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Effect of Feeding Regimen and Age on Lipid Metabolism in Broiler Breeder Hens and Progeny

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Effect of Feeding Regimen and Age on Lipid Metabolism in Broiler Breeder Hens and Progeny

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Doctor of Philosophy in Poultry Science

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ABSTRACT

Age of hens and feed allocation can affect egg yolk lipid compositions which in turn directly relate to chick quality. A series of experiments were conducted to determine the effects of age and feeding programs on lipid metabolism in breeder hens and progeny. In the first trial, the rate of yolk absorption and fatty acid profiles in the yolk were compared among 28, 35, and 49wk old hens. The results revealed that the rate of yolk absorption was higher, but the absolute amount of fatty acids absorbed was lower in young hens than in the old hens. The second trial was hypothesized that young breeder hens synthesis egg yolk lipids from amino acids. The results indicated that both glucogenic and ketogenic amino acids as well as glucose were used as a substrates for *de novo* lipogenesis (DNL) in young hens. The third experiment was set to compare the rate of DNL between young (28wk) and old (40wk) breeder hens as well as their progeny. The results suggested that the rate of DNL was higher in young hens than the old ones. At 7d of age, progeny from old breeder hens illustrated the higher rate of DNL than those chicks from the young hens. The fourth trial was conducted to determine the partitioning of lipids into adipose tissue of 7-day-old chicks. At hatch, yolk-derived lipid was the main lipid in adipose tissue, while the feed-derived and DNL lipids play a bigger role after hatch. The fifth trial was conducted to determine the effect of palmitic acid supplementation. Palmitic acid showed positive effect on hatchability at early phase of production cycle with increasing of monounsaturated fatty acids in fresh yolk. The las trial was set to test the effect of various feeding programs on egg production and progeny growth as well as economic traits. The slower feed increase before peak production with feed adding at peak, and the normal feed increases to peak production with feed adding at 35wk of age showed the best egg production (169 and 171 eggs/HH, respectively) and economic trait expressing as cost for producing a dozen of eggs

($1.21 and $1.22/dozen eggs), whereas the practical feed withdraw program showed the worst effects (148 eggs/HH and $1.29/dozen eggs).

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INTRODUCTION

The final goal of broiler breeder industry is to have the highest number of a good quality salable chicks which are depended on many factors such as, genetic, age, nutrition, and management. The genetic selection and breeding have gained the desirable characteristics of both broiler breeder hens, roosters, and broiler chicks. While breeder hens have been improved to produce more eggs, hatchability and livability of the progeny did not much improve. Broiler breeder industry is still facing the low hatchability and growth performance of progeny form young breeder hens as well as a lack of persistency of lay. Age of hens and feed allocation can affect egg yolk lipid compositions which in turn directly relates to chick quality. Chicks from young breeder flocks are not preferable for the broiler farms since it has been well known that chicks from young breeder flock have poor growth rate and higher early life mortality. Therefore, the hatchability and growth of progeny from young breeder hens need to be improved.

Feed allocation is the key of success for broiler breeder industry. Breeder hens need to accumulate adequate both fleshing and fat reserve before turning to liberty maturity. Improper feed allocation can cause adverse effect for the whole production period. Not enough fleshing at the time of photo stimulation can delayed the onset of lay, decrease peak production, and cause high mortality during transitional period to peak production. Over feeding can also impair reproductive performance by increasing double hierarchies and causing fatty liver hemorrhagic syndrome (FLHS). In the commercial practice, feed is usually withdrawn after peak production to prevent the over body weight of breeder hens and to save feed. The problem is most of the breeder flocks achieve peak production, but cannot maintain the persistency of lay (Mabbett, 2012). Therefore, the pattern of feed allocation during laying period need to be modified in order to have the best reproductive performance.

Lipids play the important roles in broiler breeder industry. Egg yolk contains 33% of fat (O'Sullivan et al., 1991). Chick embryo receive 90% of total energy from the β-oxidation of egg yolk fatty acids, and use egg yolk lipids for structural membrane synthesis (Noble and Cocchi, 1990). Fatty acids in the diet can affect the fatty acid profiles of the yolk. One of the most common fatty acid is palmitic acid, the primary product for de novo lipogenesis (DNL). Although the positive effect on feeding palmitic acid in Japanese quail was reported by Vilchez et al. in 1990, there was no study regarding feeding palmitic acid in broiler breeder hens. Therefore, it is interesting to be tested for the positive effects of this fatty acid in broiler breeder hens. A better understanding of lipid metabolism will gain insight into many problems encountering the broiler breeder industry. The use of stable isotopes is very convenience for metabolic studies. The objectives of this dissertation are to understand the age and feed allocation effects on lipid metabolism in breeder hens and progeny.

I. LITERATURE REVIEW

Lipid metabolism

Biological significances and classification of lipids

Lipids are referred to a group of small molecules which are water insoluble but dissolvable in nonpolar solvents such as ether, chloroform, and benzene. Lipids are significantly important in many aspects. They serve as energy storage similar to glycogen in liver and triglycerides (TAG) in adipose tissues. They are the structural components in cell membranes, myelin, and fat pad. Some of the vitamins and hormones are lipids such as vitamins A, D, E, K, sex hormones, glucocorticoids, prostaglandins, and aldosterone. Lipids also function as receptors, signaling molecules, antigens, sensors, biological detergents, electrical insulators, and membrane anchors for proteins.

There is no internationally accepted system to classify the lipids. Based on the component structures, lipids can be classified into three main categories: simple, complex, and derived lipids (Figure 1). Simple lipids are esters between fatty acids and various types of alcohols. Fats and waxes are two forms of simple lipids. Esterification of fatty acids and glycerol yields fats which is called oils in the liquid state. The good example of fats is triglycerides. Waxes can be formed by esterification of fatty acids with higher molecular weight mono hydroxyl aliphatic alcohols. Cholesterol esters, vitamin A and D esters are the examples of waxes. Complex lipids are referred to esters of fatty acids with other compounds in addition to alcohols, which include phospholipids, glycolipids (glycosphingolipids), and other complex lipids such as sulfolipids, amino lipids, and lipoproteins. Phospholipids is lipids containing a phosphoric acid residue such as lecithin, cephalins, and choline. In glycolipids, fatty acids form ester bonds with sphingosine and carbohydrate. Derived lipids are lipids obtained from hydrolysis of simples and complex

lipids, which include fatty acids, glycerol, steroids, fatty aldehydes, ketone bodies, hydrocarbons, lipid-soluble vitamins, and hormones (Clarenburg, 1992; Botham and Mayes, 2012).

Transportation and storage of lipids

Lipids received from food digestion, synthesis by the liver, and lipolysis of adipose tissue must be transported among tissues and organs for use and storage. In order to transport lipids which are water insoluble, associating nonpolar lipids (triglycerides and cholesteryl esters) and amphipathic lipids (phospholipids and cholesterol) with proteins is made to form lipoproteins which are soluble in water. There are two major forms of lipoproteins that facilitate the transportation of lipids: chylomicrons and lipoproteins (Figure 2). Lipids are mobilized out from the intestines as chylomicrons and from the liver as very low density lipoproteins (VLDL) to various tissues for oxidation and to adipose tissue for storage. In a period of negative metabolic balance, lipids are mobilized from adipose tissue by the action of hormone sensitive lipase (HSL), form of lipase in adipose tissue, as free fatty acids (FFA) and bound to serum albumin for transportation.

Triglycerides of chylomicrons and VLDL as well as albumen-bounded FFA are hydrolyzed by lipoprotein lipase (LPL), located on the walls of blood capillaries, to yield glycerol and FFA which is then transported into various tissues. In liver, hepatic lipase which is bound to the sinusoidal surface of liver cells only react with chylomicron remnant and high density lipoprotein (HDL), but not chylomicrons and VLDL (Bender, 2012). Fatty acids can either diffuse or bound to membrane fatty acid transporters in order to be taken up into the cells.

Lipid synthesis

The primary sites for fatty acid synthesis are liver and adipose tissue. In avian species, lipids *de novo* synthesis takes place mainly in the liver, slightly occurs in adipose tissue, and does not exist in the ovary (Salas, 2011). Palmitic acid is the primary product of fatty acid synthesis which occurs in the cytoplasm where the needed enzymes are located. However, primary precursor of fatty acid synthesis is acetyl-CoA, formed from pyruvate in the mitochondria, which cannot move across the mitochondria membranes to cytoplasm by itself. In order to move acetyl-CoA from inside mitochondria to the cytoplasm, citrate is formed from acetyl-CoA and oxaloacetate and can be translocated to the cytoplasm by the tricarboxylate anion carrier in the inner mitochondria membrane (Figure 3). In cytoplasm, citrate then is cleaved back to oxaloacetate and acetyl-CoA, which is catalyzed by enzyme adenosine triphosphate (ATP)-citrate lyase. Oxaloacetate must be converted to malate in order to return back to mitochondria by the reaction of enzyme cytosolic-malate dehydrogenase. Malate then will be converted back to oxaloacetate inside the mitochondria catalyzed by mitochondria-malate dehydrogenase. In addition a part of malate generated in cytosol can be oxidized to pyruvate and carbon dioxide by malic enzyme and pyruvate then can move across mitochondria membrane into the mitochondrial matrix.

The next step of fatty acid synthesis is conversion of acetyl-CoA to malonyl-CoA which is catalyzed by acetyl-CoA carboxylase. First, this enzyme utilizes bicarbonate as a source of carbon dioxide and bounds to biotin by hydrolysis of ATP. Then the biotin-bound carboxyl group of the enzyme is transferred to acetyl-CoA and regenerate enzyme-bound biotin and malonyl-CoA.

The last step of fatty acid synthesis is catalyzed by fatty acid synthase by adding 7 molecules of malonyl-CoA into one molecule of acetyl-CoA, which is requiring 14 molecules of NADPH and 14 molecules of H^+. The primary product is one molecule of palmitic acid (C16:0), 8 of CoA, 7 of CO_2, 14 of NADP, and 6 of H_2O. The reaction is complex and repeats itself (Figure 4) (condensation, reduction, dehydration, and reduction) until the 16-crbon palmitate (saturated fatty acid) is formed.

The synthesis of fatty acids longer than palmitate and unsaturated fatty acids is achieved by elongation and desaturation. Elongation of fatty acids takes place in the endoplasmic reticulum (ER), mitochondria, and peroxisomes. One of which, elongation in ER is the most active. The substrates for elongation can be either saturated or unsaturated fatty acyl-CoAs. The sequence of reactions are very similar to those catalyzed by fatty acid synthase, except the primers are long-chain acyl-CoAs instead of acetyl-CoA. The elongation in ER uses malonyl-CoA as the elongating group while in mitochondria uses acetyl-CoA instead.

In fatty acid desaturation, the direct oxidative desaturation is catalyzed by a complex of enzymes located in the ER. The first double bond is commonly introduced to a saturated acyl chain at the Δ9 position by enzyme Δ9 desaturase (also called SCD-1: stearoyl-CoA desaturase-1). In addition, polyunsaturated fatty acids occur by introducing double bonds into the chain at the Δ5 and Δ6 positions by Δ5 and Δ6 desaturases, but double bonds cannot take place beyond Δ9 position because of the lack of Δ12 and Δ15 desaturases. This is the reason why linoleic acid (C18:2) and linolenic acid (C18:3) cannot be synthesized by the animals and need must be provided in the diet (Sul, 2012).

Lipid breakdown

Most tissues break down fatty acids for energy need, which is varied among the tissues depending upon physiological conditions and the rate of break down is usually high during starvation and exercise. Liver and muscle tissues are the main places for lipid β-oxidation. However, the brain tissues and red blood cells cannot utilize fatty acids because of the limited blood-brain barrier and the lack of mitochondria, respectively. Once fatty acids are taken up into the cells, they bound to a fatty acid-binding proteins and are activated to a fatty acyl-CoA before being oxidized for energy production.

Since β-oxidation of fatty acids takes place in mitochondrial compartment which is impermeable for nonesterified long-chain fatty acids and their fatty acyl-CoA derivatives, carnitine facilitates the translocation of those molecules. Fatty acylcarnitine is formed from fatty acyl-CoA and carnitine by the catalyzing of enzyme Carnitine palmitoyltransferase 1 (CPT 1) which occurs in the outer mitochondrial membrane. Then the enzyme carnitine-palmitoylcarnitine translocase catalyzes transport of fatty acylcarnitine across the inner mitochondrial membrane. Upon arriving the mitochondrial matrix, fatty acylcarnitine is reesterified back to fatty acyl-CoA and free carnitine, which is catalyzed by enzyme carnitine palmitoyltransferase 2 (CPT 2). CPT 1 located in the outer mitochondrial membranes is the tissue-specific enzyme consisting with three isoforms: CPT 1-A (liver), CPT 1-B (muscle), and CPT 1-C (brain), while CPT 2 is the ubiquitous protein located in the inner mitochondrial membranes (Bonnefont et al., 2004).

The oxidation of fatty acids (Figure 5) inside the mitochondria involves a series of reactions (oxidation, hydration, oxidation, and cleavage) which reveres the fatty acid synthesis. The first step of β-oxidation is catalyzed by enzyme acyl-CoA dehydrogenases which different members

are differently specific for long-chain (>12 carbons), medium-chain (6 to 12 carbons), and short-chain (<6 carbons) acyl-CoAs. Flavin adenine dinucleotide (FAD) is used as the electron donor in this step, which in turn generates the trans-double bond enoly-CoA and the FADH2. In the second step, the hydration of the *trans* double bond created in the first step, which is catalyzed by the enoyl-CoA hydratase yielded L-3-hydroxyacyl-CoA (or D-3-hydroxyacyl-CoA when the *cis* double bond enoyl-CoA is hydrolyzed). The next two final steps start by oxidation of hydroxyacyl-CoA to generate ketoacyl-CoA which then is cleaved by 3-ketoacyl-CoA thiolase to yield acetyl-CoA and fatty acyl-CoA that has two carbon shorter. The β-oxidation will shorten two carbons from the fatty acid chain for each cycle of reactions and one molecule each of acetyl-CoA, $FADH_2$, NADH, and hydrogen ion are produced. Therefore, the oxidation of palmitic acid which requires seven cycles of reaction series yields a maximum of 35 ATPs (Sul, 2012).

Interconvertibility of metabolic fuels

Our bodies as well as animals will try to maintain balanced metabolic status. Therefore, the body will store excess energy which can later be readily used as needed. In the fed state, excess carbohydrate (from the diets) from energy need and formation of glycogen reserves in liver and muscle will be used to synthesis fatty acids and triglycerides in adipose tissue and liver (which can be then exported in form of very low density lipoprotein: VLDL). In turn, during starvation, lipolysis of adipose tissue yields fatty acids and glycerol, which only glycerol can be used for glucose synthesis. In contrast, excess fatty acids cannot be used for gluconeogenesis because the reaction forming Acetyl-CoA from pyruvate is irreversible. In addition, for every unit of Acetyl-

CoA (2C- atom) entering the Krebs cycle will yield 2 carbon dioxides before oxaloacetate is formed. However, the final step of β-oxidation of the odd number fatty acids yields (relatively rare) propionyl CoA which can be a precursor for gluconeogenesis (Figure 6).

In term of amino acids (Figure 7), an excess from requirement for protein synthesis can be catabolized into acetyl-CoA (2C), pyruvate (3C), and four or five-carbon intermediates such as oxaloacetate, fumarate, succinyl-CoA, and α-ketoglutarate. Most of which except Acetyl-CoA can be used for gluconeogenesis so that these amino acids are classified as glucogenic amino acids. Only lysine and leucine that yield Acetyl-CoA and cannot be used for glucose synthesis, which is classified as ketogenic amino acids. Moreover, there are four amino acids that yield both kinds of intermediates, which are phenylalanine, tyrosine, tryptophan, and isoleucine (Bender and Mayes, 2012).

Hormones regulating fatty acid metabolism

There are two main hormones that control level of blood glucose which is the main fuel for most cells. Insulin and glucagon play a key role in controlling blood glucose level. In the fed state, an increased concentration of blood glucose enhances the secretion of insulin from the β- islet cells of pancreas, which is responsible to take up glucose into muscle and adipose tissue for energy purpose. In response to an elevated insulin level, the glucose transporters (GLUT) migrate to the cell surface to take up glucose. In contrast, in the fasting state when insulin level falls those glucose transporters are in intracellular vesicles so that glucose uptake decreases.

The uptakes of glucose into skeletal muscle and liver have special features. In muscle, the increased concentration of cytoplasmic calcium ions in response to nerve stimulation also

enhances the migration of glucose transporters regardless to insulin level. Also, glucose is taken up into the liver cells independently from insulin. An excess of glucose from energy requirement is converted to glucose 6-phosphate (catalyzed by enzyme glucokinase) which is then used for glycogen synthesis. Some part of glucose may also be used for synthesis of lipids which are then exported from liver in form of very low density lipoprotein (VLDL). Insulin promotes glycogen synthetase and inhibits glycogen phosphorylase in both liver and skeletal muscle while stimulates glucose uptake and inhibits the release of free fatty acids in adipose tissue.

Glucagon acts in the opposite manner to insulin. In the fasting state, the concentration of blood glucose decreases resulting in low level of insulin secreted and less glucose taken up by skeletal muscle and adipose tissue. In this condition, glucagon is secreted from α-cell of the pancreas into the liver and inhibits glycogen synthetase while activates glycogen phosphorylase to generate glucose. In addition, the low level of insulin as well as high level of glucagon stimulates hormone sensitive lipase to release glycerol and fee fatty acids from adipose tissue, which are further used for gluconeogenesis in the liver and for metabolic fuel in heart, skeletal muscle, and liver, respectively (Bender and Mayes, 2012).

Significance of breeder age to broiler breeder industry

Effect of hen age on hatchability and progeny performance

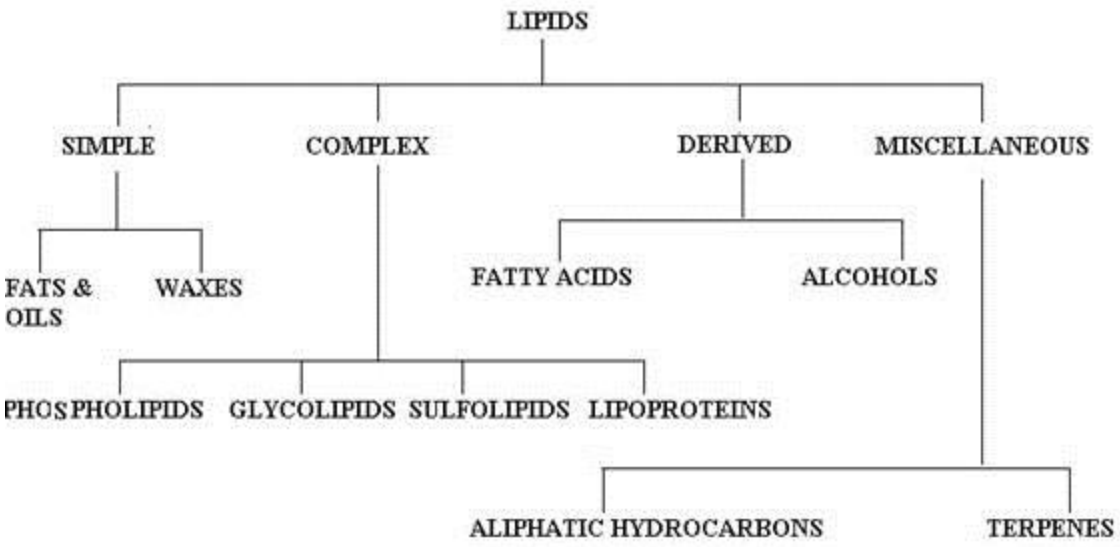
It has been documented that young breeder flocks produce smaller eggs with lighter chick weights, lower hatchability, and higher mortality (Whiting and Pesti, 1983; Wyatt et al., 1985, Burke, 1992; Suarez et al., 1997). Hatchability is low at the beginning of lay than later in production cycle (Smith and Bohren, 1975; Mauldin, 1989). Based on the field study from three

Dutch hatcheries during 2004 to 2006, hatchability of broiler breeder flocks increased from 66% at 25wk to 86% between 31 and 36wk and decreased to 50% at 65wk of age (Yassin et al., 2008). During incubation, eggs from younger flock showed higher weight loss and lower hatchability due to increased early and late death (Roque and Soares, 1994). Age of hens directly relates to internal egg composition or ratio, egg weight, and shell quality, which in turn affect hatchability (Vieira and Mora, 1998). Chick body weights at hatch and at 14 d of rearing were higher in eggs from older hens (45wk old) as compared to those from younger (35wk old) hens (Tona et al., 2004). Also the positive relations were shown among egg weight, weight at embryonic day 18 (E18), embryo: egg weight ratio, and parental age (O'Sullivan et al., 1991).

Effect of hen age on egg components

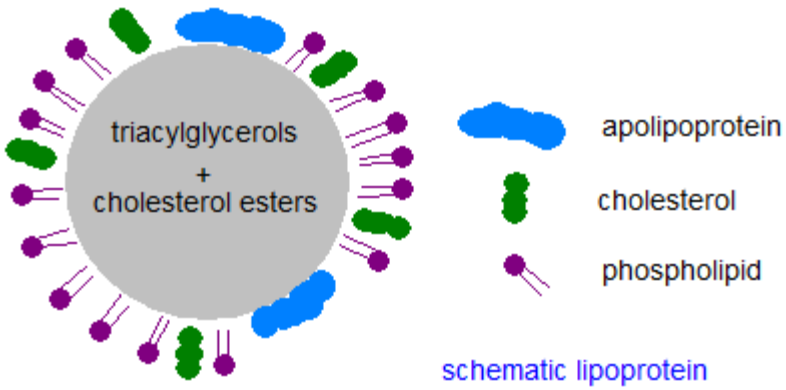
Egg weight and yolk size increase significantly as hens aged while albumen content increases slightly, leading to increased yolk: albumen ratio (O'Sullivan et al., 1991). The bigger egg size from hens in the same age is mainly due to an increase in albumen content (Vieira and Moran, 1998). It has been reported that lipid transfer from yolk sac to embryo of young hens was less efficient than those of older hens (Noble, 1987; Yafei and Noble, 1988). Yadgary et al. (2010) reported that eggs from 30wk old hens had lower fat content than eggs from 50wk old hens. Fresh yolk of 36wk old broiler breeder hens showed the higher palmitoleic acid levels as compared to the yolk from 51 and 64wk old hens (Latour et al., 1998). Chicks from young hens showed lower levels of blood cholesterol, low & high density lipoprotein cholesterol than those chicks from old hens (Latour et al., 1996).

Figure 1: Classification of lipids



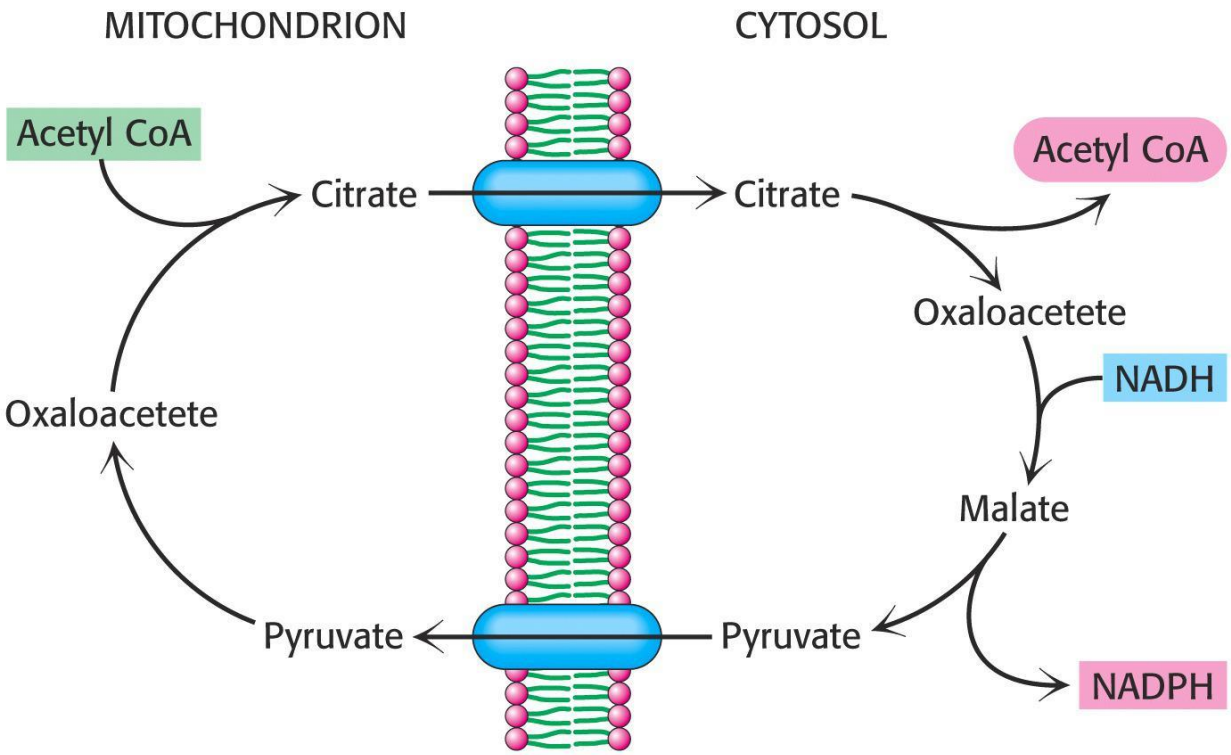
Source: http://biosiva.50webs.org/lipids.htm

Figure 2: Schematic of lipoprotein



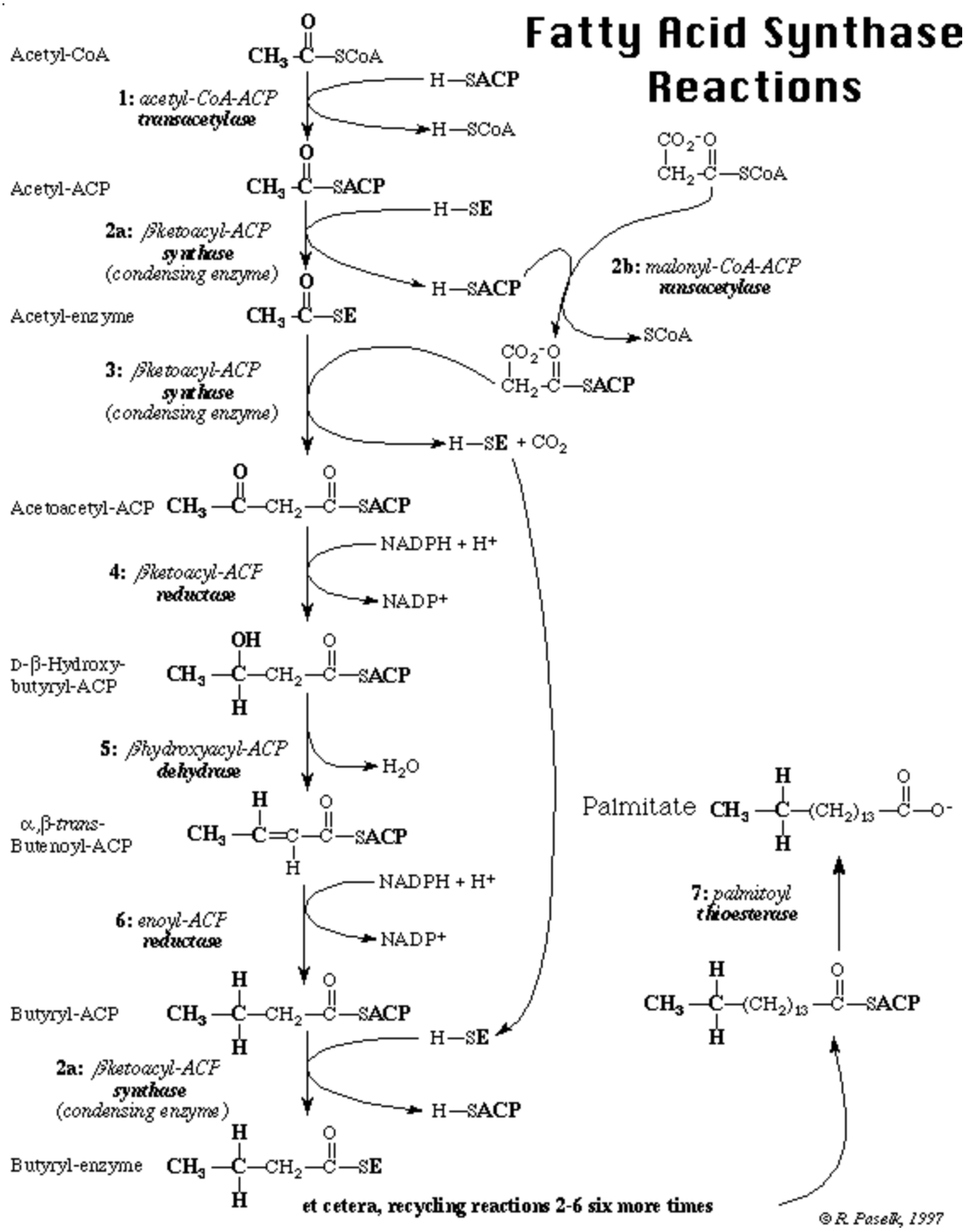
Source: http://lipidlibrary.aocs.org/Lipids/lipoprot/index.htm

Figure 3: Source of acetyl-CoA for fatty acid synthesis



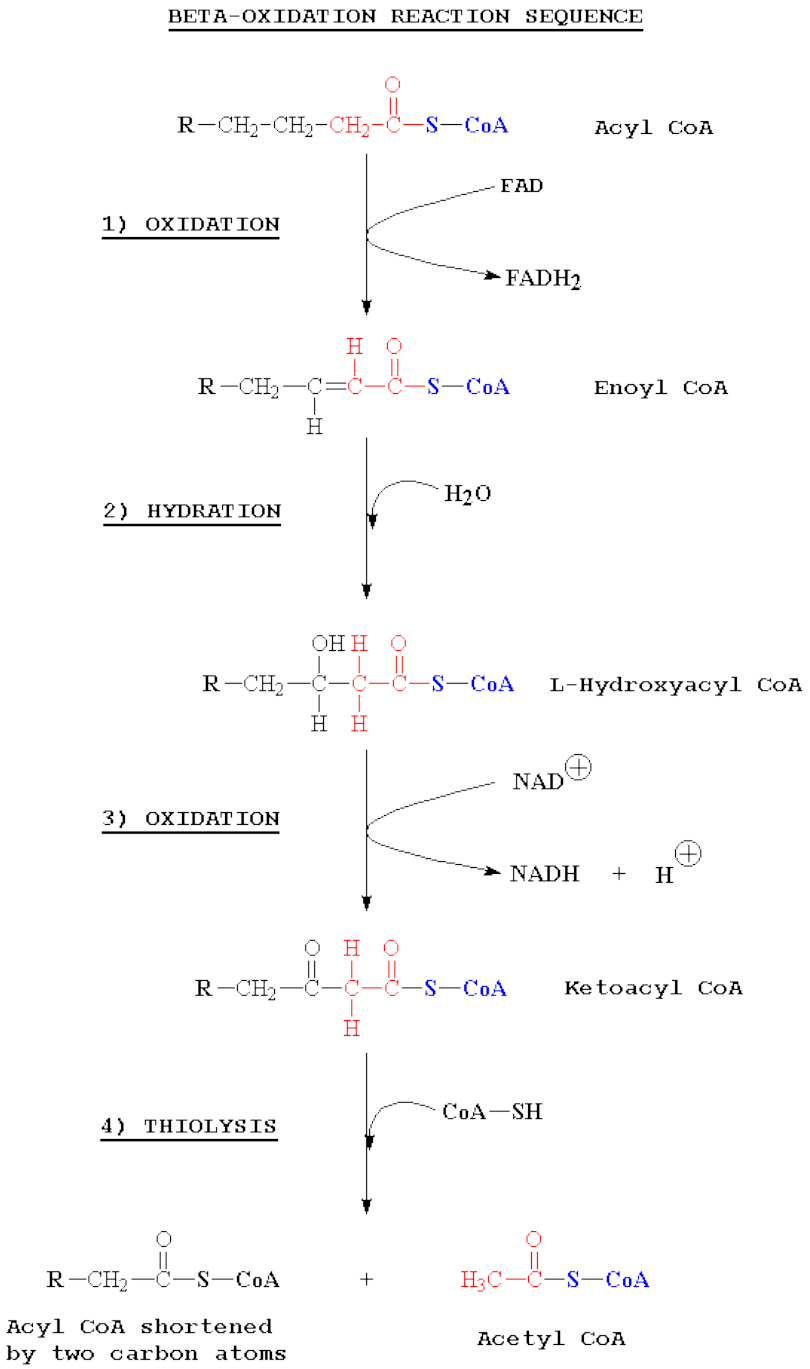
Source: http://pharmaceuticalintelligence.com/tag/fatty-acids/

Figure 4: Fatty acid synthase reactions



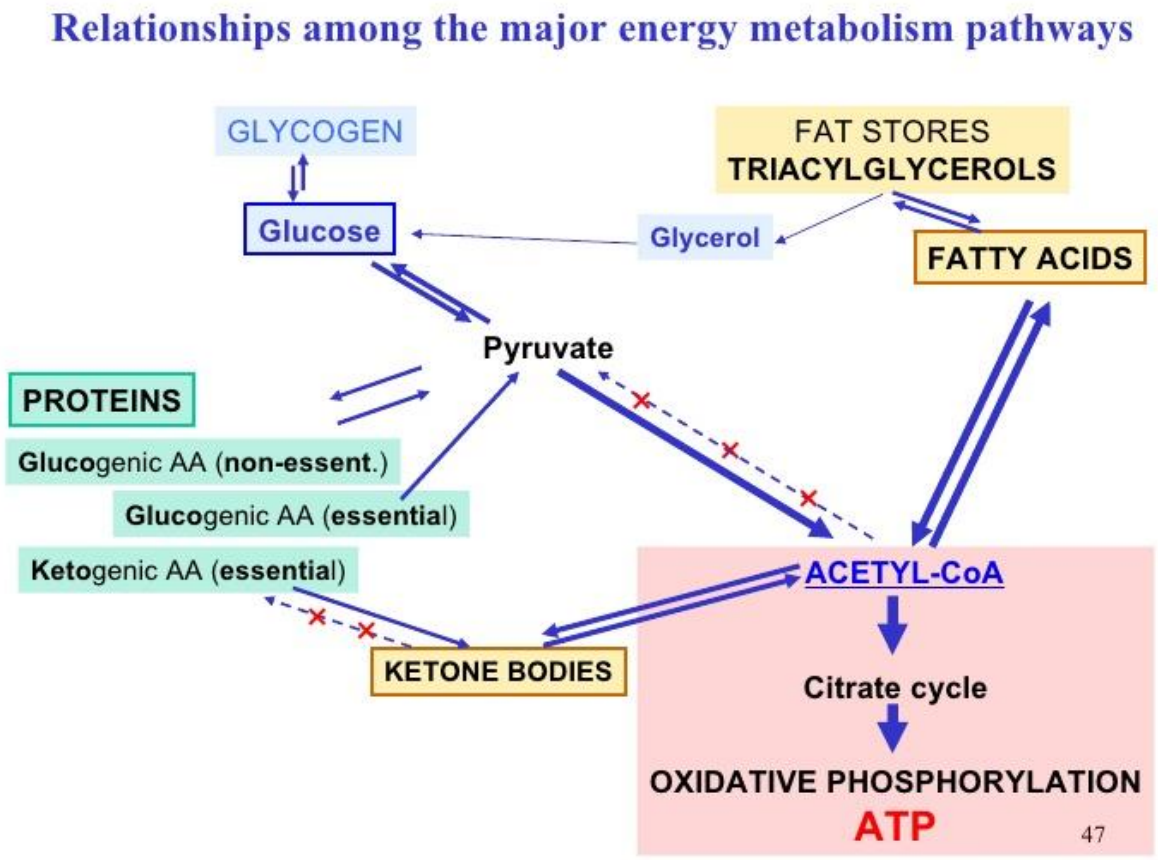
Source: http://users.humboldt.edu/rpaselk/BiochSupp/PathwayDiagrams/FAbioSynRxns.gif

Figure 5: Beta oxidation reaction sequence



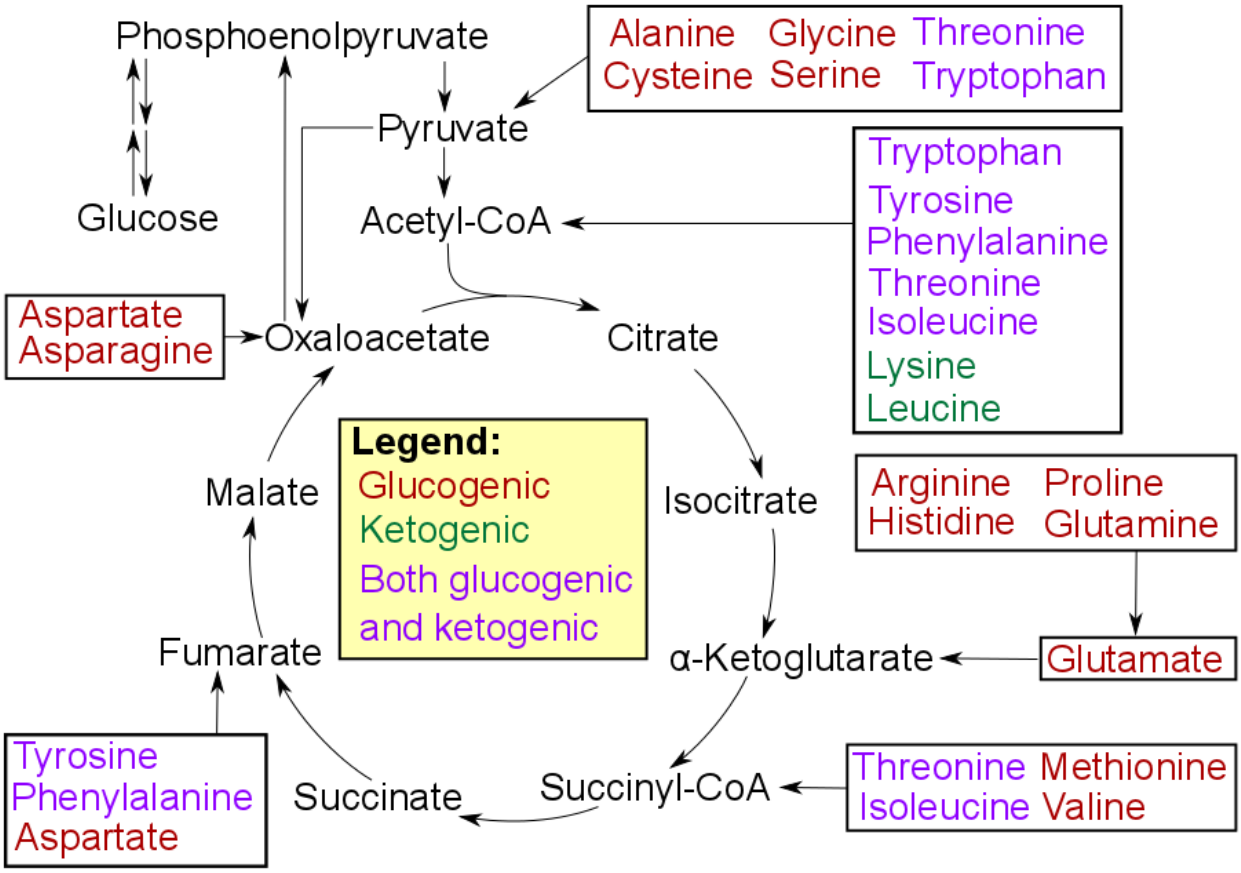
Source:https://www.uic.edu/classes/phar/phar332/Clinical_Cases/vitamin%20cases/carnitine/FA%20catab%20review.htm

Figure 6: Relationships among the major energy metabolism pathways



Source: http://www.slideshare.net/MUBOSScz/cac-9323454

Figure 7: Amino acid catabolism



Source: http://commons.wikimedia.org

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II. Effect of breeder age on yolk fatty acid composition and yolk absorption during embryonic development

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ABSTRACT

The objectives of the present study were to determine the effect of breeder hen age on yolk absorption and fatty acid profiles in yolk at different stages of development (fresh yolk, transfer day; E17, and at hatch). Hatching eggs (n=125) were obtained from breeder hens (Cobb 500) in the same flock at 28, 35, and 49wk of age. Egg weights were selected with a range of 5g differences for each age: 53-57g, 58-62g, and 66-70g for 28, 35, and 49wk hens, respectively. Twenty five eggs (or newly-hatched chicks) were used for each sampling, and 6 yolks were saved for fatty acid determination. Fatty acid compositions in blood plasma were also determined (n=6). As expected, yolk weights in all stages, and yolk free body weight at hatch showed the same patterns as their original egg weights, which significantly increased by age (P<.0001). Yolk absorption from E1 to E17 significantly increased (P<0.05) by age (23.52, 25.02, and 28.04% for 28, 35, and 49wk, respectively). However, the rate of yolk absorption from E17 to hatch slowed down by age, resulting in the lowest (P<.0001) total yolk absorption in embryo from 49wk hens, as compared to those from 28 and 35wk hens (65.14 vs. 75.90 and 75.48%, respectively). Fatty acid concentrations (mg/g dry yolk) decreased throughout embryo development period (P<.0001). Fatty acid concentrations in fresh yolk increased from 28wk to 35wk and reduced again at 49wk of age (P<.0001), which is in agreement with fatty acid contents in plasma. In addition, concentrations of fatty acids were higher in yolk from older hens at E17 and at hatch (P<.0001). Although percent of total yolk absorption was lower in 49wk-old hens, the total amounts of fatty acids being absorbed were higher (P<.0001) in older hens (4.87, 6.11, and 6.53g for 28, 35, and 49wk, respectively). The higher amount of residual yolk at hatch together with its higher concentrations of fatty acid contents in chicks hatched from older hens may result in the better growth rate as has been documented. In conclusion, the rate of yolk absorption was

higher in embryo from younger hens, but the concentration of yolk fatty acids and the quantitative size of available yolk for embryo was larger from older breeders thus providing more fat for embryo gain and livability.

Keywords: yolk absorption, fatty acid profiles, breeder age, embryonic development

INTRODUCTION

During development process, embryo receives all nutrients from albumen and yolk. Although dry matter of albumen contains more than 90% of protein and 7.8% of carbohydrate, but fresh albumen also contain about 90% of water, which makes it the poor source of nutrient (Freeman and Vince, 1974). In the other hand, fresh yolk contains about 50% water, 33% fat, 15% protein, and less than 1% carbohydrates (O'Sullivan et al., 1991). The dry matter of yolk accounts for 70.6% of total egg dry matter (Romanoff and Romanoff, 1949), which makes egg yolk the main source of nutrient for embryo development (Romanoff, 1967) . However, the nutrient percentages in fresh yolk can vary depending on genetic strains, hen age, and egg weight (Vieira and Moran, 1998; Sahan et al., 2014). Embryos receive energy mainly from yolk via the yolk sac membrane and the surrounding vascular system for tissue growth during embryonic development (Nobel and Cocchi, 1990; Vleck 1991). In the first few days of development, embryo utilize carbohydrate as the energy source, then protein is used in the middle phase of development (Romanoff, 1967; Freeman and Vince, 1974). The rate of nutrient transport from yolk to embryo tissue increases extremely in the last week of incubation (Speake et al., 1998).

Hen age also affected growth rate and livability of progeny (Tona et al., 2004). The problem of a low growth rate of progeny from young breeder hens still remains. A better understanding of dynamic changes of yolk fatty acids in different ages of breeder hens may help to solve this problem. Many studies have been conducted to determine embryo yolk absorption and fatty acid compositions in fresh yolk (Yadgary et al., 2010; Nangsuay et al., 2011). However, few research studies have been conducted to determine the effect of breeder hen age on yolk fatty acid profile during different stages of embryonic development. The objectives of this study were to

investigate dynamic changes of yolk fatty acid concentrations from fresh egg, embryonic day 17 (E17; transfer day), and at hatch in 3 different ages of hens: 28, 35, and 49wk of age.

MATERIALS AND METHODS

Stock and Management

Hatching eggs (125eggs/age) were obtained from breeder hens (Cobb 500) in the same flock at 28, 35, and 49wk of age. Breeder hens were fed a standard Breeder diet containing 2,915kcal ME/kg and 15.5% CP (Table 1). The rearing condition and management was followed Cobb Breeder Management Guide (Cobb-Vantress, 2008). Egg weights were ranged within 5g difference for each age: 53-57g, 58-62g, and 66-70g for 28, 35, and 49wk hens, respectively. Twenty five eggs (or hatched chicks) were used for each sampling for fresh yolk, E17 yolk, and residual yolk at hatch. For each sampling time, 6 yolks were saved (-20 ^{0}C) for fatty acid determination.

Fatty acid concentration determination

Yolk fatty acid profiles were determined on dry matter basis using direct methylation method described by Wang et al (2000). In brief, 50mg of dry yolk were weighed into screw-capped tube. Then, 0.5ml (2.0mg/ml) of tridecanocid acid (C13:0) was added to each tube as internal standard followed by 1ml of methanol, and 3ml of 3N-methanolic HCl. The capped tubes were then vortexed and put in water bath at 95 ^{0}C for 1 hour. After cool-down for 15 minutes at room temperature, 1ml of saturated NaCl was added to each tube followed by 2ml of hexane. Tubes

were centrifuged at 3,500 rpm for 5 minutes. Fatty acid methyl esters (FAMEs), the clear layer on top, were taken into GC vial for fatty acid analysis. FAMEs were separated and quantified by GC-2010 Plus gas chromatography (Shimadzu, Columbia, MD) using capillary column SP-2340 (Sigma-Aldrich, St. Louis, MO) and helium gas as a carrier. The injector and flame ionization detector (FID) were set at 240 ^{0}C. Column temperature was started at 140 ^{0}C with 3 min holding, and increased 5 ^{0}C/min to 170 ^{0}C and held for 35 min. The column temperature was then increased 8 ^{0}C/min to 220 ^{0}C and held for 15 min. Injection volume was 1µl and split ratio was set at 1:50.

The concentration (mg/g) of fatty acids in egg yolk was calculated as the equation described below (Wang et al., 2000).

Fatty acid concentration (mg/g) $= [\text{R. F. x peak area of a given fatty acid}]/\text{sample weight (g)}$

Where:

R.F. stands for response factor, and was calculated as follow:

$$\text{R.F.} = \frac{\text{concentration of internal standard (mg/ml)}}{\text{peak are of internal standard}}$$

All procedures in this study were conducted in accordance with the guide for the care and use of laboratory animals of the National Institutes of health, and was approved by the University of Arkansas Institutional Animal Care and Use Committee (protocol # 13002).

Statistical Analysis

The analysis of variance (ANOVA) was performed using SAS software (SAS 9.4). Differences among treatment means of interested parameters were separated using Duncan multiple range test at $P<0.05$.

RESULTS

As shown in Table 2, yolk weights and yolk free body weight at hatch showed the same patterns as their original egg weights which significantly increased by age ($P<.0001$) in all stages of development. Yolk absorption from E1 to E17 significantly increased ($P<0.05$) by age (23.52, 25.02, and 28.04% for 28, 35, and 49wk, respectively). However, the rate of yolk absorption slowed down by age from E17 to hatch (68.29, 66.82, and 51.29% for 28, 35, and 49wk, respectively), resulting in the lowest ($P<.0001$) total percentage of yolk absorption in embryo from 49wk hens, as compared to embryo from 28 and 35wk hens (65.14 vs. 75.90 and 75.48%, respectively). The absolute amounts (gram) of dried yolk being absorbed during incubation process were shown in Table 3. Although the rate of yolk absorption was lower in older hens, the total amount of yolk being absorbed was higher. The same scenario was shown when the total milligrams of fatty acid being absorbed at different stages of incubation were calculated (Figure 1). The fatty acid concentrations (mg/g dried yolk) in fresh yolk, residual yolk on E17 and at hatch were presented in Table 4. The concentration of each fatty acid decreased continuously throughout developing period. Breeder hen age significantly affected fatty acid compositions ($P<.001$) in all stages of development. The concentrations of fatty acids in fresh yolk, residual yolk at E17, and embryo yolk sac increased by hen age. The concentrations of fatty acids in

blood plasma presented in mg/ml was illustrated in Table 5. As what showed in the yolk, the concentration of blood plasma fatty acids increased from 28wk to the highest level at 35wk, and then decreased at 49wk of age.

DISCUSSION

Yolk content increased more than 40% in the eggs from 30wk hens as compared to 50wk old hens (Yadgary et al., 2010). This result is in agreement with the present study which yolk weight increases about 50% from 28wk to 49wk old hens (Table 2). Previous studies indicated that the rate of yolk absorption from E1 to E15 (Yadgary et al., 2010) or E18 (Nangsuay et al., 2011) was lower in embryo from younger hens, which showed the same scenario with the current study. In the current study, the rate of yolk absorption for E17 to hatch was higher in young hens, which is in agreement with Sahan et al. (2014) reporting the higher relative yolk absorption in young flock from E18 to hatch. However, Yadgary et al, (2010) found that the certainly same amount of fat was absorbed at hatch for embryo from both 30 and 50wk old hens. On the other hand, it had been proposed that embryo from young hens (25wk old) had much lower ability to take up fat into their tissue as compared to those embryo from old hens (41wk of age), which might lead to the higher late death in the last period of incubation in embryo from young hens (Noble et al., 1986; Yafei and Noble, 1990). Also, Sahan et al. (2014) found that the absolute amount of yolk being absorbed at E18 and at hatch was higher in older (52wk old) breeder hens as compared to younger ones (36wk old). These results are in agreement with the present study that absolute amount (mg) of fatty acids being absorbed was higher in the embryo from older hens although the total rate of yolk absorption was lower (Table 2 and 3). This makes sense because yolk is the

main source of energy for embryo during incubation process (Speake et al., 1998). Therefore, when the energy need reaches the certain point, the bigger eggs with bigger yolk content should have more potential afford to provide extra energy to embryo for growth resulting in the higher chick weight at hatch. As previously proposed, embryo used fat from yolk sac for small intestine development and body growth (Noy and Sklan, 1999; Meijerhof, 2009), it is possible that chicks hatched from older hens stored larger yolk sac with higher concentration of fatty acids in that yolk, which can be further used for growth and livability. This may result in the better growth rate of chicks from older hens as has been well known documented (Tona et al., 2004).

The major fatty acids in fresh yolk were C16:0, C16:1, C18:0, C18:1, and C18:2, which is in agreement with Sahan et al. (2014). The concentration of each fatty acid as well as the total concentration were affected by hen age, which is in corresponding to the previous studies (Nielsen, 1998; Latour et al., 2000; Yalcm et al., 2008). Although the relative amount of fatty acids being absorbed from E1 to hatch was higher in old hens, the percentage of fatty acid absorption was higher in young hens because of the fact that young hens produce smaller egg yolk with lower fatty acid concentration (Figure 1). This contributed to the total percentages of fatty acid absorption throughout the incubation period were approximately 91.39, 87.54, and 75.71% for eggs from 28, 35, and 49wk old hens, respectively. In this high percentage of total fatty acid absorption in 28wk old hens (91.39%), C16:0 and C18:0 itself accounted about 91.52 and 91.75%, respectively (data not shown). These percentages indicated the potential positive effect if young hens can produce higher concentration of fatty acid in their yolks, which can contribute to the higher fatty acid left for growth of the progeny after hatch.

It can be concluded that age of breeder hens affected the yolk fatty acid profile and yolk absorption during embryonic development. The smaller yolk size leaded to the higher percentage

of yolk absorption in young hens, but the absolute amount of yolk as well as fatty acid being absorbed was still lower than in old hens. With the bigger residual yolk size at hatch and the higher concentration of fatty acid in that residual yolk, it might be a reason why chicks from older hens grow better than those chicks from younger ones.

Table 1: Experimental diet and Nutritional value

Ingredient (%)		Nutrient (%)	
Corn grain	66.3	*Calculated:*	
Soybean meal	23.1	ME (kcal/kg)	2860
Fat	1.3	Crude Protein	15.5
Limestone	6.78	Dig Lysine	0.76
Dicalcium Phosphate	1.81	Dig Met+Cys	0.67
Salt	0.17	Dig. Methionine	0.42
Alimet	0.19	Dig. Threonine	0.52
Choline	0.10	Dig. Tryptophan	0.16
Vitamin premix	0.07	Dig. Arginine	0.95
Sodium premix	0.10	Crude fat	3.38
Mineral premix	0.08	Calcium	3.25
		Phosphorus-Non	0.41
		Sodium	0.2
		Analyzed:	
		Crude Protein	15.1
		Crude fat	4.83
		Ash	10.27
Total	100.0	Calcium (ppm)	33664

Table 2: Effect of hen age on yolk weight and yolk absorption at different stages of development

Age (wk)*	Egg weight (g)	Fresh yolk (g)	E17 yolk (g)	Hatch yolk (g)	Yolk free BW (g)	%Fresh yolk	%Yolk absorption		
							E1 to E17	E17 to hatch	Total
28	55.01[c]	15.06[c]	11.49[c]	3.63[c]	34.41[c]	27.39[c]	23.52[b]	68.29[a]	75.9[a]
35	60.43[b]	17.86[b]	13.30[b]	4.36[b]	36.74[b]	29.55[b]	25.02a[b]	66.82[a]	75.5[a]
49	68.56[a]	22.64[a]	16.39[a]	8.00[a]	43.23[a]	33.02[a]	28.04[a]	51.29[b]	65.1[b]
SEM	0.361	0.209	0.18	0.152	0.304	0.184	0.634	0.849	0.57
P-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.039	<.0001	<.0001

[a,b,c]Means within the same column with different superscripts differ significantly
*n=25 for each sampling time for each age

Table 3: Effect of hen age on absolute amount of yolk being absorbed at different stages of development

Age (wk)	Egg weight (g)	Dried fresh Yok (g)	Dried E17 yolk (g)	Dried Hatch yolk (g)	Yolk free BW (g)	%Fresh yolk	gram dry yolk absorbed		
							E1 to E17	E17 to hatch	Total
28	55.01[c]	7.77[c]	5.73[c]	1.84[c]	34.41[c]	27.39[c]	2.04[b]	3.89[b]	5.93[b]
35	60.43[b]	9.21[b]	6.96[b]	2.18[b]	36.74[b]	29.55[b]	2.26[a]	4.78[a]	7.04[a]
49	68.56[a]	11.28[a]	8.74[a]	4.32[a]	43.23[a]	33.02[a]	2.55[ab]	4.42[ab]	6.96[a]
SEM	0.361	0.209	0.18	0.152	0.304	0.184	0.634	0.849	0.57
P-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.035	<.0001	<.0001

[a,b,c]Means within the same column with different superscripts differ significantly
*n=25 for each sampling time for each age

Table 4: Effect of hen age on selected fatty acid profiles at different stages of development

Stage of development	Fatty acids[1]	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C20:3n6	C20:5n3	Total
		mg/g dry yolk								
Fresh yolk										
	28wk	3.97[a]	277.80[b]	38.52[a]	89.72[b]	434.88[b]	117.87[c]	22.25	10.61[b]	1027[b]
	35wk	4.24[a]	307.44[a]	38.24[a]	98.15[a]	490.91[a]	141.16[a]	22.53	12.94[a]	1154[a]
	49wk	3.51[b]	270.23[b]	27.19[b]	93.63[b]	430.85[b]	137.22[b]	23.27	13.78[a]	1049[b]
	SEM	0.12	5.88	1.9	1.42	10.21	3.62	0.34	0.51	20.47
	P-value	0.003	<.0001	<.0001	0.019	<.0001	<.0001	0.509	0.002	<.0001
E17 yolk										
	28wk	3.54[b]	198.20[b]	28.77[a]	61.85[b]	313.70[b]	84.27[c]	11.23[b]	3.4[c]	714.3[c]
	35wk	3.03[c]	196.61[b]	21.56[b]	64.43[b]	329.97[b]	90.18[b]	12.4[b]	4.36[b]	734.2[b]
	49wk	3.72[a]	231.79[a]	22.15[b]	77.44[a]	387.52[a]	127.16[a]	16.5[a]	6.5[a]	874.6[a]
	SEM	0.1	5.78	1.19	2.45	11.45	6.71	0.84	0.46	25.32
	P-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Hatched yolk										
	28wk	2.31[b]	99.28[c]	12.08[b]	31.21[c]	255.07[c]	53.30[c]	3.63[c]	0.37[b]	373.0[c]
	35wk	2.66[a]	152.09[b]	14.49[a]	45.87[b]	285.71[b]	72.94[b]	7.33[b]	1.25[b]	608.8[b]
	49wk	2.63[a]	163.31[a]	14.91[a]	56.97[a]	354.68[a]	103.77[a]	11.36[a]	3.58[a]	747.9[a]
	SEM	0.07	9.88	0.59	3.76	14.85	7.38	1.14	0.52	24.97
	P-value	0.0261	<.0001	0.0018	<.0001	<.0001	<.0001	<.0001	0.005	<.0001

[a,b,c]Means within the same column with different superscripts differ significantly

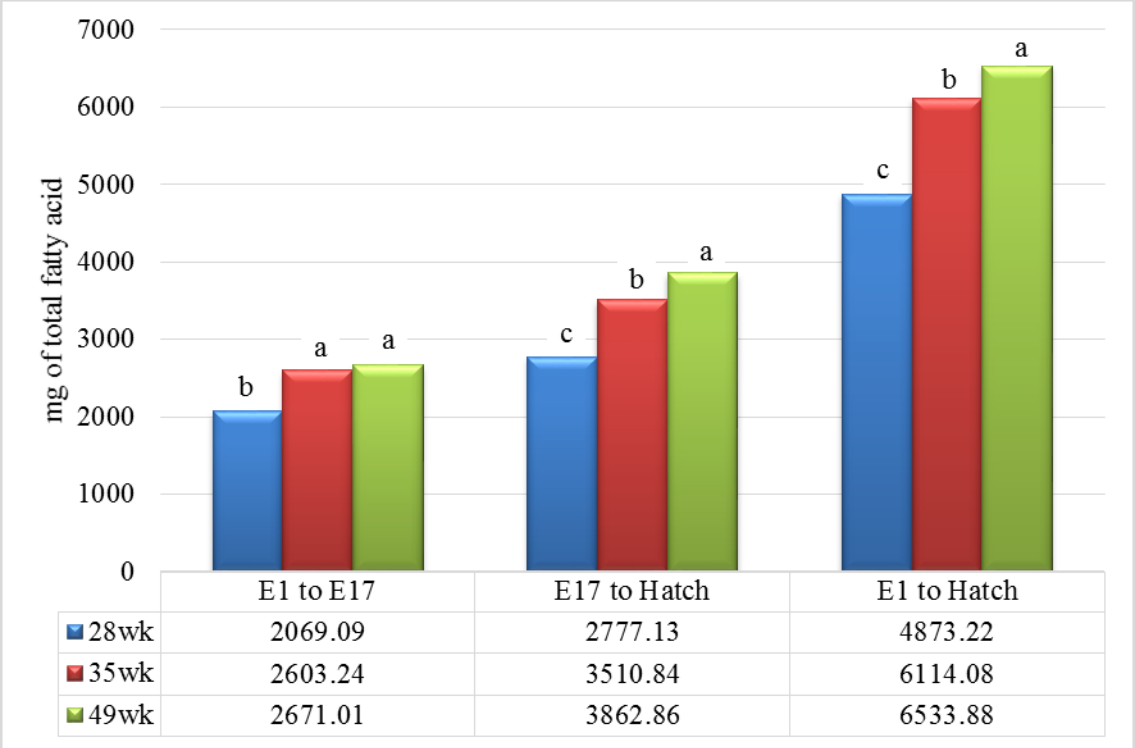
[1]n=6 for each sampling time for each age

Table 5: Effect of hen age on fatty acid concentration in blood plasma

Age (wk)	Fatty acids (mg/ml of blood plasma)[1]									
	C6:0	C8:0	C10:0	C16:0	C16:1	C18:0	C18:1n9c	C18:2n6c	C20:3n6	Total FA
28	3.75	1.85	2.07	27.4[b]	3.83	8.33[c]	37.92[c]	14.26[b]	1.99[c]	103.7[c]
35	4.98	2.4	2.74	40.49[a]	4.49	13.99[a]	68.05[a]	19.78[a]	2.84[a]	171.7[a]
49	6.83	3.58	2.83	29.87[b]	4.45	12.22[b]	59.91[b]	18.95[a]	2.51[b]	138.3[b]
SEM	0.79	0.394	0.41	1.693	0.145	0.649	3.37	0.697	0.093	6.972
P-value	0.3	0.19	0.73	0.0003	0.108	<.0001	<.0001	<.0001	<.0001	<.0001

[a,b,c]Means within the same column with different superscripts differ significantly
[1]n=6 for each sampling time for each age

Figure 1: Effect of hen age on total amount of fatty acid being absorbed at different stages of development



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III. The study of de novo lipogenesis in young hens

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ABSTRACT

The aim of this study was to determine whether the none-lipid precursors: glucose, alanine, and leucine can be used for lipid synthesis in young breeder hens at 25wk of age. Eighteen Cobb 500 breeder hens were divided into three groups of six hens each, one group of which were dosed with one of carbon (^{13}C) isotopes: U-^{13}C Glucose, L-Alanine, and L-Leucine for 14 days. Four hens in each group were dosed with 50mg/hen/day isotopes, and other two were used as control. After 14d of dosing, eggs were collected for two consecutive days at 3 different times: Day 1&2, 5&6, and 9&10 in order to have enough number of samples. On Day 10 after dosing, all hens were killed and abdominal fat pad samples were taken. For each time of sampling, six-dosed and four-control eggs from each group were used for isotope determination. Fatty acid methyl esters (FAME) were extracted from dried yolk and abdominal fat, and the isotope enrichment in palmitic acid was analyzed using the gas chromatography equipped with mass spectrometer (GC-MS). The abundance values of fragments *m/z* (mass per charge ratio) 270, 272, 274, and 276 were monitored to represent the ^{12}C-palmitic acid (M), $2\cdot^{13}C$-palmitic acid (M + 2), $4\cdot^{13}C$-palmitic acid (M + 4), and $6\cdot^{13}C$-palmitic acid (M + 6), respectively. The enrichment of isotope-derived palmitic acid synthesized from U- ^{13}C glucose and L-^{13}C alanine significantly decreased ($P<.0001$) from Day 1 (0.578 vs. 0.381%, respectively) to Day 5 (0.505 and 0.171%, respectively) and were negligible on Day 10 (0.032 and 0.047%, respectively). In contrast, the enrichment of labeled palmitic acid derived from L-^{13}C leucine in egg yolk significantly increased ($P<.0001$) from Day 1 to Day 10 (0.112, 0.207, and 0.355%, respectively). The concentrations of labeled palmitic acid were in the same trends with the enrichment percentages for all sampling times. There were no differences in both percentage of enrichment and concentration ($mg\cdot g^{-1}$) of labeled palmitic acid in abdominal fat pad between dosed and control

hens. These results indicated that besides glucose, breeder hens use amino acids as the precursors for *de novo* lipogenesis, and the mechanisms of using glucogenic and ketogenic amino acids as a precursor for fatty acid synthesis may be different.

Keywords: *de novo* lipogenesis, labeled isotopes, fatty acid synthesis, glucogenic, ketogenic

INTRODUCTION

In the broiler breeder industry, it is critical for breeder hens to have enough fleshing score (score 3-4 out of 5) and pelvic fat (more than 95% of the flock) before receiving photo stimulation, which contributes to a good peak production and persistency of lay, high start hatchability and livability of first-week progeny as well as low mortality of breeder hens during peak production (Bakker, 2015). Pelvic fat can be determined by touching the pelvic bones and estimate the amount of fat present (Harper, 2008). The de novo lipogenesis (DNL) and the synthesis of triglycerides are important factors in fat deposition and efficiency of animal production (Murphy, 2006).

DNL can take place in both liver and adipose tissue. In pig and cow, DNL occurs mainly in adipose tissue (Ballard et al., 1969), but for human and avian species it mainly occur in the liver (O'Hea and Leveille, 1969a) and very low levels in the adipose tissue (Goodridge, 1968). DNL is less active in human even in the conditions of excess carbohydrate (Schwarz et al., 1995; Diraison et al., 2003). On the other hand, DNL seems to be more active in animals. In swine, more than 80% of fatty acids in adipose tissue comes from DNL (O'Hea and Leveille, 1969b). In rodent, about 50% of fatty acids in liver and adipose tissue comes from DNL (Brunengraber et al., 2003).

The precursors for DNL can be either glucose or acetyl-CoA, which can be differently preferable among species. In rodents and swine, glucose is the main substrate of DNL in adipose tissue (Dunshea et al., 1998), whereas ruminants use acetate as the substrate for DNL in both liver and adipose tissue (Ballard et al., 1969). Acetyl-CoA is obtained from glucose, and is used as the precursor for palmitic acid synthesis as the primary product of DNL, which take place in the

cytoplasm (Botham and Mayes, 2006). The DNL from glucose substrate has been reported to occur highly in young breeder hens, reduced by hen age (Salas, 2011). Another possible substrate for DNL is amino acids which can come from both food and breakdown of muscle tissue in term of protein turnover. An excess of amino acid for protein synthesis can be converted into acetyl-CoA which is the substrate for DNL (Murray et. al., 2012).

In 1990, Geraert et al. studied DNL from glucogenic amino acids: L-U-^{14}C alanine and glutamic acid comparing between lean and fat chickens. They found that, in fed state DNL from labeled alanine as well as glutamate was greater in fat chicks than those lean ones. This data suggests that DNL can occur in any physiological status. The study of protein turnover in broiler breeder pure line and broiler breeder hens illustrated that the protein degradation rate increased significantly when the hens enter sexual maturity, and gradually decreased after peak production (Vignale, 2014). The same result was reported by Ekmay (2011) that protein degradation in breeder hens was high at beginning of lay and after 45wk of age. These results were in consistent with the body composition study conducted by Salas (2011) showing that lean mass of broiler breeder hens was steady from beginning of lay until after 45wk of age. These observations suggest that breeder hens may use muscle protein for egg formation from beginning of lay through after 45wk of age. Ekmay (2011) also reported that during early of lay, a majority of lysine found in egg albumen came from endogenous origin. The question still remain whether hens can use the product of protein turnover for egg yolk formation or not. There is no evidence to confirm that hens use amino acids for lipogenesis in egg yolk. The objective of the present study was to determine the use of amino acids and glucose as the precursors for fatty acid *de novo* synthesis in the very young breeder hens.

MATERIALS AND METHODS

Experimental procedure

At the beginning of lay (25wk old, estimated time), 18 Cobb 500FF (fast feathering) breeder hens were individually housed in the cage (47 cm × 30.5 cm × 47 cm) containing an individual feeding trough and nipple drinker. Hens were fed a commercial breeder diet containing 15.5% CP and 2860 kcal/kg ME as shown in Table 1 of Chapter II, and the lighting program was 13 hours a day. All hens were restricted fed every day based on target egg production according to the Cobb Breeder Management guide (Cobb-Vantress, 2008). Hens were divided into three groups of six hens each, one of which were dosed with one of carbon (^{13}C) isotopes: U-^{13}C Glucose, L-Alanine, and L-Leucine (Cambridge Isotope Laboratories, Inc., Andover, MA). Four hens in each group were dosed with 50mg/hen/d isotopes for 14d and other two were used as control. Isotopes were diluted in distilled water (50mg/ml) and the solutions were then individually pipetted on top of the feed that the hens received each day. In order to have enough yolk samples, the eggs were saved in two consecutive days at three different times: Day 1&2, 5&6, and 9&10 to represent the samples on Day 1, 5, and 10 after dosing respectively. Eggs and yolks were individually weighed. Yolk samples were save in the screw-capped cups and stored at -80 ^{0}C freezer for further analysis. For each time of sampling, six-dosed and four-control eggs from each group were used for isotope determination. All forms of isotopes were intended to be the precursors for *de novo* fatty acid synthesis, which can be detected in palmitic acid as the primary product of lipid synthesis in animals.

FAME preparation and isotope enrichment determination

Prior to analysis, yolk samples were lyophilized using the freeze dryer Genesis SQ 12 EL (The Virtis Compay, Gardiner, NY) and then homogenized using a common coffee grinding machine. The preparation of fatty acid methyl esters (FAME) was on the procedure described by Eder (1995). In brief, yolk samples were individually weighed (approximately 100-120 mg) into a 15.0ml screw-capped test tube. Then 0.5ml of C13:0 internal standard solution (2.0mg glyceryl tritridecanoate (Sigma-Aldrich, St. Louis, MO) dissolved in 1.0ml n-hexane) was added to each tube following by 2.0ml of 0.2M metanolic-KOH. Tubes were tightly capped, vortexed, and digested in a heating block at 50 ^{0}C for 45 minutes. During digestion, tubes need to be continuously vortexed 2-3 times per minute. After cooled down, 1.0ml of saturated NaCl solution was added to each tube following by 1.0ml of *n*-hexane. The tubes were again capped, vortexed, and centrifuged at 3000 rpm for 5 minutes. After centrifugation, the hexane layer containing FAME was separated on top of the aqueous layer. Then approximately 200µl each of the hexane layer was transferred to two GC vials equipped with insertions and tightly capped. One of the GC vial was analyzed for isotope enrichment and another one was used to determine the fatty acid concentration. The Supelco 37 Component FAME Mix (Sigma-Aldrich, St. Louis, MO) was used to determine the standard retention time for each fatty acid.

An Agilent 7890A gas chromatograph equipped with Agilent 5975C mass spectrophotometer and Agilent 7683B series auto injector (Agilent Technologies, Santa Clara, CA) was used to separate FAME and measure isotope enrichment. One microliter of FAME dissolved in *n*-hexane was injected (split 1:200) and the needle was automatically washed with methanol and acetonitrile between each injection. A silica capillary column (Agilent 19091S-433, 30m x 0.25mm x 0.25µm) was used with helium gas as a carrier (ultrahigh purity grade) at flow rate

1.0ml/min. The GC oven temperature was programed in series starting at 100 ^{0}C (hold 1 min), then was raised at 6 ^{0}C/min to 180 ^{0}C, followed by increasing of 3 ^{0}C/min to 200 ^{0}C (hold 15 min), and end up with 10 ^{0}C/min to 230 ^{0}C (hold 10 min). The total run time was 49 minutes. The mass selective detector (MSD) was operated under EI and SIM modes. The abundance values of fragments *m/z* (mass per charge ratio) 270, 272, 274, and 276 were monitored to represent the ^{12}C-palmitic acid (M), 2·^{13}C-palmitic acid (M + 2), 4·^{13}C-palmitic acid (M + 4), and 6·^{13}C-palmitic acid (M + 6), respectively (Salas, 2011). The abundance values of the natural occurring of those ^{13}C fragments received from control yolks were subtracted from the treatment yolks.

A GC-2010 Plus gas chromatograph equipped with the flame-ionization detector (FID) and AOC-20i auto injector (Shimadzu, Columbia, MD) was used to determine the fatty acid concentration. One microliter of FAME dissolved in *n*-hexane was injected (split 1:100) and the needle was automatically washed with *n*-hexane between each injection. A silica capillary column SGE BPX70 (50m x 0.22mm x 0.25µm) was used with helium gas as a carrier (ultrahigh purity grade) at flow rate 0.5ml/min. The GC oven temperature started at 180 ^{0}C (hold 2 min), then was raised at 5 ^{0}C/min to 200 ^{0}C, followed by /83 ^{0}C/min to 220 ^{0}C (hold 8 min). The total run time was 58.5 minutes. The concentration of fatty acid was calculated as described previously in Chapter II.

All procedures in this study were performed in accordance with the guide for the care and use of laboratory animals of the National Institutes of health, and was approved by the University of Arkansas Institutional Animal Care and Use Committee (protocol # 13002).

Statistical Analysis

Data were separately analyzed DNL from each labeled isotope. The analysis of variance (ANOVA) was performed using SAS software (SAS 9.4) to compare interested parameters among different sampling times. Differences among treatment means of interested parameters were separated using Duncan multiple range test at $P<0.05$.

RESULTS

The non-lipid substrates: U- ^{13}C glucose, L-^{13}C alanine, and L-^{13}C leucine were used as the precursors of DNL, which was measured in form of palmitic acid in this study. After 14 d of dosing, eggs were collected at three different times for the next 10 d. The DNL of palmitic acid from labeled glucose, alanine, and leucine were shown in Table 1 and Figure 1. The egg yolk lipid synthesis from U- ^{13}C glucose and L-^{13}C alanine significantly decreased from Day 1 to Day 5 and were negligible on Day 10. The enrichment of alanine into palmitic acid seemed to be lower than glucose, but at Day 10 there was higher concentration of alanine than glucose. In contrast, the enrichment as well as concentration of labeled palmitic acid derived from L-^{13}C leucine increased significantly from Day 1 to Day 10. After 10 d of egg collection (Day 10), all hens were killed and their abdominal fat pad were collected and analyzed for isotope enrichment. As shown in Table 2, there were no difference in both percentage of enrichment and concentration ($mg \cdot g^{-1}$) of labeled isotopes in abdominal fat pad. These results suggested that the mechanisms of using glucogenic and ketogenic amino acids as a precursor for fatty acid synthesis were different.

DISCUSSION

The purpose of this trial was to illustrate that hens entering sexual maturity use not only glucose but also amino acids for the synthesis of egg yolk lipids. As we can see from Table 1 and Figure 1, although the efficiency of converting amino acids into palmitic acid seemed to be lower than the conversion of glucose, the results can confirm that young hens use fatty acids not only for protein synthesis but also lipid genesis. The conversion of glucogenic amino acids (alanine and glutamic acid) into lipid in fat and lean chickens was reported by Geraert et al. (1990) who found that, in fasted state, lean chickens had significantly higher DNL from amino acid than the fat ones. The authors concluded that part of dietary amino acids was diverted from protein synthesis towards lipogenesis. This result suggested that DNL from amino acids may be high in young hens since they do not accumulate much fat as the old hens do. In this study, the lower enrichment and concentration of alanine-derived palmitate as compared to glucose-derived one may be due to the fact that alanine is the 3-carbon molecules whereas glucose contains 6 carbons. Also, the metabolic fates of amino acid received form the diet can be either used for protein synthesis or converted to metabolic intermediates, especially pyruvate. Pyruvate can then be converted into acetyl-CoA for energy purpose or fatty acid synthesis, or used for gluconeogenesis (Margaret and John, 2013). This may be a reason why the enrichment of alanine into palmitic acid was lower than the conversion of glucose into palmitic acid.

The study in metabolic fates of leucine in fat and lean chickens conducted by Memier et al. (1986) revealed that fat chickens illustrated significantly higher lipogenesis and protein synthesis than lean chickens. In this study, the incorporation pattern of leucine into palmitic acid was opposite to alanine and glucose. While the enrichment of alanine and glucose into palmitic acid decreased by days after dosing, the enrichment of leucine into palmitic acid increased (Table 1

and Figure 1). The possible reason for this scenario may be due to the fact that leucine is ketogenic amino acid which cannot be used for gluconeogenesis as alanine. The carbon skeleton of leucine can be converted into acetyl-CoA or acetoacetate which can then be used for energy purpose or fatty acid synthesis (Margaret and John, 2013). It is also possible that most part of leucine was used for protein synthesis and fat store in adipose tissue rather than used for lipid synthesis in the egg yolk as can be seen from the higher concentration of leucine-derived palmitic acid in the abdominal fat at the end of the experiment (Table 2) as compared to the concentration of glucose and alanine-derived palmitic acids.

The results from this study also suggested that protein turnover plays a crucial role in egg formation in the young hens. Egg formation is the long process which takes up to 25 to 26 hours (Jacob, 2015) so that it is possible that hens will rely on the product of protein turnover for egg formation. Watford (1994) reported that the muscle releases large quantities of alanine during postabsorptive state, and the rate of release increases during early starvation. The result from this study is in agreement with man previous studies. Vignale (2014) reported that the protein degradation rate increased significantly when the hens enter sexual maturity, and gradually decreased after peak production. Ekmay (2011) proposed that protein degradation in breeder hens was high at beginning of lay and after 45wk of age. The result from the high protein turnover rate can be seen from the plateau of lean mass from beginning of lay until after 45wk of age (Salas, 2011). In conclusion, young breeder hens use both glucose and amino acids as a substrate of *de novo* lipogenesis (DNL) for egg yolk lipids.

Table 1: *De novo* lipogenesis from glucose, alanine, and leucine in egg yolk of very young hens

Treatment	Parameter	Day after dosing			SEM	*P*-value
		Day 1	Day5	Day 10		
U-^{13}C Glucose	%enrichment	0.578[a]	0.505[a]	0.032[b]	0.078	<.0001
	mg·g^{-1}	0.874[a]	0.764[a]	0.041[b]	0.119	<.0001
	mg·egg^{-1}	5.578[a]	5.234[a]	0.338[b]	0.769	<.0001
L-^{13}C Alanine	%enrichment	0.381[a]	0.131[b]	0.080[b]	0.039	<.0001
	mg·g^{-1}	0.576[a]	0.208[b]	0.131[b]	0.057	<.0001
	mg·egg^{-1}	4.041[a]	1.768[b]	0.858[b]	0.413	0.0004
L-^{13}C Leucine	%enrichment	0.112[c]	0.207[b]	0.355[a]	0.031	<.0001
	mg·g^{-1}	0.169[c]	0.313[b]	0.537[a]	0.046	<.0001
	mg·egg^{-1}	0.713[c]	1.416[b]	2.466[a]	0.219	<.0001

[1]values were subtracted from the control group

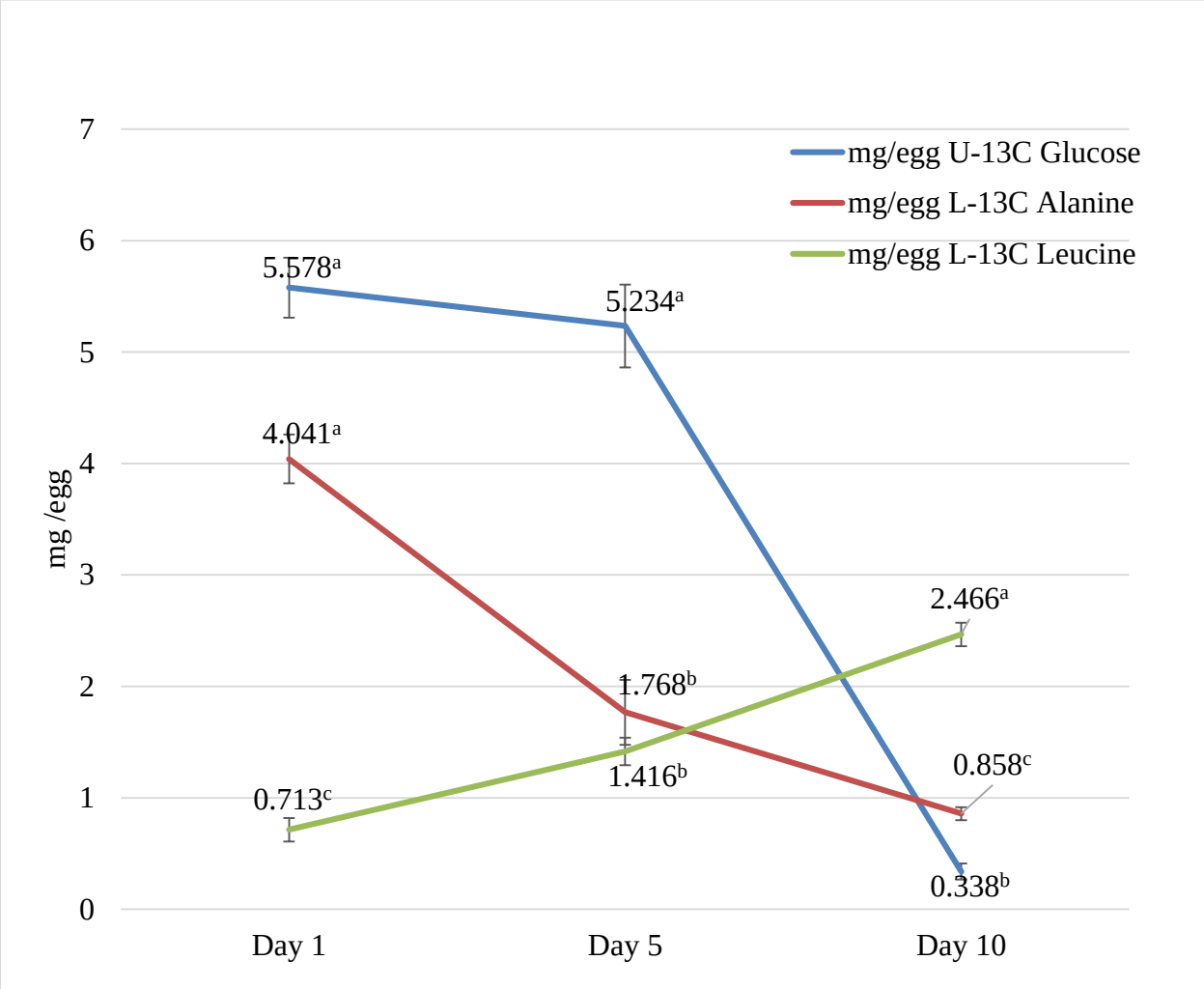
[a,b,c] Means within the same row with different superscripts differ significantly

Table 2: *De novo* lipogenesis from glucose, alanine, and leucine in abdominal fat pad of very young hens

Parameter	Treatment			SEM	*P*-value
	U-^{13}C Glucose	L-^{13}C Alanine	L-^{13}C Leucine		
%enrichment	0.032[b]	0.463[a]	0.511[a]	0.066	<.0001
mg·g^{-1}	0.078[b]	1.126[a]	1.236[a]	0.161	<.0001

[1]values were subtracted from the control group

Figure 1: Concentration (mg/egg) of palmitic acid derived from labeled glucose, alanine, and leucine



[a,b,c] Means within the same line with different superscripts differ significantly

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IV. Comparison of *de novo* lipogenesis between young and old breeder hens and their progeny

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ABSTRACT

The objectives of this experiment were to compare the rate of *de novo* lipogenesis (DNL) between young and old breeder hens as well as their progeny using labeled isotope. Two sets of experiments were conducted. In experiment 1, fifteen hens from each 28 and 40wk old were individually housed in the battery cage to represent hens from young and old breeders, respectively. Twelve hens from each age were dosed with 40mg/hen/d of U-^{13}C Glucose for 14d and the other three hens were used as control. After 14d-period of isotope dosing, their eggs were saved at three different times: Day 1, Day 7, and Day 14. For each time of egg sampling, three hens from treatment and one hen from control group were killed, and their body weight and fat pad were recorded. Fat samples were also taken. The enrichment of ^{13}C received from glucose in palmitic acid the yolk and abdominal fat was detected by the GC-MS. Although there were no significantly differences in the enrichment percentage and the concentration per gram (of dried yolk) of glucose-derived palmitic acid on Day 1, the total concentration per egg (whole yolk) was significantly higher ($P<0.05$) in 40wk than 28wk old hens. However, on Day 7 the concentration of palmitic acid synthesized from labeled glucose decreased approximately half way in the yolk from 40wk hens, while there was no change in 28wk hens with slightly increased concentration per egg. At Day 14 of egg collection, the enrichment percentage as well as the concentration of isotope-derived palmitic acid was slightly detectable, and did not significantly differ between two groups of hens. Unlike in the yolk, only abdominal fat of young hens (28wk) was detected the glucose-derived fat in the noticeable amount, which decreased continuously by days of sampling, and presented in a very small amount on Day 14. In experimental 2, ten other hens from each age (28 vs. 40wk) were randomly selected and were artificially inseminated (AI), and all eggs were saved for hatching. At hatch, eight chicks from each group were raised for 7 d,

five of which were orally dosed with 40mg/bird/d of U-^{13}C Glucose, the rest were used as control. At Day 7, all chicks were killed by CO_2 gas and subcutaneous adipose tissue of each bird was taken. The enrichment of labeled glucose into palmitic acid was measured by the GC-MS. Chicks from older hens showed higher enrichment percentage and concentration ($mg \cdot g^{-1}$) of labeled palmitic acid than those chicks from younger hens. This result indicated the higher DNL rate in 28wk hens than 40wk old hens, and the higher rate of DNL in adipose tissue of the chicks from older breeder hens than those chicks from the younger breeders.

Keywords: *De novo* lipogenesis (DNL), young and old breeder hens, labeled isotope, progeny, enrichment

INTRODUCTION

The main goals for broiler breeder industry are good egg production, long persistency of lay, good hatchability, and high livability and growth rate of progeny. The reproductive performance is directly related to hen physiological condition which is contributed from management, breeding, diseases, and nutrition (Kennard and Chamberlin, 1939). Lipids are the main component of egg yolk accounting for about 33% (O'Sullivan et al., 1991), and embryos receive energy mainly from yolk for tissue growth during embryonic development (Nobel and Cocchi, 1990). The results in Chapter II proposed that young hens had lighter yolk weight with lower concentration of fatty acids in the yolk than old hens, resulting in the lower hatched weight and slower growth rate. These observations suggested that lipids are important for egg formation and posthatch progeny growth. It is highly recommended from the breeder company to perform light stimulation only when hens have enough fleshing and fat reserve (Bakker, 2015). Salas (2011) postulated that at the beginning of lay, young hens mainly relied on DNL for yolk lipid formation, thereafter egg yolk lipids were mainly made from dietary fat and body fat reserve. This result is quite reasonable because young hens have less fat reserve than old hens so that they still need to build up more fat reserve as well as synthesize of yolk fats. However, the author did not compare the DNL in abdominal fat pad between young and old breeders. Also, the question still remains whether the DNL in the progeny from young and old breeder hens is the same or not. The better understanding on how different lipids are made between young and old breeder hens will lead to a better nutritional management as well as enhanced reproductive performance. To gain insight into this obstacle, the study of DNL in young and old breeder hens by using labeled isotope was conducted. The objectives of this experiment were to compare the age effect

on the DNL between young (28wk old) and old (40wk old) breeder hens as well as their progeny at 7d posthatch.

MATERIALS AND METHODS

Experimental procedure

Two sets of experiments were conducted to determine DNL in breeder hens and their progeny, respectively. In experiment 1, two sets of hens (Cobb 500FF) from different ages (28 and 42wk) were used. Fifteen hens from each age were individually housed in the cage (47 cm × 30.5 cm × 47 cm) containing an individual feeding trough and nipple drinker. The lighting program was 13 hours a day. Commercial breeder feed containing 15.5% CP and 2,860kcal/kg ME as shown in Table 1 of Chapter II was used and hens were restricted fed every day based on target egg production according to the Cobb Breeder Management guide (Cobb-Vantress, 2008). Twelve hens from each age were dosed with 40mg/hen/day of U-^{13}C Glucose (Cambridge Isotope Laboratories, Inc., Andover, MA) for 14 days and the other three hens were used as control. U-^{13}C Glucose was diluted in distilled water (40mg/ml) and the solution was individually pipetted on top of the feed that the hens received each day. After 14d-period of isotope dosing, their eggs were saved at three different times: Day 1, Day 7, and Day 14. In addition, for each time of egg sampling, three hens from treatment and one hen from control group were killed by CO_2 gas, and their body weight, fat pad weight, and fat pad samples were recorded and taken. Eggs as well as yolks and albumens were individually weighed. Yolk samples were save in the screw-capped cups and stored at -80 ^{0}C freezer for further analysis. The objective of this experiment was to compare the rate of *de novo* fatty acid synthesis between young (28wk old) and old (40wk old)

hens. The enrichment of ^{13}C received from glucose was intended to be converted into palmitic acid in the egg yolk and abdominal fat pad.

In experimental 2, ten other hens (Cobb 500FF) from each age (28 vs. 40wk) were randomly selected and were artificially inseminated (AI) with $2x10^6$ cells/ 50µl of pooled semen (Ekmay, 2011) two days in the row. Two days after AI, hatching eggs were saved for the next 6 days. All hatching eggs were then set into the incubator Jamesway PS500 (Jamesway, Indian Trial, NC) using the commercial practice: incubator condition 99.6 ^0F 56% RH with egg turning once an hour, and hatcher condition 98 ^0F 53% RH. At hatch, eight chicks from each group were wing-banded and raised for 7d, five of which were orally dosed with 40mg/bird/d of U-^{13}C Glucose (Cambridge Isotope Laboratories, Inc., Andover, MA) dissolved in 0.3ml distilled water, the rest were used as control. Chicks from each group were placed together in the cage (91cm length x 30cm width x 30cm high) installed with nipple drinker and feed trough. Chicks were fed *ad libitum* by commercial starter feed containing 2,988kcal/kg ME and 21.0% CP (Table 1) and reared under commercial management practice (Cobb-Vantress, 2013). On Day 7, all chicks were killed by CO_2 gas and individually weighed. The subcutaneous adipose tissue of each bird was taken from the same area (under the wings surrounding the breast). The weights of adipose tissue were recorded and the sample were save in -80 ^0C freezer for further analysis.

FAME preparation and isotope enrichment determination

Prior to analysis, yolk and fat samples were lyophilized using the freeze dryer Genesis SQ 12 EL (The Virtis Compay, Gardiner, NY). Yolk samples were then individually homogenized using a common coffee grinding machine. A small piece of each fat sample was cut and chopped on top

of the clean microscope slide. Approximately 100 -120mg of yolk samples and 50mg of fat samples were used for FAME preparation using the same procedure as described previously in Chapter III.

The Agilent 7890A gas chromatograph equipped with Agilent 5975C mass spectrophotometer and Agilent 7683B series auto injector (Agilent Technologies, Santa Clara, CA) was used to measure isotope enrichment. The concentrations of fatty acids was analyzed by the GC-2010 Plus gas chromatograph equipped with the flame-ionization detector (FID) and AOC-20i auto injector (Shimadzu, Columbia, MD). The procedures for setting up those instruments were described previously in Chapter III. The abundance values of fragments *m/z* (mass per charge ratio) 270, 272, 274, and 276 were monitored to represent the ^{12}C-palmitic acid (M), 2·^{13}C-palmitic acid (M + 2), 4·^{13}C-palmitic acid (M + 4), and 6·^{13}C-palmitic acid (M + 6), respectively (Salas, 2011). The abundance values of the natural occurring of those ^{13}C fragments received from control yolks were subtracted from the treatment yolks.

All procedures in this study were performed in the same way with the guide for the care and use of laboratory animals of the National Institutes of health, and was approved by the University of Arkansas Institutional Animal Care and Use Committee (protocol # 13002).

Statistical Analysis

The Student's T-test was performed using SAS software (SAS 9.4) to compare interested parameters between two ages of hens at each sampling time. Differences between treatment means of interested parameters were separated using Duncan multiple range test at $P<0.05$.

RESULTS

The rates of DNL in egg yolk, hen abdominal fat pad, and progeny adipose tissue were compared between young (28wk old) and old (40wk old) hens using the incorporation of labeled glucose into palmitic acid in egg yolk as well as fat tissue. As shown in Table 2 and Figure 1, the enrichment percentage of glucose-derived palmitic acid and its concentrations decreased gradually from Day 1 to Day 7, and were slightly present on Day 14. The rate of reduction of the enrichment percentage seemed to be faster in 40wk hens, as compared to 28wk ones. Although there were no significantly differences in the enrichment percentage and the concentration per gram (of dried yolk) of glucose-derived palmitic acid on Day 1, the total concentration per egg (whole yolk) was significantly higher ($P<0.05$) in 40wk hens compared to 28wk hens due to the higher yolk size (data not shown). However, on Day 7 the concentration of palmitic acid synthesized from labeled glucose decreased approximately half way in the yolk from 40wk hens, while there was no change in 28wk hens with slightly increased concentration per egg because of bigger yolk size (data not shown). At Day14 of egg collection, the enrichment percentage as well as the concentration of isotope-derived palmitic acid was slightly detectable, and were not significantly differ between two groups of hens.

The enrichment percentage and the concentrations of isotope-derived palmitate in the abdominal fat pad are presented in Table 3 and Figure 1. Unlike in the yolk, only abdominal fat of young hens (28wk old), but not old hens (40wk old), was detected the glucose-derived fat in the noticeable amount. From Day 7 to 14, the enrichment as well as the concentrations of labeled palmitic acid in abdominal fat of young hens decreased continuously, and present in a very small amount on Day 14. In the old hens, the levels of isotope-derived palmitic acid were extremely low for all those sampling times.

The DNL in the adipose tissue of the progeny from both 28 and 40wk hens were also compared (Table 4). After 7d of labeled glucose dosing (40mg/b/d), chicks from older hens showed higher enrichment percentage and concentration ($mg \cdot g^{-1}$) of labeled palmitic acid than those chicks from younger hens. This result indicated the higher lipid synthesis rate in chicks from older breeder hens.

DISCUSSION

The metabolic fates of glucose can be either oxidized into H_2O and CO_2 for energy purpose or used to synthesis of many other products such as glycogen, non-essential amino acids, *de novo* lipogenesis, and anaerobic glycolysis (Tappy et al., 1995). In this experiment, the age effect on DNL was compared between 28wk and 40wk hens using U-^{13}C glucose as a substrate. After 14d-period of dosing, the enrichment and concentration of labeled palmitic acid decreased by days of sampling, which is in agreement to the result in Chapter III. On Day 1 of egg collection, the incorporation of labeled glucose into palmitic acid did not differ between groups. However, when the yolk size was taken into account, the total milligrams of glucose-derived palmitate per total yolk was higher in older hens. The rate of reduction of glucose isotope enrichment into egg yolk palmitic acid seemed to be faster in older hens. As can be seen from Day 7 of egg sampling, the enrichment percentage as well as milligram of labeled palmitate per egg decreased almost haft way in 40wk hens, while 28wk hens showed the same enrichment with slightly higher concentration of labeled palmitate per egg because of a bigger yolk size (data no shown). This data suggested that DNL for egg lipid in young hens is higher than in old hens. This observation is consistent with results from Salas (2011) who proposed that young breeder hens use DNL as a

main source of egg yolk lipids while old breeder hens mobilized more dietary and body fat in to egg yolk.

Another interesting aspect is the difference on how young and old breeder hens deposit labeled palmitate into their abdominal fat pads. As shown in Table 3, the deposition of labeled palmitic acid in the abdominal fat was significantly high in young hens, whereas the deposition was extremely low in old hens. This observation suggested that young hens had a higher demand to build up more amount of fat reserve than old hens. Buyes et al. (2004) proposed that the rate of glucose oxidation increased with age of broilers, indicating the less DNL in older chicks. What telling us is young hens need to share the product of DNL into both egg yolk and fat store. This may be a reason why eggs from young hens contain less concentration of lipids than those eggs produced by older hens as has been reported in Chapter II.

The comparison of DNL in adipose tissue of progeny from young and old breeder hens was also conducted (Table 4). The result revealed that baby chicks have ability to synthesis lipids from glucose. The positive relations between excess carbohydrate intake and lipogenesis in broiler chicks (Tanaka et al., 1983; Donaldson, 1985) and Turkey poults (Rosebrough and Steele, 1982) have been reported. Leclercq (1984) reported the positive relationship between the body size and the number of adipocytes in broiler chicks, and the author concluded that the increase of fat in adipose tissue was mainly due to hepatic lipogenesis. This observation is in agreement with the present study finding the higher rate of DNL in progeny from old hens than those birds from young breeders since chicks hatched from older breeders had bigger body size than those chicks hatched from younger ones. Geraert et al. (1990) also reported that lipogenesis form glucogenic amino acids into adipose tissue was greater in fat than in lean birds. On the other hand, the study conducted by Latour et al. (1996) indicated that the serum of embryo at E18 from young breeder

hens (26wk old) exhibited higher cholesterol, high and low density lipoprotein cholesterol, but lower glucose than those embryo from older hens (36 and 48wk old). Furthermore, the serum of newly hatched chick from 51wk hens showed the highest level of high density lipoprotein cholesterol and the lowest level of triglycerides than those chicks from 36 and 64wk hens. These observations implicated that the lipid constituents were higher in serum of chicks from young hens. However, Noble and Cocchi, 1990 reported the lower ability of chicks from young hens to absorb liver lipids, as compared to chicks from old hens. Also, the study conducted by Latour et al. (1996) did not measure the concentration of lipids in adipose tissue of the chicks, which can be varied depending upon how efficient the birds can absorb liver lipids into their adipose tissue. In mature vertebrates, lipoprotein lipase (LPL) is responsible for taking up triglycerol-rich lipoproteins from dietary or hepatic sources into adipose tissue (Bensadoun and Kompiang, 1979), but the level of LPL is very low at hatch (Speak et al., 1993). It is possible that the DNL in liver of chicks from young hens is higher than the chicks form old hens, but the naturally low level of LPL in the blood system of chicks from young hens leads to the accumulation of lipoproteins than those chick from old hens. In conclusion, age affects DNL in breeder hens and their progeny. Young breeder hens exhibit higher rate of DNL and deposit more of lipids into both egg yolk and abdominal fat than old breeder hens. The progeny from the young breeder hens accumulate lower DNL product into their adipose tissue, as compared to progeny from old breeder hens.

Table 1: Broiler starter diet composition and nutritional content

Ingredient (%)	Broiler starter
Corn grain	52.27
Soybean meal	38.79
Ark Poultry Fat	4.12
Dicalcium Phosphate	2.25
Limestone	0.86
Salt	0.38
Methionine	0.35
Lysine	0.28
Chol Cholride-60	0.20
Ark vitamin premix	0.20
Threonine	0.15
Ark Trace Mineral	0.10
Kemin Mold Curb	0.05
Selenium	0.02
Monsanto Sanoquin 6 etho	0.02
Total	100.0
Calculated nutrient (%)	
ME (kcal/kg)	2988
Crude Protein	21.0
Calcium	1.0
Non-phytate P	0.5
Total P	0.73
Lysine	1.19
Methionine	0.52
Met + Cys	0.89
Analyzed nutrient (%)	
Crude Protein	20.7
Crude Fat	5.52
Ash	5.83
Calcium (ppm)	10860
Phosphorus (ppm)	6722

Table 2: The enrichment percentage and concentration of palmitic acid derived from labeled glucose in egg yolks of young and old breeder hens

Day after dosing	Parameter[1]	Age of hen		SEM	*P*-value
		28wk	40wk		
Day 1	%enrichment	0.220	0.237	0.009	0.3677
	$mg \cdot g^{-1}$	0.363	0.373	0.014	0.7413
	$mg \cdot egg^{-1}$	2.706	3.774	0.202	0.0014
Day 7	%enrichment	0.218	0.113	0.025	0.0225
	$mg \cdot g^{-1}$	0.361	0.178	0.042	0.0175
	$mg \cdot egg^{-1}$	3.018	1.842	0.317	0.0412
Day 14	%enrichment	0.038	0.025	0.03	0.8462
	$mg \cdot g^{-1}$	0.064	0.039	0.049	0.8301
	$mg \cdot egg^{-1}$	0.551	0.410	0.463	0.8978

[1]values were subtracted from the control group

Table 3: The enrichment percentage and concentration of palmitic acid derived from labeled glucose in abdominal fat of young and old breeder hens

Day after dosing	Parameter[1]	Age of hen		SEM	*P*-value
		28wk	40wk		
Day 1	%enrichment	0.493	0.064	0.097	0.0003
	$mg \cdot g^{-1}$	1.139	0.138	0.227	0.0003
	$mg \cdot fat^{-1}$	78.619	16.906	14.075	0.0006
Day 7	%enrichment	0.415	0.032	0.086	<.0001
	$mg \cdot g^{-1}$	0.962	0.069	0.201	<.0001
	$mg \cdot fat^{-1}$	66.41	8.497	13.046	<.0001
Day 14	%enrichment	0.081	0.021	0.014	<.0001
	$mg \cdot g^{-1}$	0.188	0.045	0.032	<.0001
	$mg \cdot fat^{-1}$	12.975	5.523	1.683	0.0001

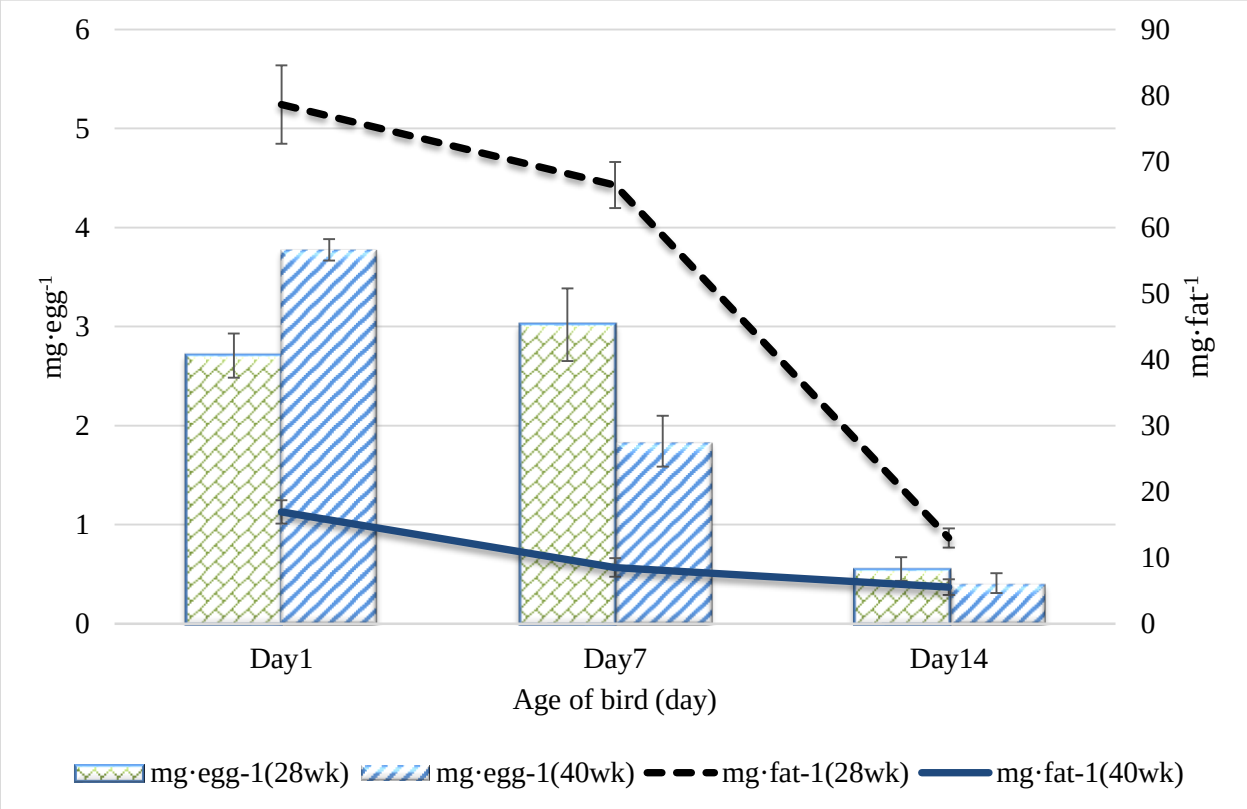
[1]values were subtracted from the control group

Table 4: The enrichment and concentration of palmitic acid derived from labeled glucose in adipose tissue of 7d old progeny hatched from young and old breeder hens

Parameter[1]	Age of hen		SEM	*P*-value
	28wk	40wk		
%enrichment	0.927	1.458	0.112	0.0056
mg·g^{-1}	3.145	4.087	0.187	0.0016

[1]values were subtracted from the control group

Figure 1: The concentration of labeled palmitic acid in total egg yolk and abdominal fat pad of young and old breeder hens (data were taken from Table 2 and 3)



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V. The sources of lipids partitioning to adipose tissue of chicks during first week posthatch

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ABSTRACT

The objective of this study was to determine the sources of lipid in the adipose tissue of chicks during first week posthatch. Six broiler breeder hens (Cobb 500FF; 25wk of age) were used in this trial. Hens were individually housed in the battery cage. Four hens were dosed with 50mg/hen/day of U-^{13}C Linoleic acid (C18:2) ethyl ester dissolved in 1.0ml of corn oil for 14 days, and the other two hens were dosed with 1.0ml/hen/day of corn oil for 14 day and used as a control. On Day 13 and 14, all hens were artificially inseminated, and two yolk samples from treatment hens, and one from control hens were randomly collected on Day 1, 3, and 6 of the 6-day egg collection. The rest of the eggs were set into the incubator. At hatch, three chicks from each group were randomly selected for adipose tissue sampling. The rest of the chicks from each group (6 control and 8 treatment birds) were wing-banded and placed in group in the floor pen installed with nipple drinker and feed trough for 7 days. Chicks hatched from the hens dosed with U-^{13}C Linoleic acid were orally dosed with 50mg/bird/day of U-^{13}C Glucose and 50mg/bird/day of ^{31}D Linoleic methyl for 7 days. On Day 3 and 7 of growing, three control and 4 treatment chicks were kills by CO_2 gas. Chicks were individually weighed, and the subcutaneous fat as well as residual yolk were collected. The subcutaneous adipose tissue of each bird was taken from under the wings and surrounding the breast, and the samples were save at -80 ^{0}C freezer for further analysis. The U-^{13}C Linoleic ethyl ester was intended to represent the source of lipid receiving from the egg yolk, while the ^{31}D Linoleic methyl ester was expected to show the part of lipid coming from the feed. In addition, U-^{13}C Glucose was proposed the lipid gaining from *de novo* lipogenesis. The enrichment percentage and the concentration of U-^{13}C LA decreased significantly (P<.0001) from fresh yolk to Day 3, and were undetectable on Day 7 posthatch. The enrichment and concentration of U-^{13}C LA which represented the lipid derived

from egg yolk were significantly reduced (P<.0001) from hatch to 7d of age. On the other hand, the partitioning of ^{31}D-LA and ^{13}C-PA (represented lipids from feed and DNL, respectively) were significantly increased (P<0.01) from hatch to 7d of growing. This result indicated that chicks utilize lipids from each source in different manners.

Keywords: U-^{13}C Linoleic acid, DNL, ^{31}D-LA, residual yolk, baby chicks

INTRODUCTION

Avian and mammalian species are different in many aspects of carbohydrate and lipid metabolism. They can resist to acute hypoglycemia (Houpt, 1958) as well as low-carbohydrate and high-fat diet (Renner, 1964). The blood glucose level of avian is about two-time higher than that of mammals (Leveille et al., 1975). Unlike mammals, avian embryo are relied on nutrient deposited in the eggs for the whole period of incubation, which directly affects hatching quality (Uni et al., 2012). It has been well known that embryo from young hens have smaller body size and residual yolk, which directly affect final body weight (Nielsen, 1998; Tona et al., 2004; Nangsuay et al., 2011). The data from Chapter II revealed that residual yolk of progeny from young breeder hens was relatively low in lipid content. Baby chicks use residual yolk for energy and tissue development in the first few days after hatch (Noble and Ogunyemi, 1989; Huang et al., 2008). Right after hatch, residual yolk can be either used for maintenance or intestinal growth. The reduction of body within 48 hours after hatch, before feed is available, is mainly due to yolk utilization. The first few days after hatch is the transitional period that chicks must adapt from yolk sac dependence to exogenous feed utilization. The presence of feed in the gastrointestinal tract stimulates the absorption of yolk material into the small intestines (Noy and Sklan, 2001). The partitioning of lipid from feed, body fat, and *de novo* synthesis (DNL) into egg yolk has been studied in broiler breeder hens by Salas (2011). The author found that at the beginning of lay, the lipids accumulated into egg yolk were from DNL, and as hen aged the main sources of lipids in the yolk came from dietary and body fat reserve. However, there is no evidence on how lipids are partitioning into adipose tissue of newly hatched chicks. A better understanding on how chicks built up their body fat at the beginning of life will provide insight into how important among the yolk-derived, feed, and DNL lipids to newly hatched chicks. In

order to gain knowledge on that mechanism, three forms of labeled isotopes were used in this study: U-^{13}C Linoleic acid, ^{31}D - Linoleic acid, and U-^{13}C glucose. The purpose of this study was to determine the partitioning of lipids from three sources: yolk-derived, feed, and DNL, into adipose tissue of baby chicks from one to seven days of age.

MATERIALS AND METHODS

Experimental procedure

The objective of this study was to determine the sources of lipid in the adipose tissue of baby chicks during the first week posthatch. Six broiler breeder hens (Cobb 500FF; 25wk of age) were used in this trial. Hens were individually housed in the battery cage (47 cm × 30.5 cm × 47 cm) containing an individual feeding trough and nipple drinker. Hens received commercial breeder feed containing 15.5% CP and 2,860kcal/kg ME as shown in Table 1 of Chapter II, and feed was restricted based on target egg production according to the Cobb Breeder Management guide (Cobb-Vantress, 2008). Four hens were dosed with 50mg/hen/d of U-^{13}C Linoleic acid (C18:2) ethyl ester (Cambridge Isotope Laboratories, Inc., Andover, MA) dissolved in 1.0ml of corn oil for 14 days, and the other two hens were dosed with 1.0ml/hen/d of corn oil for 14 day and used as a control. The isotope dissolved in corn oil as well as corn oil was pipetted on top of the feed that the hens received each day. On Day 13 and 14, all hens were artificially inseminated with $2x10^6$ cells/50µl of pooled semen (Ekmay, 2011). All hatching eggs were collected for hatching in the next 6 days. Two yolk samples from treatment hens, and one from control hens were randomly collected on Day 1, 3, and 6 of collection. The rest of the eggs were set into the incubator Jamesway PS500 (Jamesway, Indian Trial, NC) using the commercial practice:

incubator condition 99.6 ^{0}F 56% RH with egg turning once an hour, and hatcher condition 98 ^{0}F 53% RH.

At hatch, three chicks from each group were randomly selected for adipose tissue sampling. Chicks were individually weighed and killed by CO_2 gas. The subcutaneous adipose tissue of each bird was taken from under the wings and surrounding the breast. The weights of adipose tissue as well as residual yolk were recorded and the samples were save in -80 ^{0}C freezer for further analysis. The rest of the chicks from each group (6 control and 8 treatment birds) were wing-banded and placed in group in the floor pen (91cm length x 30cm width x 30cm high) installed with nipple drinker and feed trough for 7 days. Chicks were fed *ad libitum* with commercial starter feed containing 2,988kcal/kg ME and 21.0% CP as used for the trial in Chapter IV, and were reared under commercial management practice (Cobb-Vantress, 2013). Chicks hatched from the hens dosed with U-^{13}C Linoleic acid were orally dosed with 50mg/bird/day of U-^{13}C Glucose and 50mg/bird/day of ^{31}D Linoleic methyl ester (Cambridge Isotope Laboratories, Inc., Andover, MA) for 7 days. U-^{13}C Glucose was dissolved in 0.5ml distilled water, and ^{31}D-Linoleic methyl ester was dissolved in 0.5ml corn oil. The control chicks were also dosed with 0.5ml corn oil. On Day 3 and 7 of growing, three control and 4 treatment chicks were kills by CO_2 gas. Chicks were individually weighed, and the adipose tissue as well as residual yolk were collected using the same basis as described previously. The U-^{13}C Linoleic ethyl ester was intended to represent the source of lipid receiving from the egg yolk, while the ^{31}D Linoleic methyl ester was expected to show the part of lipid coming from the feed. In addition, U-^{13}C Glucose was proposed the lipid gaining from *de novo* lipogenesis.

FAME preparation and isotope enrichment determination

The preparation of the FAME, analysis of fatty acid concentration, and measurement of isotope enrichment were the same as what have been described in Chapter III and IV. U-^{13}C Glucose was expected to be used for palmitic acid synthesis as the product of DNL. Therefore, the abundance values of fragments *m/z* (mass per charge ratio) 270, 272, 274, and 276 were monitored to represent the ^{12}C-palmitic acid (M), 2·^{13}C-palmitic acid (M + 2), 4·^{13}C-palmitic acid (M + 4), and 6·^{13}C-palmitic acid (M + 6), respectively (Salas, 2011). For linoleic acids, the abundance values of fragments *m/z* 294, 312, and 325 were monitored representing ^{12}C-linoleic acid (M), 18·^{13}C-linoleic acid (M+18), and $^{2}D_{31}$-linoleic acid (M + 31), respectively. The abundance values of the natural occurring of those ^{13}C and ^{2}D fragments received from control samples were subtracted from the treatment samples. The concentration of fatty acid in egg yolk and adipose tissue was determined by the gas chromatography equipped with the FID as previously described in Chapter II.

All procedures in this study were performed following the guide for the care and use of laboratory animals of the National Institutes of health, and was approved by the University of Arkansas Institutional Animal Care and Use Committee (protocol # 13002).

Statistical Analysis

The analysis of variance (ANOVA) was performed using SAS software (SAS 9.4) to compare interested parameters among different sampling times. Differences among treatment means of interested parameters were separated using Duncan multiple range test at $P<0.05$.

RESULTS

The enrichment and concentration of U-^{13}C LA in fresh yolk, residual yolk at hatch and Day 3 posthatch were shown in Table 1. The enrichment percentage and the concentration of U-^{13}C LA decreased significantly (P<.0001) from fresh yolk to Day 3, and were undetectable on Day 7 posthatch. The partitioning among two forms of labeled linoleic acids and glucose into adipose tissue of chicks from hatch tills 7d of age were shown in Table 2 and Figure 1. The enrichment and concentration of U-^{13}C LA which represented the lipid derived from egg yolk were significantly reduced (P<.0001) from hatch to 7d of age. On the other hand, the partitioning of ^{31}D-LA and ^{13}C-PA representing lipids from feed and DNL, respectively were significantly increased from hatch to 7d of growing. There were no partitioning of those fatty acids at hatch because the labeled ^{31}D-LA and U-^{13}C glucose were administered after hatch. This result indicated that baby chicks utilize lipids from each source in different manners.

DISCUSSION

In the first few days of life, chicks utilize nutrients from the left over residual yolk (Noble and Ogunyemi, 1989). The data from Hang et al. (2008) illustrated that baby chicks utilized all residual yolk around Day 6 of life. In the present study, the amount of residual yolk sac at Day 7 after hatch was relatively small which can be assumed as undetectable. The enrichment and concentration of yolk-derived linoleic acid in residual yolk decreased extremely from hatch to Day 3 and was undetected on Day 7 since the very tiny left over residual yolk sac (Table 1). This observation showed the importance of residual yolk for early growth of baby chicks. As expected, the adipose tissue of hatched chicks was detected the enrichment of yolk-derived

linoleic acid, suggesting that lipid mobilization mechanism exists during embryonic development period. After chicks were fed, the mobilization of feed-derived linoleic acid as well as glucose-derived palmitic acid into adipose tissue started, and the accumulation increased by age (Table 2 and Figure 1). On the contrary, accumulation of yolk-derived linoleic acid in to adipose tissue of the baby chicks reduced by age. It is possible that chicks may use lipids in residual yolk for maintenance or gastrointestinal growth at the first priority as reported by Noy and Sklan (2001). Newly hatched chicks that did not receive feed for 48 hours after hatch decreased body weight about 7.8%, while the weight of the small intestine still increased by 80% (Noy and Sklan, 1999). Also, the less available of residual yolk as the chicks aged with the bigger size of the adipose tissue may result in the lower concentration of yolk-derived linoleic acid in adipose tissue.

The increase of labeled palmitic acid in adipose tissue of baby chick from at Day 3 and 7 after hatch is in consistent with the result in Chapter IV showing the exist of DNL in baby chicks from both young and old breeder hens. Liver plays a key role for *de novo* lipogenesis in avian species (Leveille et al., 1975; Borron and Britton, 1977). Noy and Sklan (1999) reported that the absorption of carbohydrate and protein increased immediately after hatch, which can bring up more substrate for DNL in the liver.

The experiment conducted by Noy and Sklan (1999) by feeding a bolus of labeled glucose, methionine, or oleic acid to the newly hatched chicks found that, at hatch, the absorption of fatty acids was upper 80% and higher than that of glucose and methionine. Then, the rates of absorption for all substrates increased with age and were more than 80% by Day 4 of life. This observation is in agreement with the result in the present study showing the increased accumulation of feed-derived linoleic acid in adipose tissue of the chicks from hatch to 7d of life.

In summary, at hatch yolk-derived lipids are the main source of lipids in adipose tissue of the baby chicks, while lipids from feed and DNL play a bigger role after hatch. After hatch, chicks seem to use yolk-derived lipids for the growth of gastrointestinal tract and energy purpose rather than body fat reserve.

Table 1: The enrichment and concentration of U-^{13}C Linoleic acid in fresh and residual yolk at hatch and Day 3 posthatch

Treatment	Parameter	Day of experiment			SEM	*P*-value
		Fresh Yolk	Hatch RY	Day3 RY		
U-^{13}C Linoleic acid (Yolk)	%enrichment	2.53^{a}	0.64^{b}	0.25^{c}	0.298	<.0001
	mg·g^{-1}	2.47^{a}	0.29^{b}	0.05^{c}	0.325	<.0001
	mg·egg^{-1}	16.93^{a}	0.51^{b}	0.02^{b}	2.348	<.0001

^{a,b,c}Means within the same row with different superscripts differ significantly

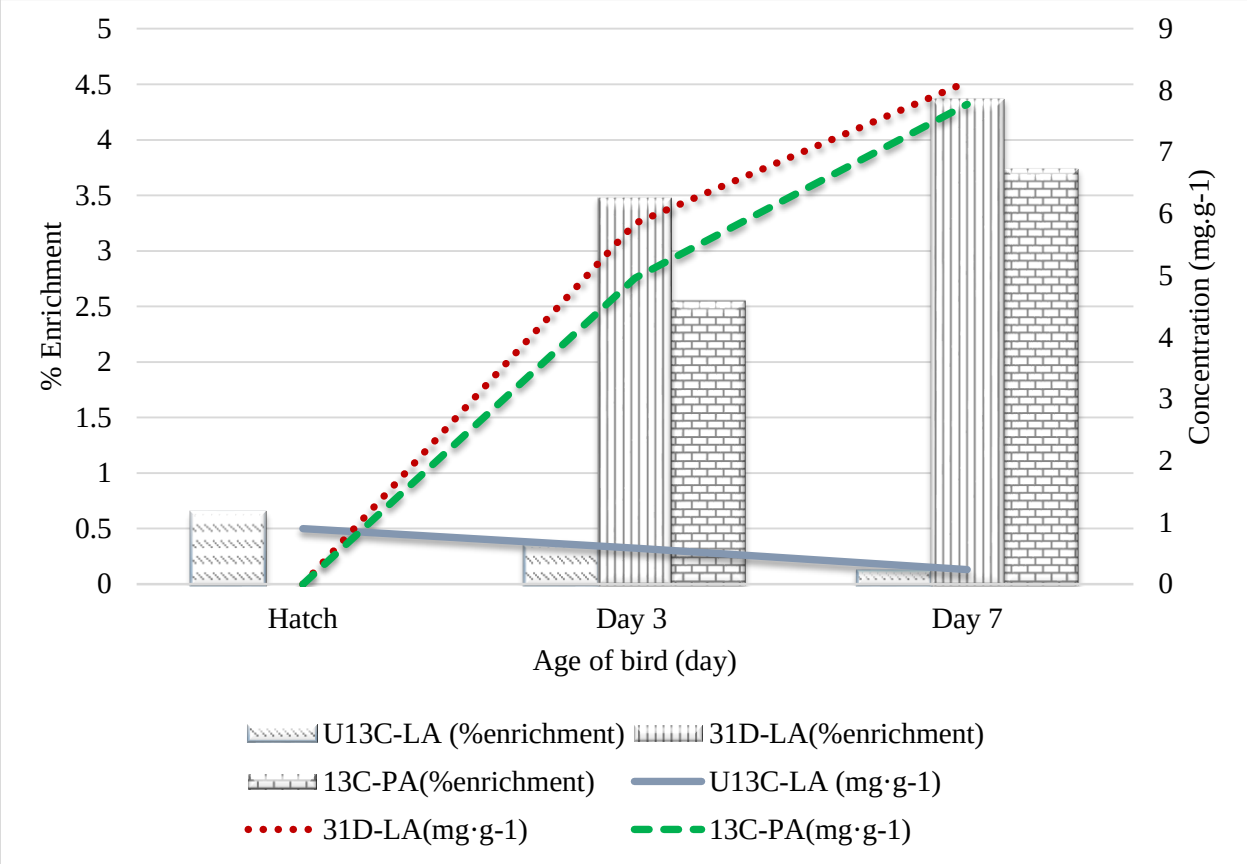
Table 2: The partitioning of differently labeled fatty acids in adipose tissue of baby chicks from hatch tills 7d of age

Treatment	Parameter	Day of experiment			SEM	*P*-value
		Hatch	Day 3	Day 7		
U-^{13}C Linoleic acid (Yolk)	%enrichment	0.646a	0.347b	0.127c	0.064	<.0001
	mg·g^{-1}	0.898a	0.581b	0.238c	0.078	<.0001
^{31}D - Linoleic acid (Feed)	%enrichment	NA	3.484b	4.371a	0.16	<.0001
	mg·g^{-1}	NA	5.845b	8.139a	0.392	<.0001
^{13}C Palmitic acid (DNL)	%enrichment	NA	2.556b	3.739a	0.231	0.0026
	mg·g^{-1}	NA	4.959b	7.778a	0.524	<.0001

^{a,b,c}Means within the same row with different superscripts differ significantly

^{1}values were subtracted from the control group

Figure 1: The enrichment and concentration of different isotope-derived fatty acids in adipose tissues (data were taken from Table 2)



of baby chicks during first week posthatch

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XI. Effect of palmitic acid supplementation on hatchability, reproductive performance, yolk fatty acid profiles, and progeny growth

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ABSTRACT

The objectives of the present study were to determine the effect of supplementation of 3.0% of palmitic acid in the diet on hatchability, egg production, and progeny performance of broiler breeder hens. A total of 90 Cobb-500 pullets (18wk of age) were raised in group on the floor pens (35 pullets / pen). Pullets were divided into two groups, each of 45 birds. Two feed rations were prepared containing the same energy and protein levels. Basal diet were formulated and then corn starch and Palmit 80 (containing 80% free palmitic acid) were added to make the control and treatment diets, respectively. The experimental period lasted from 18 to 45wk of age. At 21wk of age, pullets were placed into individual cage and photo stimulation program was applied. Egg production, hatchability traits, progeny performance, and egg yolk lipid profiles were compared at different ages. The Student's T-test were performed to compare means of interested parameters, and Chi-square test was used for hatchability traits (SAS 4.0). Cumulative egg production at 45wk of age was not significantly different between control (89.17 eggs/hen) and palmitic acid supplemented groups (87.12 eggs/hen). The body weights of hens from 23 to 40wk of age were not significant difference between treatments. Palmitic acid supplemented feed showed positive effects on fertility and percent hatch of fertile at 27 and 35wk of age, but there was no difference at 40wk of age. The ten-day growth performances of progeny as well as egg weights were tested at 27 and 35wk of ages. No significant difference was detected except for O-day body weight at 35wk hens, which was significantly higher in progeny from palmitic acid supplemented hens. The same scenario was also shown at 40wk of age by which progeny from palmitate fed hens had heavier ($P<0.05$) body weight at hatch which contributed from higher ($P<0.05$) residual yolk at hatch. Hens received palmitic acid (C16:0) supplemented feed did not produce higher amount of palmitic acid in their eggs (fresh yolk), but did generate higher

(P<0.05) amount of MUFA, especially oleic acid (C18:1) which contributed to the higher (P<0.05) total FA concentration per gram of dried yolk as well. However, the concentration of each fatty acid in the residual yolk at hatch did not differ between treatments.

Keywords: palmitic acid, Palmit 80, egg production, hatchability, lipid profile

INTRODUCTION

While the genetic selection and improvement is trying to enhance egg production and progeny growth rate at the same time, the hatchability and livability of embryo from young breeder hens are still low. McNaughton et al. (1978) reported the higher mortality in progeny hatched from 29wk old than 58wk old hens. The field study revealed that the average hatchability of 25wk old breeder hens was about 20% less than that of hens at peak production (Yassin et al., 2008). Age of hens affects egg quality which in turn relates to hatchability (Yassin et al., 2008). It has been reported that the ability to take up fat into the body tissues of embryo from young hens lower than those embryo from old hens, which might lead to the higher late death in the last period of (Noble et al., 1986; Yafei and Noble, 1990). The type of lipids in the diet can affect the lipid profiles in the egg yolk (Vilchez et al., 1991; Oliveira et al., 2010). The previous study in Chapter II showed that the huge amount of palmitic acid was utilized (about 64.0% of palmitic acid content in the fresh yolk) by the embryo from young hens (28wk of age). Also, supplementation of 3.0% palmitic acid in the diet has been reported to enhance egg production, hatchability, and livability of progeny in Japanese quails (Vilchez et al., 1990). However, the positive effect of palmitic acid supplementation needs to be calcified in broiler breeder hens. A little improvement of hatchability and livability of progeny in the very young breeder hens can worth a thousand of dollars for the industry. The objective of the present study were to determine the effect of supplementation of 3.0% of palmitic acid in the diet on hatchability, egg production, and progeny performance of broiler breeder hens.

MATERIALS AND METHODS

A total of 90 Cobb-500 pullets (18wk of age) were raised in group (35 pullets / pen) on the floor pens (2.0x4.7x2.0 m^3). Pullets were divided into two groups, each of 45 birds. Two breeder feed rations were prepared containing the same energy (2,921kcal/kg ME) and protein (15.5%) levels (Table 1). Basal diet were formulated and then corn starch and Palmit 80 (containing 80% palmitic acid; Global Agri-Trade Corporation, CA) were added to make the control and treatment diets, respectively. The experimental period lasted from 18 to 45wk of age. At 21wk of age, pullets were placed into individual cage (47 x 30.5 x 47 cm^3) equipped with feeder and nipple drinker. The photo stimulation program was applied at that time. All management practices were followed the Cobb Breeder Management Guide (Cobb-Vantress, 2008). Maximum feed allocation was peak at 390kcal/hen/day at 70% peak production without withdrawal. Egg production, hatchability traits, hen weight, egg weight, and progeny performance were compared at 27, 35, and 40wk of age. Fatty acid profile of fresh yolk was also compared between two groups of hens.

At 27 and 35wk of age, hens were artificially inseminated with 50µl (containing $2x10^6$ cells) of pooled semen (Ekmay, 2011) two days in the row. Eggs were collected for hatch, and hatchability and 10d-progeny growth performance were compared at 27, 35, and 40wk of age. The commercial starter feed containing 2,988kcal/kg ME and 21.0% CP was used to grow the progeny (Table 2). All management practices were followed the Cobb broiler management guide (Cobb-Vantress, 2013) The fatty acid profiles of the yolk was determined using the same procedure as previously described in Chapter III.

All procedures in this study were performed as recommended by the guide for the care and use of laboratory animals of the National Institutes of health, and was approved by the University of Arkansas Institutional Animal Care and Use Committee (protocol # 13002).

Statistical Analysis

The Student's T-test were performed to compare means of interested parameters, and Chi-square test was used for hatchability traits (SAS 4.0).

RESULTS

The accumulate egg production per hen at 45wk of age was not significantly different between treatments, which was slightly higher in control group (89.17 vs. 87.12 eggs/hen; SEM 1.364, data not shown). The body weights of hens from 23 to 40wk of age was shown in Table 3 with no difference between treatments. Palmitic acid supplemented feed showed positive effects on fertility and percent hatch of fertile at 27 and 35wk of age, but there was no difference at 40wk of age (Table 4).

The 10d growth performances of progeny as well as egg weights were tested at 27 and 35wk of ages (Table 5). No significant difference was detected except for O-day body weight at 35wk hens, which was significantly higher in progeny from palmitic acid supplemented hens. The same scenario was also shown at 40wk of age by which progeny from palmitate fed hens had heavier ($P<0.05$) body weight at hatch which contributed from higher ($P<0.05$) residual yolk at hatch (Table 6).

The fatty acid profiles in fresh yolk and residual yolk at hatch were also compared between the treatments at 40wk of age (Table 7). Hens received palmitic acid (C16:0) supplemented feed did not produce higher amount of palmitic acid in their eggs (fresh yolk), but did generate higher ($P<0.05$) amount of MUFA, especially oleic acid (C18:1) which contributed to the higher ($P<0.05$) total FA concentration per gram of dry yolk as well. However, the concentration of each fatty acid in the residual yolk at hatch did not differ between treatments.

DISCUSSION

Hen body weight did not differ throughout the experimental period (Table 3), which indicated that hens in both groups received the same amount of nutrients. Also, egg production was not significantly different between groups, suggesting that hens in both groups receive the same nutritional level even though the feed ingredients were different. Supplementation of 3.0% palmitic acid in the diet seemed to improve the hatchability in young hens, but not old hens (Table 4). The experiment conducted by Vilchez et al. (1990) also found that the supplementation of 3.0% palmitic acid in the diet of Japanese quail breeders improved hatchability. The study in rats indicated that saturated fatty acids promote liver lipoprotein synthesis and lipid storage (Beynen and Katan, 1985). As follow the hen body and egg weight, the 10d body weight of the progeny from both groups of hens did not significantly different. Also, all other growth parameters did not differ between groups (Table 5). At 40wk of age, hens fed palmitic acid supplemental diet seemed to have slightly bigger egg size, which might contribute to the significantly higher chick weight, and the yolk free body weight at hatch (Table 6). However, the O-day body weight of the progeny from palmitate supplemented hens was also

significantly higher although their original egg weight was slightly lower than hens fed control diet. It may be other factors affecting this phenomenon.

Interestingly, the fatty acid profiles (Table 7) in fresh yolk at 40wk of age were significantly different between groups. The hens received palmitic acid showed the higher concentration of monounsaturated fatty acids (MUFA), especially oleic acid (C18:1). This observation is in agreement to Vilchez et al. (1990) who found that the Japanese quail breeders received palmitic acid supplementation exhibited higher level of palmitoleic and oleic acids in their plasma and egg yolk. It is possible that hens use palmitic acid as a precursor for desaturation and elongation to form oleic acid (Botham, 2012). The total fatty acid content in the fresh yolk of palmitate supplemented hens was significantly higher than that of control hens, while the total fatty acid concentration in the residual yolk at hatch was the same. This observation suggested that the total fatty acid absorption into embryo tissue during incubation period is greater in palmitic acid supplemented hens, which may contribute to the higher yolk free body weight at hatch. In summary, palmitic acid supplementation improve hatchability in young hens without affecting egg production and progeny growth performance.

Table 1: Breeder diet composition and nutritional content

Ingredient (%)	Breeder control	Breeder + palmitic acid
Corn grain	58.4	58.4
Soybean meal	23.3	23.4
Limestone	7.2	7.2
Arkansas Poultry fat	2.29	2.29
Dicalcium Phosphate	1.83	1.83
Salt	0.48	0.48
Methionine 98.5%	0.26	0.26
Breeder Vitamin Premix	0.20	0.20
Lysine	0.10	0.10
Chol Cholride-60	0.10	0.10
Ark Track Min	0.10	0.10
Selenium Premix-60%	0.02	0.02
Monsanto Sanoquin 6 etho	0.02	0.02
Corn Starch	5.70	
Sand		1.95
Palmit 80		3.68
Total	100.0	100.0
Calculated nutrient (%)		
ME (kcal/kg)	2921	2921
Crude Protein	15.5	15.5
Calcium	3.25	3.25
Non-phytate P	0.41	0.41
Total P	0.66	0.66
Lysine	0.87	0.87
Methionine	0.50	0.50
Met + Cys	0.77	0.77
Analyzed nutrient (%)		
Crude Protein	15.7	15.6
Crude Fat	3.71	7.69
Ash	10.22	11.54
Calcium (ppm)	29277	30019
Phosphorus (ppm)	5860	5631

Table 2: Broiler starter diet composition and nutritional content

Ingredient (%)	Broiler starter
Corn grain	52.27
Soybean meal	38.79
Ark Poultry Fat	4.12
Dicalcium Phosphate	2.25
Limestone	0.86
Salt	0.38
Methionine	0.35
Lysine	0.28
Chol Cholride-60	0.20
Ark vitamin premix	0.20
Threonine	0.15
Ark Trace Mineral	0.10
Kemin Mold Curb	0.05
Selenium	0.02
Monsanto Sanoquin 6 etho	0.02
Total	100.0
Calculated nutrient (%)	
ME (kcal/kg)	2988
Crude Protein	21.0
Calcium	1.0
Non-phytate P	0.5
Total P	0.73
Lysine	1.19
Methionine	0.52
Met + Cys	0.89
Analyzed nutrient (%)	
Crude Protein	21.5
Crude Fat	5.60
Ash	5.83
Calcium (ppm)	10172
Phosphorus (ppm)	7152

Table 3: Hen body weight at different ages

Treatment / Age	Hen body weight (g)					
	23wk	25wk	28wk	30wk	35wk	40wk
Control feed	2561.89	2931.36	3357.5	3509.32	3746.82	3881.74
Palmitic acid feed	2594.11	2985.44	3404.33	3531.44	3765.67	3908.41
SEM	40.69	35.92	38.105	40.813	28.98	46.71
P-value	0.5769	0.2927	0.3899	0.704	0.7471	0.6858

Table 4: Hatchability at different ages of hens

Treatment	27wk		35wk		40wk	
	%fertile	% hatch of fertile	%fertile	% hatch of fertile	%fertile	% hatch of fertile
Control feed	75.2[b]	70	77.5[b]	77	96.51	94.36
Palmitate feed	80.2[a]	73.3	89.2[a]	87.2	95.97	93.61
SEM	2.512	NA	3.624	NA	0.6114	1.211
P-value	0.0492	NA	0.0174	NA	0.6919	0.7921

Table 5: Ten day progeny growth performance at 27 and 35wk of hen ages

Age/Treatment	EW (g)	O-day BW (g)	10d BW (g)	ADG	FCR
27wk of age					
Control feed	51.89	37.46	228.48	19.6	1.17
Palmitate feed	52.94	37.91	222.01	18.5	1.31
SEM	0.449	0.211	4.148	0.587	0.051
P-value	0.2479	0.2865	0.4377	0.4113	0.1607
35wk of age					
Control feed	63.85	41.8[b]	263.27	22.11	1.19
Palmitate feed	63.24	43.04[a]	265.21	22.16	1.21
SEM	0.544	0.197	1.673	0.164	0.008
P-value	0.5787	0.0015	0.6191	0.8795	0.3184

Table 6: Egg and chick traits at hatch from 40wk old hens

Treatment	Fresh egg data				Hatch data			
	EW (g)	Yolk weight (g)	%Yolk	%DM	BW (g)	YFBW (g)	%RY	%DM
Control feed	64.93	19.29	29.77	51.79	47.27	39.73	5.46	47.70
Palmitate feed	65.29	18.88	28.94	52.34	48.3	40.83	6.58	48.19
SEM	0.52	0.192	0.268	0.410	0.235	0.447	0.186	0.875
P-value	0.7289	0.2784	0.124	0.5571	0.0293	0.2253	0.002	0.7999

Table 7: Fatty acid profiles of fresh yolk and residual yolk at hatch at 40wk of age

Treatment	Concentration of fatty acid (mg/g of dry yolk)													
	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:1	C20:3	C24:1	Total FA	SFA	MUFA	PUFA
Fresh yolk														
Control feed	2.26	141.54	21.01	44.68	229.85	68.09	1.13	1.06	11.35	5.23	533.51	190.74	258	84.82
Palmitate feed	2.27	141.22	20.65	46.38	242.06	67.53	1.21	1.05	10.92	5.97	544.47	192.5	268.6	83.23
SEM	0.056	1.028	0.287	0.964	3.468	1.266	0.035	0.033	0.183	0.203	1.976	1.375	2.241	1.316
P-value	0.9649	0.8848	0.5734	0.4079	0.0348	0.6003	0.683	0.9889	0.262	0.0586	0.0231	0.5536	0.043	0.3693
RY at hatch														
Control feed	1.57	91.74	13.03	29.02	160.51	39.46	0.63	0.69	5.78	1.52	342.77	119.88	175.6	46.85
Palmitate feed	1.73	91.05	13.17	28.83	162.48	40.71	0.65	0.68	5.57	1.46	344.98	120.9	176.3	47.74
SEM	0.102	2.391	0.548	0.472	2.645	1.450	0.030	0.074	0.153	0.075	4.857	4.993	7.246	1.941
P-value	0.4631	0.8953	0.9108	0.8523	0.204	0.6916	0.773	0.9955	0.5153	0.7306	0.8352	0.9272	0.962	0.8362

RY = residual yolk; FA = fatty acid; SFA = saturated fatty acid; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acid

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XII. Effect of various feeding programs during laying period for broiler breeder hens on reproductive performance

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ABSTRACT

The objective of the present study was to determine the effect of different feeding programs during laying period (21-65wk of age) on reproductive performance of broiler breeder hens. A total of 324 breeder females (Cobb 500) were randomly assigned into 6 treatments (54 birds each) and placed in the individual battery cages (47x47x30.5cm3). Feeding program 1, 2, 4, 5, and 6 provided 390kcal ME/kg/hen/day at 60% egg production while program 3 provided peak feed (390kcal ME/hen/day) was applied at 75% egg production. Feed was not withdrawn after peak production except for program 2. Feed was withdrawn for program 2 breeders after peak feed based on commercial practice (total of 8.21% of peak feed). Moreover, feeding amounts were increased to 415kcal ME/hen/day at 75% egg production for program 4 and at 32, 35, and 40wk of age for programs 3, 5, and 6, respectively. The accumulated eggs per hen housed (eggs/HH) of hens that received feeding program 2 was significantly lower ($P<0.05$) than hens in other feeding programs (148 vs. average 160.6 eggs/hen), while hens in feeding programs 3 and 5 showed the best egg numbers per hen (169 and 171 eggs/HH, respectively). The hatch of fertile at 30, 35, 45, 55, and 65wk of age varied between 85 to 88%, and were not significantly different. Starting from 35wk of age to the end of the experiment, hens in program 2 had significant lower BW than hens in other programs ($P<0.05$). In addition, from 45 to 65wk of age, EW and chick BW at hatch produced by hens in feeding program 2 were significantly lower than EW and chick BW from breeders on other programs ($P<0.01$). Also, YFBW at hatch, BW and ADG at 7d after hatch of chicks from hens in feeding program 2 were significantly lower ($P<0.05$), as compared to other feeding programs. In conclusion, feed withdrawal after peak production can be detrimental to egg production and persistency of lay as well as progeny performance.

INTRODUCTION

As hens age, egg production, fertility, and hatchability decrease (Fasenko et al., 1992). Current broiler breeder hens have been shown to accumulate lean mass from 45-65 wk while body fat content is reduced. Lipids in egg yolk are primarily produced from *de novo* synthesis at the beginning of lay whereas feed and fat mobilization have been shown to play a bigger role in providing yolk lipids in older hens (Salas, 2011). This is in agreement with breeder skeletal protein synthesis and degradation observed in both pure-line and PS breeders (Vignale, 2014). The rate of protein synthesis was high from 22 to 24wk of age, and dropped from 24 to 44wk of age before increasing again after 44wk of age. Protein degradation in skeletal muscle in breeders increases from first egg through peak to 37wk and then declines as egg production drops. Breeders are using protein from lean mass for egg production during beginning of lay through peak production and then may be using fat calories for egg production and lean mass gain during the later stages of production. Hess and Lien (2009) reported that slow feed withdrawal during 41-50wk of age yielded more eggs. Feed withdrawal after peak production may not provide enough energy to the hens for lean mass gain and egg production. NRC (1994) recommends 400-450kcal ME/h/d for laying breeder hens raised on floor. However, caged hens need 20% less MEm than hens grown on floor (Sakomura, 2004). Salas (2011) has previously shown that standard BW caged-breeder hens fed energy levels ranging from 330 to 480kcal ME at peak produced the highest number of hatching eggs when fed 390kcal ME. Most breeder flocks achieve peak egg production, but cannot maintain persistency of lay (Mabbett, 2012). Feed allocation during laying period need to be addressed in order to achieve best reproductive performance. The objectives of the present study were to investigate the effect of different

feeding programs during laying period (21-65wk of age) on reproductive performance and economic trait of broiler breeder hens as well as progeny growth performance

MATERIALS AND METHODS

A total of 324 Cobb-500 pullets were raised on floor-pen (2.0x4.7x2.0 m3) based on standard management guideline (Cobb-Vantress, 2008). At 21wk of age, each pullet was randomly placed into individual battery cage (47x30.5x47 cm3). The completely randomized block design (CRBD) was applied. Six different feeding programs were randomly applied to each block. Each feeding program had 6 blocks, each of 9 hens. All feeding programs use the same breeder feed containing 15.5% CP and 2,860kcal/kg ME as shown in Table 1. Feeding program 1, 2, 4, 5, and 6 provided 390kcal ME/hen/day at 60% egg production while program 3 provided peak feed (390kcal ME/hen/day) was applied at 75% egg production (Table 2 and Figure 1). All feeding programs did not withdraw feed after peak production except program 2 which feed was withdrawn after peak based on commercial practice (total of 8.21% of peak feed) as shown in Table 3 and Figure 1. Moreover, feeding amounts were increased to 415kcal ME/hen/day at 75% egg production (program 4) and at 32, 35, and 40wk of age for programs 3, 5, and 6, respectively (Fig 1). Egg production, hen BW, EW, hatch of fertile, cost per dozen eggs, chick BW at hatch were determined. At 60wk of age, all hens were artificially inseminated with 50µl (containing $2x10^6$ cells) of pooled semen (Ekmay, 2011). Eggs were saved for hatching using the same protocol and equipment as previously described in Chapter II. The 7d growth performance of the progeny from each feeding programs were compared. The commercial starter feed containing

2,988kcal/kg ME and 21.0% CP was used as shown in Table 2 of Chapter XI. All management practices were followed the Cobb broiler management guide (Cobb-Vantress, 2013).

All procedures in this study were performed as suggested by the guide for the care and use of laboratory animals of the National Institutes of health, and was approved by the University of Arkansas Institutional Animal Care and Use Committee (protocol # 13002).

Statistical Analysis

Analysis of variance (ANOVA) was performed using SAS software (SAS 9.3). Differences among treatment means of interested parameters were separated using Tukey's HSD at $P<0.05$. Chi-square test was performed to compare hatchability among treatments ($P<0.05$).

RESULTS

As shown in Figure 2 and Table 4, the accumulated eggs per hen housed (eggs/HH) of hens received feeding program 2 was significantly lower ($P<0.05$) than those hens in other feeding programs (148 vs. average 160.6 eggs/hen), while hens in feeding programs 3 and 5 showed the best egg numbers per hen (169 and 171 eggs/HH, respectively). The hen day egg production (eggs/HD) showed the same trend as the eggs per hen housed. In term of cost per dozen eggs, hens in treatment 2 still showed the highest cost followed by hens in treatments 4 and 6. The cheapest cost per dozen eggs still showed in treatment 3 and 5. Livability did not differ among treatments. The hatch of fertile did not affect by the feeding program from 30wk to the end of the experiment (Table 5).

Egg weight directly reflected chick BW at hatch (Table 6). Eggs produced by hens in feeding program 2 were significantly lower ($P<0.05$) than those eggs in other feeding programs (Fig 3). The growth performance of the progeny from the 60wk old hens in each feeding program were shown in Table 7. Yolk free body weight (YFBW) at hatch, BW and ADG at 7d after hatch of chicks from hens in feeding program 2 were significantly lower ($P<0.05$), as compared to other feeding programs.

DISCUSSION

Broiler breeders nowadays differ from the past decade. They have bigger body size and gain lean mass all the way through the end of flock. The bigger body size will contribute to the higher need of energy for maintenance. Salas (2011) suggested that the metabolized energy at 390kcal per hen per day yield the best egg production. However, in the broiler breeder industry feed is usually withdrawn after peak egg production, which might lead to inadequate energy for egg production. In this experiment, treatment 2 showed the lowest reproductive performance including egg production, egg weight, chick weight and growth, and cost per dozen eggs. The feeding pattern in this treatment was adopted from the commercial practice by withdrawing feed after peak egg production. Compared to treatment 1 which the feeding pattern is closest to treatment 2 except no feed withdraw after peak production, treatment 2 still showed the lower reproductive performance than treatment 1. This suggested that feed withdrawal after peak impairs persistency of lay. In addition, additional energy is needed after peak egg production to sustain persistency of lay and provide energy for maintenance and lean mass growth.

The study by Salas (2011) indicated that breeder hens use body fat reserve and lipids from feed as the main sources of lipids putting in the egg yolk. The results from Chapter IV also implicated that older hens need more feed to produce lipids into their eggs since the DNL slowed down by age. In agreement to those observations, hens in treatment 5 which were added daily energy consumption from 390 to 415kcal/hen/day showed the best reproductive performance. However, feed must be added at the right time. The increase of feed too early (treatment 4) or too late (treatment 6) did not show the positive effect.

Since the commercial practice usually encounters with high mortality rate, double hierarchies, and multiple ovulation from the beginning of lay to peak production when feed allocation rate is too fast in this period (Leksrisompong et al., 2014), the feeding program 3 was conducted to solve the problem. In feeding program 3, feed increase rate was slowed down to provide peak feed at 75% egg production instead of 60% egg production as other treatments. Then feed allowance was increased again at peak egg production and held for the whole laying cycle. The result showed the positive effects of the slow feed increment program.

The growth performance of progeny from the hens in each feeding program seemed to reflect the hen body weights and original egg weights. Chicks from feeding program 2 show the numerical less efficient than other feeding programs. It has been well known that chicks hatched form smaller eggs will have less performance than those chicks hatched form bigger eggs (Kosin et al., 1952; Wyatt et al., 1985; Lourens et al., 2006). In conclusion, feed withdrawal after peak production can be detrimental to egg production and persistency of lay as well as progeny performance. Increasing feed at the right time such as at 32 or 35wk of age (instead of withdrawing feed) significantly increased egg numbers.

Table 1: Experimental diet and Nutritional value

Ingredient (%)		Nutrient (%)	
Corn grain	66.3	*Calculated:*	
Soybean meal	23.1	ME (kcal/kg)	2860
Fat	1.3	Crude Protein	15.5
Limestone	6.78	Dig Lysine	0.76
Dicalcium Phosphate	1.81	Dig Met+Cys	0.67
Salt	0.17	Dig. Methionine	0.42
Alimet	0.19	Dig. Threonine	0.52
Choline	0.10	Dig. Tryptophan	0.16
Vitamin premix	0.07	Dig. Arginine	0.95
Sodium premix	0.10	Crude fat	3.38
Mineral premix	0.08	Calcium	3.25
		Phosphorus-Non	0.41
		Sodium	0.2
		Analyzed:	
		Crude Protein	15.3
		Crude fat	4.87
		Ash	10.31
Total	100.0	Calcium (ppm)	33604

Table 2: Feed allocation patterns from 5% to peak production of each treatment

Treatment 1,2,4,5,6				Treatment 3			
Stage	%Production	%Increase	kcal/h/d	Stage	%Production	%Increase	kcal/h/d
	before 5%		329		before 5%		329
1	5%	5%	333	1	5%	2.50%	331
2	13%	5%	335	2	13%	5.00%	334
3	21%	10%	342	3	21%	5.00%	337
4	29%	10%	348	4	29%	7.00%	341
5	37%	15%	356	5	37%	7.00%	345
6	45%	15%	366	6	45%	8.50%	351
7	53%	20%	378	7	53%	10.00%	357
8	61%	20%	390	8	61%	15.00%	365
				9	69%	20.00%	377
				10	75%	20.00%	390
	Total	100%			Total	100.00%	

Table 3: Reduction pattern of feed allocation for treatment 2

Week after peak	Wk of age	kcal/h/d 390	% Reduction	lb/100b/d
1	33	383	1.83%	0.5
2	34	376	1.73%	0.5
3	35	374	0.70%	0.2
4	36	371	0.71%	0.2
5	37	368	0.71%	0.2
6	38	366	0.72%	0.2
7	39	363	0.72%	0.2
8	41	360	0.73%	0.2
9	42	358	0.73%	0.2
End	65	358	8.21% (total)	

Table 4: Reproductive performance of each feeding program

Treatment	Eggs/HH	Eggs/HD	Cost/dozen eggs ($)	Livability (%)
1: normal	157[ab]	177[ab]	1.22	77%
2: normal/withdraw	148[b]	166[b]	1.29	83%
3: slow + 32wk	169[a]	182[a]	1.21	80%
4: normal +75%	158[ab]	175[ab]	1.27	79%
5: normal + 35wk	171[a]	181[a]	1.22	81%
6: normal + 40wk	161[ab]	175[ab]	1.25	81%
SEM	2.479	1.746	0.018	2.041
P-value	0.0398	0.0169	0.7113	0.7424

Table 5: Percent hatch of fertile (HOF) of each feeding program at different ages of hens

Treatment	Week of age (%)					
	30	35	45	55	65	Average
1 - normal	91	92	89	87	82	88
2 - normal/withdraw	86	90	86	87	85	87
3 - slow + 32 wks	93	88	81	82	85	86
4 - normal + 75%	89	87	79	81	87	85
5 - normal + 35 wks	88	92	89	84	75	86
6 - normal + 40 wks	91	91	85	88	79	87
P-value	0.225	0.464	0.801	0.729	0.468	

Table 6: Egg and chick weights of hens from each feeding programs at different ages

Age (wk)	30		35		45		55		65	
Feeding program	EW	Chick BW	EW	Chick BW	EW	Chick BW	EW	Chick BW	EW	Chick BW
1: normal	57.5	41.2	62.18	44.0	67.37	47.9	70.28	51.0	72.08	51.7
2: normal/withdraw	57.8	41.6	61.59	43.5	65.36	47.0	68.45	49.1	70.71	50.2
3: slow + 32wk	58.43	41.2	62.19	44.3	67.75	48.2	70.7	51.2	72.81	52.3
4: normal +75%	58.15	41.4	62.38	43.6	66.84	48.4	70.68	51.4	73.12	52.2
5: normal + 35wk	58.31	41.2	62.62	43.9	67.85	47.7	71.76	51.4	73.54	52.9
6: normal + 40wk	58.47	41.3	62.15	44.0	68.08	49.1	71.82	51.0	74.25	53.3
SEM	0.206	0.086	0.207	0.082	0.257	0.084	0.318	0.085	0.375	0.362
P-value	0.713	0.818	0.596	0.169	0.005	0.003	<.0001	<.0001	0.007	0.003

Table 7: Growth performance of progeny from hens in each feeding program

Treatment	YFBW* at hatch (g)	BW 7d (g)	ADG 7d (g)	FCR 7d
1:normal	45.22[a]	189.80[a]	18.65[a]	1.14
2:normal/withdraw	41.76[b]	169.20[b]	15.96[b]	1.19
3:slow+32wk	44.59[a]	188.20[a]	18.73[a]	1.00
4:normal+75%	45.26[a]	187.20[a]	18.70[a]	1.14
5:normal+35wk	44.79[a]	187.80[a]	19.06[a]	1.00
6:normal+40wk	44.68[a]	186.60[a]	18.74[a]	1.11
SEM	0.269	2.145	0.312	0.025
P-value	0.001	0.037	0.026	0.211

Figure 1: Feeding programs from 20 to 42wk of age

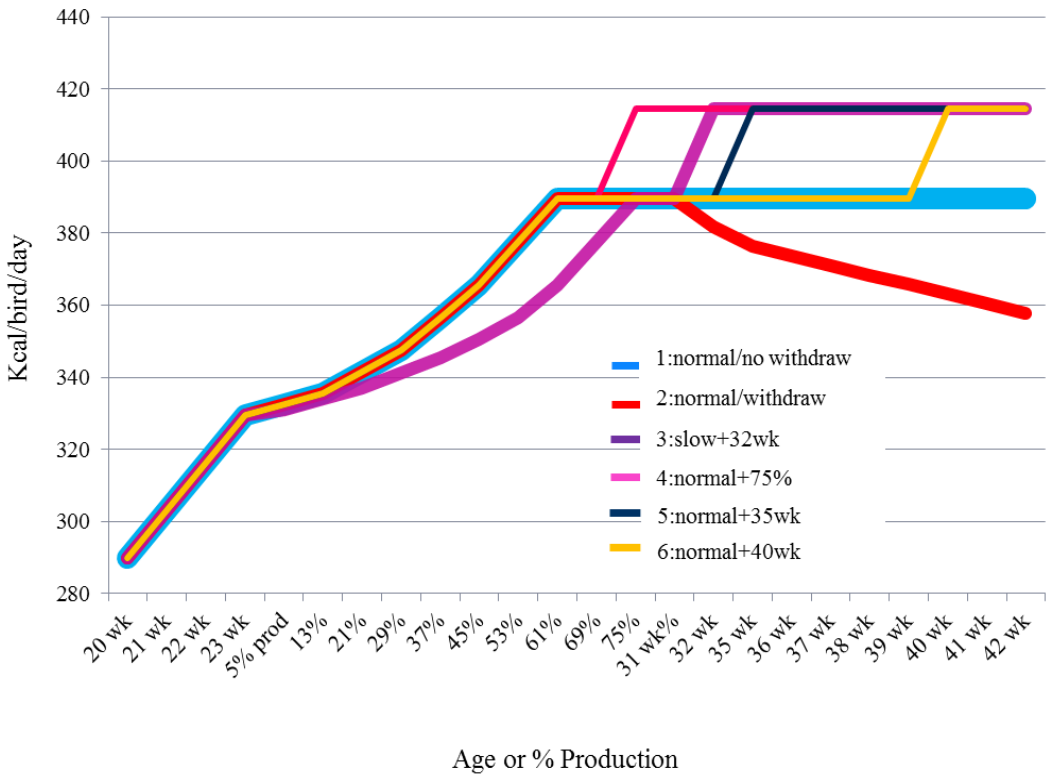


Figure 2: Egg production of each feeding programs as compared to Cobb's standard

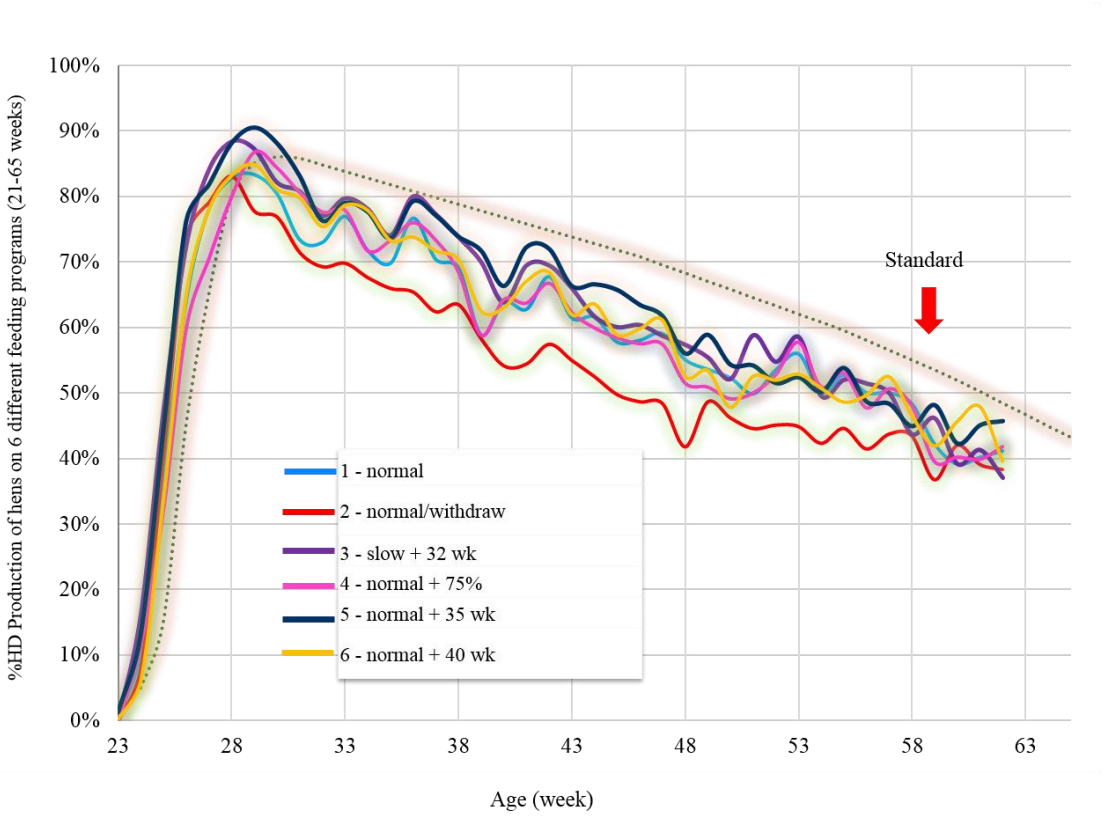
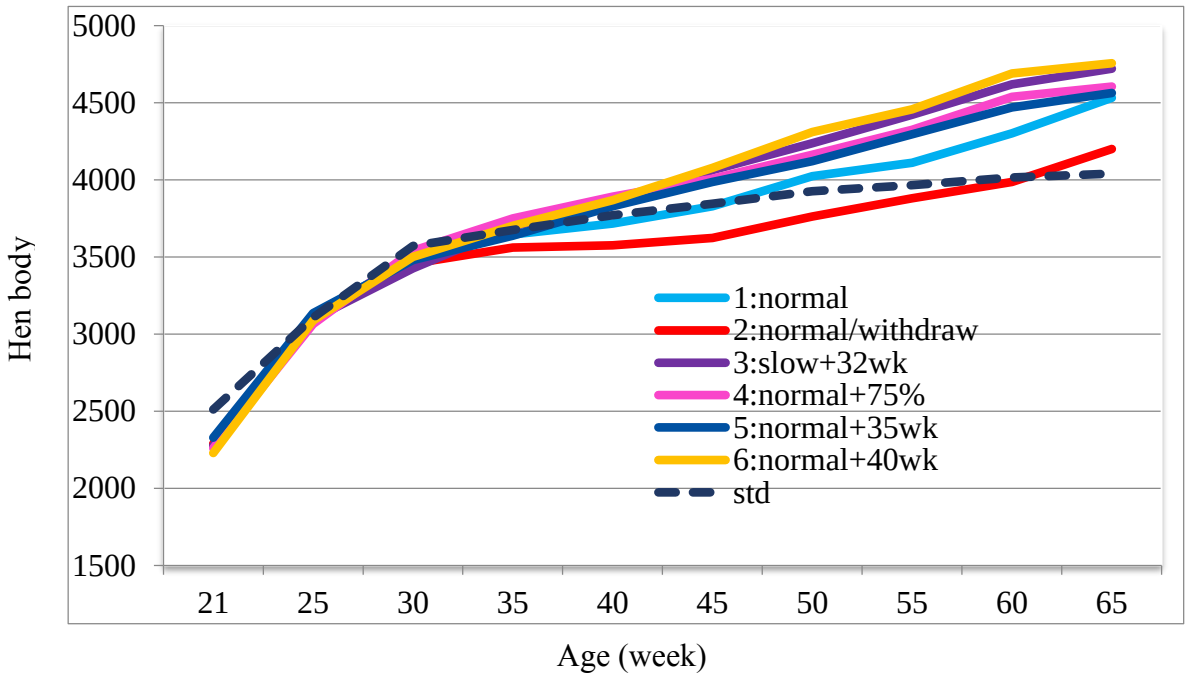


Figure 3: Hen body weights at different ages as compared to Cobb's standard



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CONCLUSION

Effects of age and feeding program on the metabolism of lipid in breeder hens and progeny have been studied in many aspects. Eggs from young breeder hens contain less nutritional values than those eggs from old breeder hens, which might result in the lower growth rate of progeny. The studies of *de novo* lipogenesis (DNL) illustrate that young hens have higher rate of DNL than old hens. At the beginning of lay, all non-lipid substrates are used for DNL including glucose and both ketogenic and glucogenic amino acids. The progeny from the young breeder hens have less ability to deposit the products of DNL into the body fat tissue as compared to those progeny from old breeder hens. At hatch, yolk-derived lipids are the main source of lipids in adipose tissue of the baby chicks. Then, the yolk lipids are used for energy purpose and gastrointestinal growth after hatch, and lipids from feed and DNL play a key role in the deposition of adipose tissue lipids. Palmitic acid supplementation increases the levels of monounsaturated fatty acids (MUFA) especially oleic acid, and has positive effect on hatchability at early production period. The slow feed increase during photostimulation to peak production with feed adding at 32wk and the normal feed increase with feed adding at 35wk of age enhance egg production and economic traits. Feed withdrawal after peak production impairs egg production and persistency of lay.

APPENDIX

UNIVERSITY OF ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Craig N. Coon

FROM: Carol Rodlun, Program Manager
Institutional Animal Care
And Use Committee

DATE: February 15, 2013

SUBJECT: IACUC Modification Request **APPROVAL**
Expiration date: **July 31, 2015**

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** the modification request (to add the use of metabolism chambers) to Protocol **#13002- "Evaluation of broiler breeder feeding regimes for pure-line and commercial type stock during rearing and production phases and calcium requirement during the production period."** You may implement this modification immediately.

In granting its approval, the IACUC has approved only the modification request provided. Should there be any additional changes to the protocol during the research, please notify the IACUC in writing [via the Modification Request Form] **prior** to initiating the changes.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

cc: Animal Welfare Veterinarian

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