with 10% tallow had greater SLA than poults fed the diet with 10% soy oil only at 5 days of age. By 9 days, SLA did not differ between tallow- or soy oil-fed poults, and by day 21 tallow-fed poults had lower SLAs. In other words, an interaction between the type of fat and age
occurred for SLA. Pancreas size, as represented by its dry weight, was greater for tallow-fed poults than for soy-oil fed poults, and these differences became greater as poults aged. TLA was greater for tallow-fed poults at 5 and 9 days, but no differences occurred by 21 days of age. From the results obtained in this research, it cannot be concluded whether the effect of diet on pancreatic lipase activity was due to the type of fat itself or to feed intake. Tallow-fed poults had a greater feed intake than soy oil-fed poults at all ages. The greater SLA in tallow-fed poults was expected because it has been shown that in in vitro studies that lipase hydrolyzes unsaturated fats (i.e., soy-oil) at a faster rate than saturated (i.e., tallow) ones. Hulan and Bird (1972) found that addition of fat into diets for chickens increased pancreatic lipase activity. Several other researchers (Gidez, 1973; Corring, 1980; Ouagued et al., 1980) have found similar results in mammals. Saraux et al. (1982) reported, however, that the degree of unsaturation of the dietary fat had no impact on pancreatic lipase in rats. At present, it cannot be concluded whether the type of dietary fat i.e., degree of unsaturation, has any influence on pancreatic lipase in turkeys.

Effect of 17β-estradiol Dipropionate on Pancreatic Lipase Activity

The purpose of treating poults with 17β-estradiol dipropionate (E2) was to increase lipase activity in the pancreatic gland. Increased number of zymogen granules in the cytosol of pancreatic acinar cells was found by Grossman et al. (1983) when rats were treated with E2. It was postulated, therefore, that the increased number in zymogen granules could result in an
increase in lipase activity. In preliminary research reported herein, poult injected with 5.5 mg E2/mg of BW had greater lipase activity than non-treated poult, but undesirable side-effects, including rectal prolapses, hyperirritability, cannibalism, and mortality occurred. In a succeeding experiment, doses (from 0 to 2 mg E2/kg of body weight) of E2 caused consistent increases in lipase activity, but these effects did not reach the 10% level of significance. Also, a numerical increase was observed in the number of zymogen granules in acinar cells from poult treated with E2, but this increase was not significantly different from the controls. No effect of E2 on performance was noted in any of the experiments conducted. It seems reasonable to conclude that E2 increased lipase activity, but only when the dose was too high to be considered of practical application. When a lower dose (0.02 mg E2/kg of body weight), which would not have undesirable side-effects was used, the increase in lipase activity was lower than the variability of the experiment and thus not statistically significant. In summary, E2 may play an essential role in maintaining the normal secretory processes of the pancreas as reported by Grossman et al. (1983) in rats, but it does not have a profound influence on pancreatic lipase activity in the turkey.

Retention of Dietary Fat. Effect of 17β-estradiol Dipropionate and the Type of Dietary Fat

Apparent fat retention from the diet averaged 85.3, 84.8 and 93.5% at 5, 9, and 21 days of age, respectively. No change in fat retention occurred between 5 and 9 days, but by 21 days fat retention increased significantly,
irrespective of the diet. Results for fat retention, thus, paralleled those for SLA in the pancreas because SLA remained relatively constant until 14 days and then increased by 21 days of age. In fact, a positive relationship ($R^2 = .92$) was found between apparent fat retention and SLA. Similar changes in fat retention with age were observed by Whitehead and Fisher (1975) and Salmon (1977), who reported that most of the increase in retention of dietary fat in turkeys between 1 and 6 weeks of age occurred by 21 days of age.

Interestingly, retention of fat from tallow was greater than from soy-oil in 5-day-old poults. No reference has been found in the scientific literature on retention of dietary fat by poults younger than 1 week, but, in general, it is accepted that saturated fats like tallow, are utilized less efficiently by the young poult than unsaturated fats like soy-oil. It could be speculated, then, that pinocytosis of the fat droplets into the intestinal cells was taking place in the newborn poult. The pinocytosis pathway was reported to be an important alternative pathway until the adult micellar absorption appeared (Berendsen and Blanchette-Mackie, 1979) in the rat.

When the research reported herein was designed, an increase in the utilization of dietary saturated fat with E2 treatment was hypothesised. Contrary to what was expected, treatment with E2 did not affect retention of either saturated or unsaturated fat from the diet at any age.

Extrapolating from the in vitro results of lipase activity in pancreas, it seems that pancreatic lipase is not limiting for the utilization of dietary fat in the young turkey. In order to illustrate this point, the following example is
given for the 9-day-old poult. Assume that TLA per pancreas is 1.3 meq OA/min, as determined in the research reported herein. These 1.3 meq OA/min are equivalent to .65 meq of glycerol trioleate (GTO)/min if only the fatty acids in positions one and three of the GTO molecule are hydrolyzed. Now, consider a diet with 10% total fat, and an average feed intake of 16 g/poult per day. With this feed intake, a total of 5.42 meq of GTO will be consumed per poult daily (GTO's Mw = 885.4). Thus, because TLA is 1.3 meq/min, 5.42 meq of GTO will be hydrolyzed into monoglycerides and free fatty acids in 8.4 min. This would be if lipolysis occurred as efficiently *in vivo* as *in vitro*. Considering, however, that lipolysis *in vivo* may be only 25% as efficient, about 30 min would be needed for lipase to totally hydrolyze the 5.2 meq of ingested GTO. A much longer period than 30 min of interaction lipase-substrate should be expected because turkeys, as other poultry, are considered "constant nibblers". The 25% efficiency, however, could be reduced more if we consider that pancreatic lipase might be degraded in the small intestine by proteolytic enzymes. Proteolytic enzymes are secreted in important amounts by the pancreas of the young turkey. Furthermore, no protease inhibitors have been described in the intestinal tract of the young turkey.

In summary:

1- Pancreatic lipase activity in nutritional studies should be determined by using a long-chain fatty acid acylglycerol substrate.
2- Specific lipase activity in the pancreas is very low in the 1-day-old poult, it increases rapidly with the beginning of food ingestion, and then it
increases slowly until about 14 days of age. Lipase activity increases rapidly between 14 and 21 days of age.

3- Lipase activity does not seem to be the limiting factor for the utilization of dietary fat. The possible increase in lipase activity in the pancreas by 178-estradiol dipropionate treatment did not result in a better utilization of dietary fat. More likely, a combination of lipase, colipase, and bile salts, together with the development of the intestinal absorptive processes will, determine the degree of utilization of dietary fat by the young poult.
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