EFFECT OF AGING ON WHOLE BODY COMPOSITION, PROTEIN SYNTHESIS AND DEGRADATION RATE OF BREAST AND LEG MUSCLES IN MEAT- AND EGG- TYPE CHICKENS

By

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ABSTRACT

EFFECT OF AGING ON WHOLE BODY COMPOSITION,
PROTEIN SYNTHESIS AND DEGRADATION RATE
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A technique was developed to measure the rate of protein synthesis in muscles of chickens. This technique was then used to estimate protein degradation.

The composition of whole body, breast and leg muscles were studied in egg- and meat- type chickens, 10 to 45 days of age. The weight of abdominal fat and total body fat increased more rapidly than whole body weight of these chickens, 5 to 6 weeks of age.

The protein turnover study was conducted when the chickens were 4-6 weeks of age. At that time the nutrient energy appeared to have shifted toward production of fat rather than protein. The changes in protein over a measured period of time in relationship to average amount of total protein (fractional accretion rate or FAR, as %/day) were 7.7, 6.3 and 4.4 for meat-type chickens at 4, 5, and 6 weeks of age, respectively. The FARs for egg-type chickens of the same ages were 6.1, 5.4 and 3.4 %/day, respectively. The FARs of protein in breast or leg muscles of both egg- and meat-type chickens, generally declined as the animals aged from 4 to 6 weeks of age.

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Age had no effect on the fractional synthesis rates (FSR, %/day) in muscles of the leg or breast of meat-or egg-type chickens between 4 to 6 weeks of age. However, the FSRs of meat-type chickens were much higher than those of egg-type chickens in both leg (33.0 vs 17.0) and breast muscles (57.0 vs 28.0), respectively.

In egg-type chickens, overfeeding for 4 days increased the FAR of protein in breast muscles, but showed no change in leg muscles. Overfeeding appeared to produce a decline in FSR of leg muscles, and seemed to enhance the rate of protein synthesis in breast muscles.

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CHAPTER I

Introduction

The efficient production of meat is the principal of meat animal production. This improvement in feed efficiency for poultry and other animals has come about through selection for gain, proper nutrition, management and health programs. However, this improvement in gain may be fat rather than lean tissue. Therefore, carcass composition of animals has become an important consideration in this decade, because of the quantity of animal fat consumed by humans.

Considerable research has been conducted to explain the flow of nutrients toward fat and protein deposition in an animal. Endocrine systems are the most likely candidates to regulate the flow of nutrients and determine body composition (Bergen 1974; Bauman et al., 1982; Ronald and Stephen 1982). Protein production in a cell consists of protein synthesis and degradation (protein turnover). Protein accretion is the result of the difference between the rate of synthesis and the rate of degradation (Waterlow and Stephen 1968; Millward 1970; Maruyama et al., 1978; and Millward et al., 1981). The effect of endocrine or other factors on protein turnover could be examined if a measurement of protein synthesis and breakdown, in vivo, can be accomplished.

The primary effort of this research was to provide a technique to measure the rate of protein synthesis and degradation in muscles of

chicken. Almost all studies on carcass composition indicated that, age had a significant effect on water, fat and protein composition (Kubena et al., 1972; Bergen 1974; Edwards 1981). This study was conducted to describe the aging effect on protein turnover of breast muscles (pectoralis superficial + pectoralis subclavis), leg muscle (gastrocnemius + peroneous longus) and body composition of fast and slow growing chickens. The effect of over-feeding on the turnover of these two muscle types was also studied.

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CHAPTER II

Literature Review

The Composition of Growth

The composition of growth is an important criterion in evaluating efforts to enhance the growth of an animal for meat production. During growth, there is not only skeletal growth and protein accretion, but also fat accretion (Kubena et al., 1972; Bergen 1974). Searle and Mc Graham (1972) showed that, in an animal beyond a certain body weight, fat becomes a large and constant fraction of weight gain. Bergen (1974) concluded that, the process of protein accretion and fat accretion occurs concomitantly during early growth; whereas in later growth the rate of protein accretion becomes negligible. In addition, animals which are laying down a greater proportion of fat are likely to be less efficient in feed utilization (Kubena et al., 1972).

Factors Affecting the Composition of Growth

Fattening in birds is influenced by genetics (Fowler 1958; Biondini et al., 1968; Mc Carthy 1974; Griffiths et al., 1977; Stewart and Muir 1982), environment (Kleiber and Dougherty 1934; Winchester and Kleiber 1938; Kubena et al., 1972), age (Lepore and Marks 1971; Kubena et al., 1972; Kubena et al., 1974) and nutrition (Summers et al., 1965; Edwards and Hart 1971; Bartov et al., 1974; Griffiths et al., 1977). In a literature review by Demby and Cunningham (1980), the water

content of chicken meat varied from 63% to 75%, the protein content from 17% to 23.5% and fat content from 1.0% to 17.4%. It is often recognized that female broilers contain more fat than male broilers (Edwards and Denman 1975; Twining et al., 1978). Goodwin et al., (1969) analyzed broilers from different strains and found variations in both protein and fat content. The amount of protein varied from 65% to 69% and fat from 28% to 32.5% in dry weight of meat, that contained from 70% to 72.5% water. Tzeng and Becker (1981) showed that the percent of abdominal fat of carcass increased with age up to about 70 days in male broilers. At that age it comprised about 4% of carcass weight. Furthermore, it has also been found that changing the calorie/protein ratio of a diet greatly influences the fat content in an animal (Summers et al., 1965; Goodwin et al., 1969). Thus chemical composition of a chicken can be influenced by a number of factors.

Non Nutritional Factors

Genetic

Improvement in feed efficiency in poultry and other animals has come about as a correlated response to selection for gain (Fenton and Dowling, 1953; Mickelsen et al., 1955; Stewart and Muir, 1982). However, the increased gain may be fat rather than lean tissue. Studies with rats (Mickelsen et al., 1955) and with mice (Fenton and Dowling, 1953; Fenton and Marsh, 1956) have indicated strain differences in their body composition, particularly the amount of fat in the carcass. Rapid growing mice have shown a higher contribution of fat to body weight gain as compared to strains of slow growing mice (Fowler,

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1958; McCarthy 1974; McPhee and Neill, 1976). Biondini et al., (1968) demonstrated that mice selected for rapid growth, produced 73% more carcass fat than controls, while protein declined 15% as rate of gain increased. Robinson and Bradford (1969) also found that rapid weight gain of mice was associated with large increases in the percent of fat. Mitchell et al., (1926; 1931) noted that White Plymouth Rock cockerels contained a greater percent of fat in their carcasses at every weight than White Leghorn cockerels. Hunt (1965), reported that the body nitrogen: H_2O ratio which is an index of body composition was different between two strains of chickens studied. Furthermore, Campion et al., (1982) studied the whole body composition in 3 lines of Japanese quail, and found that at 56 days of age the light weight line (control) contained 16.5% protein and 9.3% fat, while heavy weight lines produced 20.6% protein and 13.0% fat.

Environment

The temperature extremes that fowl can tolerate depend on complex relationships, many of which are not well understood. Several investigators have demonstrated that, the environmental temperature influences the body composition of chicks. Kleiber and Doupherty (1934), Olsen et al., (1972), and Swain and Farrel (1975) reported an increase in the fat content of a chick's carcass and a decrease in moisture content, as the rearing temperature of the chicks was increased. However , Adams et al., (1962) and Mickelberry et al., (1966) found no significant differences in fat or moisture content of carcasses of chicks reared at temperatures of 21.1^0 or $29.^0$ C.

However, these investigators observed a trend toward higher ether extract content in the birds reared at 29.4^{0} C. Similarly, Kubena et al., (1972) showed the same trend in chickens reared at 18.3^{0} C versus 29.4^{0} C, with no differences in carcass protein content. Swain and Farrel (1975) also showed that the carcass protein did not change with increasing rearing temperatures of chicks. A trend toward carcasses with higher fat and protein, and lower moisture in broilers up to 6 weeks of age reared at 32^{0}C as compared to 22^{0}C was observed by (Husseiny and Creger, 1980). Farrel and Swain (1977) showed that chickens reared at a low temperature had lower nitrogen retention as a percent of body weight than those at high temperature. Cold environmental conditions decreased the carcass fat deposition in ducklings (Scott et al., 1959).

Size and Age

Almost all investigators agree that, in the growing animal, the protein and fat content (%) of the carcass increases and the moisture content (%) decreases as the animal ages (Kubena et al. 1972; Bergen 1974). However, in a mature animal (post growth) protein accretion ceases whereas fat accretion continues (Zucker and Zucker, 1963, Bailey and Zobrisky, 1968; and Searle and McC Graham 1972). These investigators also reported that, fat accretion starts early and as the animal reaches maturity its fat content exceeds protein content. Kubena et al. (1974) noted that the broiler increased its absolute quantity of abdominal fat with increasing age up to 9 weeks. Similarly, the amount of fat in carcass increased as the bird increased in size (Edwards et

al., 1973). Tzeng and Becker (1981) observed that, the growth of total deposited fat and lean meat had an accelerating phase from hatch to 5 weeks in male broilers. The total deposited fat had a steeper linear growth from 5 to 9 weeks. The total lean meat had a straight linear growth from 5 to 10 weeks of age. Lepore and Marks (1971) studied the age effect on carcass water, fat and protein composition of quail. As the birds aged from 2 to 8 weeks, moisture content declined, fat content increased. The % of carcass protein increased to 4 weeks and then decreased to 8 weeks. Edwards (1981) showed also an increase in fat content of carcass with increasing age in quail up to 7 weeks of age. More recently Campion et al. (1982) observed a decrease in protein and an increase in fat as a percent of body weight in light weight quail from 28 to 56 days of age.

Nutritional Factors

Calorie: Protein ratio

Mendel (1923) was the first to report that an animal first eats to satisfy its energy needs. Fraps (1943) reported that, the body composition could be altered through manipulation of dietary protein and energy. Since these initial studies, the relationship between carcass fat and moisture has been clearly established (Marion and Woodroof 1966; Velu and Baker 1974; Twining et al., 1978). Fattening also occurs when the amount of energy consumed by the birds is above its requirement for growth and maintenance (Bartov 1979). Seaton et al., (1978) observed an increase in carcass fat and a decrease in carcass moisture with an increase in dietary energy level, while

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carcass protein was uneffected. This is in contrast to the reports of Summers et al., (1965) and Velu and Baker (1974) which indicated that the body protein (%) decreased with an increase in dietary energy level. Decreasing dietary energy level but maintaining the same protein concentration reduced carcass fat content (Hill and Dansky, 1954; Bartov et al., 1974). Griffiths et al. (1977) reported that, the amount of abdominal fat in male broilers exposed to early caloric restriction was reduced when the calorie:protein (cal:p) ratio of the diet was lowered. High dietary energy level, per se, was not the major responsibility for the excess energy consumption, as long as the protein level in the diet was balanced to produce the optimum ratio of calories to protein, or to essential amino acids (Bartov et al., 1974; Scott et al., 1976). The extra energy consumed by the birds fed a cal:p ratio above optimum increased fat deposition (Donaldson et al., 1956; Combs, 1964; Summers et al., 1965; Velu et al., 1971; Bartov and Bornstein, 1976a, 1976b). Yeh and Leveille (1969) observed a reduction in the rate of hepatic lipogenesis in chicks fed a diet containing a higher protein concentration. An increase in carcass protein and a decrease in carcass fat was reported in chicks fed diets with increasing levels of protein (Summers et al., 1965). On the other hand, Bixler et al. (1969) have shown that body fat of poultry increased when fed low protein diets. Furthermore, reducing the cal:p ratio by addition of either low or high quality protein to the diet effectively reduced the weight of abdominal fat (Griffiths et al., 1977).

Amino Acids

Bartov (1979) stated that the effect of single amino acid deficiency on voluntary food intake and body composition is different from that of a low protein diet, although both result in retarded growth. Chicks fed a diet with a normal protein concentration but severely deficient in a single essential amino acid had a decrease in feed consumption and near cessation of growth (Edwards et al., 1956; Maruyama et al., 1975; Maruyama et al., 1978). Arginine is an indispensible amino acid for growth as well as maintenance of nitrogen balance in the adult rooster (Fisher et al., 1958). Unlike the rat, neither proline nor glutamic acid singly or in combination exert any sparing effect on the arginine needs of the chickens. Seaton et al., (1978) showed that the carcass fat decreased and moisture increased as the percent of protein or lysine in the diet increased. Almquist (1954), explained that, when a diet deficient in amino acids was fed, other amino acids accumulate in the blood and impair appetite. This theory has been proved in chickens for tryptophan, lysine and methionine (Fisher and Shapiro 1961) and for leucine, isoleucine, valine, arginine and lysine (Hill and Olsen, 1963). Khalil et al., (1968) reported that, the excessive addition of methionine to the diet fed to the animal depressed food consumption and growth rate and lowered carcass fat content. Furthermore, the diet with an excessive addition of tyrosine decreased feed intake and weight gain (Harper et al., 1970; Ip and Harper 1973) and had no effect on body composition of Single Comb White Leghorn SCWL chicks (Yanaka and Okumura, 1982).

Dietary Fat

Studies on the effect of dietary fat on growth and carcass composition of chickens are conflicting. Fat incorporated into isocaloric diets had no effect on carcass composition or abdominal fat pad weight (Baldini et al., 1957; Edwards and Hart, 1971; Fuller and Rendon, 1977). However, increasing dietary fat level by substitution for carbohydrate on a weight basis increased carcass fat content (Donaldson et al., 1956). The body fat increased and body protein decreased (%) with an increase in caloric density of a diet fed to one-week old chicks (Velu and Baker 1974). Griffiths et al., (1977) demonstrated that the isocaloric diet formulated at 0, 3, 6 and 9% fat levels fed to broiler chicks had no effect on carcass composition or abdominal fat pad weight. Contrastingly, Rand et al., (1957) and Carew et al., (1964) observed that tissue energy gain per unit of metabolizable energy (ME) intake increased as the level of corn oil increased in the isocaloric diet. They viewed that, fat enhanced the utilization of ME.

Diet containing added fat reduced hepatic lipogenesis in chicks (Leveille et al., 1975; Shapira et al., 1978). They pointed out that the decrease in hepatic lipogenesis is at least partly due to the decrease in dietary carbohydrate. The reduced lipogenesis does not necessarily affect fat content. Dietary fat itself can be a direct source of body fatty acids.

Starvation and Overfeeding

Leveille et al. (1975) determined that fasting the bird for a short period of time depressed lipogenesis; whereas refeeding following the hunger increased it. Restricting the energy intake of chicks in the 0-3 week growth period was shown to have no effect on abdominal fat pad weight when measured at 8 weeks of age (Griffiths et al., 1977). However, restricting food intake from 6-8 weeks reduced carcass fat content and body weight at eight weeks of age.

Overfeeding due to force-feeding increases the rate of hepatic lipogenesis and the fat content of both liver and carcass (Shapira et al., 1978). These investigators also reported that administering an isocaloric quantity of glucose or oil in over-fed chicks results in similar increases in weight and lipid content of carcass and adipose tissue, but liver weight and fat content are increased more by the glucose supplementation than by corn oil. Polin and Chee (unpublished data) noted an absolute increase in protein content of chickens, 2 weeks of age, which were over-fed (force-fed) for a 4 day period. Carcass fat also increased somewhat. Glick et al., (1982) observed a higher protein gain in liver and gastrocnemius muscle of over-fed rats compared to control But, they did not determine the fat content of individual tissue or whole body.

Exogenous Biochemicals

Drugs have been used to improve the feed efficiency and promote weight gain of birds. The subcutaneous implants of diethylstilbestrol (DES) pellets were used to fatten cockerels (Lorenz 1943). Lorenz

(1954) stated that the estrogen increased hepatic lipogenesis and caused lipemia and body fat deposition. Wesley et al., (1965) injected 6-week old chickens with 10 mg of estradiol-17 -monopalmitate (EMP), and noted a highly significant strain, response in yield of carcass. Except, for one strain, the feed conversion was improved by the EMP treatment. Broilers treated for 7 weeks with EMP showed improved feed efficiency and weight gain as well as an increase in fat and a decrease in moisture content of light and dark muscle by EMP treatment (York & Mitchell 1969). Mickelberry (1968) showed that, the administration of EMP resulted in a significant increase in meat on breast and a decrease in breast bone as a percentage of a rooster's body weight. The use of EMP also produced marked anabolic and lipogenic effects in meat-producing animals (Mattox and Junkman, 1966). More recently, 19-oxo-androstenedione was used to enhance growth and fat deposition in poultry (Johnston et al., 1980).

May (1980) demonstrated that, broilers fed diet containing 1 ppm T_3(3,,5,3'-triiodothyronine) had poorer weight gain and feed efficiency than controls. Carcass moisture increased and carcass fat decreased and carcass protein remained uneffected in birds given the T_3 treatment. Including 1 ppm T_4 (Thyroxine) in the diet, had no effect on moisture and protein content of broilers. May (1980) concluded that T_3 treatment produced a greater effect on body composition than does T_4.

Bergen (1974) stated that the main problem in any effort to increase protein deposition in animals is to describe the mechanism whereby energy is directed to fat synthesis rather than protein

synthesis.

Muscle Protein Synthesis and Regulation of Protein Turnover

Labelled isotopes of natural compounds was first used to study protein metabolism in an experiment by Sprinso and Rittenberg (1949). They injected a single dose of ^{15}N-glycine and calculated the turnover rate of the metabolic pool from cumulative abundance of ^{15}N in the urine over a period of 30 hours. This work along with the earlier work of Borsook and Jeffreys (1935) and Shoenheimer et al. (1939) showed that there was a very rapid protein turnover in the whole body. The proteins of sketetal muscle including those of myofibril exhibited considerable turnover (Waterlow & Stephen, 1967).

Turnover as defined by Waterlow et al. (1978) is a process of replacement of a particular substance. Zak et al. (1979) described the protein turnover as the continuous processes of protein breakdown and synthesis. Waterlow & Stephen (1968), showed that the rate of protein turnover in muscle is somehow lower than in other tissues. They also pointed out that, the rates of protein turnover may vary between different muscle types. Protein turnover has been shown to be more rapid in cardiac muscle than in skeletal muscle (Kimata and Morkin 1971; Garlick et al., 1973, 1975). Millward et al. (1978) reported that there was a higher rate of protein synthesis in red muscles (soleus and diaphragm) compared with predominantly white glycolytic muscles (plantaris, gastrocnemius) in the adult rat. Millward et al. (1976a) stated that the rates of protein synthesis and protein breakdown are equal in the steady state situation (no growth). In the

growth state, the rate of protein turnover can be calculated by subtracting the net change of protein mass or accretion from the protein synthesis rate (Millward, et al., 1976a).

The turnover of protein was thought to be a wasteful process (Millward et al., 1975; Young and Pluskal, 1977; Laurent and Millward, 1980). Net protein accretion during rapid growth was only a small portion of the total amount of muscle protein synthesized (Millward et al., 1975; Millward, 1980). Sola et al. (1973) indicated that new fibres being formed during muscle growth, de novo, may not reach maturity. Some were likely to have been "wasted" with degradation of their contents including nuclei. This contributed to the increased protein breakdown. Fibre proliferation also occurred as a result of fiber splitting, which appeared to be a common feature of growth (Reitsma, 1970; Edgerton, 1970). The split fibers associated with newly formed lysosomes caused the mitochondrial degeneration during rapid muscle growth (Morton & Rowe, 1974). This is also consistant with the increased breakdown of protein. Laurent et al. (1978a) reported that the anterior Latissimus dosi muscle has a higher rate of protein degradation than the posterior muscle in the growing fowl. Earlier work of Stauber et al. (1977) showed that the activities of cathespin D and other lysosomal enzymes were higher in the anterior than posterior muscle. This raises the possiblity that the relative turnover rates in the two muscles reflects the relative concentration of the proteolytic system which degrades muscle proteins.

Dreyfus et al. (1960) and Morkin (1970) stated that the myofibrilar proteins have a finite lifespan. Other studies suggested a

random turnover of myofibrilar proteins (Goldberg, 1969a; Millward et al., 1976b).

Factors Affecting Protein Turnover

Tissue growth is a complex process in which the size and number of functional units of the tissue increase with often morphological or biochemical changes (Goss, 1964). In other words, growth is the net result of the rate of protein synthesis exceeding that of protein degradation (Waterlow and Stephen, 1968; Millward, 1970; Morgan, 1974; Millward et al., 1975; Laurent et al., 1978b; and Millward et al., 1981). Regulation of the growth of muscle proteins could be exerted through changes in either protein synthesis or breakdown (Waterlow & Stephen, 1968). In some cases muscle growth is associated with an elevated rate of protein synthesis (Millward et al., 1981). During the rapid growth of very immature animals degradation is more rapid than in adults yet less than synthesis (Millward, 1980). There are numerous studies on the regulation and physiology of protein turnover. But the mechanism of body protein regulation at the molecular level remains obscure. Nevertheless, the information on the mechanism of protein turnover will be discussed in here.

Hormones

The growth process causes changes in the flow of nutrients to different tissues according to the availability of nutrients, and according to the genetic code (Bauman et al., 1982; Ronald & Stephen 1982). Ronald & Stephen (1982) proposed that a higher order of

endocrine regulation may be provided by homeostatic mechanisms that direct the flow of nutrients to support the physiological or developmental process of highest prevaling priority. Bergen (1974) suggested that the hormonal systems are likely candidates for the mechanism that regulates the diversion of calories to fat away from protein synthesis or vice versa. Waterlow & Stephen (1968) stated that endocrine hormones probably caused changes in protein synthesis in rats during starvation or the feeding of a protein depleted diet. The hormonal effects on nitrogen metabolism could be separated into 2 classes: those that are anabolic, for example, growth hormone (Evans and Simpson 1931; Bergen 1974; Goldberg et al., 1980; Bauman et al., 1982), insulin (Munro 1964; Rannels et al., 1975; Ronald and Stephen, 1982) testosterone (Kochakian, 1966; Buresova and Gutman, 1971; Grigsby et al., 1976) and those that are catabolic, for example glucocorticoidal steroids (Alleyne and Young, 1966, 1967; Goldberg and Goodman, 1969; and Goldberg et al., 1980) and thyroxine (Goldberg et al., 1980).

The somatrophic effects of growth hormone (GH) have been known since the classical work of Evans and Simpson (1931) who demonstrated in rats an increased body weight gain after chronic administratisn of bovine GH. Machlin (1972) reported that when pigs received a daily injection of porcine GH for an 8-week period, daily gain increased 16% and a marked improvement in feed efficiency occurred. The growth hormone treatment resulted in an increased protein accretion and a decreased lipid deposition. Goldberg et al. (1980) reported that muscle isolated from hypophysectomized rats had lower rates of protein synthesis and breakdown than their normal controls. They suggested

that lack of GH is primarily responsible for the lower rate of protein synthesis, although GH did not effect overall protein catabolism.

Insulin was shown to have pronounced effects on carbohydrate and protein metabolism (Munro, 1964; Ronald and Stephen, 1982). Insulin may promote lipid deposition by increasing both adipocyte membrane permeability to glucose and a subsequent metabolism of glucose to α-glycerophosphates thereby stimulating fatty acid estrification (Ronald and Stephen, 1982). Insulin was also observed to have immediate effects on muscle protein synthesis by increasing amino acid incorporation into a large number of different proteins (Manchester, 1970; Goldberg et al., 1980). Studies with animals have demonstrated a wasting of skeletal muscle to be a prominent feature of diabetes mellitus which is reversible by the administration of insulin in vivo (Rannels et al., 1975; Waterlow et al., 1978). Waterlow et al., 1978 also stated that the principle acute effect of insulin is to modulate the rate of muscle protein synthesis by increasing the translational efficiency of ribosomes. There is a long term effect which involves increasing the capacity for protein synthesis by increasing the concentration of ribosomes (Jefferson, 1980).

Other investigators showed that insulin stimulated protein synthesis and inhibited protein breakdown in the isolated muscle and cultured cell (Goldberg & John, 1976; Ballard et al., 1980). Studies in vitro indicated that the level of insulin is probably the most important factor regulating protein balance in skeletal muscle (Rannels et al., 1975; Goldberg et al., 1980; Hugden and Smith 1982; Ballard and

Francis, 1983). Rannel et al., (1975) reported that insulin inhibited protein degradation in the perfused rat heart. However, the concentration requirement for insulin was high (200 μ units/ml of perfusate) before significant inhibition was observed. Later a report by Hugden and Smith (1982) revealed that, in the presence of glucose, insulin significantly inhibited protein degradation in the perfused rat heart at concentrations as low as 50 μ units/ml. They concluded that, the inhibition of protein degradation occurs over the normal range of plasma concentrations of insulin in vivo and that the presence of glucose may be at least in part necessary for this effect of insulin.

Kochakian (1966) demonstrated a marked depression in growth and lack of muscle development, when male rodents were castrated, but, upon exogenous administration of testosterone normal growth was obtained (Kochakian et al., 1964; Kochakian, 1966). Testosterone administration also increased the incorporation of radioactive amino acids into muscle protein of castrated guinea pigs (Kochakian et al., 1964) and intact male rabbits (Grigsby et al., 1976). Kochakian (1966) reported that various muscles showed a different response to androgen. Target muscles or muscles with receptors for androgen such as levator ani muscle seem to be more responsive to testosterone than non-target muscles such as diaphram (Buresova and Gutman, 1971).

Treatment of animals with thyroxine produces a catabolic effect (Demartino and Goldberg, 1978; Flaim et al., 1978). Some investigators have obtained evidence that the enhancement of protein degradation by thyroid hormones is associated with an increase in the content of the various proteases (Demartino & Goldgerg, 1978). They also reported

that, the treatment of a hypothyroid animal with T_3 (triiodothyronine) or T_4 (tetraiodothyronine) caused a 2 to 3 fold increase in the activity of cathepsin B and D. Activities of these proteasis are suggested to be controlled partially by thyroid hormone. Thyroidectomy decreased the level of these enzymes in skeletal muscle by approximately 50% of controls.

High circulating concentrations of glucocorticoid steroids are shown to have a catabolic effect on the body, inhibiting growth in children and young animals (Waterlow et al., 1978). Young (1970), Millward et al., (1976a, 1976b), Rannels et al., (1978), and Odedra & Millward (1982) stated that the suppression of protein synthesis in rats is the main effect of glucocorticoids in muscle. However, McGrath and Goldspink (1982) demonstrated that, the glucocorticoid hormones decreased the rate of both protein synthesis and protein degradation in perfused soleus muscles. Generally, good agreement exists from a variety of experiments, both in vitro and in vivo, in ascribing to steroids an inhibitory action of muscle protein synthesis (Wool & Weinshelbaum, 1959; Goldberg, 1969b; Shoji and Pennington, 1977; Thomas et al., 1979).

Anabolic steroids were reported to increase growth rates, especially for female and castrated male animals, and also to improve the efficiency by which nutrients are converted into muscle protein (Vernon and Buttery, 1978; Heitzman, 1979). In vitro studied showed that, the anabolic agents such as trenbolone, dietylstilboesterol and testosterone did not alter rates of intracellular protein breakdown and did not interfere with the glucocorticoid-induced catabolic response

(Ballard and Francis 1983). Wangsness et al., (1981) proposed that anabolic steroids, via a secondary hormone change, modify the response of endogenous hormone(s) and thus change growth rate. These researchers reported that after implantation of trenbolone acetate or zerdnol plasma insulin and somatomedins increased and thyroxine concentration decreased. Furthermore, Vernon and Buttery (1978) showed that both muscle protein synthesis and breakdown are decreased after injection of trenbolone acetate into female rats, but the accumulation of carcass nitrogen was greater than control animals. Thus the rate of protein degradation was depressed more than the rate of protein synthesis.

Age and Species

Young animals, pigs (Reeds et al., 1980) lambs (Solts et al., 1973), chicken (Maruyama et al., 1978) and rats (Waterlow and Stephen 1968), synthesize protein at a higher rate per unit of metabolic body weight (W .75) than older animals. The body size of an animal affects the total protein metabolism of the body (Munro, 1969). Garlick et al., (1976) studied the rate of protein synthesis in large and small animals. The fractional rate of protein synthesis was 2.5 times faster in the rat leg muscle than that in the comparable muscle of the pig. Millward et al., (1975) showed that the fast growth in the young rat was the result of a rapid rate of muscle protein synthesis as well as a rapid rate of breakdown, but the former outpacing the latter. The

fractional synthesis rates of 29, 11 and 5 %/day were observed at 23, 65 and 130 days of age, respectively. The protein breakdown in the brain during development was 2-2.5 times greater in rat 2 days of age than in one at 30 days of age (Dunlop et al., 1978). The breakdown of brain protein was approximately 65% of the synthesis rate during the more active phase of growth. Mulvany (1981) reported a decrease in the rate of protein synthesis and breakdown (%/day) in longissimus, semi-tendinosis and brachialis muscles in pigs of 45 kg as compared to those from pigs of 22 kg body weight. Millward ad Waterlow (1978) pointed out that, the rate of protein breakdown was faster in slow growing strains than in fast growing strains. For slow growing rats the protein synthesis and breakdown rates were 28 and 23 %/day, respectively, at 20 days of age and only 5 and 5 %/day at 350 days of age. However, in the fast growing rats the rate of protein synthesis and breakdown were 16 and 9 %/day, respectively at 20 days and only 4 and 4 %/day at 300 days of age.

Maruyama et al., (1978) measured the rate of protein synthesis and degradation in leg muscle, breast muscle and whole body in the chickens by infusion of ^{14}C-tyrosine. They observed a 38 %/day fractional synthesis rate (FSR) in breast, and a 24 %/day rate in leg muscle of chicks one week of age. But by 2 weeks of age, the FSR in breast muscle was much less and almost the same as that of leg muscle (22 VS 24%). They also noted that, the rate of protein synthesis and breakdown in the leg muscle of slow and fast growing birds were similar at 2 weeks of age.

Substrate Supply (Amino Acids)

Rate of growth and development of chicks (Jeppeson and Grau, 1948; Bragg et al., 1971; Bragg and Akinwande, 1973) and rats (Grau and Kamei, 1950; Middleton, 1959) were markedly impaired when wheat protein or wheat gluten was fed as the sole source of dietary protein. When they supplemented the wheat protein or wheat gluten with an optimal quantity of lysine, the growth was equal to that of wheat-soy or a casein diet containing equal dietary protein. Thus the supply of amino acids does affect the mechaism of protein biosynthesis. Yokogoshi et al., (1977) supplemented a protein-free diet with methionine and threonine, and found that the catabolism of proteins in liver and muscle was slightly reduced as compared with that of rats fed the protein-free diet. Munro (1968) reported that during a short term, study the supply of amino acids will control the aggregation of liver polysomes and protein synthesis even in the absence of nuclear RNA synthesis. Therefore, he concluded that the supply of amino acids regulated protein synthesis by a cytoplasmic mechanism. However, in long term, the amino acids regulated liver protein synthesis by affecting the number of ribosomes that actively synthesize proteins and by influencing the rate of synthesis of new ribosomes (Wannemcher et al., 1968, Wannemcher et al., 1971). When the dietary amino acid supply is restricted to a growing rat, muscle protein synthesis declined (Howarth, 1972). Waterlow & Stephen (1968); Garlick et al., (1975) reported a marked decrease in protein synthesis in rat muscle in response to starvation or protein-deprivation. Reduced liver protein

synthesis has occurred in response to starvation (Henshaw et al., 1971; McNurlan et al., 1979) and protein deprivation (Conde & Scornik, 1976; McNurlan & Garlick, 1981). In contrast, other studies have suggested that liver protein synthesis was maintained under starvation or protein-deprivation (Waterlow & Stephen, 1968; Haider & Tarver, 1969; Peters and Peters, 1972; and Garlick et al., 1975). Apparently conflicting conclusions arise because different studies have imposed conditions of deprivation which differ in severity, differ in methods of assessing protein synthesis, differet in populations of liver protein and also in the methods used (McNurlan and Garlick 1981). Certainly protein synthesis in liver is more resistant to nutritional deprivation than that in muscle. But Glick et al., (1982) suggested that severe nutritional deprivation probably results in a reduction in the rate of a liver's protein synthesis. Cahill et al., (1972) and Goldberg & Chang (1978) studied the regulation and significance of amino acid metabolism in skeletal muscle of fasted rats. They concluded that, in fasting, the mobilization of amino acids stored in the muscle protein helped provide the organism with gluconeogenic percursors. It was surprising to find out that the rate of protein synthesis in liver and muscle protein was reduced when rats were over-fed for 4 days (Glick et al., 1982). However, they did not show the rate of protein accretion in liver and muscles of over-fed vs control rats which would have helped to understand the rate of protein degradation and/or protein turnover.

The involvement of leucine in the regulation of protein turnover in muscle was first observed by Miller (1961). He noted that (^{14}C)

leucine was degraded at the same rate by normal and hepatectomized animals. Subsequently, Manchester (1965), Oddessey & Goldberg (1972), and Fulks et al., (1975) demonstrated that isolated rat muscle can rapidly degrade branched chain amino acids. They concluded that the rate of protein synthesis was increased and protein breakdown was decreased by the addition of branched chain amino acids, particularly by leucine in a muscle incubate. This property of leucine at least in vitro, has be related to the fact that branced chain amino acids are extensively oxidized by muscle at rates comparable to their rates of incorporation into protein (Rannels et al., 1974; Fulks et al., 1975; Li and Jefferson 1978; Chua et al., 1979). Sherwin (1978) reported an improvement in nitrogen balance of fasting patients given intravenous infusions of leucine. By contrast, intravenous injection of leucine in the fed or starved rat had no effect on protein synthesis in gastrocnemius muscle, heart muscle or jejunal serosa (Garlick et al., 1981). The lack of an effect of leucine in vivo was contributed to the secretion of insulin in response to the injection of leucine. More recently, Garlick and Clugston (1981) demonstrated there is a decrease in the relative rate of oxidation of leucine at night (fasting). They viewed this to imply that leucine was stored during the day, and then withdrawn from stores and oxidized at night. Garlick and Clugston (1981) in their communication with Fern et al., (unpublished data) showed that the accumulation of amino acids during feeding occurs by storage as tissue protein and not an expansion of the free leucine pool. Furthermore, Simon et al., (1978) reported that the calculated rate of muscle protein synthesis from infusing U-^{14}C-leucine was twice

that of infusions of U-^{14}C-lysine into pigs. In conclusion, the *in vitro* results that leucine exerts stimulatory effect on muscle protein synthesis are not in agreement with the *in vivo* results. It is possible that the conditions of incubation or perfusion *in vitro* are never achieved *in vivo*.

Methods of Measuring Protein Turnover

In Vivo

Indirect Approach

Skeletal muscle is probably a major tissue involved in whole body protein metabolism. However, there is little information on the mechanisms responsible for the maintenance of protein content in skeletal muscle and the contribution of protein turnover to overall body protein metabolism in muscle under various nutritional and hormonal conditions (Young, 1970; Millward *et al.*, 1976a; Young and Pluskal, 1977).

Actin and myosin, the contractile proteins of skeletal muscle are methylated following peptide bond synthesis, and produce N -methylhistidine, 3-methylhistidine, (3 MeHis) (Young and Munro 1978). During intracellular breakdown of these proteins, the 3-MeHis is released and excreted in the urine. The 3-MeHis is not reutilized for protein synthesis and therefore could be an indicator of protein turnover (Asatoor & Armstrong, 1967; Reporter, 1969). However, the function of methylated amino acid in actin and myosin is not understood (Paik & Kim, 1971).

In the context of muscle myofibrilar protein turnover, it appeared

that 3 MeHis offered a good opportunity for developing a useful technique to study muscle protein breakdown in the whole organism (Young and Munro, 1978). It presumbly arises as products of the breakdown of body proteins that contain it. But the urinary output of this amino acid may also be dependent on the composition of the diet (Bilmazes et al., 1978). 3-MeHis is present in the globular head of the myosin heavy chain (Huszar & Elzinga, 1971), but it is absent in the myosin of the muscle of fetus cardiac muscle (Kuehl & Adelstein, 1970; Huszar, 1972). On the other hand, the content of 3-MeHis is constant in the actins isolated from all sources so far examined (Trayer et al., 1968; Pollard & Weihing 1974). In order to validate the use of 3-MeHis as a quantitative index of muscle protein breakdown it was necessary to establish that it was not reutilized for protein synthesis. That was studied by showing that muscle tRNA fails to bind (charge) with 3-MeHis. In vitro studies with skeletal muscle showed no tRNA charging by 3-MeHis (Young et al., 1970). In vivo administration of labelled 3-MeHis did not result in the tRNA extracted form (Young et al., 1972). Second, the metabolism of the 3-MeHis released by turnover of actin and myosin has to be known. Cowgill and Freeburg (1957) found that most of the radioactivity following the administration of (^{14}C) methyl-3-MeHis to rat, rabbit and chicken appeared in the urine. The radioactivity was also recovered in the TCA soluble supernatant of muscle. Condon & Asatoor (1971) reported that, changes in the kidney function may alter the steady state level of 3-MeHis in cirrculating blood. They suggested that, there is a constant body 3-MeHis pool and that kidney clearance is constant during the period of measurement of

uniary 3-MeHis. Addition of 3-MeHis to the free amino acid pool by breakdown of actin and myosin proteins will be followed by its quantitative elimination in the urine. The problems with this method arose when Haverberg et al., (1975) reported that there were protein-bound 3-MeHis in other tissues as well as skeletal muscle. They found that the mixed proteins in skeletal muscle, diaphram, heart, liver, stomach, kidney, lung, spleen, testes, brain and blood serum contained detectable levels of protein-bound 3-MeHis. But, the total amount in other organs was quite small as compared with that in the skeletal mass. In contrast, the contribution of each tissue source to 3-MeHis in the urine depends on its turnover rate as well as its size (Millward et al., 1980). The production rates of 3-MeHis from skin, gastrointestinal and skeletal muscles accounted for less than half the observed excretion rate (Millward et al., 1980). Skeletal muscle accounted for only 25% of total excretion. Nishizawa et al., (1977) also reported that the 3-MeHis from skin and intestine has accounted for about 10% of the total body 3-MeHis pool. Furthermore, Fisher et al., (1975) observed a reduction in 3-MeHis content of chicken muscle under protein depletion. They also showed a considerably higher concentration of 3-MeHis in the muscle protein of well nourished chickens than those in muscles of the rat (Haverberg et al., 1975), pig (Rangeley & Lawrie, 1976), and sheep (Rangeley & Lawrie, 1976). Also the value was higher than that reported earlier for chicken thigh muscle (Asatoor and Armstrong, 1967). These experiments raised a serious doubt about the assumptions that skeletal muscle is the source of the 3-MeHis in the urine of an animal. The other factor which

limited the use of this method is that, this measurement estimated overall protein metabolism in the body, and not the turnover rates for individual organs and individual proteins which may differ (Fisher et al., 1975; Nishizawa et al., 1977).

Direct Approach

The participation of intracellular proteins in intermediary metabolism was first suggested by Borsook and Keighley (1935). However, the nature of intracellular proteins was proposed by Schoenheimer (1939) after labelled tracers became available for protein studies. The use of radioactive tracer had shown that all macromolecules in living cells except nuclear DNA, undergo continuous breakdown and resynthesis (Zak et al., 1979). These processes were called intracellular turnover.

The principles of chemical kinetics in protein turnover were reviewed by Reiner (1953), Jardetsky & Barnum (1958), Russell (1958), Zilversmit (1960), and Koch (1962). However, the precursor-product analysis to follow protein turnover was rarely attempted because it was assumed that the radioactive precursors of proteins were rapidly removed from the organism. Therefore, the estimates of protein half-life were based on the measurement of decay of the protein radioactivity. This approach leads to a gross overestimate of protein half-lives, because tracer amino acids are reutilized (Zak et al., 1979). The reutilization of CPM of the tracer amino acid is dependent on its precursor pool specific activity. Thus, to study protein synthesis the specific activity of the precursor pool during the time

course of the incorporation period must be described (Bergen 1975).

The choice of a proper compartment for analysis of precursor amino acid is difficult. The most obvious approach is the measurement of specific radioactivity in the immediate protein precursor. Berg, (1956a; 1956b) pointed out that prior to incorporation of an amino acid into the polypeptide chain, it is activated by ATP and attached to specific tRNA molecule in a reaction catalyzed by aminoacyl-tRNA synthetase. However, Ilan and Singer (1975) and Vidrich et al. (1977) described that the amino acyl-tRNA studies are technically difficult due to the very small concentration of tRNA in most cells. Kimata & Morkin (1971) and Poole (1971) used the free intracellular amino acids instead of amino acyl-tRNA. But Kipnis et al. (1961) Rosenberg et al. (1963), Hider et al. (1971) and Adamson et al. (1972) reported that extracellular amino acids are incorporated into protein molecules in preference to the intracellular compartment free amino acids. Thus, there is a controversy regarding the charging of tRNA and its source of amino acids to be from either intracellular or extracellular amino acid pools. Airhart et al., (1974) have proposed that tRNA is charged from a labile amino acid pool located in the membrane and associated with the amino acid transport system. Both intracellular and extracellular amino acids contribute to this pool, and the relatiave proportions of the contribution depend on the bidirectional flux of amino acid across the membrane. On the other hand, Hod and Hershko (1976) in their studies of cultured hepatoma cells reported that tRNA is charged at two separate sites, one localized at the membrane and charged from the extracellular amino acid pool, whereas the other is localized within

the cell and charged from the intracellular pool of amino acids. Further, Khairallah and Mortimore (1975) concluded that, the total tissue pool approach does present a useful estimate that is not generally too different from the "real" precursor pool.

The fractional turnover rate is the fraction of tracer amino acid in the protein molecule that is replaced per unit time by the same amino acid from the precursor pool (Zak et al., 1979). The fractional turnover rate can be derived by tracer techniques from simultaneous measurement of radioactivity in the precursor pool and in the protein molecule.

Single Administration of Radioactive Amino Acid

The most convenient experiment to measure fractional turnover rates employs the use of a single administration of radioactive tracer amino acid (Haider & Tarver, 1969). The tracer specific radioactivity is then determined at intervals over a period of time in both the precursor pool and the protein molecule (Henshaw et al., 1971). A radioactivity peak in the precursor amino acid pool is reached soon after injection and then falls rapidly (Zilversmit, 1960). In the protein of the tissues, the radioactivity rises to a maximum and then declines (Zilversmit, 1960). To avoid a rapid fluctuation in specific activity after a single injection, attempts are made to maintain a constant specific activity of the free amino acid in the precursor pool. If the precursor specific radioactivity is constant throughout the experiment, the calculation of synthesis rate is straight-forward (Garlick and Millward, 1972). However, in practice a short time

elapses before a constant level is reached. There are two approaches to provide a steady state situation. Garlick and Millward (1972) suggested that, the measurements should last long enough so that the time to reach a steady state is negligible as compared to the entire period over which measurements are taken. Alternatively, the injection of a large quantity of labelled and unlabelled amino acid (a flooding dose) results in a rapid rise and a slow decline in precursor specific activity lasting as long as 20 minutes (Henshaw et al., 1971) or 10 minutes (Garlick et al., 1980). The advantage of this method is that a single measurement at the end of the experiment is sufficient to define the entire time-course of specific activity. Thus, the rate of protein syntheis can be calculated (Henshaw et al., 1971; Garlick et al., 1980).

Continuous Administration of Radioactive Amino Acid

An alternative approach to the single injection of tracer that is frequently used for protein turnover measurement, either in the whole body or individual tissues, consists of constant administration of radioactive amino acid (Garlick et al., 1973; Laurent et al., 1978b). Waterlow and Stephen (1968), Gan & Jeffay (1971), and Garlick et al. (1973) suggested that the tracer can be infused directly into circulation. Some other investigators, gave the tracer through food or drink (Swick & Handa, 1956; Swick, 1958; Maruyama et al., 1978). In either case, an equilibrium eventually develops in which the influx of radioactive amino acid is balanced by its metabolism or excretion, or both (Zak et al., 1979). The constant specific activity in the

precursor pool is reached within the first 1 or 2 hours with intravenous infusion (Garlick et al., 1973) and within days when the tracer is included in the food (Swick & Handa, 1956; Swick, 1958). The equilibrium is maintained for many hours (Gan & Jeffay, 1967; Waterlow and Stephen, 1967). Finally, the specific activity rises once again, because of recycling of label from protein degradation (Waterlow & Stephen, 1967; Garlick, 1969). For measurements in any tissue one must know the time course of free amino acid specific activity in the blood as well as the protein and precursor pool in that tissue at the end of the infusion period (Garlick et al., 1973). The complete assumptions and mathematical equations for calculation of the fractional turnover rate for the constant infusion method is explained by Garlick et al. (1973), and Zak et al. (1979).

Protein Turnover Study In Vitro

Many investigators have studied protein metabolism in a preparation in which the tissue or organ is perfused with physiological solution (Jeanne et al., 1973; Odessey and Goldberg, 1972). Clarke (1957) and Buse et al. (1972) have studied the perfused heart while Miller (1962) experimented with the liver. Boyd and Jefferson (1979) utilized perfused rat hemicorpus while Spydevold (1979) and Huston et al. (1978) perfused rat hindquarter. However, in some studies part of a tissue may not be fully oxygenated due to the size of the preparation, therefore there may be a population of dead or dying cells (Odessey et al., 1974) The latter also pointed out that the amino acids transported across the membrane of perfused tissue may be

metabolized due to the preparation. Several studies have been performed on tissue homogenates to overcome the problems of amino acid transport. Studies were performed on homogenates of liver (Dawson et al., 1967; Dohm et al., 1976), heart (Dohm et al., 1976), and skeletal muscle (Paul and Abibi, 1976; 1978). "The physiological relevance of all perfusion studies must be questioned since the cytoplasmic environment is impossible to be duplicated" (Schneible, 1980). Also, the proposed action of the various hormones are often very speculative, when derived from in vitro work and are probably often not applicable to the in vivo situation (Bergen, 1975).

CHAPTER III

Experimental Procedures

A. Introduction and Concepts

Experiment #1

During early growth, the protein and lipid content of the whole body increases as the animal ages (Kubena et al., 1972). However, in later ages protein accretion ceases whereas fat accretion continues (Zucker and Zucker, 1963, Bailey and Zobrisky, 1968; Searle and Mc Graham, 1972).

Experiment #1 was conducted to determine the relationship of protein, ether extract (lipid) and moisture in whole body, breast muscles (pectoralis superficial + pectoralis subclavis muscles) and leg muscles (gastrocnemius + peroneous longus muscles) in male and female meat-type chickens at 10, 17, 24, 31, 38 and 45 days of age.

Experiment #2

The body composition was shown to be different between breeds and strains of chickens (Mitchell et al., 1926; 1931; Hunt, 1965). This experiment was designed to determine the relationship of protein, lipid and moisture content in whole body, breast muscles (pectoralis superficial + subclavis muscles) and leg muscles (gastrocnemius + peroneous longus muscles) in male and female Single Comb White Leghorn (SCWL) chickens at 12, 19, 26, 33, 47, 61, and 82 days of age.

Experiment #3

Muscle growth is the result of the rate of protein synthesis exceeding that of protein degradation (Waterlow and Stephen, 1968; Morgan, 1974; Millward et al., 1975; Laurent et al., 1978b). In other words, the protein accretion (A) is the difference between protein synthesis (S) and breakdown (B) or A = S - B. The A is usually measured by starting with two groups of animals of equal weight and, killing one group at a certain age, and sacrificing the other group a few days later. The desired tissue(s) was analyzed for protein from 2 groups of animals of equal weight, age or sex killed a few days apart. The difference between the two groups would be the A for those few days. If S is measured in the animal, then B is obtained by calculation with the above equation.

The determination of tissue protein synthesis (S) in vivo is complicated by amino acid reutilization. Amino acids that are released during the intracelluar breakdown of proteins can be reutilized for protein synthesis within the cell (intracellular recycling) or they may be transported to other cells in the same muscle, or to other organs where they may enter the pathways of protein anabolism (intercellular recycling).

Single injection (Haider and Tarver, 1969; Henshaw et al., 1971; Garlick et al., 1980) and continuous infusion (Waterlow and Stephen 1967, Garlick et al., 1973; Glick et al., 1982) of radioactive amino acid are the two techniques which have been used to measure the S in individual tissue(s) and/or whole body. This experiment was designed

to develop a modified method of the two approaches for measuring the S in individual tissue(s) and/or whole body.

Experiment #4

The results from experiment #1 showed that, there was an increase in protein content and a decrease in fat content per unit of weight gain up to 31 days of age in male meat-type chicken. The fat content per unit of weight gain drastically increased while the protein content decreased from 31 until 45 days of age. Experiment #4 was conducted to measure the rate of protein turnover (synthesis and degradation) in pectoralis superficial + pectoralis subclavis muscles (breast muscles) and gastrocnemius + peroneous longus muscles (leg muscles) at 28, 36, and 43 days of age in male chickens, and to conceptualize, whether the decrease in protein content per unit of body weight gain at a later age, as measured in experiment #1, was due to a change in protein synthesis and/or breakdown.

Experiment #5

Millward and Waterlow (1978) pointed out, that the rate of protein degradation was faster in the slow growing strains than fast growing strains of rats. Similarly, Maruyama et al., (1978) demonstrated that, the rate of protein breakdown in leg muscles was higher in slow growing than rapid growing birds at 2 weeks of age. However, the rate was similar in breast muscles of two breeds studied at the same age. Experiment #2 indicated an increase in protein content and fat content per unit of weight gain from 28 to 40 days of age in light breed (SCWL) chickens. Experiment #5 was designed to measure the protein turnover

rate in pectoralis superficial + pectoralis subclavis muscles (breast muscles) and gastrocnemius + peroneous longus muscles (leg muscles) at 28, 36 and 43 days of age in SCWL chickens. The results of experiment #5 could be compared with experiment #4, to determine if there is any difference in the rate of protein turnover in two breeds studied at 3 different ages; also, to determine whether the increase in protein content per unit of weight gain from 28 to 40 days of age is due to an enhanced protein synthesis and/or depressed rate of degradation.

Experiment #6

A marked reduction in protein synthesis in muscle tissues of rats have been reported in response to starvation (Waterlow and Stephen, 1968; Garlick et al., 1975). Glick et al., (1982) were first to report a decrease in protein synthesis rate (%/day) in muscles of rats which were over-fed for 4 days. They suggested that overnutrition seems to be associated with a reduced rate of protein synthesis similar to those in starvation. Polin and Chee (unpublished data) demonstrated that the absolute protein content had increased when chickens were over-fed during a period of 4 days. Experiment #6 was conducted to find out whether the responses in the rate of protein synthesis in chickens appear to occur as observed in rats. also, whether the increase in protein content in over-fed chickens is due to a change in protein synthesis and/or changes in degradation rate.

B. General Procedure

Male and female heavy breed or meat-type chicks (Hubbard White Mountain) were obtained from the Fairview Hatchery, Inc., Remington, Indiana. Male and female egg-type, Single Comb White Leghorn (SCWL), chicks were from stock kept by the Animal Science Department, Michigan State University, East Lansing, Michigan.

Meat-type chicks were used for experiments #1, #3 and #4 and SCWL chicks for experiments #2, #5 and #6. In all experiments, the chicks were raised on practical-type diets. The practical-type diet (Table 1) for meat-type chickens was used for experiments #1,#3 and #4, while that for SCWL chickens (Table 2) was used for experiments #2, #5, and #6.

All experiments were conducted under identical environmental and housing conditions. Chicks up to 4 weeks of age were kept in electrically heated battery brooders with wire floors. The room was lighted for 16 hours a day and had a temperature of 21 $\pm$ 2°C during the rearing and experimental periods. They were transferred into growing batteries 100 x 80 x 30 cm (l x w x h) and raised until the termination of experiments. Feed intake and weight gain were measured for every group according to the experimental design. When anesthesia was used, the chickens were infused via brachial vein with approximately 1.5 ml pentobarbital solution per kg of body weight. The concentration was 25 mg pentobarbital per ml of saline.

Table 1. Composition of Diets for Experiments #1, #3, and #4

Ingredients	g/kg
Corn #2, yellow	482.2
Soybean meal (48%)	365.0
Alfalfa leaf meal (17%)	68.0
Corn oil, stable	42.0
DL-Methionine	0.8
Calcium phosphate	22.0
Limestone	9.0
Choline chloride, 50%	3.5
Salt	3.5
Vitamin mix[1]	3.0
Mineral mix[2]	0.5
Selenium mix[3]	0.5
Crude protein	23.4%
Metabolizable energy	3.05 Kcal/g

1 Supplied the following per kg of diet; Vitamin A. 11,000 I.U; Vitamin D_3, 1,100 I.C.U.; Vitamin E, 11 I.U.; Vitamin K, 2.2 mg; Thiamin, 2.2 mg; Riboflavin, 4 mg; Panthothenic acid, 14.1 mg; Nicotinic acid, 31.5 mg; Pyridoxine, 4 mg; Biotin, 0.1 mg; Folic acid, 1.3 mg; Choline, 13.2 mg, Vitamin B_{12}, 0.01 mg; and Antioxidant (Ethoxyquin), 125 mg.

2 Supplied the following per kg of diet: Manganese, 60 mg; Zinc, 40 mg; Iron, 30 mg; Copper, 5 mg; Iodine, 0.5 mg.

3 From Calcium Carbonate Company - supplied as 0.1 mg/kg of diet.

Table 2. Composition of Diets for Experiments #2, #5 and #6

Ingredients	g/kg
Corn, No. 2 Yellow	502.1
Soybean Meal (48%)	310.0
Alfalfa leaf meal (17%)	50.0
Wheat bran	60.0
Corn oil, stable	40.0
DL - Methionine	0.9
Limestone	5.0
Dicalcium phosphate	22.0
Salt	3.0
Choline Chloride, 50%	3.0
Vitamin Mix[1]	3.0
Mineral Mix[2]	0.5
Selenium Mix[3]	0.5
Crude protein	21.2%
Metabolizable energy	2.99 kcal/g.

1 supplied the following per kg of diet: Vitamin A, 11,000 I.U.; Vitamin D_3, 1,100 I.C.U.; Vitamin E, 11 I.U.; Vitamin K, 2.2 mg; Thiamin, 2.2 mg; Riboflavin, 4 mg; Panthothenic acid, 14.1 mg; Nicotinic acid, 31.5 mg; Pyridoxine, 4 mg; Biotin, 0.1 mg; Folic acid, 1.3 mg; Choline, 13.2 mg; Vitamin B_{12}, 0.01 mg; and Anioxidant (Santoquin), 12.5 mg.

2 supplied the following per kg of diet: Manganese, 60 mg; Zinc, 40 mg; Iron, 30 mg; Copper, 5 mg; Iodine, 0.5 mg.

3 from Calcium Carbonate Company - supplied as 0.1 mg/kg of diet.

C. The Experiments

Experiment #1

Sixty male meat-type chickens, 3 days of age, were weighed, banded and sorted into 6 groups of 10 birds each. They were given feed and water ad libitum. Feed intake and weight gain were measured weekly. At 10 days of age ten birds were starved overnight (16 hours) and then killed using excess CO_2. They were divided into two subgroups of 5 birds each. The leg and breast muscles from the right side of the chicks, and abdominal fat of one subgroup were excised and weighed. The leg or breast muscles were each pooled for proximate analysis as described in Analytical Procedures. The carcasses of the other subgroup of 5 birds were pooled for analysis of Protein, ether extract and moisture in whole body according to Analytical Procedures. The procedures used for the first group were followed for the 2nd, 3rd, 4th, 5th and 6th groups which correspond to 17, 24, 31, 38, and 45 days of age, respectively.

The same experimental design was used for sixty female meat-type chicks starting when they were 3 days of age.

Experiment #2

Ninty-eight mixed sex, SCWL chicks, 5 days of age, were weighed, banded and sorted into 7 groups of 14 birds each. They were given feed and water adlibitum. Feed intake and weight gain were measured weekly. One group was starved overnight (16 hrs) and sacrificed with excess

CO_2 at 12 days of age and then divided into two subgroups of 7 birds each. The chicks in one subgroup were sexed and then leg and breast muscles from right side and abdominal fat excised and weighed. The leg and breast muscles of each sex were pooled for proximate analysis as described in Analytical Procedures. The other subgroup of 7 birds were sexed and pooled separately for whole body analysis of protein, lipid and moisture according to Analytical Procedures.

The procedures used for the first group were followed for the 2nd, 3rd, 4th, 5th, 6th and 7th groups which correspond to 19, 26, 33, 47, 61 and 82 days of age, respectively.

Experiment #3

Fifteen male meat-type chicks, 18 days of age, were divided into 5 groups of 3 birds each. The average body weight of the chicks was 307 grams. The birds were anesthesized as described in the General Procedure and restrained on their back on a V shaped wooden structure. A cotton vest was placed over the bird's chest and the wings extended through holes in the vest so that the brachial veins were accessible. The vest was tied to the V structure with 4 strings that were attached to the vest. The brachial vein was cannulated as well as the carotid artery, each with a 25 cm. polyethylene tube (I.D. 0.86 mm) from Becton, Dickson Company. The cannula from the brachial vein was connected to a 20 guage needle which was in turn connected to a 20 ml syringe containing radioactive leucine. This syringe was driven by a Harvard Infusion Pump according to a program (Table 3) developed in a series of preliminary experiments, to obtain a constant specific

radioactivity of leucine in blood and muscle throughout the experiment. The infused radioactive solution was prepared in order to infuse approximately 35 μCi of 3H-leucine + 110μg of "cold" leucine/100 gm body weight by 30 minutes of infusion (Appendix A Table 1). The cannula in the carotid artery was used for withdrawing blood samples during and at the end of the infusion time. The experimental design is presented in Table 4. The birds were killed by infusion of 2 ml, 10% KCl/bird into the carotid artery at the end of infusion time. A part of leg and a part of breast muscles from right side were excised, weighed to the nearest 10th of a gram, put in polyethylene bags and placed between layers of dry ice. The time to kill a bird and remove the muscles and place them under dry ice took about 2 minutes. The muscle samples were stored at -30°C. The remaining portions of leg and breast muscles from right side were excised and weighed. The 3H-leucine specific radioactivity in the precursor pool of leg and breast muscles and blood samples were measured according to the Specific Radioactivity Assay. The 3H-leucine incorporation into the proteins of two muscles were determined as described in the Total Incorporation Radioactivity.

Experiment #4

The experimental design is presented in Table 5. Four birds on day 28, 36 and 43 of age were anesthesized according to the General Procedure, and cannulated in the brachial vein as described in Experiment #3. They were infused with a solution containing a blend of 3H-leucine and "cold" leucine according to the infusion program (Table

Table 3. Infusion Program for Individual Bird

Pump* Position	Infusion Time (Min) From - to	Infusion Rate With 20 ml Syringe ml/min.
5	0-1.25	0.97
8	1.25-20	0.097
9	20-30	0.0398
Total	30	3.31

*Infusion/withdrawal pump, Model 950, Harvard Apparatus

Table 4. Experimental Design

Infusion Time (Min)	No. of Birds	Average Body Wt. (gm)	μCi 3H-leucine* Infused/Bird
0	3	323	0.00
3	3	302	48.38
10	3	307	72.15
20	3	304	102.09
30	3	301	116.00

*Infused solution contained 35 μCi ^{3}H-leucine + 110 μg "cold" leucine per ml of saline.

3). The solutions were designed to obtain approximately 35μ Ci 3H-leucine + 110 μg "cold" leucine/100 gm body weight by 30 minutes of infusion (Appendix A Table 2,3, and 4). The blood samples were taken into heparinized syringes from non cannulated brachial vein at 3, 10, 20 and 30 minutes of infusion and were counted for ^{3}H as described in Plasma Specific Radioactivity. At the end of the infusions the birds were sacrificed by introduction of 2 ml of 10% KCl into the bird's brachial vein. The process of measuring the leucine specific activity in the precursor pool and the incorporated radioactivity in the leg and breast muscles were as described in the Experiment #3. The other birds in this experiment were killed by excess CO_2 according to the experimental design (Table 5). The crops were emptied and the birds were weighed. The leg and breast muscles from the right side were excised and weighed individually. The leg and breast muscles and whole body were analyzed for N, lipid and moisture for each bird as described in the Analytical Procedures.

Table 5. Design of Experiment #4

	Periods								
	I			II			III		
Sacrificed age (day)	26	28	30	34	36	38	41	43	45
No. of Birds	5	4	5	5	4	5	5	4	5

Experiment #5

The experimental design is shown in Table 6. Four birds on day 28, 36 and 43 were anesthesized as described in General Procedures and cannulated in the brachial vein according to the Experiment #3. They were infused with radioactive solution according to the infusion program (Table 3). The solutions were prepared to infuse approximately 35 μCi ^{3}H-leucine +110 μg cold leucine/100 gm body weight by 30 minutes (Appendix A Table 5, 6 and 7). Blood samples were withdrawn into heparinized syringes from a non-cannulated brachial vein at 3, 10, 20 and 30 minutes of infusion and were counted for ^{3}H as described in the Plasma Specific Radioactivity. The chickens were killed by infusion of 2 ml, 10% KCl per bird into brachial vein to 30 minutes of infusion. The leg and breast muscles were removed and analyzed as explained in Experiment #3.

Table 6. Design of Experiment #5

	Periods								
	I			II			III		
Sacrificed age (day)	26	28	30	34	36	38	41	43	45
No. of Birds	20	4	20	16	4	16	12	4	12

The other birds in experiment #5 were killed by excess CO_2 according to the experiment design (Table 6). The crops were emptied and the whole body weighed. The leg and breast muscles from the right side were excised and weighed. The samples of muscles and whole carcasses were each pooled to have 4 of each type for every age. These samples of muscles and of whole carcasses were analyzed for N, lipid and moisture as described in the Analytical Procedures.

Experiment #6

Thirty SCWL chicks, 32 days of age were divided into 6 groups of 5 birds each. Three groups of 5 birds each served as controls which were fed ad libitum while the other 3 groups were force-fed, 4 times a day, an amount of feed equal to 138% of the control group's intake of the previous day. One group of controls and one force-fed group were sacrificed at day 33, while the other two groups, one control and one force-fed were killed by excess CO_2 at 37 days of age. Their crops were emptied and the birds weighed. The leg and breast from the right side were excised and weighed. The leg and breast muscles were analyzed for protein according to the Analytical Procedures. The third group from control and force-fed chickens were weighed, anesthesized according to General Procedure and cannulated in the brachial vein as described in Experiment #3. They were infused with radioactive solution according to the infusion program (Table 3). The infused solution was prepared to infuse approximately 5 μCi. ^{14}C-leucine + 110 μg cold leucine/100 gm body weight by 30 minutes of infusion (Appendix A, Table 8). Blood samples were obtained from a brachial vein at

infusion times of 3, 10, 20 and 30 minutes. The chickens were then killed by infusion of 2 ml, 10% KCl per bird into brachial vein. The two muscles were trimmed according to the Experiment #3. The precursor pool of ^{14}C-leucine specific radioactivity and incorporated ^{14}C-leucine in the muscle samples were determined as described in Specific Radioactivity Assay and Total Incorporated Radioactivity, respectively.

D. Specific Radioactivity Assay

Specific Activity of Leucine in Muscle's Pool of Free Amino Acids

The dansyl chloride, thin-layer chromatography (DNS-TLC) used to determine the radioactive specific activity of the amino acid was a modification of that by Airhart et al., 1979. The procedure was performed as follows:

Sample Preparation

One gram leg or breast muscles from a chick which was infused with either L- (3,4,5-^{3}H) leucine (Research Products International Corp., Mount Prospect Illinois, with specific activity of 50 Ci/mmol.) or L-(U-^{14}C)-leucine (ICN Pharmaceuticals, Inc. Chemical and Radioisotope, Irvine, California, with specific activity of 300 μCi/mmol.) was weighed and placed into a sterile polypropylene, 50 ml centrifuge tube. Nine ml saline was added to the sample and homogenized for 90 seconds with a Tekmar high speed homogenizer. Two ml of homogenized muscle was poured into a 20 ml disposable culture tube with addition of 2 ml 10% Trichloroacetic acid (TCA). The sample was centrifuged at 5000 rpm for 10 minutes. Then the supernatant was decanted into another 20 ml tube. The precipitate was depelleted and two ml of 5% TCA was added to it.

The sample was then vortexed followed by centrifugation. The supernatant was added to the first collection. These processes with 5% TCA were repeated once more. The precipitate was then washed with 2 ml diethyl ether (2x) and saved for measuring the radioactive amino-acid incorporated into the muscle's proteins.

The supernatant was brought to 2 ml in a vaccum oven at 60-70°C and 500-600 mm Hg of vaccum. The supernatant was then washed three times with 2 ml of diethyl ether. The supernatant was adjusted to a pH of 7 with ammonium hydroxide or hydrochloric acid using a pH Meter 125 (Science Products, Medfield, Massachusetts).

Column Preparation

The Dowex - 50w resin of hydrogen form (Sigma Chemical Company, St. Louis, Mo.) with 8% cross-linked and 100-200 dry mesh, was used for crude separation of leucine from other amino acids, mostly basic types. The preparation of the column was based on a procedure by Thompson et al. (1959). Dowex -50 w was soaked in water for 12 hours and then an equal volume of resin and water was stirred in a beaker. The fines were removed by decanting the supernatant after 30 minutes, and the process repeated once more. The resin was heated for 16 hours at 96°C with 2 volumes of 1N NaOH. The activated resin was then poured into a glass column 0.7 cm diameter x 10 cm length. The column was drained and washed with 2 column volumes of distilled deionized water (DDW). The column was then treated with 5 column volumes of 6 N HCl and then washed with DDW until the effluent was free of chloride ion as tested with one drop of silver nitrate.

The pH adjusted sample was poured on the top of the column and allowed to drain into the resin. The the column was washed with 15 ml of 2N NH_4OH to elute the non-basic amino acids. A standard of ^{14}C-leucine and non radioactive supernatant, from a non-infused bird was passed through a prepared column with eluted fractions to determine at what volumes the radioactivity was eluted (Figure 1).

Based on the standard effluents, the first 7 ml of effluent was discarded and then a 3 ml fraction was collected (Figure 1). The collected effluent was reduced to 0.2 ml in a heated vaccum oven or N-evaporator. This sample was used for dansylation.

Dansylation Procedures

50 l of prepared sample was pipetted into a 9 ml test tube and the pH was adjusted to 9-10 with addition of either sodium bicarbonate or sodium carbonate using 1-2 l amounts on pH paper. Twenty l of prepared ^{14}C- or ^{3}H dansyl chloride[1] (Appendix A Table 9, 10, 11, 12, and 13) with a known specific activity was added to the sample, the contents gently shaken and placed in a 37^{0}-$40^{0}C$ incubator oven until the sample went colorless. The dansylated sample was dried down in a N-evaporator.

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1. (methyl -^{14}C) dansyl chloride (Amersham Corporation, Arlington Heights, Illinois, with specific activity of 109 Ci/mmol.) was used when the sample contained ^{3}H-leucine. (G-^{3}H) dansyl chloride (Amersham Corporation, Arlington Heights, Illinois; with specific activity of 12 Ci/m mol.) was used when sample contained ^{14}C-leucine. Unlabeled dansyl chloride was provided from Fisher Scientific, Livonia, Michigan.

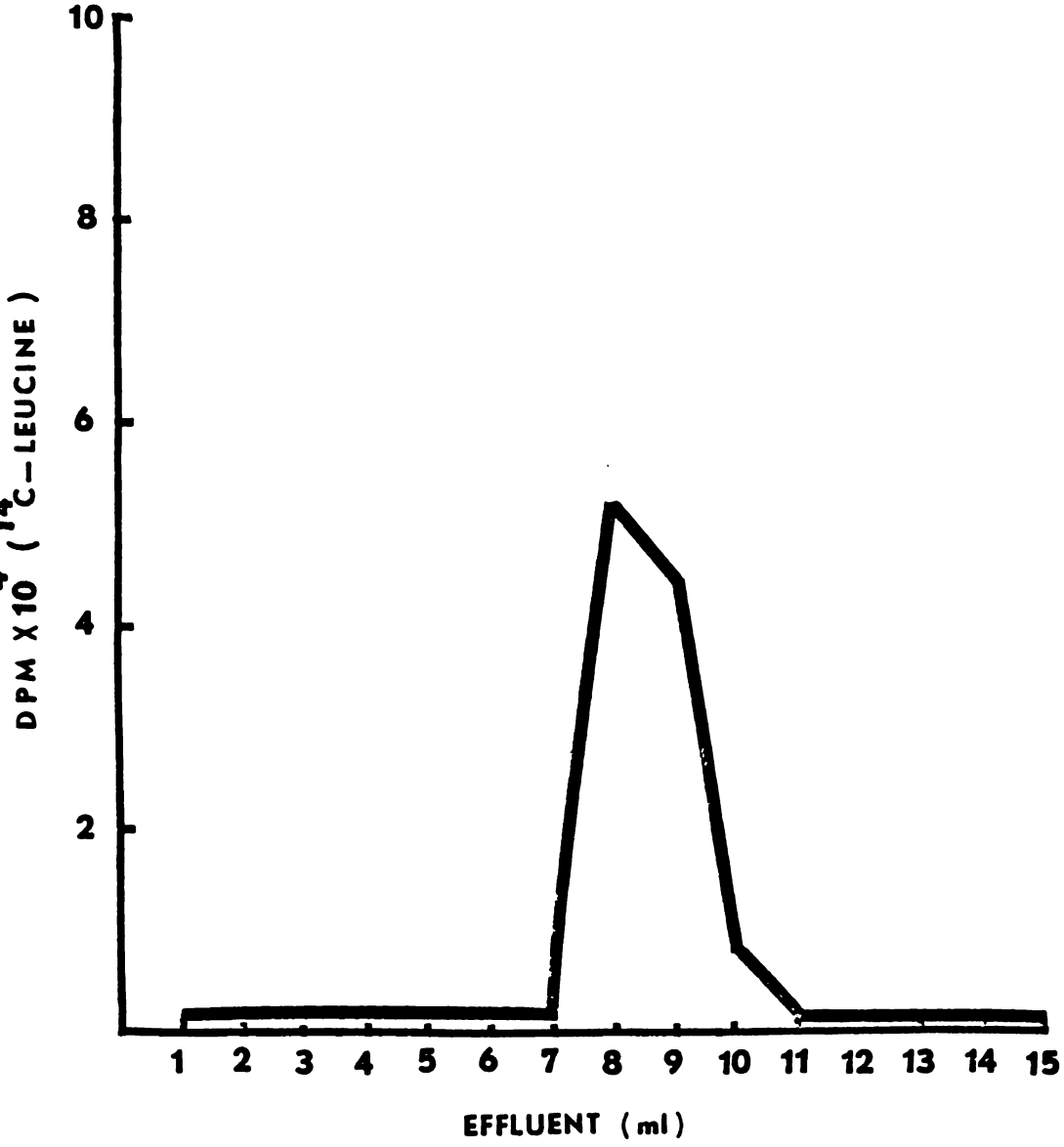

Figure 1. Standard curve for leucine collection from Dowex 50 column.

Extraction of Salts by Ethyl Acetate

The bicarbonate added to the sample to adjust the pH to 9-10 previous to dansylation introduces excess salt into the sample. The presence of excess salt interferes with spotting and compromises the chromatographic separation of the dansyl-amino acids. Thus, 200 of water-saturated ethyl acetate was added to the N-dried dansylated sample, vortexed and centrifuged at 3000 rpm for 20 minutes. The supernatant was decanted into another 8 ml test tube. Another 200 of water-saturated ethyl acetate was added to the precipitate, gently shaken, centrifuged again and the supernatant added to the previous collection. This washing process with the precipitate was repeated once more. The collected supernatant was dried down in a ventilated oven at 50^{0}C.

Thin Layer Chromatography (TLC)

The Cheng-Chin polyamide sheet 15 x 15 cm (Pierce Chemical Co. Rockford, Illinois) was cut into 4 pieces of 7 1/2 x 7 1/2 cm. The plates were labelled and marked lightly with a pencil at a point about 1 cm from left side and bottom of the plate (Figure 2). Twenty µl of acetone: DDW (1:1) was added to the dried supernatant and the tupe gently shaken. A disposable glass micro-pipette (Scientific Manufacturing Industries, Emeryville, California) was used to spot the sample. The plates were positioned on a flat surface and touched at the pencil dot with a micropipette containing the amino acid-dansyl

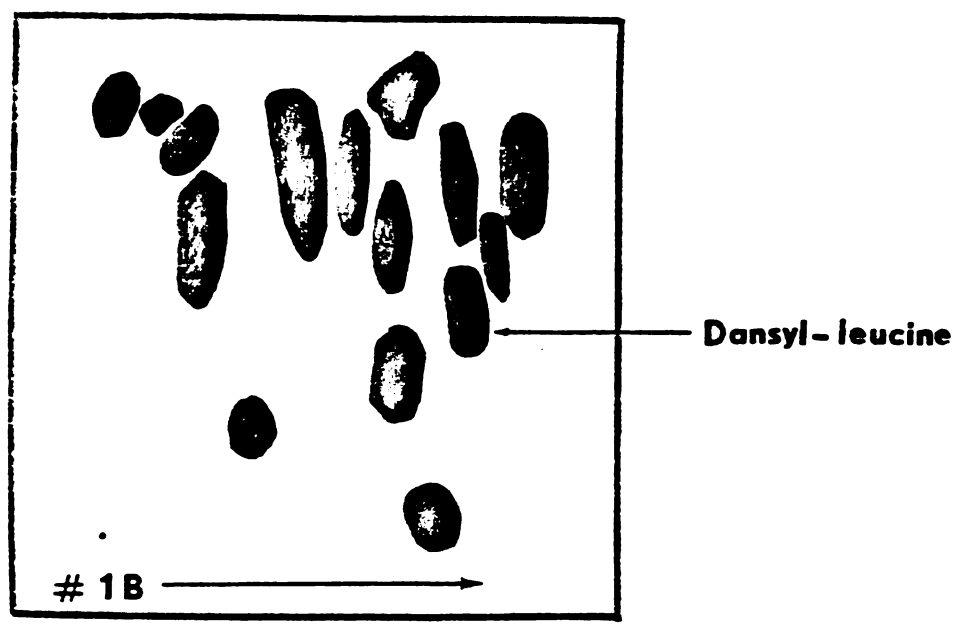

Figure 2. A pictoral view of the polyamide plate shows the dansyl-leucine location among the other dansyl-amino acids, following chromatography.

sample so that a small wet area appeared. The wet area was partially dried under a stream of cool air from a hair dryer. Then, another spot was applied to the same area. In this fashion, every sample was entirely spotted on two plates. Every attempt was made to keep the spot as small as possible, and to avoid rubbing or scratching the plate with the micro-pippette. The spotted plates were placed in a chromatographic tank containing water: formic acid (100:2) and set at an angle so that they leaned against the wall of the tank. The plates were allowed to run in the first dimension for 30 minutes or until the (solvent overran the plates). The plates were dried and checked under the UV light to make sure the amino-acids-dansyl complex was chromatrographed near the top of the plates. Most of the plates were chromatographed a second time for about 15 minutes to assure good resolution. Then the plates, after drying, were chromatographed at right angles to the second run. The solvent used for the second run was benzene:acetic acid (90:10) and the tank was kept under a hood and was completely sealed with grease. The plates were allowed to be in the second tank until the solvent front was near the top edge (about 20 minutes). Then, the plates were taken out and dried under a steam of air. A standard of dansyl-L-leucine (Sigma Chemical Company, St. Louis, Mo.) was spotted on a plate and chromatographed in both tanks as described for the samples. The dansyl-leucine spot as detected by comparison to the standard dansyl-leucine spot detected under a UV light.

Counting

The dansyl-leucine spots were cut out of the polyamide sheets and combined so that two from each sample were placed into an eight-ml counting vials for liquid scintilation counting (Sargent Welch Scientific Company). Into the vials was pipetted 0.2 ml of unisol^{TM} -solubilizing tissue fluid (Isolab Incorporated, Akron, Ohio). The Polyamide was wiped out from the scissor between the samples. The vials were kept overnight at room temperature to allow the complex of amino-acids-dansyl to dissolve. Then, 5 ml of unisol-complement^{TM} (Isolab Incorporated, Akron, Ohio) was poured into every vial and left for 24 hours at room temperature and then counted in channel 9 of an Isocap-300 for both ^{3}H and ^{14}C in order to count a minimum 3000 CPM for either ^{3}H or ^{14}C. Except for experiment #3, duplicates of each sample were assayed. In each assay, standard curves were established using 30 of the 8 ml vials which contained 2 small parts from a non radioactive polyamide plate (similar to leucine-dansyl spot). The ^{3}H-leucine (about 1350 dpm/vial) was added to 10 vials and ^{14}C-dansyl chloride (1340 dpm/vial) was added to another 10 vials, and the third 10 vials were used as background. Carbon tetrachloride (CCl_4) was used as a quenching agent at the level of 0, 10, 20, 30 and 40 per vial in duplicates. The process of adding Unisol^{TM} and unisol-complement^{TM} and counting was the same as described for the samples. The relationship between efficiencies and quenched external standard ratio (Q-ESR) for ^{14}C, ^{14}C spill, ^{3}H and ^{3}H spill were determined using regression analysis (Snedecor and Cochran, 1968) and presented in Figure 3.

Plasma Specific Radioactivity

The plasma specific activity was only measured for experiment #3, and this provided the information that specific activity was constant in the blood at 3, 10, 20 and 30 minutes of infusion time. One ml of blood was withdrawn into heparinized syringes from the infused birds at 3, 10, 20 and 30 minutes of infusion time (Table 4). The blood samples were centrifuged at 3000 rpm for 5 minutes, and their plasmas stored at $-30^{0}C$. Ten l of plasma were counted in the ^{14}C and ^{3}H channel of the Isocap-300 to determine the plasma radioactivity level. One hundred l of plasma were used for measuring the leucine specific activity, as outlined according to the procedure muscle homogenates as described in the section on "Specific activity of leucine in muscle's pool of free amino acids".

E. Total Incorporated Activity

One ml Unisol^{TM} was added to the diethyl ether washed precipitate from the Specific Radioactivity Assay and the sample was allowed to stand at room temperature for 24 hours to obtain a complete digestion of proteins. These digested samples were decanted into 20 ml scintillation vials, then the tubes were washed with 10 ml of Unisol-complement^{TM} and this in turn was added to the vials. After standing at room temperature for 48 hours the vials were counted in either the ^{3}H and/or ^{14}C channel of Isocap-300 depending on whether the birds were infused with ^{3}H-leucine or ^{14}C-leucine.

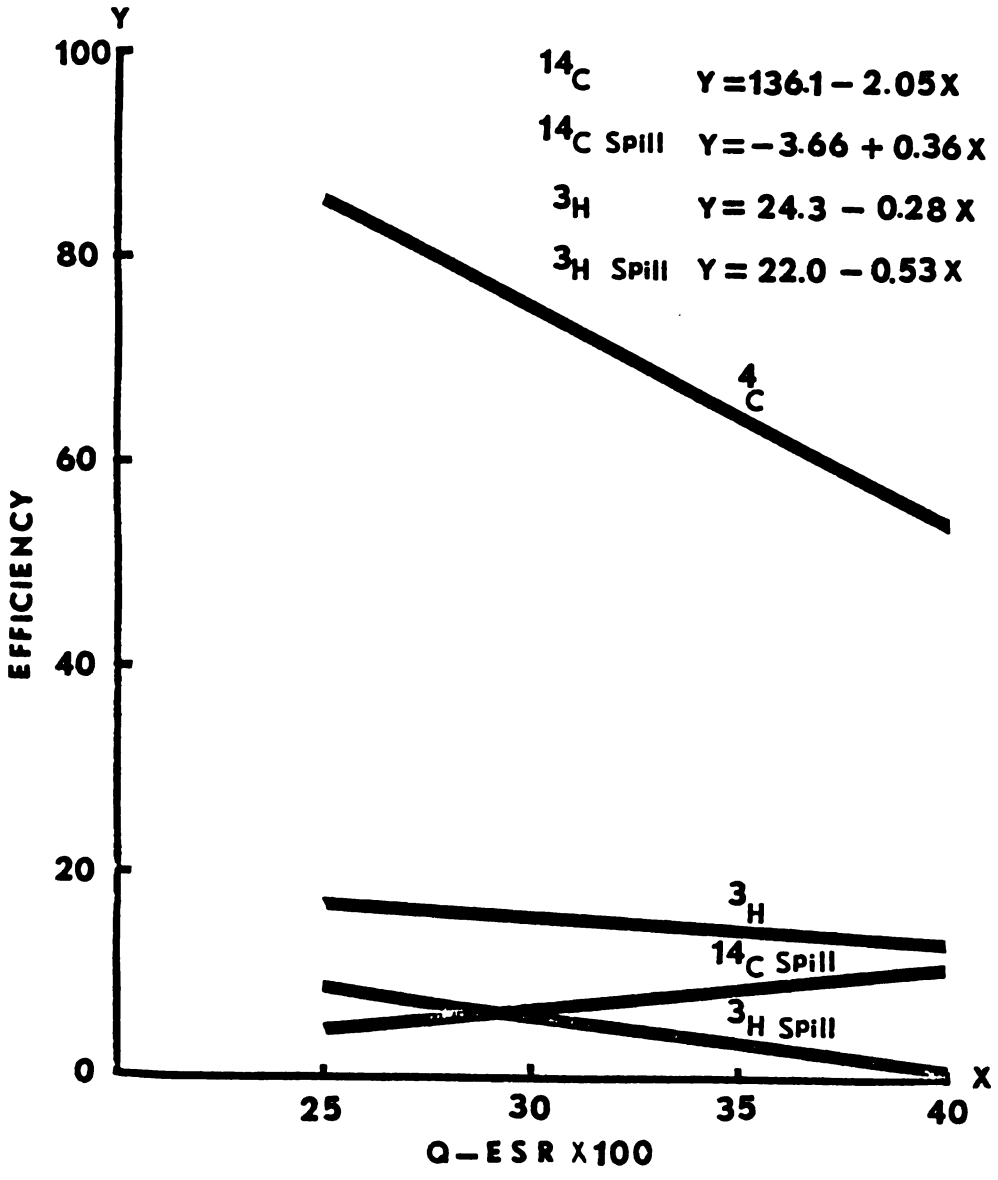

Figure 3. Standard curves for calculation of ^{14}C and ^{3}H dpm from simultaneous counting of the two isotopes.

F. Analytical Procedures

1. Determination of Leucine Concentration in Muscle Proteins

One gram of muscle from the birds which were not infused with radioactive solution were treated similarly to those used in the Specific Radioactivity Assay. The precipitates after the wash with diethyl ether were further washed with ethanol, acetone and ether successively to obtain a dry white powder. Fifty mg of the dry white powder were weighed out and placed in hydrolysis tubes with 20 ml of 6N HCl. A stream of N was used to displace the air in the tube, and the tube capped tightly. The samples were hydrolyzed in an autoclave for 18 hours. The concentration of leucine was determined with a Technican TSM-1 Auto Amino Acid Analyzer (Bergen and Potter, 1975). The percent protein in 100 mg of dry white powder was determined by Colorimetric Method (AOAC, 1980).

2. Sample Preparation and Proximate Analysis

The muscle samples and whole body carcasses were frozen at -30^{0}C. The whole body was cut into small pieces by an electric band saw. Then, a Hobart 3 H.P. meat grinder was used to grind the pieces into hamburger consistency (including feathers). The grinding was repeated 5 times to achieve a uniformly ground sample, from which a grab-sample was taken for determination of % moisture. The frozen muscle samples were cut into small pieces with a knife and these were ground with a small kitchen meat grinder until a uniform sample was prepared. The ground samples were weighed and put in tared aluminum trays. These were placed in a ventilated oven at 65^{0}C until no change appeared in

the weight of the samples. The dried samples were blended in a blender to provide powdered samples which were used for protein and lipid analysis.

Protein concentration was determined by a colorimetic method (AOAC, 1980).

The powdered samples were analyzed for fat content by petroleum ether extraction (AOAC, 1975).

G. Statistical Analysis

Experimental data were analyzed statistically by the analysis of variance (Gill, 1978), and the means were compared by Tukey's test (Gill, 1978). Regression analysis (Snedecor and Cochran, 1968) was used whenever applicable to obtain the correlation between different measurements.

CHAPTER IV

Results

Experiment #1

The live body weights, breast muscles (pectoralis superficial + pectoralis subclavis from right side), leg muscles (gastrocnemius + peroneous longus muscles from right leg) and abdominal fat (A.F.) weights were increased curvilinearly ($P < 0.01$) on a semi-log scale as the birds aged (Tables 7 and 8 and Figures 4 and 5). The live body weight, breast muscles, leg muscles and A.F. were almost similar in both male and female meat-type chickens at 10 and 17 days of age, when the daily feed intakes were about equal (Table 7). These criteria except A.F. developed to be greater in the male than in the female chickens from 24 to 45 days of age (Table 7). The absolute A.F. weight was heavier in the male as compared to female chickens at 45 days of age, however the A.F. as a percentage of live body weight was greater in the female than male chickens (Table 7). The numerical values for breast and leg muscle weights as a percentage of live body weights increased quadratically ($P < 0.01$) in both male and female meat type chickens (Table 7 and 8).

The % moisture in the whole body, breast muscles and leg muscles declined linearly ($P < 0.01$) as the male or female chickens aged (Table 9 and 10). The % lipid in the whole body, breast and leg muscles were not changed in the male chickens as they aged (Table 9). However, it

declined linearly ($P < 0.05$) in the breast muscles and increased linearly ($P < 0.05$) in the whole body and did not change in the leg muscles as the female chicken aged (Table 9 and 10).

The individual live body weight, breast muscles, leg muscles and abdominal fat weight at 10, 17, 24, 31, 38 and 45 days of age are presented in Appendix B Table 1.

The fractional accretion rate of protein or FAR[1] (%/day) in the whole body, breast muscles and leg muscles were 11.4, 18.0 and 12.0 at 13 days of age, respectively in male meat-type chickens (Table 11). The FAR (%/day) was decreasing linearly in the whole body ($p < 0.01$), breast muscles ($p < 0.01$) and leg muscles ($p < 0.05$) of the male chickens as they aged (Table 12 and Figure 6). The FAR measured in the whole body or breast muscles declined linearly ($p < 0.05$) in the female chicken as they grew older (Table 11 and Figure 7). The FAR in the leg muscles was approached to be linearly significant ($p < 0.10$) in the female chickens as they aged (Table 12). The numerical FAR in the breast muscle was higher than whole body or leg muscles at 13 days of age for both male and female chickens. However, the FAR in the whole body and either of the two muscles tended to be similar at 20, 27, 35, and 41 days of age with exception of the FAR for leg muscles at 34 days of age (Table 11). The fat gain per unit of weight gain was 10 and 20 percent and the protein was 17 and 12 percent for 13 and 41 days of age, respectively, in the male chickens (Table 11). The protein deposition per unit of weight gain in the whole body tended to be

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1-See appendix B, page 179 for FAR calculation.

Table 7. Feed Intake, Live Body Weight and Weights of Pectoralis Superficial + Pectoralis Subclavia (Breast Muscles From Right Side), Gastrocnemius + Peroneous Longus (Leg Muscles From Right Leg) and Abdomini Fat (A.F.) or Breast Muscles, Leg Muscles or A.F. as a Percentage of Live Body Weight in Male and Female Meat-Type Chicken at 10, 17, 24, 31, 38 and 45 Days of Age (Experiment #1).

	MALE						Level[a] of Significance
Age (day)	10	17	24	31	38	45	
No. of Birds	5	5	5	5	5	5	
Feed Intake (g/d/b)	37	60	89	110	130		
Live Body (g)	101. (± 16.2)	240.5 (±34.3)	470.8 (±54.0)	816.0 (±76.3)	1274. (±197.7)	1659 (±94.8)	Q**
Breast Muscles (g)	2.9 (±0.54)	10.6 (±7.1)	23.1 (±3.83)	46.23 (±9.40)	70.5 (±9.37)	92.64 (±9.37)	Q**
Breast Muscles (g) / Live Body (g) x100	2.9 (±0.40)	4.4 (±.41)	4.9 (±0.46)	5.7 (±0.37)	5.6 (±0.75)	5.6 (±0.30)	Q**
Leg Muscles	2.54 (±1.10)	6.1 (±0.86)	14.1 (±7.32)	27.7 (±7.83)	41.8 (±7.0)	60.64 (±3.39	Q**
Leg Muscles (g) / Live Body (g) x100	2.5 (±0.60)	2.5 (±0.20)	3.0 (±0.24)	3.4 (±0.18)	3.3 (±0.35)	3.7 (±0.11)	
A.F.	0.14 (±0.06)	1.54 (±1.78)	3.5 (±1.45)	7.7 (±8.68)	12.96 (±8.92)	22.85 (±8.92)	Q**
A.F. (g) / Live Body (g) x100	0.14 (±0.07)	0.64 (±0.30)	0.73 (±0.26)	0.95 (±0.18)	1.46 (±0.84)	1.38 (±0.51)	Q**

	FEMALE						Level[b] of Significance
Age (day)	10	17	24	31	38	45	
No. of Birds	5	5	5	5	5	5	
Feed Intake (g/d/b)	36	57	81	99	110		
Live Body (g)	102.6 (± 17.9)	232. (±28.0)	478.6 (±43.7)	752.0 (±56.9)	903.0 (±170.5)	1340 (±95.0)	Q**
Breast Muscles (g)	3.12 (±1.10)	11.0 (±1.78)	28.7 (±3.41)	41.5 (±1.43)	50.4 (±12.30)	88.2 (±17.7)	Q**
Breast Muscles (g) / Live Body (g) x100	3.0 (±0.70)	4.7 (±.44)	5.14 (±0.34)	5.5 (±0.44)	5.6 (±0.61)	6.3 (±0.30)	Q**
Leg Muscles (g)	2.28 (±0.72)	6.05 (±1.23)	14.3 (±1.51)	24.15 (±1.47)	28.7 (±5.68)	45.23 (±8.49)	Q**
Leg Muscles (g) / Live Body (g) x100	2.2 (±0.38)	2.6 (±0.22)	3.0 (±2.20)	3.2 (±0.30)	3.2 (±0.22)	3.4 (±0.23)	Q**
A.F. (g)	0.30 (±0.17)	1.50 (±0.37)	4.6 (±2.95)	7.32 (±2.17)	11.50 (±2.69)	20.3 (±7.56)	Q**
A.F. (g) / Live Body (g) x100	0.29 (±0.17)	0.67 (±0.15)	0.95 (±0.51)	0.98 (±0.35)	1.29 (±0.23)	1.53 (±0.54)	Q**

1 Means (± S.D.).

a Significance level of quadratic ($p < 0.01$ = Q**) in male

b Significance level of quadratic ($p < 0.01$ = Q**) in female

Table 8. Test of Significance of Deviation From Linear Regression on Body Weight (Body Wt.), Breast Muscles (Pectoralis Superficial + Pectoralis Subclavis From Right Side), Leg Muscles (Gastrocnemius + Peroneous Longus From Leg), and Breast Muscles, Leg Muscles or Abdominal Fat Weight as a Percentage of Body Wt. (Arcsin $\sqrt{\%}$) (Experiment #1).

Source of Variation	Deviation from Regression (Mean Square)							
	MALE							
	d.f.	Body Wt.	Breast Muscle	Leg Muscle	A.F. (g)	Breast as % of Body Wt.	Leg As % of Body Wt.	A.F. as % of Body Wt.
Linearity	1	594628.6**	28119.2**	-2452.5**	-1322.12	182.0**	26.8**	141.5**
Quad.	1	8696648.0**	3075.5**	15132.6**	3158.1**	-1214.16**	-10.66**	- 79.85**
Error	27	9636.9	34.64	11.67	23.98	0.413	0.31	1.40

Cont. of above table.

	FEMALE						
d.f.	Body Wt.	Breast Muscle	Leg Muscle	A.F. (g)	Breast as % of Body Wt.	Leg As % of Body Wt.	A.F. as % of Body Wt.
	1693931.0**	- 104.8	1101.2**	-709.9**	157.1**	41.64**	97.14**
	3513234.2**	21706.5**	5158.9**	2077.6**	-100.18**	-25.88**	-48.24**
	12949.3	85.7	19.94	12.04	0.65	0.21	1.06

**p < 0.01
*p < 0.05

Table 9. Moisture, Lipid and Protein Content of Breast Muscles, Leg Muscles, and Whole Body In Male and Female Meat-Type Chickens at 10, 17, 24, 31, 38 and 45 Days of Age (Experiment #1)

Tissues	Male Age (day) 10	17	24	31	38	45	Level[a] of Significance
Whole Body							
% Moisture	73.7	71.4	69.4	69.7	68.7	66.7	L **
% Lipid	5.9	8.3	8.6	7.8	8.1	10.9	L *
% Protein	18.4	18.0	19.0	20.2	20.1	18.2	NS
Brest Muscles[2]							
% Moisture	79.1	76.7	76.0	75.7	74.1	74.0	L ***
% Lipid	1.8	0.9	1.2	0.8	1.1	0.7	NS
% Protein	17.7	21.5	21.4	21.6	23.5	23.5	L **
Leg Muscles[3]							
% Moisture	78.3	77.9	77.0	76.8	76.0	75.8	L ***
% Lipid	3.3	2.3	2.6	3.6	4.1	3.4	NS
% Protein	16.9	17.4	19.0	18.4	19.0	20.0	L ***

Tissues	Female Age (day) 10	17	24	31	38	45	Level[b] of Significance
Whole Body							
% Moisture	72.8	69.5	68.7	68.1	66.6	64.5	L ***
% Lipid[1]	6.2	9.4	8.7	9.7	10.7	13.2	L **
% Protein[1]	18.4	18.3	19.1	19.5	20.0	19.6	NS
Brest Muscles[2]							
% Moisture	77.5	76.0	76.0	75.8	73.9	74.5	L ***
% Lipid	1.8	1.3	1.4	1.2	1.2	1.1	L **
% Protein	19.5	21.4	21.5	20.5	24.0	23.5	L **
Leg Muscles[3]							
% Moisture	77.7	76.8	76.6	76.8	75.8	75.4	L ***
% Lipid	4.0	3.2	3.6	3.5	4.0	4.1	NS
% Protein	17.0	18.1	18.4	18.1	19.1	19.7	L ***

1 % lipid or % Protein on wet basis.
2 Pectoralis superficial + pectoralis subclavis muscles from right side.
3 Gastrocnemius + peroneous longus muscles from right leg.
a Significance level for linearity ($p < 0.01$ = L ***, $P < 0.05$ = L**, $P < 0.10$ = L*) or not significance (NS) for male chickens.
b Significance level for linearity ($p < 0.01$ = L ***, $P < 0.05$ = L**, $P < 0.10$ = L*) or not significance (NS) for female chickens.

Table 10. Test of Significance of Deviation From Linear Regression on % Moisture (Arcsin $\sqrt{\%}$), % Lipid (Arcsin $\sqrt{\%}$) and % Protein (Arcsin $\sqrt{\%}$) on Whole Body, Breast Muscle, and Leg Muscles (Experiment #1)

Source of Variation	Deviation from Regression (Mean Square)									
	MALE									
	d.f.	Whole Body			Breast Muscles			Leg Muscles		
		$\%H_2O$	%Lipid	%Pro.	$\%H_2O$	%Lipid	%Prot.	$\%H_2O$	%Lipid	%Prot.
Linear	1	10.18***	59.25	0.01	7.132***	2.70	9.1**	2.24***	1.18	3.21***
Error	4	0.243	12.80	0.583	0.453	0.202	0.667	0.016	0.968	0.148
Total	5									

Cont. of above table. FEMALE

d.f.	Whole Body			Breast Muscles			Leg Muscles		
	$\%H_2O$	%Lipid	%Pro.	$\%H_2O$	%Lipid	%Prot.	$\%H_2O$	%Lipid	%Prot.
	14.05**	21.28**	1.18	3.46***	1.355*	4.84	1.33	0.256	2.06**
	0.231	2.02	4.116	0.116	0.112	0.582	0.047	0.304	0.079

*** $p < 0.01$
** $p < 0.05$
* $p < 0.01$

Table 11. Fractional Accretion Rate (%/day) or Percent Change of Body as a Percentage of Protein or Fat in Male and Female Meat-Type Chicken at 13, 20, 27, 34 and 41 Days of Age (Experiment #1)

	Age (day)					
	Male					Level[a] of Significance
	13	20	27	34	41	
FAR (%/day) In:						
Whole Body	11.4	9.9	8.5	6.2	2.4	L**
Breast	18.1	10.5	9.6	7.1	3.9	L**
Leg	12.1	12.6	8.6	3.6	6.1	L*
% in Whole Body as						
% Protein	17.7	20.0	21.8	19.9	20.1	Q*
%Fat	10	8.9	6.7	8.6		Q*
	Female					
FAR (%/Day) In:						
Whole Body	11.6	9.8	6.6	3.0	5.2	L*
Breast	16.9	11.0	6.6	5.0	6.9	L*
Leg	13.6	11.7	7.1	3.2	6.8	L*
% in Whole Body as						
%Protein	20.0	18.9	20.2	22.5	18.8	NS
%Fat	11.9	8.0	11.4	15.7	18.4	Q*

1. Fractional accretion rate(%/day)= change in protein/average pool of protein X100
2. Perctoralis superficial + pectoralis subclavis muscle from right side.
3. Gastrocnemius + peroneous longus muscle from right side.
4. %Δ in whole body as % protein or%fat or $\frac{\Delta \text{ in weight of protein or fat in whole body over a 7 day}}{\Delta \text{ in weight of whole body over a 7 day}} \times 100$

a significance level for linearity ($p < 0.01$ = L*, $p < 0.05$ = L*, $p < 0.10$ = L) or quadratic ($p < 0.01$ = Q*, or not significance (NS).

Table 12. Test of Significance of Deviation From Linear Regression for FAR (Arcsin $\sqrt{\%}$), (Experiment #1).

Source of Variation	df	Deviation From Regression (Mean Square)					
		Male			Female		
		Whole Body	Breast Muscle	Leg Muscle	Whole Body	Breast Muscle	Leg Muscle
Linear	1	65.53***	96.34***	48.27**	47.2**	60.17**	51.7*
Error	3	2.387	2.007	6.97	5.22	7.32	9.44
Total	4						

*** $p < 0.01$
** $p < 0.05$
* $p < 0.10$

Table 13. Test of Significance of Deviation from Regression for % $\Delta(\text{Arcsin}\sqrt{\%})$ in Whole Body As a Percentage of Protein or Fat (Experiment #1)

Source of Variation	df	Deviation From Regression (Mean Square)			
		Male		Female	
		Protein	Fat	Protein	Fat
Linear	1	-85.6*	-202.4*	1.44	-83.9
Quadratic	1	119.2*	279.1*	-0.67	121.4*
Error	2	0.63	2.21	1.85	5.3
Total	4				

* $p < 0.05$

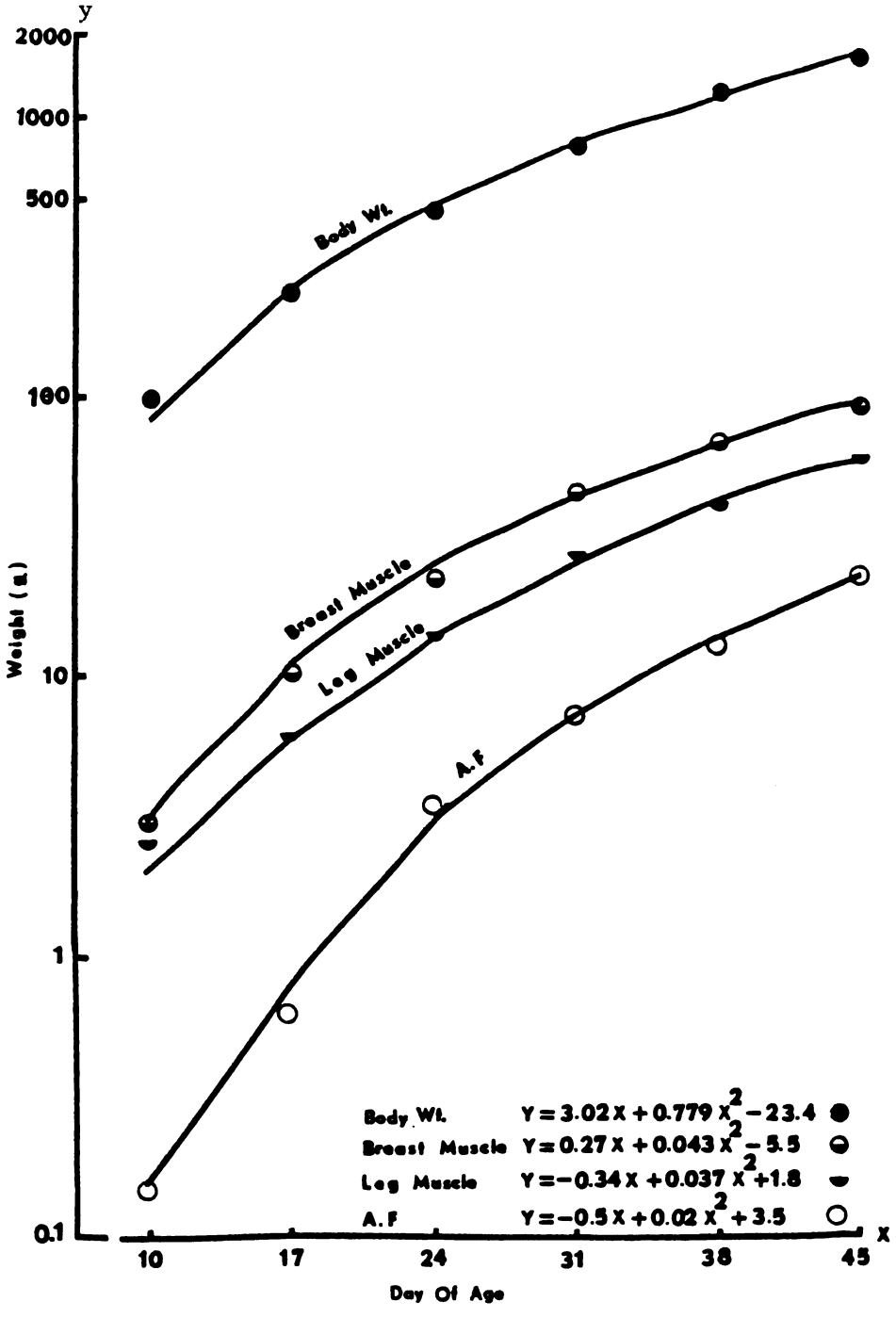


Figure 4 Relationship of body weight (Body Wt.), breast muscles (pectoralis superficial + pectoralis subclavis muscles from right side), leg muscles (gastrocnemius + peroneous longus muscles from right leg and abdominal fat (A.F.) with age of male meat-type chickens (Experiment #1). Where x = Day of age, y = weight (g).

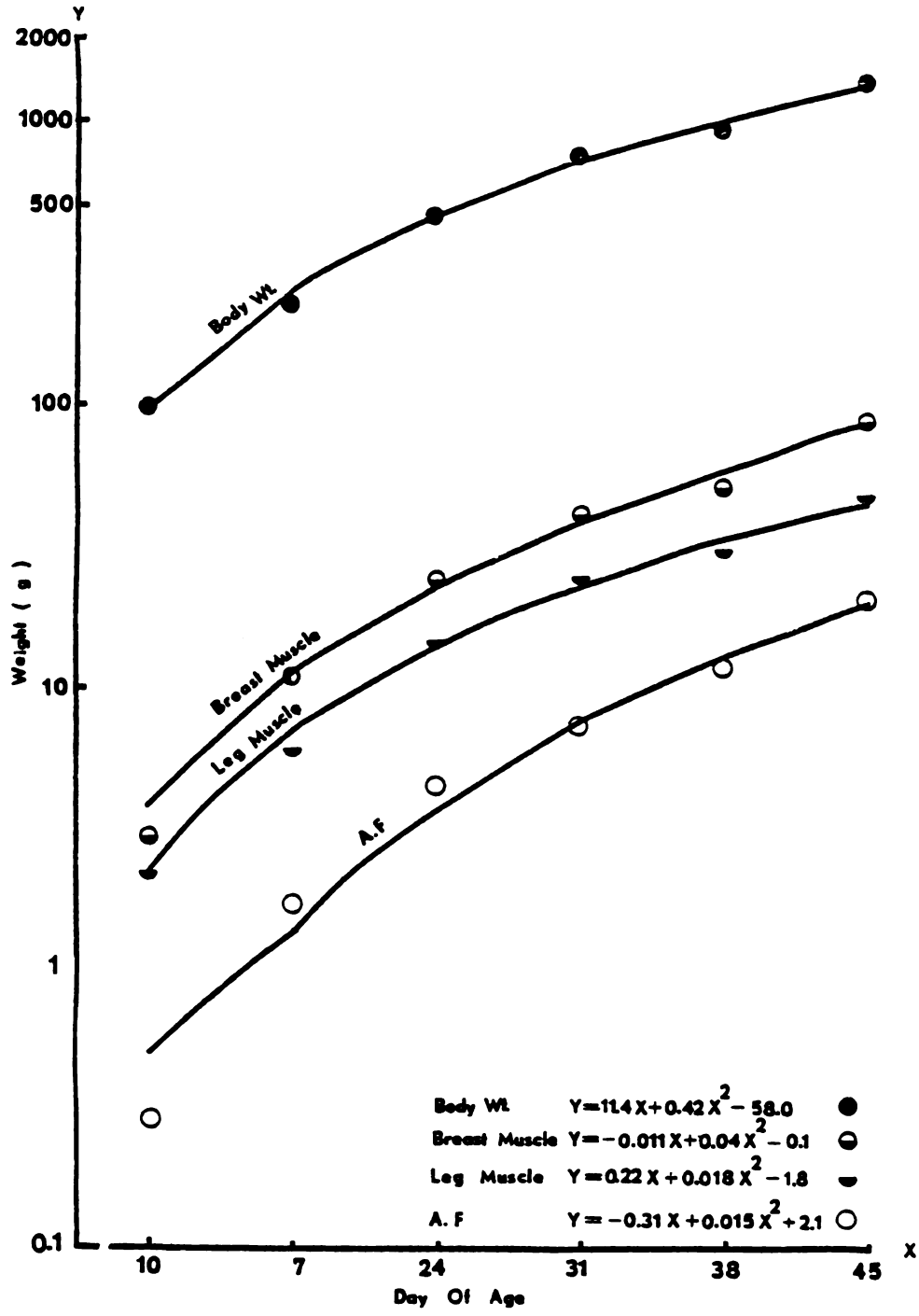


Figure 5 Relationship of body weight (Body wt.), breast muscles (pectoralis superficial + pectoralis subclavis muscles from right side), leg muscles (gastrocnemius + peroneous longus muscles from right leg) and abdominal fat (A.F.) with age of female meat-type chickens (Experiment #1) where, x = day of age, y = weight (g).

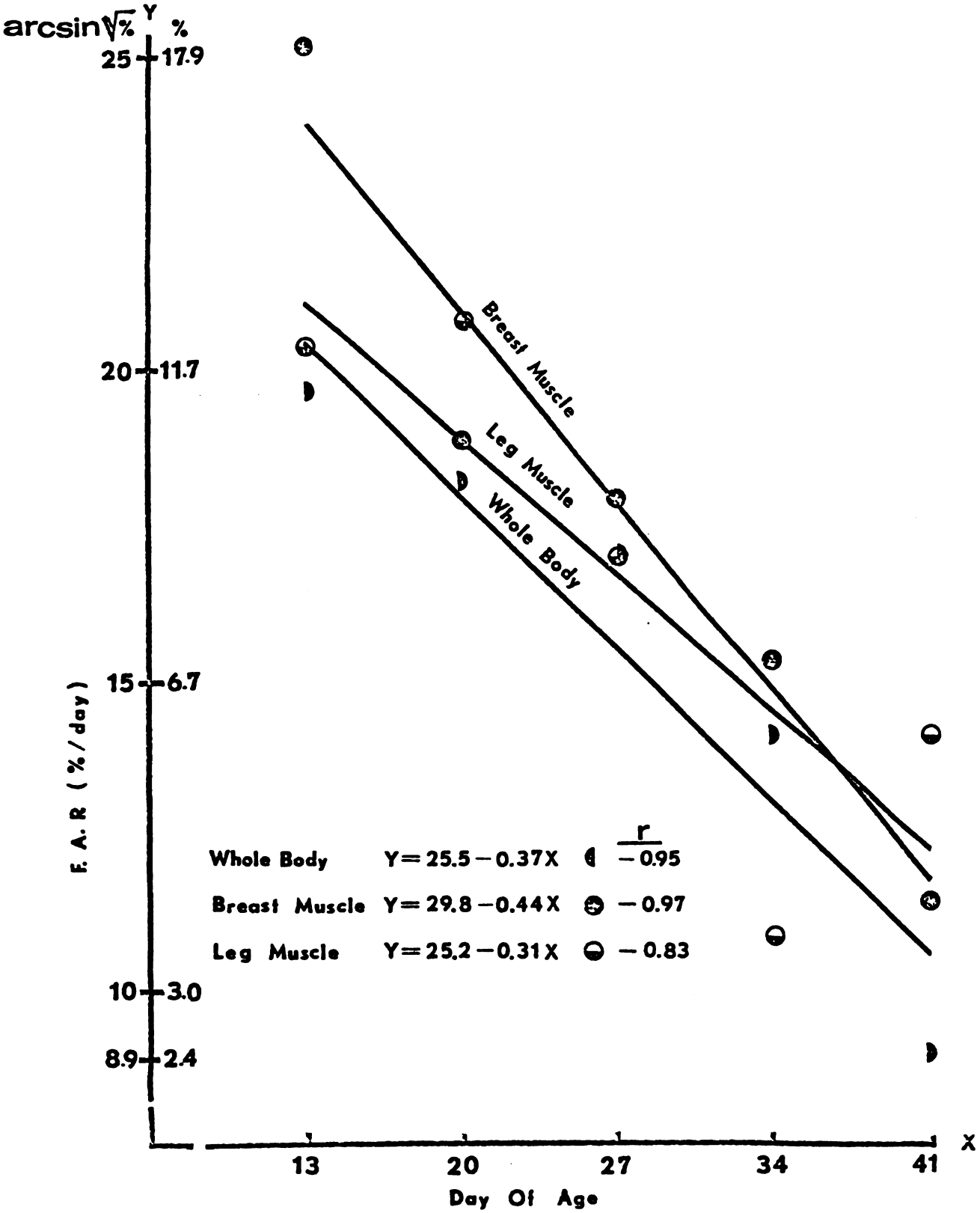


Figure 6 Relationship of fractional accretion rate (F.A.R.) as %/day with age in whole body, breast muscles and leg muscles in male meat-type chicken (Experiment #1). Where X=Day of age; Y=F.A.R. (arcsin$\sqrt{\%}$).

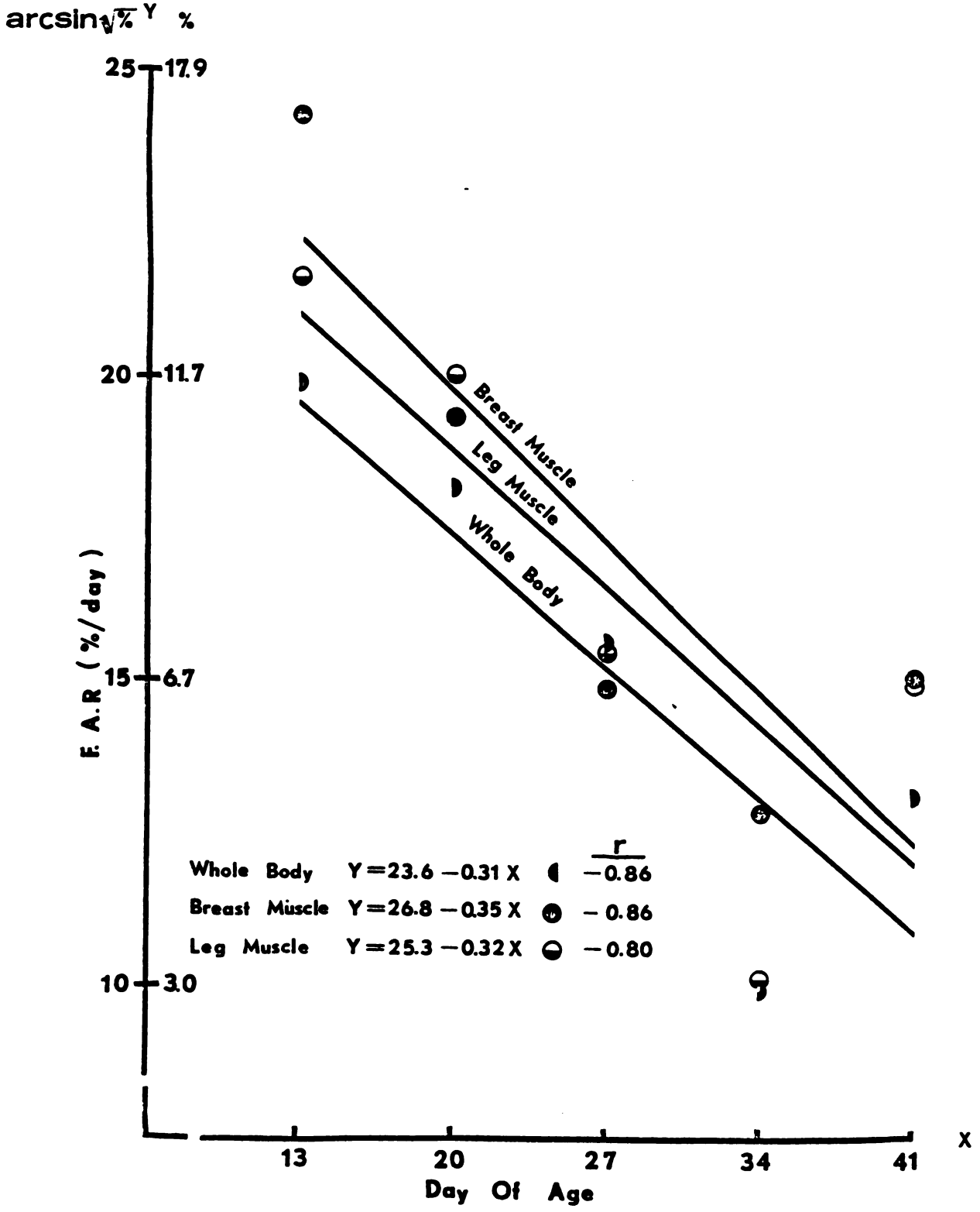


Figure 7 Relationship of fractional accretion rate (F.A.R.) as a %/day with age in whole body, breast muscles and leg muscles in female meat-type chicken (Experiment #1). Where X=Day of age; Y=F.A.R. (arcsin$\sqrt{\%}$).

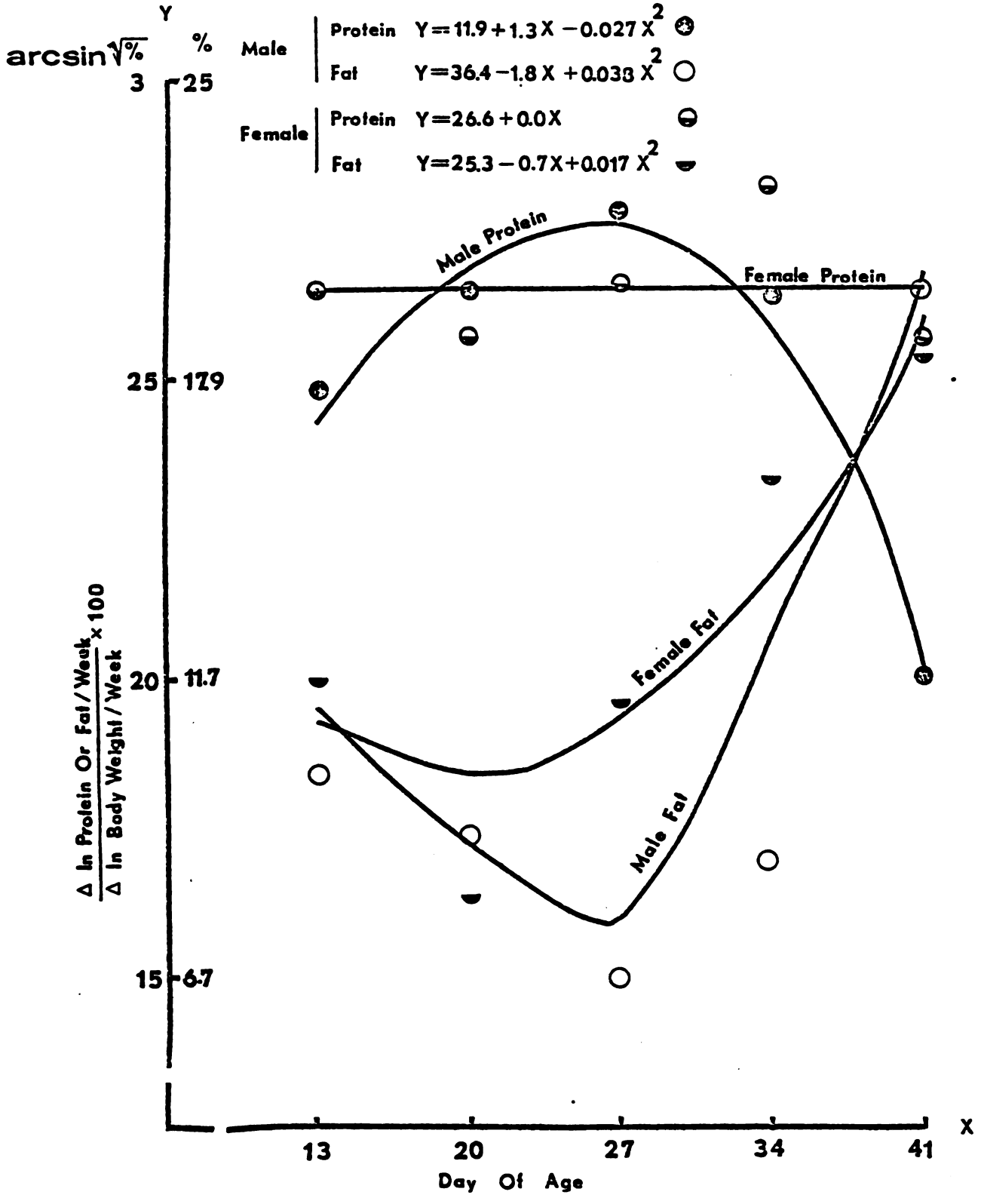


Figure 8 Relationship of age (X) to the ratio of a change in protein or fat relative to the change in body weight (Y) in male and female meat-type chicken. (Experiment #1) Where X=Day of age;

$$Y=\frac{\Delta \text{in protein or fat (g)/week}}{\Delta \text{in whole body (g)/week}} \times 100 \ (\arcsin\sqrt{\%}).$$

decreased quadratically ($p < 0.05$), while the fat gain sharply increased ($p < 0.05$) in the male chickens as they aged from 13 to 41 days (Table 13 and Figure 8). The protein gain per unit of weight gain in the whole body did not change, but fat deposition increased in the female as the animal aged from 20 days to 41 days (Table 13 and Figure 8).

Experiment #2

The live body weights, breast muscles (pectoralis superficial + pectoralis subclavis from right side), leg muscles (gastrocnemius + peroneous longus muscles from right leg) and abdominal fat (A.F.) weights and breast muscles, leg muscles and A.F. as a percentage of body weight increased quadratically ($p < 0.01$) as the birds grew older (Table 15 and Figures 9 and 10). The breast and leg muscles as a percentage of body weight were 2.7, 5.5 and 2.1, 4.0 at 12 and 82 days of age, respectively in the male Single Comb White Leghorn (SCWL) chickens (Table 14). This indicates that the breast and leg muscles were growing at a faster rate than whole body. Similarly the breast and leg muscles were growing at a rate faster than whole body in female (SCWL) chickens (Table 14). The A.F. as a percentage of whole body were 0.03, 0.82 and 0.03, 1.42 in the male and female chickens at 12 and 82 days of age, respectively (Table 14). This reveals that the A.F. is growing at a rate greater than whole body, breast muscles or leg muscles in both male and female chicken. Although, the A.F. growth rate was faster in the female than male chicken (Table 14).

The % moisture and % protein in the whole body, breast and leg muscles were increased linearly ($p < 0.05$) as the male or female

chickens aged from 12 to 82 days of age (Tables 16 and 17). The % lipid in the whole body was increased linearly in male ($p < 0.05$) and female ($p < 0.01$) chickens as they grew older (Table 17). The % lipid was constant in the breast and leg muscles as the animal aged (Table 16). The individual live body weights, breast muscles, leg muscles and A.F. weights are presented in Appendix B, Table 2.

The effect of aging on fractional accretion rate of protein (FAR) as %/day in the whole body, breast muscles, leg muscles and the deposition of protein or fat per unit of weight gain are shown in Table 17. The numerical FARs were 9.0, 15.8, and 12.5 %/day in the whole body, breast muscles and leg muscles, respectively, at 15 days of age in male Single Comb White Leghorn (SCWL) chickens. The FARs decreased continuously in the whole body and leg muscles as the male chickens aged (Table 18). The linear regression (Table 19 and Figure 11) indicated that the FARs were correlated with age, $r = -0.91$, $r = -0.81$ and $r = -0.85$ for the whole body, breast and leg muscles, respectively, in the male chickens. But these regression analysis did not include the FARs at 15 days of age which were highly above the regression lines (Figure 11). The FARs in the breast muscles or whole body did not significantly change in the female chickens as they aged (Table 19). However, the FAR in the whole body or breast muscles showed a

Table 14. Feed intake, live body weight and weights of pectoralis superficial + pectoralis subclavis (breast muscle from right side), gastrocnemius + peroneous longus (leg muscles from right leg) and abdominal fat (A.F.) or Breast muscles, Leg muscles or A.F. as a percentage of live body weight in male and female Single Comb White Leghorn (SCWL) chickens at 12, 19, 26, 33, 47, 61 and 82 days of age (Experiment #2).

	Male							
Age (day)	12	19	26	33	47	61	82	Level of significance[a]
No. of birds	4	4	3	6	6	4	3	
Feed intake (g/b/d)	17.9	26.4	39.6	50.0	54.0	62.0		
Live body(g)	70.7 (±6.13)[5]	129.0 (±30.0)	186.0 (±42.7)	286.0 (±55.2)	520.0 (±53.6)	772.0 (±127.3)	1200.0 (±88.5)	Q**
Breast muscles[2](g)	1.88 (±0.24)	4.94 (±1.77)	7.44 (±2.35)	10.3 (±3.72)	25.1 (±3.60)	37.44 (±9.35)	66.6 (±9.01)	Q**
Breast Muscle(g)X100 / Live body(g)	2.68 (±0.21)	3.75 (±0.53)	3.93 (±0.38)	3.7 (±0.81)	4.82 (±0.41)	4.8 (±0.50)	5.5 (±0.38)	Q**
Leg Muscles[3](g)	1.46 (±0.24)	3.4 (±0.77)	5.1 (±1.26)	7.23 (±1.94)	17.2 (±2.55)	25.56 (±4.86)	47.31 (±6.39)	Q**
Leg Muscle(g)X100 / Live body(g)	2.1 (±0.21)	2.7 (±0.21)	2.7 (±0.10)	2.8 (±0.26)	3.3 (±0.19)	3.3 (±0.21)	4.0 (±0.25)	Q**
A.F.[4](g)	0.02 (±0.017)	0.23 (±0.16)	0.45 (±0.06)	1.18 (±0.43)	1.74 (±1.16)	5.15 (±2.46)	9.67 (±0.83)	Q**
A.F.(g)X100 / Live body(g)	0.03 (±0.018)	0.18 (±0.10)	0.25 (±0.03)	0.45 (±0.95)	0.34 (±0.23)	0.72 (±0.46)	0.82 (±0.12)	Q**

	Female							
Age (day)	12	19	26	33	47	61	82	Level of Significance[b]
No. of birds	3	3	4	1	1	3	4	
Feed intake (g/b/d)								
Live body(g)	78.0 (±4.57)	100.0 (±22.3)	159 (±37.87)	285.0 (-)	420 (-)	600.0 (±80.0)	911.0 (±102.3)	Q**
Breast muscles[2](g)	2.45 (±0.36)	3.57 (±1.85)	6.2 (±2.03)	14.0 (-)	18.6 (-)	31.65 (±6.39)	49.2 (±10.41)	Q**
Breast Muscle(g)X100 / Live body (g)	3.2 (±0.42)	3.4 (±1.1)	3.8 (±0.56)	4.9 (-)	4.4 (-)	5.2 (±0.35)	5.4 (±0.57)	Q**
Leg Muscles[3](g)	1.58 (±0.19)	2.54 (±1.28)	4.17 (±1.11)	8.56 (-)	13.86 (-)	19.58 (±3.91)	29.91 (±4.05)	Q**
Leg Muscle(g)X100 / Live Body(g)	2.0 (±0.21)	2.5 (±0.70)	2.6 (±0.25)	3.0 (-)	3.3 (-)	3.2 (±0.21)	3.3 (±0.13)	Q**
A.F.[4](g)	0.017 (±0.012)	0.21 (±0.18)	0.62 (±0.67)	0.69 (-)	2.53 (-)	5.93 (±1.51)	12.37 (±5.1)	Q**
A.F.(g)X100 / Live body(g)	0.03 (±0.015)	0.22 (±0.21)	0.42 (±0.45)	0.25 (-)	0.6 (-)	1.0 (±0.40)	1.42 (±0.34)	Q**

[1] Feed intake for mixed sex SCWL chicken (g/b/d).

[2] Pectoralis superficial + pectoralis subclavis muscles from right side.

[3] Gastrocnemius + Peroneous longus muscles from right leg

[4] Abdominal fat (A.F) excised from after the gizzard.

[5] Mean (± S.D.)

[a] Quadratic significance level (P <0.01=Q**) for Male SCWL.

[b] Quadratic significance level (P <0.01=Q**) for Female SCWL.

Table 15. Test of significance of deviation from linear or curvilinear regresson on body weight (Body wt.), breast muscles (pectoralis superficial + pectoralis subclavis from right side), leg muscles (gastrocnemius + peroneous longus muscles from right leg), breast or leg muscle's weight as a percentage of body wt. (arcsin $\sqrt{\%}$). Experiment #2).

Source of Variation	d.f.	Deviation from regression (Mean Square)						
		Male						
		Body wt.	Breast Muscles	Leg Muscles	A.F.	As % of Body wt.		
						Breast Muscle	Leg Muscle	A.F.
Linearity	1	1325096.0**	924.0**	126.8**	-121.6**	72.39**	35.12**	72.06**
Quad.	1	2189436.6**	10227.7**	5370.36**	370.8**	-33.11**	-13.06**	-33.5**
Error	27	4152.1	23.5	8.41	1.25	0.74	0.18	0.94
Total	29							

** P <0.01

cont. of above table

	Female						
	Body wt.	Breast Muscles	Leg Muscles	A.F.	As % of Body wt.		
					Breast Muscle	Leg Muscle	A.F.
	988504.0**	2407.2**	1232.3**	-189.6**	67.13**	36.675**	133.18**
	995932.9**	3916.7**	1068.6**	629.6**	-38.67**	-24.24**	47.30**
	3356.0	28.25	5.91	5.27	0.77	0.315	0.68

Table 16. Moisture, fat, and protein in whole body, pectoralis superficial+pectoralis subclavis muscles and gastrocnemius + peroneous longus muscles in male and female (SCWL) Chicks. (Experiment #2).

	Male Age (day)							
	12	19	26	33	47	61	82	Level of Significance[a]
Whole body								
% Moisture	72.6	71.3	71.1	71.4	60.5	58.3	55.4	L**
%Fat[1]	4.8	5.7	5.0	3.9	6.1	6.1	12.4	L*
% Protein[1]	18.0	19.2	20.8	19.0	26.2	27.9	26.8	L**
Breast Muscle[2]								
% Moisture	80.7	75.2	75.6	73.9	73.9	71.7	71.3	L*
% Fat	0.9	0.7	0.44	1.2	0.7	0.9	1.0	NS
% Protein	17.2	22.7	21.6	22.4	22.8	24.4	25.6	L*
Leg Muscle[3]								
% Moisture	78.2	76.8	76.2	76.6	75.1	74.5	70.9	L**
% Fat	2.9	2.6	2.7	3.0	2.3	2.4	3.2	NS
% Protein	17.2	18.9	19.5	19.5	21.0	21.6	21.9	L**

	Female Age (day)							
	12	19	26	33	47	61	82	Level of Significance[a]
Whole body								
% Moisture	72.2	71.3	71.6	69.7	60.	58.2	55.0	L**
% Fat[1]	3.9	5.7	3.6	4.9	6.5	8.5	11.8	L**
% Protein[1]	18.2	19.2	19.6	20.9	27.4	27.5	28.9	L**
Breast Muscle[2]								
% Moisture	78.6	76.8	75.4	76.0	74.	70.6	70.0	L**
% Fat	0.8	1.1	1.4	0.6	0.7	1.4	1.44	NS
% Protein	17.0	21.9	22.7	23.5	24.3	24.1	24.6	NS
Leg Muscle[3]								
% Moisture	77.1	76.6	76.1	77.6	75.9	75.5	74.2	L*
% Fat	3.1	2.9	3.4	2.3	3.1	3.0	2.7	NS
% Protein	17.5	19.2	19.5	19.2	20.4	21.0	22.0	L**

[1]% Fat or % Protein on wet basis.

[2]Pectoralis superficial + pectoralis subclavis muscles from right side of breast.

[3]Gastrocnemius + peroneous longus muscles from right leg.

[a]Significance level for linearity ($P < 0.01$ = L**, $P < 0.05$ = L*) or not significant NS.

Table 17. Test of significance of deviation from linear regression on % moisture (arcsin $\sqrt{\%}$), % lipid (arcsin $\sqrt{\%}$), and % protein (arcsin $\sqrt{\%}$) on whole body, breast muscle and leg muscles. (Experiment #2).

Source of variation	d.f.	Deviation from linear regression (Mean Square)								
		Male								
		Whole body			Breast muscles			Leg muscles		
		$\%H_2O$	%lipid	%prot.	$\%H_2O$	%lipid	%Prot.	$\%H_2O$	%lipid	%prot.
Linear	1	107.1**	39.0*	38.92**	18.67*	0.166	14.42*	13.13**	0.013	7.30**
Error	5	2.37	4.26	1.72	1.59	2.76	1.35	0.22	0.40	0.24
Total	6									

** P <0.01
* P <0.05

cont. of above table

		Female								
		Whole body			Breast muscles			Leg muscles		
		$\%H_2O$	%lipid	%Prot.	$\%H_2O$	%lipid	%Prot.	$\%H_2O$	% lipid	%Prot.
		108.6**	54.23**	53.14**	40.92**	1.00	11.0	2.30*	0.114	5.88**
		1.98	1.55	1.60	0.34	1.03	2.0	0.18	0.51	0.121

Table 18. Fractional accretion rate (%/day) in the whole body, breast muscles and leg muscles or percent change as a percentage of protein or fat in the whole body in male and female Single Comb White Leghorn (SCWL) Chickens at 15, 22, 29, 40, 54 and 71 days of age. (Experiment #2).

	Age (day)												
	Male						Level of Significance[a]	Female					Level of Significance[a]
	15	22	29	40	54	71		15	22	29	40	54	71	
FAR[1] (%day in:														
Whole body	9.0	6.4	4.8	6.1	3.1	1.9	L**	4.3	6.7	8.9	4.5	2.5	2.2	NS
Breast Muscle[2]	15.8	5.1	5.1	6.1	3.3	2.9	NS	8.8	8.2	11.6	2.2	3.7	2.2	NS
Leg Muscle[3]	12.5	6.1	5.1	6.3	3.0	2.9	L*	7.8	7.2	9.6	3.8	2.7	2.2	L*
% change in whole body as[4]														
% Protein	20.1	24.9	15.6	35.	31.4	24.5	NS	22.7	20.3	22.5	41.1	27.7	31.6	NS
% Fat	6.7	3.4	1.8	8.8	6.1	23.8	Q*	12.1	--	6.5	9.9	13.2	18.2	Q*

[1]Fractional accretion rate (% day).

[2]Pectoralis superficial + pectoralis subclavis muscles from right side.

[3]Gastrocnemius + peroneous longus muscle from right leg.

[4]% change in whole body as % protein or % fat or $\frac{\text{change in weight of protein or fat in the whole body over a period}}{\text{change in the whole body weight over the same period.}} \times 100$

[a]Significance level for linearity ($P < 0.01$ = L**, $P < 0.05$ = L*) or Quadratic ($P < 0.05$ = Q*) or Not Significant (NS).

Table 19. Test of significance of deviation from linear regression for FAR (arcsin $\sqrt{\%}$). (Experiment #2).

Source of Variation	d.f.	Deviation from regression (mean Square)					
		Male			Female		
		Whole body	Breast muscle	Leg muscle	Whole body	Breast muscle	Leg muscle
Linear	1	50.3**	67.7	53.3*	29.8	75.0	61.73*
Error	4	1.772	13.24	4.94	6.89	11.29	4.28
Total	5						

** P <0.01
* P <0.05

Table 20. Test of significance of deviation from regression for % Δ (arcsin $\sqrt{\%}$) in whole body as a percentage of protein or fat. (Experiment #2).

Source of Variation	d.f.	Deviation from regression (Mean Square)			
		Male		Female	
		Protein	Fat	Protein	Fat
Linear	1	131.9	-395.2	165.1	-91.8
Quadratic	1	-89.72	622.5*	-112.8	140.1*
Error	3	23.52	16.52	22.28	5.52
Total	5				

* P <0.05

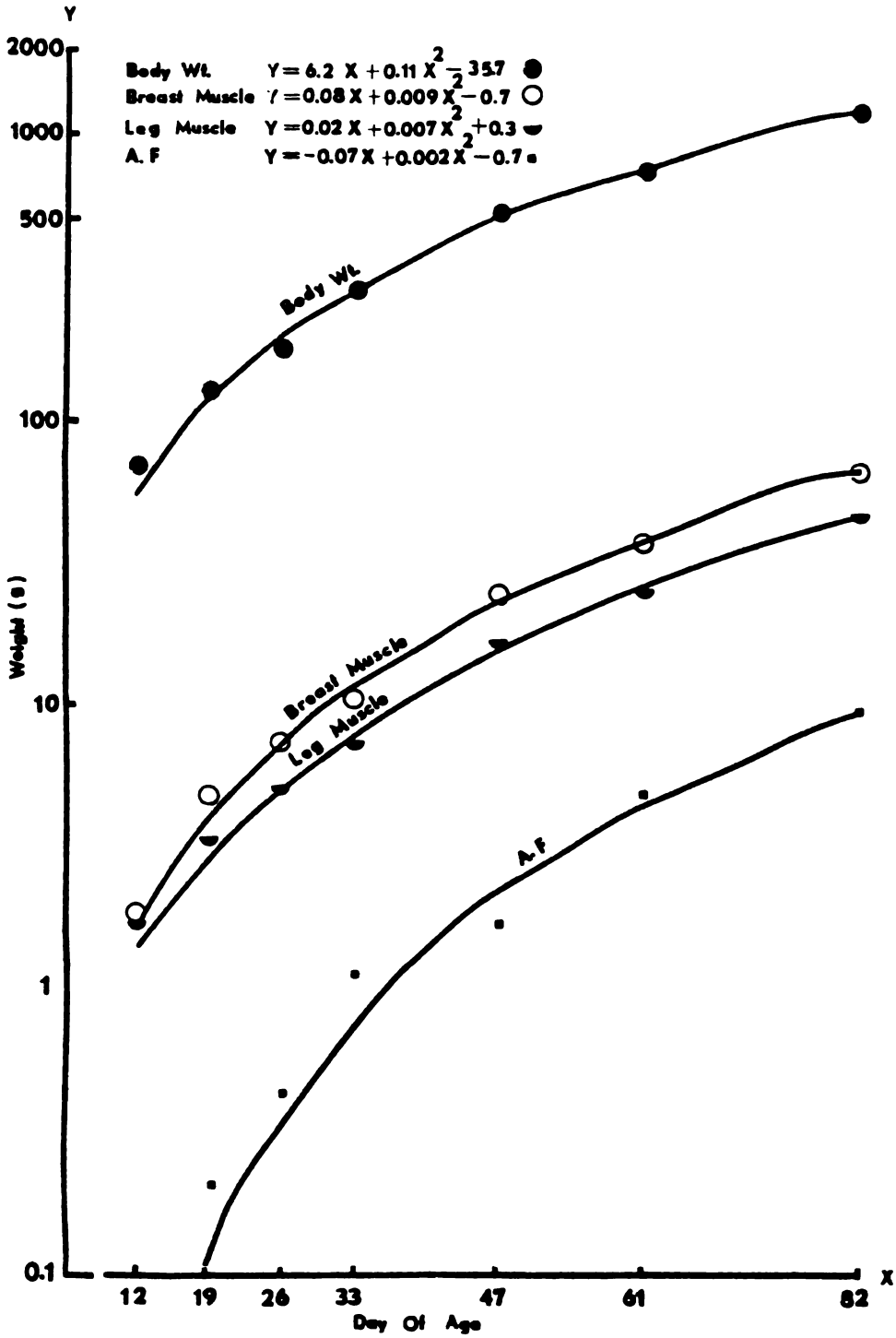


Figure 9 Relationship of body weight (Body wt.), breast muscles (pectoralis superficial & pectoralis subclavis muscles from right side), leg muscles (gastrocnemius & peroneous longous muscles from right leg) and abdominal fat (A.F.) with age of male Single Comb White Leghorn chickens (Experiment #2). Where X = Day of age; Y = Weight (g).

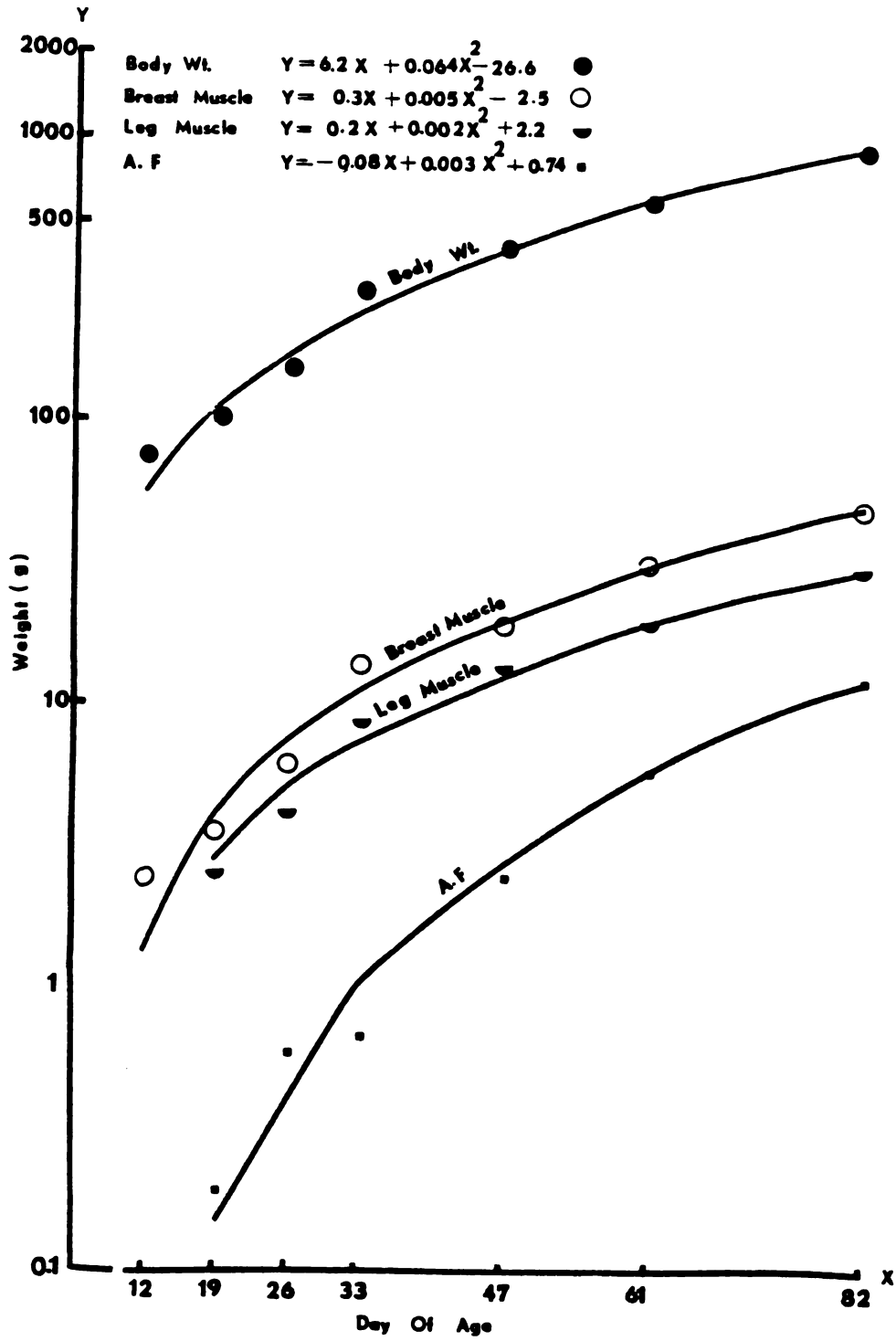


Figure 10 Relationship of body weight (Body wt.), breast muscles (pectoralis superficial & pectoralis subclavis muscles from right side), leg muscles (gastrocnemius & peroneous longus muscles from right leg) and abdominal fat (A.F.) with age of female Single Comb White Leghorn chickens (Experiment #2. Where X = Day of age, Y = Weight (g).

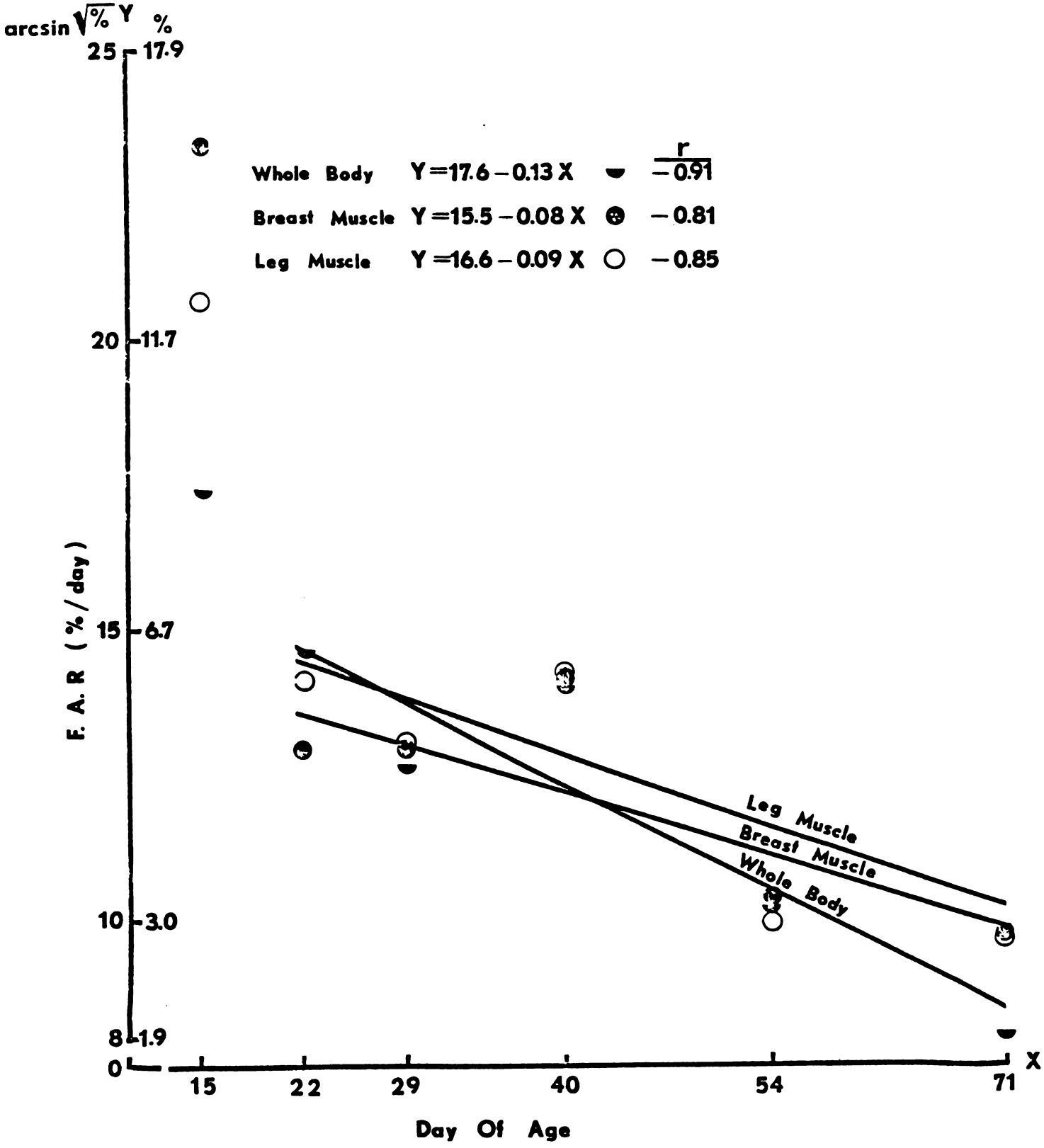


Figure 11 Relationship of fractional accretion rate (F.A.R) with age in whole body, breast muscles and leg muscles in male Single Comb White Leghorn chickens (Experiment #2). Where X=Day of age, Y=F.A.R. as %/day (arcsin$\sqrt{\%}$).

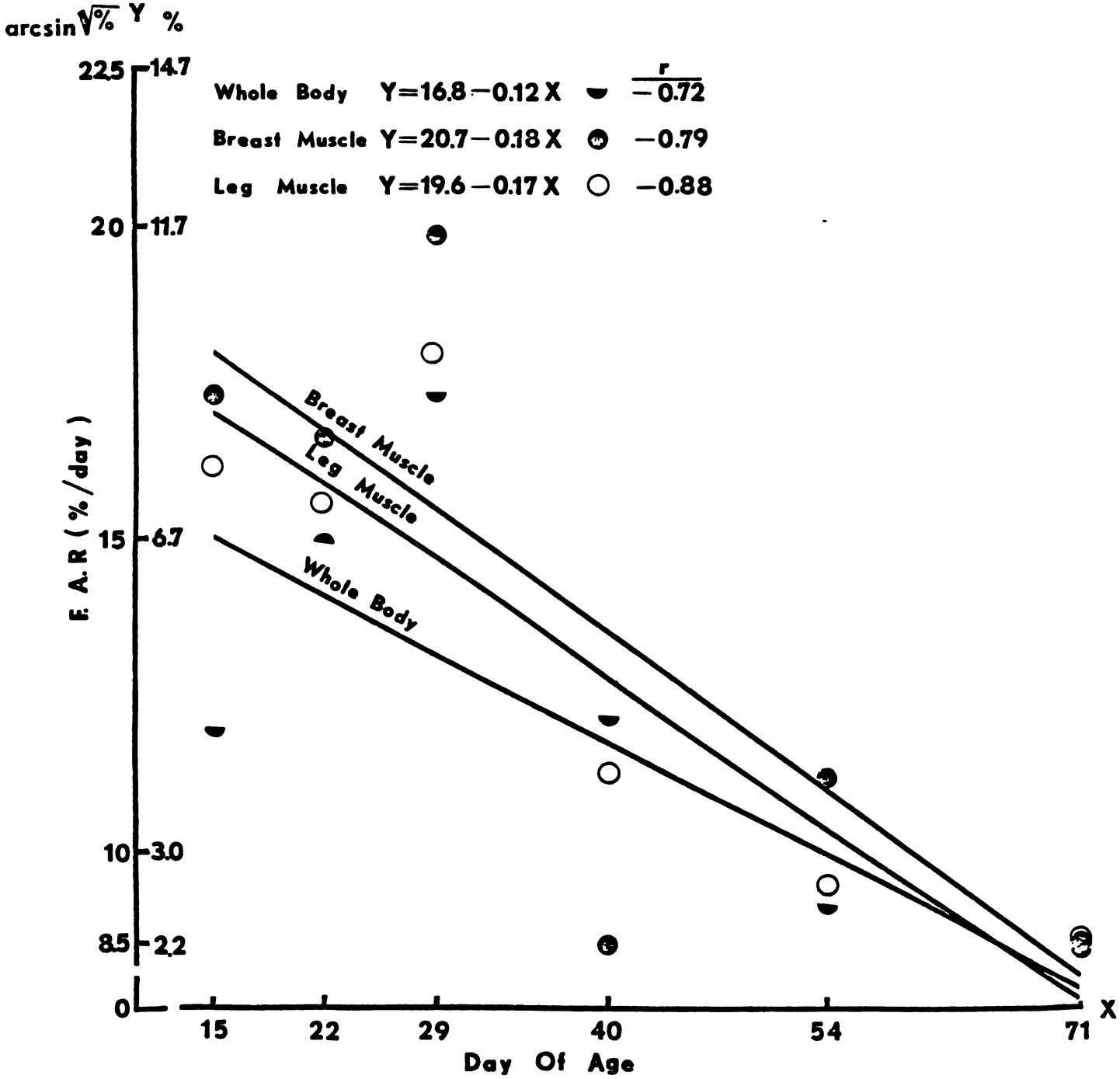


Figure 12 Relationship of fractional accretion rate (F.A.R.) with age in the whole body, breast muscles and leg muscles in female Single Comb White Leghorn chickens (Experiment #2). Where x = Day of age y = F.A.R as %/day (arcsin √%).

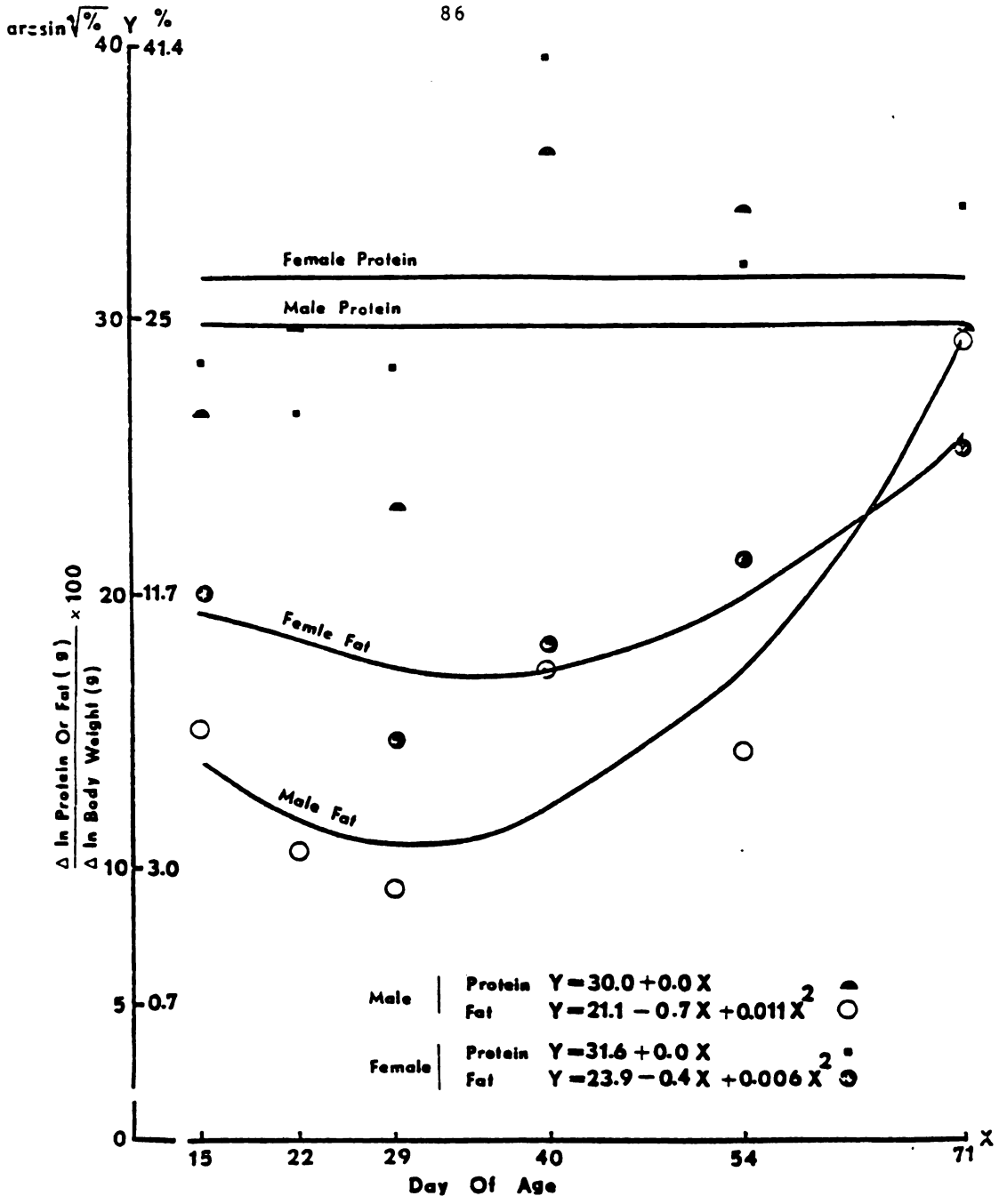


Figure 13 Relationship of age (x) to the ratio of a change in protein or fat (in 1, 2 or 3 weeks period) relative to the change in body weight (in 1, 2 or 3 weeks period) (Y) in male and female Single Comb White Leghorn (Experiment #2). Where X = Day of age,
$Y = \frac{\Delta \text{in protein or fat (g)/1, 2 or 3 weeks}}{\Delta \text{in whole body (g)/1, 2 or 3 weeks}} \times 100 \text{ (arcsin } \sqrt{\%}\text{)}$.

tendency to decline as the animal aged (Figure 12). The FAR in the leg muscles decreased significantly ($p < 0.05$) as the female chicken aged (Table 18). The numerical values of FARs for the whole body, breast and leg muscles were approximately similar in male and female chickens at 54 and 71 days of age (Table 18).

The protein deposition per unit of weight gain was constant relatively but variable in either male or female chickens as they aged (Tables 18 and 20). The protein contributed about 25% and 30% of weight change in male and female chickens, respectively (Figure 13). The fat gain per unit of weight gain was 6.7 and 24 percent in male, 12 and 18 percent in female chickens at 15 and 71 days of age, respectively (Table 18). The fat deposition per unit of weight gain increased quadratically in male ($p < 0.05$) and female ($p < 0.05$) chickens as they aged (Table 20 and Figure 13).

Experiment #3

The results for experiment #3 are presented in Table 21. The specific radioactivity (S.A.) of leucine remained fairly constant in the precursor pool of each type of muscles analyzed from chickens killed during the 30 minutes of radioactive infusion into the brachial vein (Tables 21 and 21). However the S.A. of leucine measured in the plasma was significantly higher at 20 minutes than 10 and 30 minutes of radioactive infusion and was higher than in the muscles (Table 21). Thus, the proposed infusion program (Table 3) produced a steady state level of leucine S.A. in the precursor pool of breast or leg muscles of chickens (Figure 14). This constant S.A. of leucine throughout the

Table 21. Leucine specific radioactivity in the precursor pool of breast muscles or leg muscles and plasma in response to infusion time. (Experiment #3).

S.A.[1] dpm/nmol. of leucine in precursor pool of	Infusion time (min.)				
	3	10	20	30	Ave.
Leg[2]	237.0[a](±67.0)[4]	220.0[a](±43.3)	357.0[a](±29.5)	271.0[a](±13.1)	271.0
Breast[3]	95.0[a](±38.2)	75.0[a](±17.7)	124.0[a](±28.7)	81.0[a](±4.3)	94.0
Plasma	4544.0[ab](±17.9)	479.0[a](±11.0	642.0[b](±6.08)	493.0[a](±31.1)	540.

[a,b]Means in the same row with different superscripts indicate significantly ($P < 0.01$) different specific activity from steady state.

[1]Specific radioactivity (dpm/nmol.)

[2]Leg (gastrocnemius + peroneous longus) muscles.

[3]Breast (Pectoralis superficial + Pectoralis subclavis) muscles.

[4]Mean (±S.E.)

Each datum is the average from 3 birds.

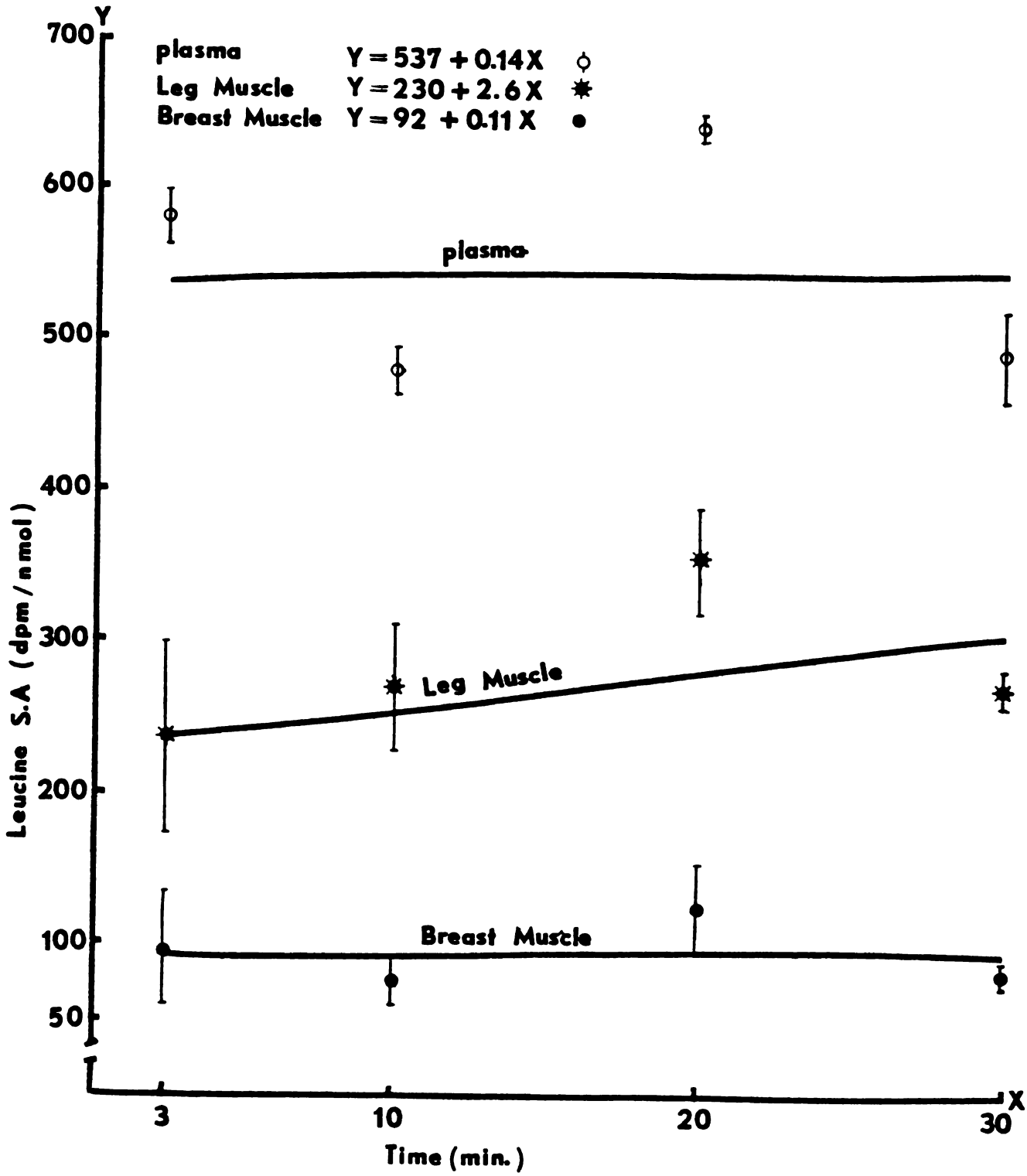


Figure 14 Time course of leucine specific radioactivity (S.A.) in the precursor pool of leg muscles, breast muscles and plasma (Experiment #3). Where X = Time of infusion in minutes, Y = Specific radioactivity (dpm/nmol).

experimental time (infusion time) could be used to calculate the synthesis rate of muscle proteins. The incorporation of the radioactive leucine into the muscle's protein must be measured at the end of the infusion program, and the percentage of leucine in the muscle's protein ws measured (Appendix B Table 3), in order to calculate the rate of protein synthesis. The individual leucine S.A. in the precursor pool of breast muscles, leg muscles and plasma of chickens at 3, 10, 20 and 30 minutes of experimental period are presented in Appendix B, Table 4.

Table 22. Analysis of Variance for Leucine Specific Radioactivity In Free Pool of Leg Muscles, Breast Muscles and Plasma (Experiment #3)

Source of Variation	d.f.	Mean Square Leg Muscle	Breast Muscle	Plasma
Treatment	3	11164.2	1462.9	16380.1*
Error	8	5556.3	1965.3	1087.6
Total	11			

*p <0.01

Experiment #4

The means of feed intake, empty crop body weight (E.C.B.Wt.), and weights of breast and, leg muscles, and the muscles' weight as a percentage of E.C.B.Wt. as well as the change in the muscles weights at different ages are shown in Table 23. The E.C.B.Wt. and the weights of the two muscles increased linearly (p <0.01) increased as the animal aged (Table 24 and Figure 15). The significant linear slopes revealed by statistical analysis indicated that, there were increases in weight

of the E.C.B.Wt., breast and leg muscles over each 4 day period used to obtain samples (Tables 23 and 24). The weights of breast muscles as a percentage of E.C.B.Wt. increased linearly ($p < 0.05$) from 5.0 to 5.7% during the time the chickens aged from 26 to 45 days. The weight of leg muscles as a percentage of E.C.B.Wt. was also increased linearly ($p < 0.01$) (Table 24). This increase in the muscle weight as a percentage of E.C.B.Wt. indicated that the muscles were growing at a rate faster than the whole body. Breast muscle weights over a 4 day period increased as the animals aged from days 26 to 45 (Figure 16). But the animals' aging had little or no effect on the leg muscles gain over a 4 day period (Figure 16). The percent moisture decreased ($p < 0.01$), % lipid and % protein increased ($p < 0.01$) in the whole body as the animal grew older (Table 26). However the % of moisture in the muscles remained constant as the chickens aged (Table 26). The % protein decreased ($p < 0.01$) in the breast muscles while it increased ($p < 0.01$) in the leg muscles of chickens aging from 26 to 45 days (Table 25). The percent lipid in the breast muscles was 0.6% at day 26 and increased linearly ($p < 0.05$) to about 1.0% at 45 days of age (Table 25). The lipid content in the leg muscles was 3.8% at day 26 and remained relatively constant to 45 days of age (Table 25).

The percent change of body as a percentage of protein or fat was higher at 34-38 days of age than 26-30 or 41-45 days (Table 27). This higher protein or fat deposition per unit of weight gain at 34-38 days indicated that, there were probably more protein and fat synthesis or less degradation and/or combination of both.

Table 23. Means of feed intake and empty crop body weight, pectoralis superficial and pectoralis subclavis muscles and gastrocnemius + peroneous longus muscles or muscles weight as percentages of E.C.B. wt. and change in muscles weight over a 4 day period in male meat-type chicken at 26; 30, 34, 36; 41 and 45 days of age. (Experiment #4).

	Age (day)									
	26	30	Sig. Level[5]	34	38	Sig. Level[5]	41	45	Sig. Level[5]	Sig. level all ages[5]
No. of birds	5	5		5	5		5	5		
Feed intake (g/bird/day)	96.0			108.0			146.0			
E.C.B. wt.[1] (g)	743[4]±27.7	991±46	L**	1141±65	1409±30	L**	1525±87	1817±20	L**	L**
Change in E.C.B.wt.(g)	248			268			292			
Breast muscles[2]	37.8±3.1	52.0±3.4	L**	63.3±10.4	79.8±7.6	L**	85.6±11.9	104±7.1	L**	L**
Breast wt.[2]/ E.C.B.wt. X100	5±0.64	5.1±0.33		5.5±0.44	5.7±0.44		5.6±0.5	5.7±0.25		L**
Change in muscle wt.(g)	14.2			16.5			18.4			
Leg muscles[3] (g)	22.2±1.0	32.2±2.8	L**	38.0±1.5	46.8±2.7	L**	52.2±4.3	61.1±5	L**	L**
Muscle wt.[3]/ E.C.B.wt. X100	3.0±0.09	3.2±0.38		3.3±0.08	3.3±0.13		3.4±0.14	3.4±0.18		L**
Change in muscle wt.(g)	10			8.8			8.9			

[1] Empty crop body weight.

[2] Pectoralis superficial + pectoralis subclavis muscles from right side of chicken.

[3] Gastrocnemius + Peroneous longus muscles from right leg.

[4] Mean (± S.D.)

[5] Linearly significant between two ages (L^{**} = $P < 0.01$)

Table 24. Test of significance of deviation from linear regression on empty crop body weight (E.C.B.wt.), breast muscles weight, leg muscles weight, breast muscles weight as a percentage of E.C.B.wt (arcs-in $\sqrt{\%}$) and leg muscles weight as a percentage of E.C.B.wt. (arcs-in $\sqrt{\%}$). (Experiment #4).

Source of Variation	d.f.	Deviation from regression (Mean Square)				
		E.C.B.wt.	Breast Muscles	Leg Muscles	Breast as % of E.C.B.wt.	Leg as % of E.C.B.wt.
Linearity	1	3749600.**	14616.6**	4971.8**	2.83*	1.291**
Error	28	6074.1	68.7	14.48	0.458	0.103
Total	29					

** P <0.01
* P <0.05

cont. of above table 23.

d.f.	Deviation from regression (Mean Square)								
	E.C.B.wt.			Breast Muscles			Leg muscles		
	Ages(day)			Ages(day)			Ages(day)		
	26&30	34&38	41&45	26&30	34&38	41&45	26&30	34&38	41&45
1	154008.1**	180096.4**	213744.4**	432.96**	685.6**	838.3**	251.6**	194.4**	199.1**
8	7304.6	2551.0	8091.2	51.97	83.3	95.16	22.1	4.79	21.63
9									

Table 25. Effect of age on moisture, lipid and protein in whole body, pectoralis superficial and pectoralis subclavis muscles and gastrocnemius and peroneous longus muscles in male meat-type chicken at 26, 30; 34, 38; 41 and 45 days of age. (Experiment #4).

	Age (day)						
	26	30	34	38	41	45	Level of Significance[4]
No. of birds	5	5	5	5	5	5	
Whole E.C.B.[1]							
% Moisture	69.8±1.29[5]	69.3±0.91	68.6±1.52	68.1±0.93	66.6±1.31	67.1±1.75	L P <0.01
% Lipid	7.6±1.36	8.1±0.97	8.1±1.52	8.5±1.22	9.8±1.58	9.5±1.86	L P <0.01
% Protein	17.6±0.40	17.6±0.50	17.9±0.41	18.7±0.42	18.6±0.25	18.6±0.50	L P <0.01
Breast muscles[2]							
% Moisture	74.0±0.61	74.3±0.36	74.3±0.46	74.0±0.25	73.9±0.55	73.9±0.31	NS
% Lipid	0.66±0.23	0.84±0.23	0.84±0.39	0.90±0.07	0.80±0.23	1.02±0.22	L P <0.05
% Protein	23.1±0.27	23.1±0.57	21.9±0.45	22.1±0.69	22.1±0.37	22.0±0.47	L P <0.01
Leg muscles[3]							
% Moisture	75.1±0.62	75.7±0.22	74.8±0.73	75.0±0.17	75.1±0.37	75.0±0.45	NS
% Lipid	3.8±0.70	3.7±0.35	4.2±0.63	3.6±0.30	3.9±0.69	4.1±0.59	NS
% Protein	18.1±0.33	18.7±0.55	19.1±0.69	19.2±0.66	19.2±0.66	19.1±0.19	L P <0.01

[1]Empty crop body.
[2]Pectoralis superficial and pectoralis subclavis muscles.
[3]Gastrocnemius and peroneous longus muscles.
[4]Significant for lineavity (L), Not Significant(NS).
[5]Mean (±S.D.)

Table 26. Test of significance of deviation from linear regression on % moisture ($\arcsin\sqrt{\%}$), % lipid ($\arcsin\sqrt{\%}$) and % protein ($\arcsin\sqrt{\%}$) on empty crop (E.C. Body), breast muscles and leg muscles. (Experiment #4).

Source of Variation	d.f.	Deviation from regression (Mean square)								
		E.C. Body			Breast muscles			Leg muscles		
		$\%H_2O$	%Lipid	%Prot.	$\%H_2O$	%Lipid	%Prot.	$\%H_2O$	%Lipid	%Prot.
Linear	1	13.38**	16.00**	4.74**	0.18	4.12*	2.36**	0.13	2.33	1.74**
Error	28	0.61	1.85	0.11	0.08	0.65	0.14	0.11	0.65	0.15
Total	29									

**P <0.01
* P <0.05

Table 27. Percent change of body as a percentage of protein or fat in male meat-type chickens at 28, 36 and 43 days of age (Experiment #4).

Percent change[1] of body due to	Age (day)		
	26-30	34-38	41-45
Protein	18.8	22.0	18.9
Fat	9.2	10.3	8.6

[1] $\frac{\text{The change in weight of protein or fat in whole body over a 4 day}}{\text{The change in weight of whole body over the same 4 days}} \times 100$

Table 28. Fractional protein accretion (FAR), synthesis (FSR) and breakdown (FBR) rates in the leg muscles or breast muscles and FAR in the whole body of male meat-type chicken at 28, 36 and 43 days of age. (Experiment #4).

Muscle and Trait	Age (day)		
	28	36	43
Leg muscle[1]			
FAR[2]	9.9	5.5	3.9
FSR[2]	35.5[a]±8.7	31.2[a]±11.6	31.5[a]±12.2
FBR[2]	25.6	25.7	27.6
Breast muscle[3]			
FAR	7.4	5.9	4.7
FSR	61.4[b]±12.72	53.2[b]±5.3	57.2[b]±16.5
FBR	54.	47.3	52.5
Whole body			
FAR	7.7	6.3	4.4

[1]Gastrocnemius + peroneous longus muscles from right leg.

[2]Expressed as %/day and FBR values obtained indirectly, FBR=FSR-FAR.

[3]Pectoralis superficial + pectoralis subclavis muscles from right side.

[a,b]Means in the same row with different superscripts are significantly ($P < 0.05$) different.
Means in the same column with different superscripts are significantly ($P < 0.05$) different.

Table 29. Analysis of variance on fractional synthesis rate or FSR (Arcsin $\sqrt{\%}$) on leg muscles or breast muscles for male meat-type chicken. (Experiment #4).

Source of Variation	Leg muscles		Breast muscles		Between two muscles at 3 ages			
	d.f.	Mean Square	d.f.	Mean Square	d.f.	Mean Square		
						28 days	36 days	43 days
Treatment	2	5.68	2	25.04	1	510.7*	345.7*	486.1*
Error	9	44.3	9	28.6	6	45.0	30.4	78.8
Total	11		11		7			

*P < 0.05

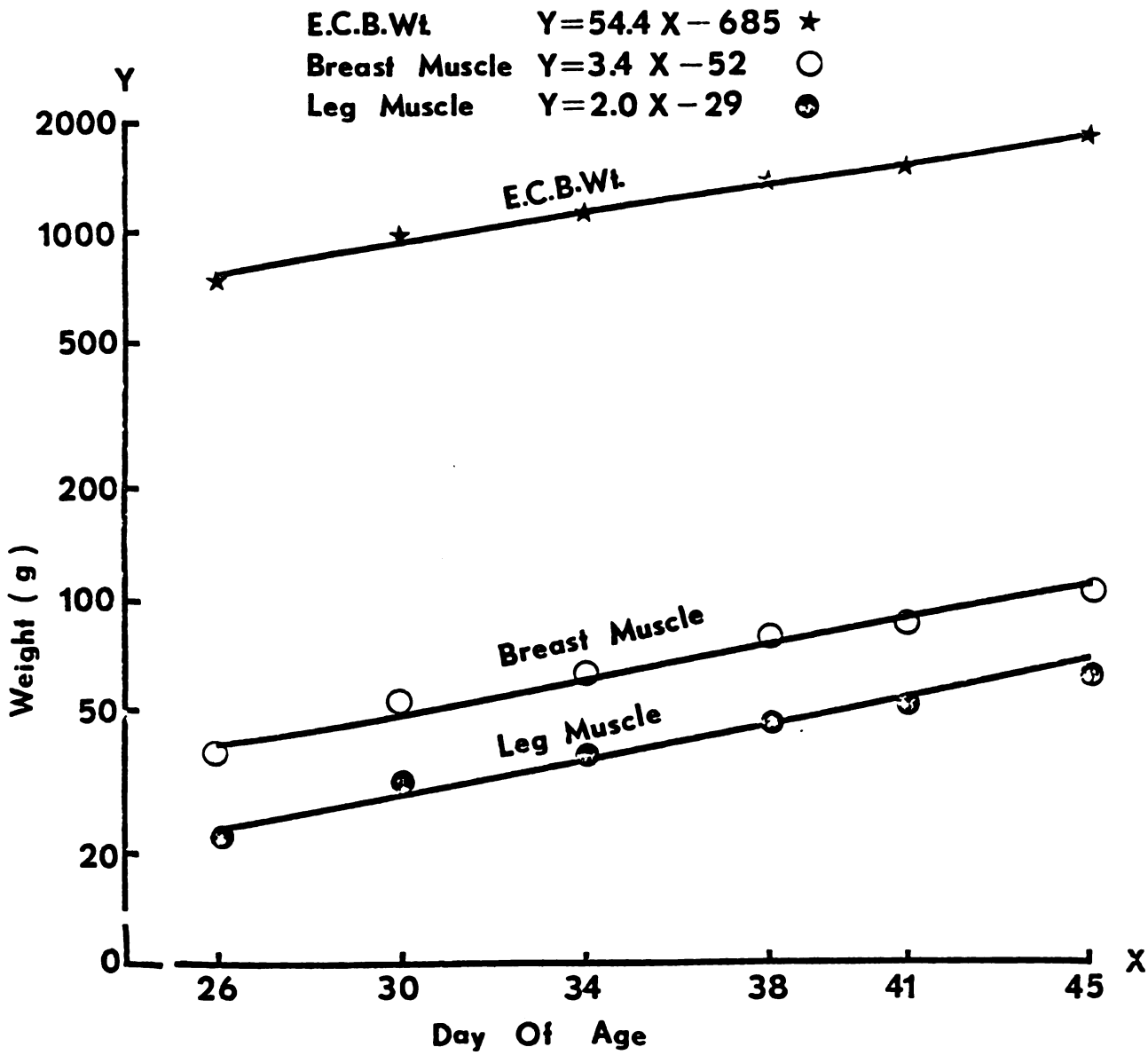


Figure 15 Relationship of empty crop body weight (E.C.B. Wt.), breast muscles (pectoralis superficial & pectoralis subclavis muscles from right side) and leg muscles (gastrocnemius & peroneous longus muscles from right leg) with age of male meat-type chicken (Experiment #4). Where X=Day of age, Y=Weight (g.).

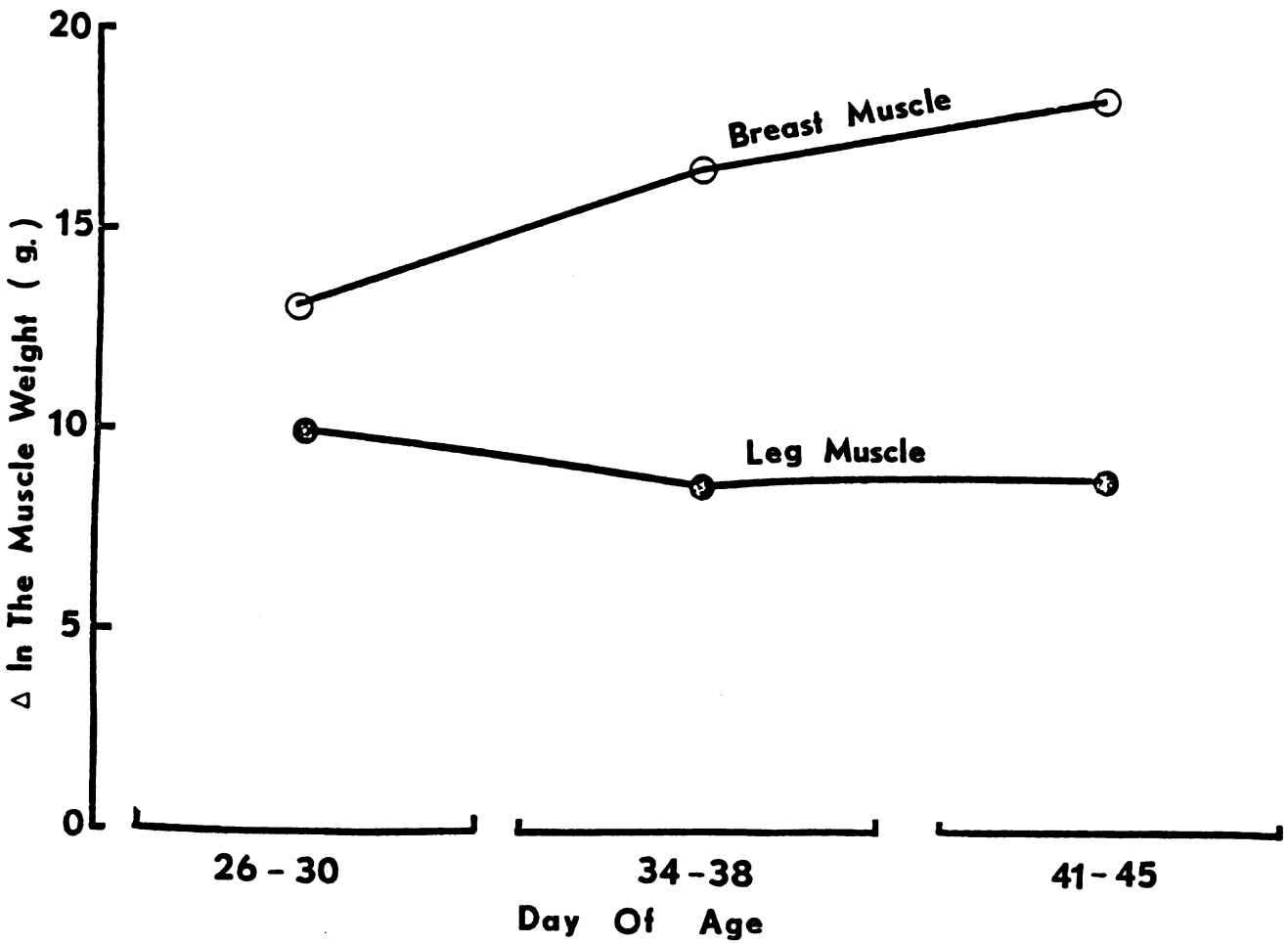


Figure 16 Net change in the weight of breast muscles (pectoralis superficial & pectoralis subclavis muscles from right side) and leg muscles (gastrocnemius & peroneous longus muscles from right leg) over a 4-day growth in male meat-type chicken (Experiment #4).

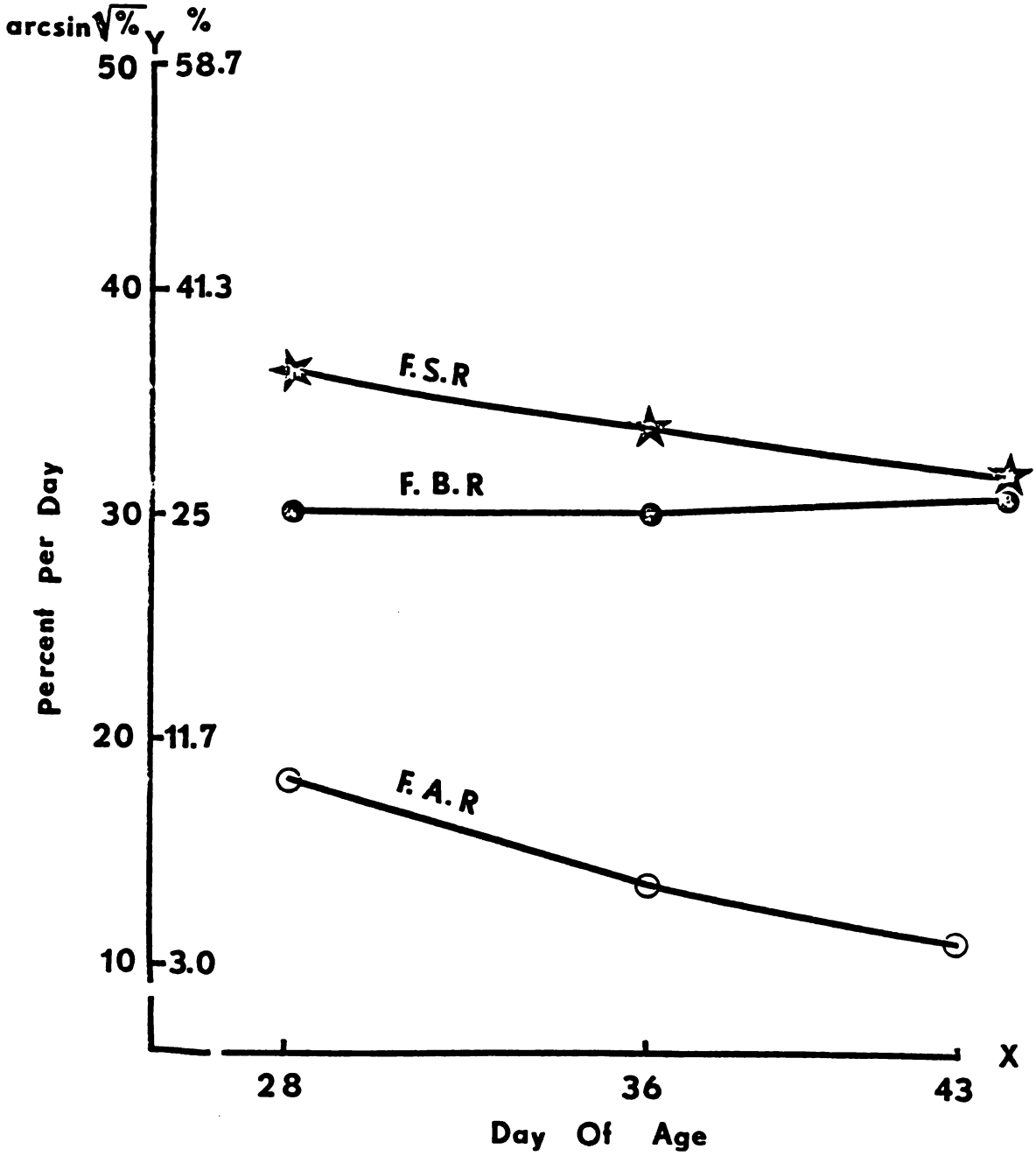


Figure 17 Relationship of fractional synthesis rate (F.S.R), fractional breakdown rate (F.B.R) and fractional accretion rate (F.A.R) in gastronemius & peroneous longus muscles (leg muscles) with age of male meat-type chicken (Experiment #4). Where X=Day of age, Y=%per day (arcsin $\sqrt{\%}$).

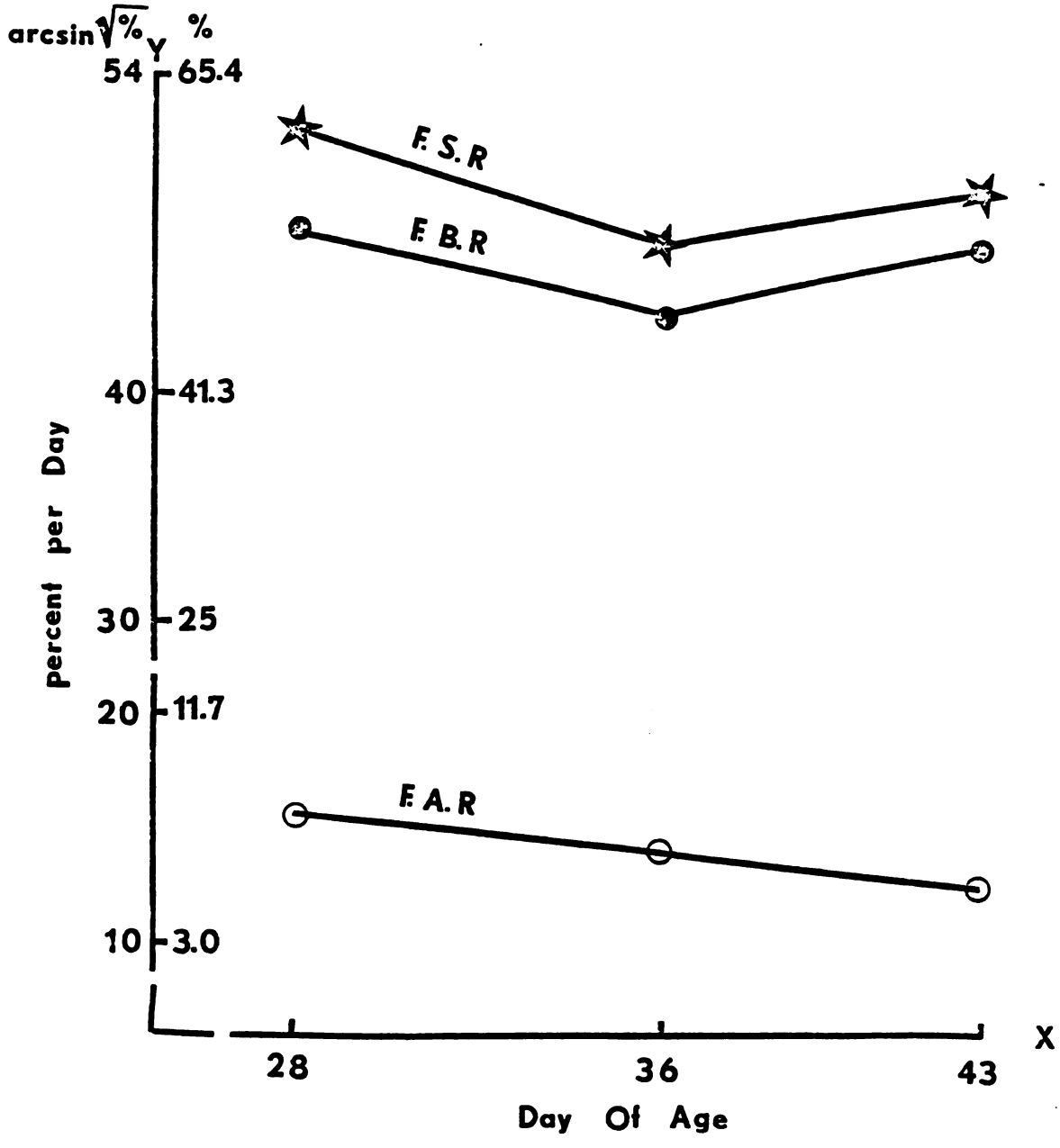


Figure 18 Relationship of fractional synthesis rate (F.S.R), fractional breakdown rate (F.B.R) and fractional accretion rate (F.A.R) in pectoralis superficial & pectoralis subclavis muscles (breast muscles) with age of male meat-type chicken (Experiment #4). Where X=Day of age, Y=%/day (aresin $\sqrt{\%}$).

The fractional accretion (FAR), synthesis (FSR)[1] and breakdown (FBR) rates of protein as a %/day in either of the two muscles of meat-type chickens at 28, 36 and 43 days of age are shown in Table 27. The numerical value for FAR was 9.9 and 7.4 %/day in the leg muscles and breast muscles at 28 days of age, respectively. The FAR decreased from 9.9 %/day to 5.5 and 3.9 %/day in the leg muscles obtained from chickens aged 28, 36 and 43 days, respectively. The FAR values were 7.4, 5.9 and 4.7 in the breast muscles of chickens at 28, 36 and 43 days of age respectively. The FAR in the leg muscles declined at a rate faster than breast muscles as the animal aged (Table 28). The FSR in the leg muscles or breast muscles were not significantly ($p > 0.05$) different at 28, 36 and 43 days of age (Table 29). The FSR (%/day) in the breast muscles was significantly ($p < 0.05$) higher than in the leg muscles at 28, 36 or 43 days of age (Table 29). The relationships of FSR, FAR and FBR in the leg or breast muscles with age of male meat-type chickens are presented in Figures 17 and 18, respectively. The differences between the FBR and FSR tend to be smaller in either of the two muscles as the animal aged from 28 to 43 days (Figures 17 and 18). Individual empty crop body weight, weights of breast and leg muscles and proximate analysis for these 3 tissues in male meat-type chickens at 26, 30, 34, 38, 41 and 45 days of age are shown in Appendix B Table 5. Individual specific activity of leucine or ^{3}H-leucine incorporation into the leg or breast muscles proteins and plasma radioactivity level at 3, 10, 20 and 30 minutes of infusion in male chickens at 28, 36, 43 days of age are presented in Appendix B Table 6.

1-See appendix B, page 180 for calculation of FSR.

Experiment #5

Table 30 contains the means of feed intake, empty crop body weight (E.C.B.Wt.), weights of leg and breast muscles from right side and change in the muscle weights over a 4-day period in mixed sex, single Comb White Leghorn (SCWL) chickens at 26, 30, 34, 38, 41 and 45 days of age. A significant slope ($p < 0.01$) for E.C.B.Wt., weight of breast and leg muscles indicate continuing growth through at the experiment (Tables 30, 31 and Figure 19). The breast muscles or leg muscles as a percentage of E.C.B.Wt. increased linearly ($p < 0.01$) as the animal aged (Table 31). Thus, breast or leg muscles were growing at a rate faster than the whole body. The increase in muscle weight (change in muscle weight) for either of the two muscles over each 4-day experimental period was greater as the birds aged from 26 to 45 days (Table 30 and Figure 20).

The percent of water in whole body or breast muscles decreased linearly ($p < 0.01$), but no such changes occurred in the leg muscles (Tables 32 and 33). The % lipid in the whole body or breast muscles did not change, while it decreased significantly ($p < 0.01$) in the leg muscles of SCWL chickens (Table 33). The percentage of protein increased linearly ($p < 0.01$) in the whole body, breast and leg muscles as the chickens aged (Table 32).

The percent change of body as a percentage of protein was highest and fat was lowest at 34-38 days of age than at 26-30 or 41-45 (Table 34). This indicated that there were possibly changes in the protein and fat synthesis or degradation and/or both during 34-38 days period.

Table 30. Feed intake, empty crop body weight (E.C.B.wt.), Pectoralis superficial and pectoralis subclavis muscles and gastrocnemius and peroneous longus muscles or muscle's weight as a percentage of E.C.B.wt. or change in weight of muscles over a 4 day period in mixed sex Single Comb White Leghorn (SCWL) Chicken at 26, 30; 34, 38; 41 and 45 days of age. (Experiment #5).

	Age (day)								
	26	30	Sig.[6] Level	34	38	sig.[6] Level	41	45	Sig.[6] Level	Sig level[6] for all ages
No. of groups[1]	4	4		4	4		4	4		
Feed Intake (g/b/d)	39.0			48.0			58.0			
E.C.B.wt.[2](g)	217.5±1.29[5]	270.±3.2	L*	322±2.94	383.±5.5	L*	480.±4.36	556±5.2	L*	L*
Change in E.C.B.wt.(g)	52.5			61.0			76.0			
Breast Muscles[3](g)	8.8±0.12	11.1±0.59	L*	13.9±0.32	17.3±0.52	L*	22.1±0.56	26.3±0.75	L*	L*
Muscle wt. / E.C.B.wt. X100	4.1±0.06	4.1±0.17		4.3±0.096	4.5±0.13		4.6±0.18	4.7±0.13		L*
Δ in muscle wt. (g)	2.30			3.40			4.2			
Leg Muscle[4](g)	5.8±0.10	7.3±0.26	L*	9.1±0.3	11.3±0.59	L*	14.6±0.34	17.3±0.33	L*	L*
Muscle wt / E.C.B.wt. X100	2.6±0.05	2.7±0.1		2.8±0.08	3.0±0.1		3.0±0.06	3.1±0.1		L*
Δ in muscle wt. (g)	1.5			2.2			2.7			

[1]Each group is the average of 3-5 birds.

[2]Empty crop body weight.

[3]Pectoralis superficial and pectoralis subclavis muscles from right side.

[4]Gastrocnemius + peroneous longus muscles from right side.

[5]Mean ±S.D.

[6]Linearly significant (L* P <0.01)

Table 31. Test of significance of deviation from linear regression on empty crop body weight (E.C.B.wt.), breast muscle's weight, (right side). leg muscle's weight (right side), breast muscle's weight (right side) as a percentage of E.C.B.wt. (arcsin $\sqrt{\%}$) and weight of leg muscles as a percentage of E.C.B.wt. (arcsin $\sqrt{\%}$). (Experiment #5).

Source of Variation	d.f.	Deviation from regression (Mean Square)				
		E.C.B.wt.	Breast Muscles	Leg Muscles	Breast Muscles E.C.B.wt.	Leg Muscles E.C.B.wt.
Linearity	1	310106.7*	864.2*	378.3*	2.9955*	2.2593*
Error	22	430.72	1.37	0.63	0.0329	0.0173
Total	23					

* P <0.01

cont. of above table 31.

	Deviation from regression (Mean Square)								
	E.C.B.wt.			Breast muscles			Leg muscles		
	Age (day)			Age (day)			Age (day)		
d.f.	26&30	34&38	41&45	26&30	34&38	41&45	26&30	34&38	41&45
1	5565.3*	7442.0*	11250.0*	10.134*	23.12*	35.28*	5.12*	9.68*	14.06*
6	5.96	18.0	23.0	0.182	0.193	0.462	0.040	0.180	0.115
7									

Table 32. Moisture, lipid and protein in whole body, pectoralis superficial + pectoralis subclavis muscles and gastrocnemius and peroneous longus muscles of Single Comb White Leghorn (SCWL) Chicken at 26, 30; 34, 38; 41 and 45 days of age. (Experiment #5).

	Age (day)						
	26	30	34	38	41	45	Level of Significance[5]
No. of groups[1]	4	4	4	4	4	4	
Whole E.C. Body[2]							
% Moisture	69.7±0.65[6]	69.7±0.57	69.4±0.69	69.2±0.79	68.9±0.72	68.1±0.29	L*
% Lipid	6.4±1.06	6.3±0.68	5.9±0.70	5.9±0.51	5.8±0.53	6.4±0.34	NS
% Protein	18.1±0.77	18.6±0.21	18.8±0.11	19.6±0.62	20.0±0.29	19.8±0.37	L*
Breast muscles[3]							
% Moisture	75.2±0.29	75.5±0.37	75.2±0.26	74.7±0.45	74.7±0.17	74.3±0.39	L*
% Lipid	0.86±0.2	0.71±0.11	0.51±0.08	0.89±0.13	0.89±0.13	1.1±0.08	NS
% Protein	21.8±0.24	21.9±0.14	21.8±0.32	22.4±0.18	22.3±0.47	22.4±0.25	L*
Leg muscles[4]							
% Moisture	76.6±0.36	77.0±0.47	77.2±0.51	76.7±0.57	76.6±0.35	76.6±0.50	NS
% Lipid	2.8±0.32	2.5±0.21	2.2±0.33	2.2±0.31	2.1±0.15	2.0±0.28	L*
% Protein	18.6±0.44	18.7±0.29	18.9±0.40	19.5±0.60	19.8±0.24	19.5±0.42	L*

[1]Every group contains 3-5 birds.

[2]Whole empty crop body.

[3]Pectoralis superficial + pectoralis subclavis muscles.

[4]Gastrocnemius + Peroneous longus muscles.

[5]Significance for linearity (L* $p < 0.01$), Not Significant (NS).

[6]Mean (±S.D.)

Table 33. Test of significance of deviation from linear regression on % moisture (arcsin $\sqrt{\%}$), % lipid (arcsin $\sqrt{\%}$) and % protein (arcsin $\sqrt{\%}$) of empty crop body (E.C. body), breasts muscles and leg muscles. (Experiment #5).

Source of variation	d.f.	Deviation from regression (Mean Square)								
		E.C. Body			Breast muscles			Leg Muscles		
		$\%H_2O$	%Lipid	%Protein	$\%H_2O$	%Lipid	%Protein	$\%H_2O$	%Lipid	%Protein
Linear	1	2.473*	0.158	5.030*	1.4096*	1.64	0.68*	0.0516	4.985*	2.037*
Error	22	0.1417	0.626	0.1377	0.0600	0.464	0.0413	0.0999	0.272	0.0951
Total	23									

* P <0.01

Table 34. Percent change of body as a percentage of protein or fat of mixed sex Single Comb White Leghorn (SCWL) Chickens at 28, 36 and 43 days of age. (Experiment #5).

Percent change[1] of body due to	Age (day)		
	26-30	34-38	41-45
Protein	20.8	23.9	18.6
Fat	6.0	5.2	9.9

[1] $\frac{\text{The change in weight of protein or fat in whole body over 4 days}}{\text{The change in weight of whole body over the same 4 days}} \times 100$

Table 35. Fractional protein accretion (FAR), Synthesis (FSR) and breakdown (FBR) rates in the leg muscles or breast muscles of mixed sex Single Comb White Leghorn chicken at 28, 36 and 43 days of age. (Experiment #5).

Muscle and Trait	Age (day) 28	36	43
Leg muscle[1]			
FAR[2]	6.1	6.2	4.4
FSR[2]	(4)[4] 17.4[a] ±2.46	(4) 17.3[a] ±5.55	(4) 17.3[a] ±1.3
FBR[2]	11.6	11.2	12.9
Breast Muscle[3]			
FAR	6.0	6.1	3.1
FSR	(4) 29.[b]0±6.8	(4) 26.[b]1±1.65	(4) 28.6[b] ±4.63
FBR	23.0	20.0	25.5
Whole Body			
FAR	6.1	5.4	3.4

[1]Gastrocnemius + peroneous longus muscles from right leg.

[2]Expressed as % day, the FBR values obtained indirectly FBR = FSR - FAR.

[3]Pectoralis superficial + pectoralis subclavis muscles from right side.

[4]No. of chickens.

[a,b]Means in the same row with different superscripts are significantly ($P < 0.05$) different.
Means in the same column with different superscripts are significantly ($P < 0.05$) different.

Table 36. Analysis of variance on fractional synthesis rate or FSR (arcsin $\sqrt{\%}$) on leg or breast muscles of Single Comb White Leghorn Chicken. (Experiment #5).

Source of Variation	Leg muscle		Breast muscles		Between Two muscles at 3 ages			
	d.f.	Mean Square	d.f.	Mean Square	d.f.	Mean Square		
						28 days	36 days	43 days
Treatment	2	0.084	2	3.639	1	122.23*	80.96*	118.66**
Error	9	7.240	9	9.662	6	11.36	9.12	4.87
Total	11		11		7			

**P< 0.01
*P< 0.05

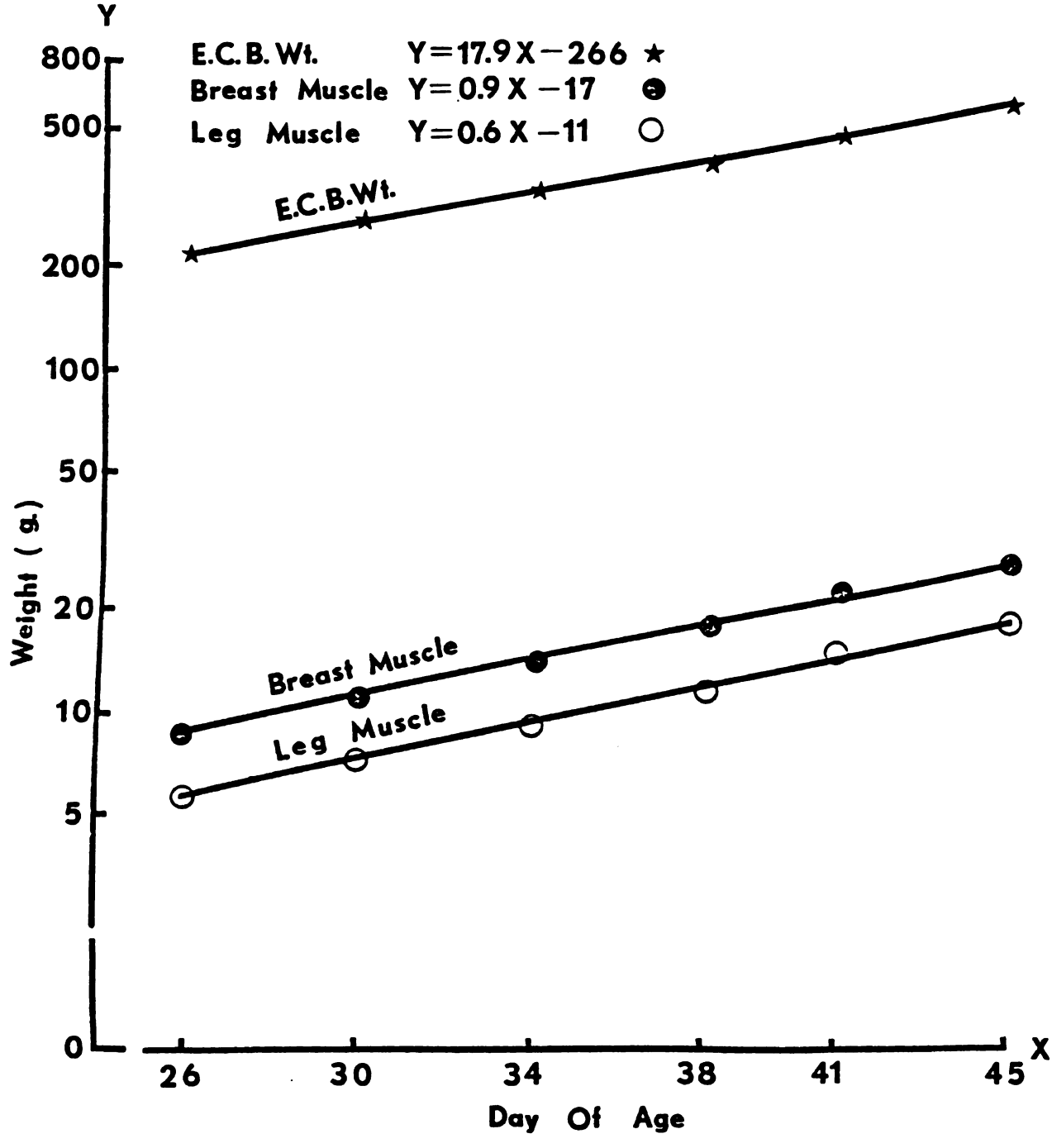


Figure 19 Relationship of empty crop body weight (E.C.B.Wt.), breast muscles weight (pectoralis superficial + pectoralis subclavis muscles from right side) and weight of leg muscles (gastrocnemius + peroneous longus muscles from right leg) with age of mixed-sex, Single Comb White Leghorn chicken. (Experiment #5). Where x = Day of age, y = weight (g).

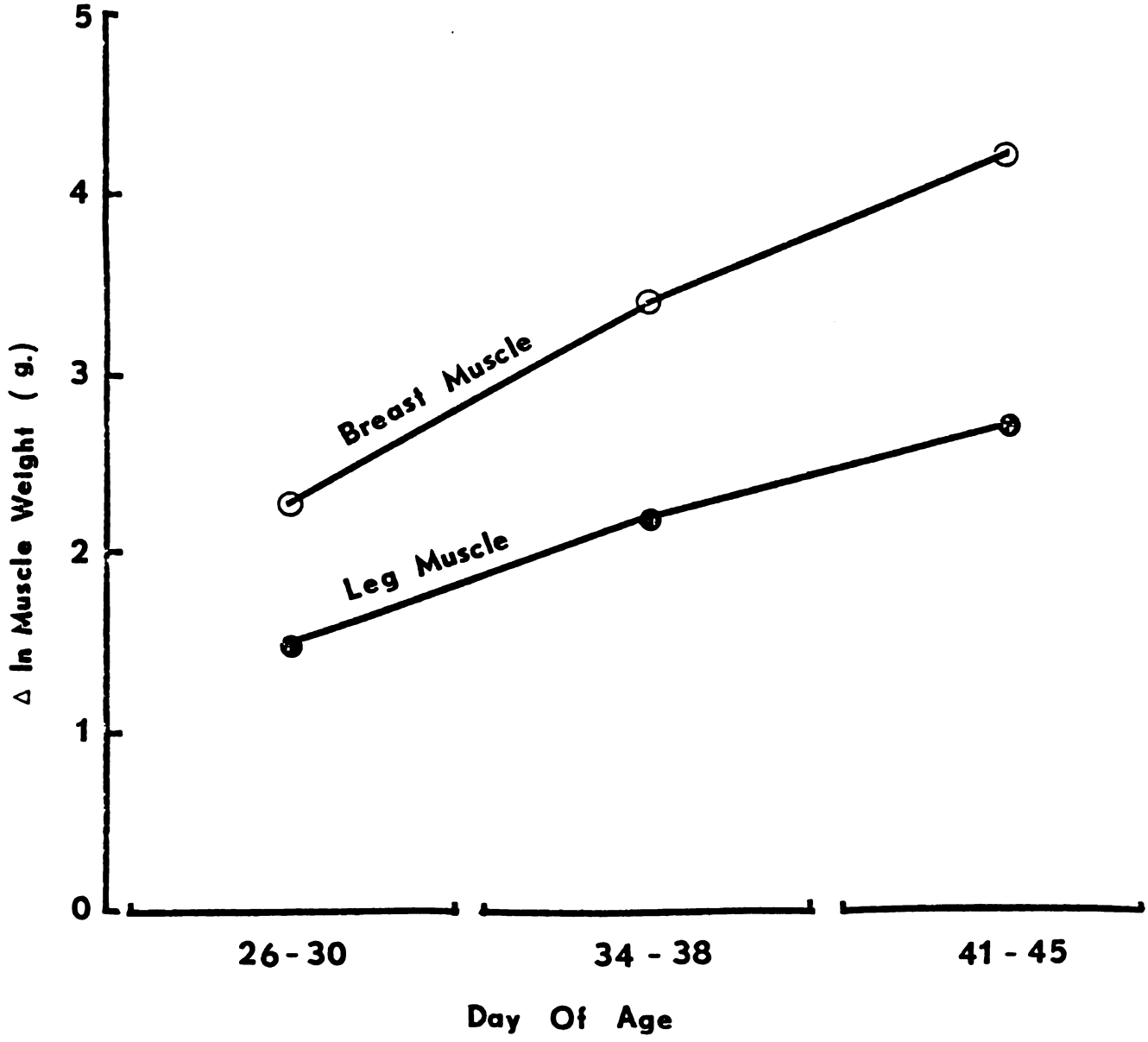


Figure 20 Net change in the weight of breast muscles (pectoralis superficial + pectoralis subclavis muscles from right side) and leg muscles (gastrocnemius + peroneous longus muscles from right leg) determined over three 4-day periods in mixed sex, Single Comb White Leghorn chicken (Experiment #5).

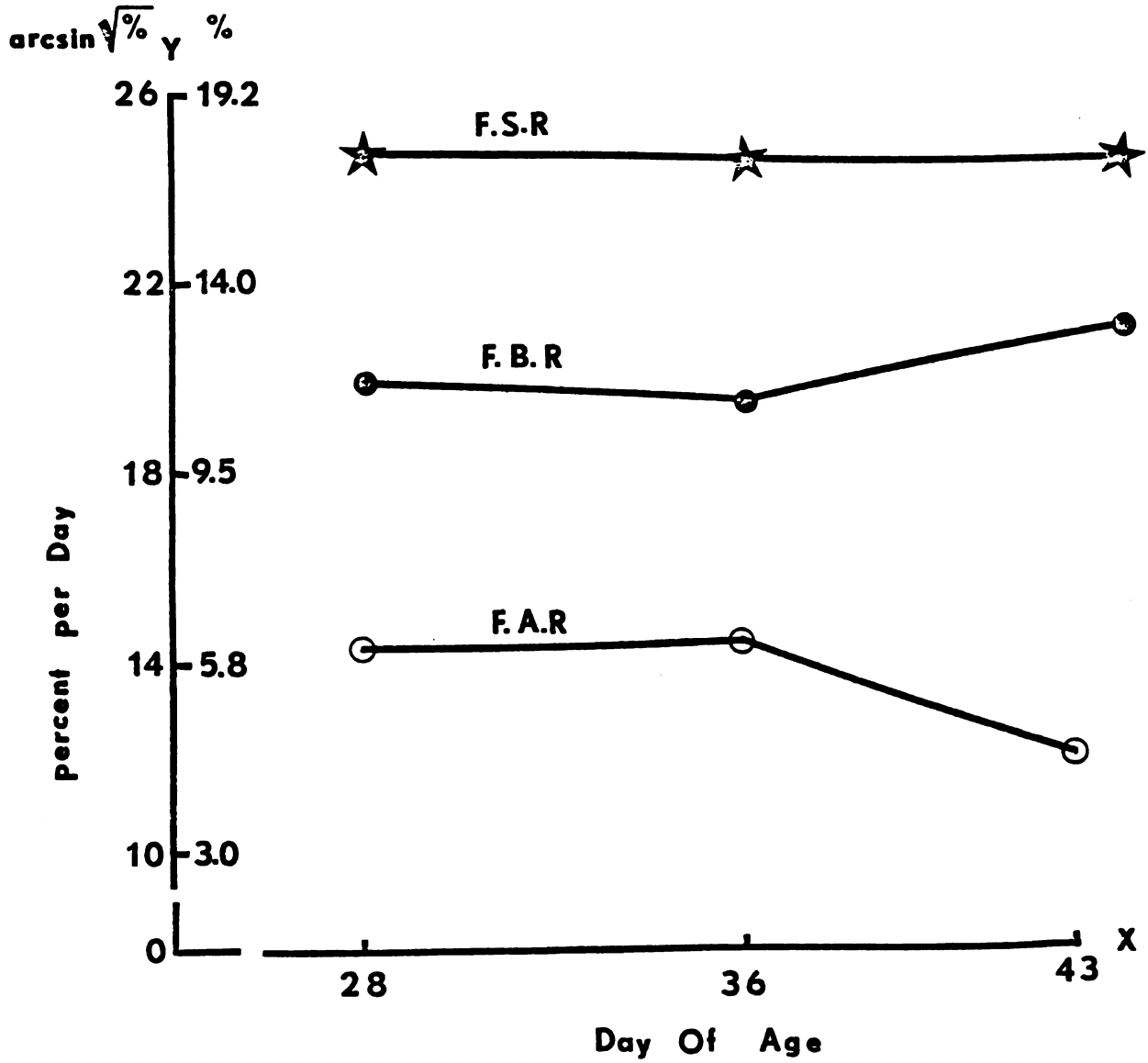


Figure 21 Relationship of fractional synthesis rate (F.S.R), fractional breakdown rate (F.B.R) and fractional accretion rate (F.A.R) in leg muscles (gastrocnemius + peroneous longus muscles from right leg) with age of Single Comb White Leghorn chicken, (Experiment #5). Where x = Day of age y = %/day (arcsin $\sqrt{\%}$).

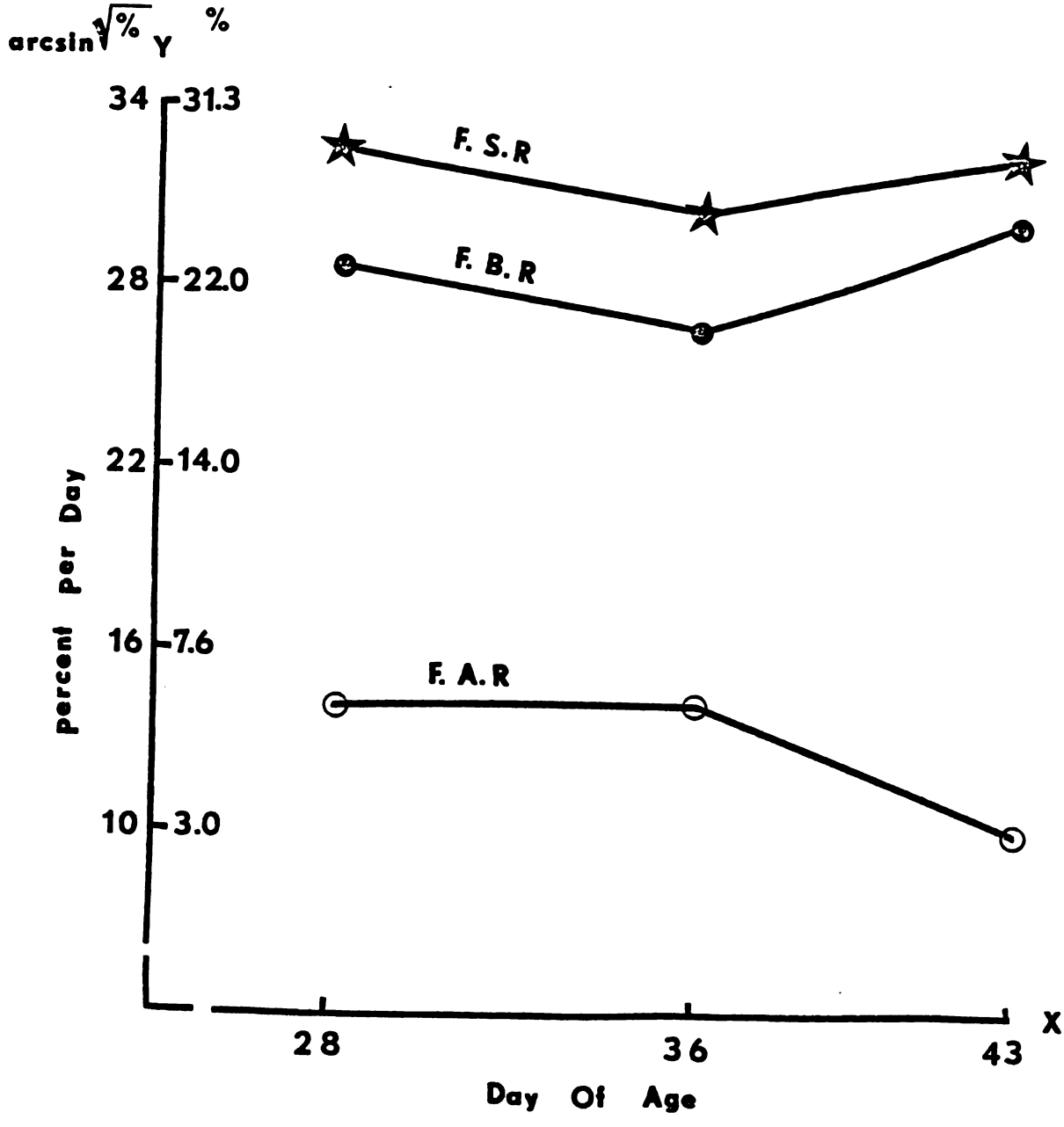


Figure 22 Relationship of fractional synthesis rate (F.S.R), fractional breakdown rate (F.B.R) and fractional accretion rate (F.A.R) in breast muscles (pectoralis superficial + pectoralis subclavis muscles from right side) with age of Single Comb White Leghorn chicken (Experiment #5). Where x = Day of age, y = %/day (arcsin $\sqrt{\%}$).

The fractional accretion (FAR), synthesis (FSR) and breakdown (FBR) rates in the breast or leg muscles of SCWL chickens at 28, 36 and 43 days of age are presented in Table 35. The numerical values for FAR in the leg muscles were 6.1, 6.2 and 4.4 at 28, 36 and 43 days of age, respectively. The FAR was about 6.0 %/day for each of the two muscles at 28 and 36 days of age. But it was much less in the breast muscles (3.1 %/day) as compared to the leg muscles (4.4 %/day) at 43 days of age (Table 35). The FSR in the leg muscles was about 17 %/day for all ages, while the FBR tended to increase as the animal aged (Figure 21). The FSR in the breast muscles was not significantly different at various ages studied, but it was higher in the breast muscles than the leg muscles at any particular age (Table 36). The numerical values for FBR in the breast muscles were 23.0, 20. and 25.5 %/day at 28, 36 and 43 days of age, respectively. The relationships of FSR, FAR and FBR in the leg and breast muscles at various ages for the SCWL chicken are shown in Figures 21 and 22. The difference between the FSR and FBR in each of the two muscles became less as the animal aged (Figures 21 and 22). Individual empty crop body weight, leg and breast muscle weights or proximate analysis for pooled of each group are shown in the Appendix B - Table 7 and 8, respectively. The leucine specific radioactivity in either of the two muscles or ^{3}H-leucine incorporation into the muscle proteins or plasma radioactivity at 3, 10, 20 and 30 minutes of infusion for individual bird at 28, 36 and 43 days of age are presented in Appendix B-Table 9.

Experiment #6

The empty crop body weight (E.C.B.Wt.) was significantly increased in control ($p < 0.01$) or force-fed ($p < 0.05$) chickens at they aged from 33 to 37 days (Tables 37 and 38). The breast muscle's weight increased significantly ($p < 0.05$) from 12.0 g. to 16.0 g. in force-fed chickens at 33 and 37 days of age, respectively. The breast muscles weight was of border signficane ($p < 0.10$) in control chickens as they aged (Table 38). A significant slope ($p < 0.05$) obtained by statistical analysis for weight of leg muscles indicated the muscle grew from 33 to 37 days of age. The relationships of E.C.B.Wt., breast or leg muscles in control or over-fed chickens with age are shown in Figure 23. The change in E.C.B.Wt. was 85.0 g. and 143.0 g. in control and force-fed chickens, respectively, over a 4-day period. The weight of breast or leg muscles were higher in force-fed than control chickens at 37 days of age (Table 37). The numerical values for protein accretion over a 4-day period were greater in each of the two muscles from over-fed than control chickens (Figure 24). The muscle's weight as a percentage of E.C.B.Wt. did not change in control or force-fed chickens during 33 and 37 days of age (Table 37 and 38). The individual empty crop body weight, weight of muscles and % protein in either of the two muscles in control or over-fed chickens at 33 and 37 days of age are presented in Appendix B Table 10.

The fractional accretion (FAR), synthesis (FSR) and breakdown (FBR) rates of protein in each of the two muscles are shown in Table

Table 37. Feed intake, empty crop body weight (E.C.B.wt.), pectoralis superficial and pectoralis subclavis muscles, gastrocnemius and peroneous longus muscles or weight of muscles as percentage of E.C.B.wt. or change in weight of muscles over a 4 day period in control or force-fed (over fed) Single Comb White Leeghorn (SCWL) chickens at 33 and 37 days of age, (Experiment #6)

	Age					
	Control		Level of Significance[4]	Force-fed		Level of Significance[4]
	33	37		33	37	
No. of birds	5	5		5	5	
Feed Intake (g/bird/day)	41			53.0		
E.C.B.wt.[1](g)	305.2±23.9	390.0±23.6	L***	322.±24.65	465.0±40.1	L**
Change in Bwt.(g)	84.8			143.0		
Breast Muscle[2](g)	12.2±1.7	15.5±2.81	L*	12.1±1.89	16.3±2.13	L**
$\frac{\text{Breast muscle (g)}}{\text{E.C.B.wt.(g)}}$ X100	4.0±0.23	4.0±0.54	NS	3.8±0.36	3.5±0.51	NS
Δin the breast(g)	3.3			4.2		
Leg Muscle[3](g)	8.4±1.11	10.6±1.51	L**	9.5±1.51	12.4±1.56	L**
$\frac{\text{Leg Muscle(g)}}{\text{E.C.B.wt.(g)}}$ X100	2.8±0.13	2.7±0.24	NS	3.0±0.24	2.7±0.23	NS
Δin the leg(g)	2.2			2.9		

[1]Empty crop body weight pectoralis

[2]Pectoralis superficial and subclavis muscles from right side

[3]Gastrocnemius and peroneous longus muscles from right leg

[4]Linearly significant (L*** = P<0.01, L** = P<0.05, L* = P<0.10) or Not Significant (NS)

[5]Mean ± S.D.

Table 38. Test of significance of deviation from linear regression on empty crop body weight (E.C.B.wt.), breast muscles (right side) and leg muscles weights (right leg) or weight of muscles (right side) as a percentage of E.C.B.wt. (arcsin $\sqrt{\%}$) in control or force-fed chickens (Experiment #6).

Source of variation	d.f.	Deviation from regression (Mean Square)					
		Control			Force-fed		
		E.C.B.wt.	Breast Muscles	Leg Muscles	E.C.B.wt.	Breast Muscles	Leg Muscles
linearity	1	17892.8***	26.9*	11.45**	45427.6***	44.1**	20.45**
error	8	566.1	5.41	1.76	1108.1	4.06	2.35
Total	9						

*** P <0.01
** P <0.05
* P <0.10

cont. of above table

	Deviation from regression (Mean Square)			
	Control		Force-fed	
	As % of E.C.B.wt.		As % of E.C.B.wt.	
	Breast muscles	Leg muscles	Breast muscles	Leg muscles
	0.027	0.60	1.243	0.518
	0.379	0.116	0.464	0.165

Table 39. Fractional protein accretion (FAR), synthesis (FSR) and breakdown (FBR) rates in pectoralis superficial + pectoralis subclavis or breast muscles and gastrocnemius + peroneous longus or leg muscles in control and force-fed chickens. (Experiment #6).

Trait	Control		Force-fed	
	Breast	Leg	Breast	Leg
FAR[1]	5.7	6.3	8.1	6.9
FSR[1]	(5)[2]25.2^{bc}±4.44	(5)20.3^{ab}±5.13	(5)29.6^{c}±8.89	(5)14.1^{a}±3.26
FBR[1]	19.5	14.0	21.5	7.2

[1]Expressed as %/day, the FBR values obtained indirectly, FBR=FSR-FAR.

[2]No. of chickens.

^{a,b,c}Means in the same row which do not have common superscripts are significantly ($P < 0.05$) different.

Table 40. Analysis of variance on fractional synthesis rate or FSR ($\arcsin\sqrt{\%}$) on breast and leg muscles of control and force-fed male Single Comb White Leghorn Chickens. (Experiment #6).

Source of Variation		Muscles
		Mean Square
Treatment	3	110.32*
Error	16	15.05
Total	19	

* $P < 0.01$

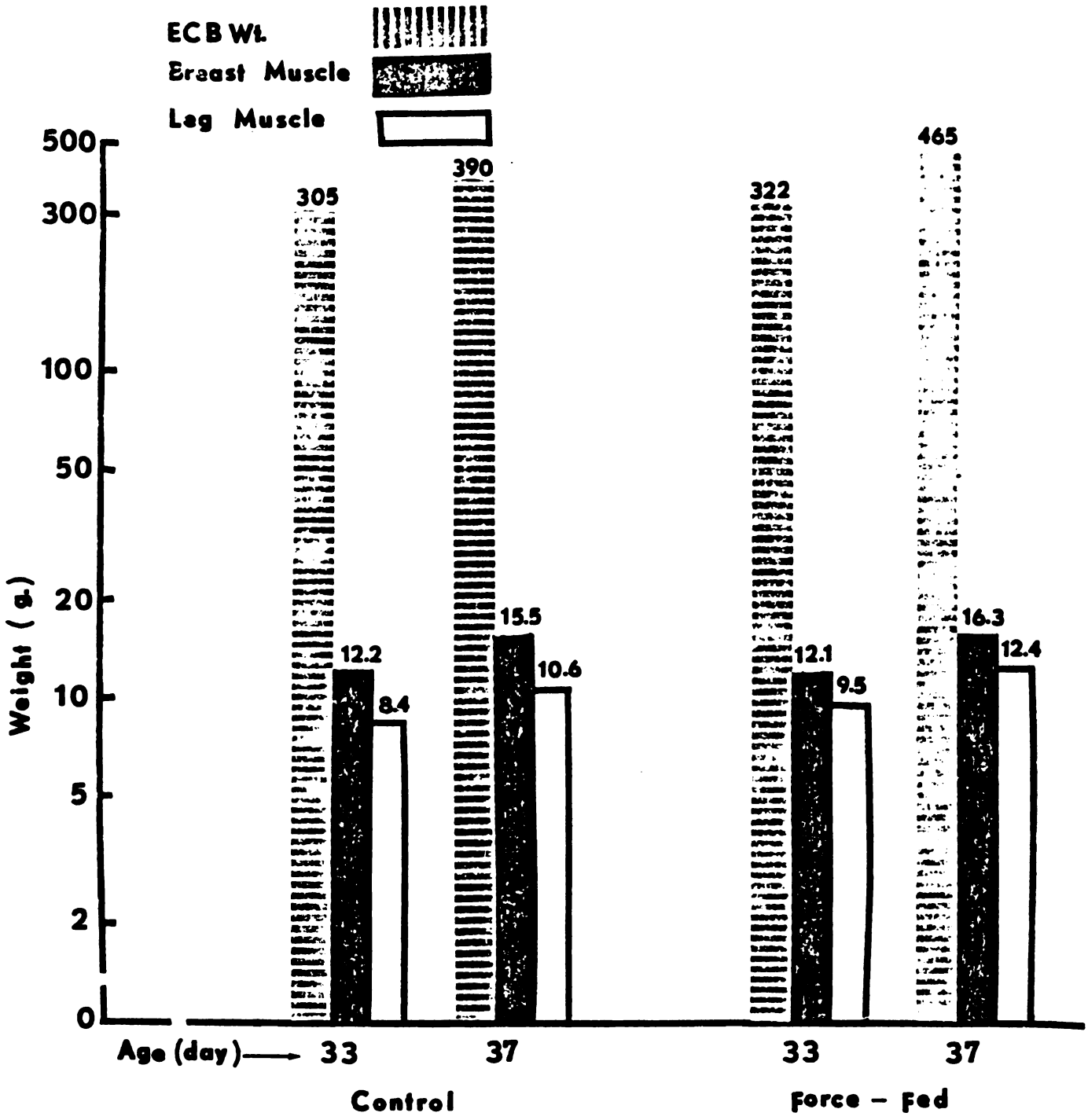
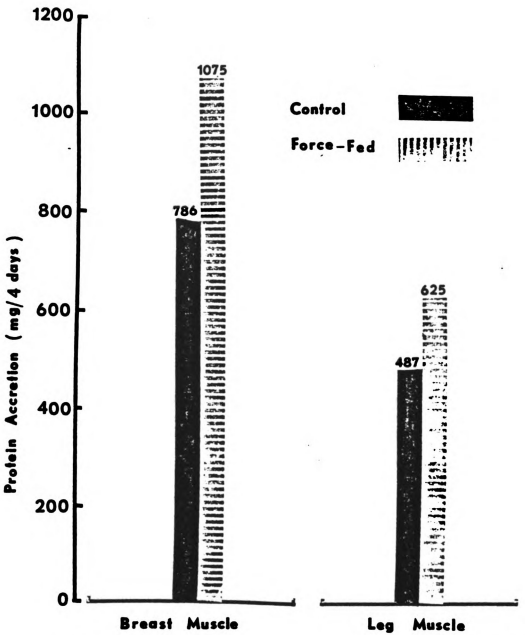


Figure 23. Relationship of empty crop body weight (ECBWt.), breast muscles (pectoralis superficial + pectoralis subclavis muscles from right side) and leg muscles (gastrocnemius + peroneous longus muscles from right leg) of control and force-fed male Single Comb White Leghorn chickens, (Experiment #6). Where x = Day of age; y = weight (g).



Protein 24 Protein accretion in breast muscles (pectoralis superficial + pectoralis subclavis muscles from right side) and leg muscles (gastrocnemius + peroneous longus muscles from right leg) of control or force-fed male Single Comb White Leghorn chicken over a 4-day period, (Experiment #6).

39. The FSR in the breast muscles or leg muscles were 25.0 and 29.6 or 20.0 and 14.0 %/day in control and force-fed male SCWL chickens, respectively. Although the FSR in the breast and leg muscles were not significantly different in control chickens, but the FSR in leg was numerically less than the breast muscles (Table 39). The statistical analysis on values of FSR for breast and leg muscles indicated that a signficant ($p < 0.01$) response occurred from dietary treatment on type of muscles in male (SCWL) chickens (Table 40). The FSR in the leg muscles was depressed while in the breast muscles enhanced in response to force-feeding (Table 39). The FSR in the breast muscles was signficantly ($p < 0.01$) greater than in the leg muscles in the force-fed chickens (Table 39). But such changes did not occur in the control chickens. The fractional accretion rates (FAR) in the breast or leg muscles were 5.7 and 8.1 or 6.3 and 6.9 %/day in control and force-fed chickens, respectively. The relationships of FAR, FSR and fractional breakdown (FBR) in the breast and leg muscles are shown in Figure 25. The FBR in the breast muscles was almost similar in control and force-fed chickens (Figure 25). But the FBR in the leg muscles of control chickens was twice that of the values for the force-fed chickens (Table 39 and Figure 25). This indicates that force-feeding depressed FSR and FBR in the leg muscles, but not in the breast muscles. The individual leucine specific activity (S.A.) in the breast or leg muscles and ^{3}H-leucine incorporation into proteins of either muscles or plasma radioactivity at 3, 10, 20, and 30 minutes of infusion in chickens are presented in Appendix B Table 11.

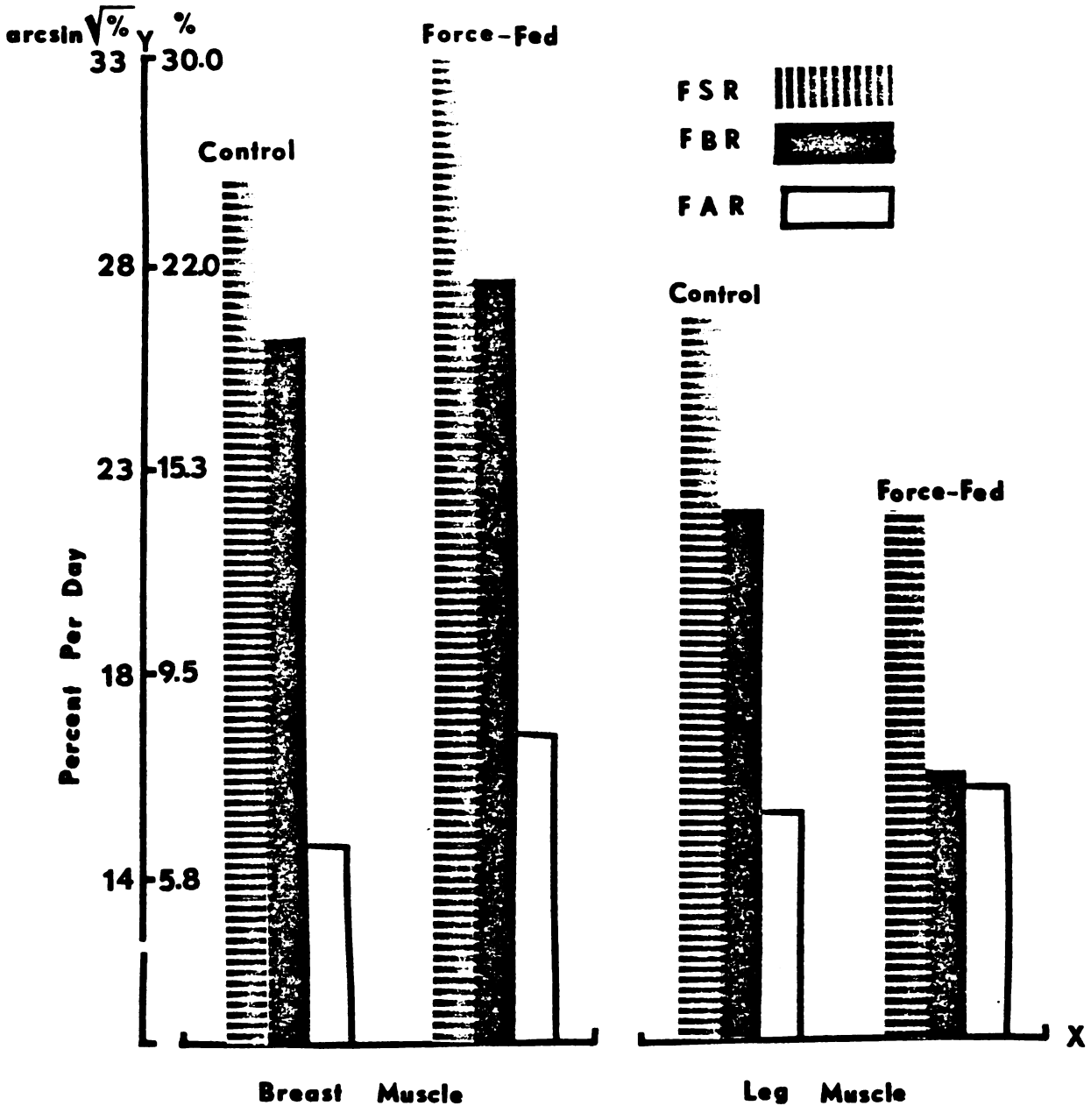


Figure 25 Relationship of fractional synthesis rate (F S R), fractional breakdown rate (F B R) and fractional accretion rate (F A R) in breast muscles (pectoralis superficial + pectoralis subclavis muscles) or leg muscles (gastrocnemius + peroneous longus muscles) of control and force-fed Single Comb White Leghorn chicken at 35 days of age, (Experiment #6).
Where x = type of muscles; y = %/day (arcsin $\sqrt{\%}$).

CHAPTER V

Discussion

Method of Protein Turnover Study

The validation of quantitative assessment of protein turnover by the use of radioactive isotopes is a most important element in the protein study. When, protein synthesis was measured as CPM incorporated per 100 mg protein isolated, the rates of muscle protein synthesis were similar for rats fed either a high quality or low quality protein; however, there was a three-fold difference in weight gain (Hsueh et al., 1975). Bergen (1975) suggested that, this approach is inappropriate and that to study protein synthesis in vivo, the specific activity of the precursor pool during the time course of incorporation must be described. This approach is even more critical if only a tracer dose with no "cold" carrier is administered, because the tracer is more rapidly reutilized.

In a method using single administration of tracer amino acid, the tracer specific radioactivity (S.A.) in the precursor pool of protein must be constant throughout the experimental period. However, in practice, a radioactivity peak in the precursor amino acid pool of protein is reached between 2 and 4 minutes postinjection, then followed by a rapid decline (Martin et al., 1977). Intraperitoneal administra-tion of (^{3}H) valine resulted in slower mixing with the result that a plateau occurred in the counting values which lasted for

about 10 minutes (Airhart et al., 1974). The S.A. of ^{14}C-lysine was measured after a single intravenous injection and was found to decline slowly over a period of 10 to 20 minutes (Henshaw et al., 1971). They assumed that the S.A. value obtained at the time of tissue removal was sufficiently close to the average for the entire 20 minute period which was used to calculate the protein synthesis. These assumptions could be questioned because the most rapid fluctuation of the S.A. of the tracer was reached in the first few minutes after administration of the tracer amino acid (Martin et al., 1977, Zak et al., 1979).

The optimum approach to study protein turnover is consistent of continuous administration of radioactive amino acid. The S.A. of the tracer in the blood and intracellular pools eventually reach a steady state in which the influx of radioactive amino acid is balanced by its metabolism or excretion or both (Zak et al., 1979). This steady state was developed within the first 1 or 2 hours with intravenous infusion (Garlick et al., 1973). The S.A. of the precursor pool and protein are measured at the end of the infusion time, then the suggested formulas (Garlick et al., 1973; Zak et al., 1979) are used to calculate the protein synthesis. Zilversmit (1960) suggested that the mathematical treatment of experimental data is simplified considerably when the precursor S.A. does not change during the course of an experiment. This criteria was met in our experiments involving muscle protein uptake of ^{3}H-leucine. The key to obtaining near constant S.A. of ^{3}H-leucine in blood and precursor pool of muscles was the infusion program that was developed. Our data demonstrated a higher S.A. of ^{3}H-leucine in the plasma than in the muscles throughout the experiment.

This was substantiated with the work of Henshaw et at. (1971) and MC Nurlan et al. (1979) that the tracer S.A. was less than that in the plasma. Therefore, the measurement of the S.A. of ^{3}H-leucine in the precursor pool and the incorporation of the tracer in the muscle proteins at the time of tissue removal is adequate to calculate the amount of protein synthesized per unit of time (FSR max.).

General Influence of Age, Sex and Stain on Body Composition

Age, sex and strains have been found to greatly influence body composition. Total carcass lipid content tended to increase as chickens aged form 1 to 6 weeks (Kubena et al., 1972; Husseiny and Creger, 1980). The body weight and absolute quantity of abdominal fat in broilers also increased with increasing age from 7 to 9 weeks (Kubena et al., 1974). Our results showed that, the weight of abdominal fat in male and female meat-type chickens almost doubled every week from 2 to 6 weeks of age. However, the whole body weight doubled weekly from 2 to 4 weeks and then increased over the next two weeks by a 1/3 fraction of whole body weight. This increase in the body weight and abdominal fat in male chickens at 5 and 6 weeks of age indicated a more rapid fat deposition than whole body growth during this period. This is in agreement with Tzeng and Becker (1981).

Summers et al. (1965) showed that six week old female broilers contained considerably more fat than males. Our data supported the results of Summers et al. (1965) and demonstrated this to be characteristic for all ages examined. The abdominal fat as a percentage of whole body was higher in the meat-type chickens than in

egg-type chickens (SCWL) at all ages studied. Therefore, sex and strain influenced the fat depot of chickens. The body growth per unit of body weight declined as the animal aged. The growth of individual muscles per unit of each muscle's weight also decreased with increasing age in both sexes and strains of chickens. This observation indicated that, the protein synthesis or degradation and/or both was (were) changing in a manner to reduce the accretion rate of muscles. Our results also revealed that the reduction in fractional accretion rate (FAR) as %/day occured when the chickens of both strains aged from 26 to 45 days. This suppression of FAR was even more pronounced in the meat-type chicken (fast growing) than in the egg-type bird (slow growing). Substantial evidence was accumulated, indicating that lean meat had an accelerating phase in the younger animal followed by a slower rate as they aged (Zucker and Zucker, 1963; Baily and Zobrisky, 1968; Searle and Mc Graham, 1972; and Tzeng and Beckder, 1981). The weight of breast muscle as a percentage of body weight increased as the male meat-type and egg type chickens aged from about 24 to 26 days of age to the period of 45 to 47 days of age. Our results also revealed that the breast muscle's weight, starting from 10 or 12 days of age increased as a percentage of body weight. This showed that, the breast muscles enlarged up to 45 days of age at a rate faster than the whole body. The weight of breast muscle was a larger proportion of whole body in the fast-growing than in the slow growing chickens. This large proportion of breast muscles is a genetic trait and is a product of genetic selection (Maruyama et al., 1978).

The weight of the leg muscles as a percentage of body weight

increased as the fast and slow growing chickens aged from 24 to 47 days. Thus, leg muscles were also growing faster than the whole body. In general, the data indicated that, whole body, breast muscles and leg muscles had a parallel curvilinear growth for both strains of chickens aged from 10 to 45 days. But, linear growth was observed over the age 24 to 45 days.

Edwards et al. (1973); and Husseiny and Creger (1980) showed that, the %water content of whole body decreased as the animal aged. Our data showed the same trend for both breeds. It (H_2O) varied from 67 to 71% of the whole body for chickens 24 to 47 days of age. Generally, the percent fat of body increased and the % moisture declined as the bird aged (Lepore and Marks 1971; Edwards et al., 1973; Kubena et al., 1974; Tzeng and Becker, 1981). This is in agreement with our experimental results. In a literature review by Demby and Cunningham, (1980), the water content of chicken meat varied from 63 to 75%. The % water of breast muscles was between 74 to 76%, a value very simllar to the water content of the leg muscles during the period of 26 to 45 days of age. The % lipid content of whole body increased from about 7.5 to 9.5% in the male, fast-growing chickens as they aged from 26 to 45 days. This is in agreement with Edwards et al. (1973) and Goodwin et al. (1969). The % lipid increased linearly in the breast muscles as the meat-type chickens aged. However, the % lipid in the breast muscles of slow-growing chickens averaging about 0.8% was constant as these chickens aged. The % lipid content in the leg muscles of fast-growing male chickens was constant at various ages examined. But, it declined in the leg muscle of slow-growing chickens as they aged

from 26 to 45 days. The % lipid of leg muscles ranged from 2.0 to 4.2% regardless of age and strains studied. Goodwin et al., (1969) analyzed meat-type chickens of different strains and found that their fat content ranged from 8 to 9.6% while their protein content ranged from 18.9 to 20.1%.

The % protein of whole body weight for chickens of both breeds increased linearly (p 0.01) as they aged. The % protein of the chickens ranged between 17.6 - 20.8% during their age of 10 to 45 days. This is similar to results from Goodwin et al., (1969). The protein content in the breast muscles ranged, overall, from 20 - 25%. In all experiments, the % protein in the leg muscle of chickens increased linearly as they aged. The % protein content in the leg muscles was greater than that in the breast muscles. In general, our results demonstrated that the weight of the whole body or individual muscle's increased linearly as the chickens aged from 24 to 45 days of age. During this time the % water declined and the % lipid and protein increased.

The percent change of body weight as a percentage of protein or fat at various periods of life should be considered, in order to determine the total body protein or fat during the specific period which the total protein per unit of body gain declines and fat deposition sharply increases. This could be a clue to study the change in protein and fat synthesis or degradation during that specific period of life which the protein and fat contribute the lowest and highest amount respectively, to the unit of body gain. Experiment #4 did not show any drastic changes in protein and fat accretion of meat-type

chickens per unit of weight gain during any 4-day period. This indicated that 4 day intervals between measurements were not sufficient time to detect these changes in weight. On the other hand, a significant change in fat deposition (increase) and protein accretion (decrease) was detected over the 4 day period of 41 to 45 days of age for slow-growing chickens.

Fat Deposition as Effected by the Chicken's Age

Nutritional and non-nutritional factors affect the degree of fatness in the animal. The excess abdominal fat in a bird is considered to be a problem of energy balance, as an interaction between energy intake, energy expenditure, energy reserve and heat loss (Maurice, 1981). Excess of energy intake in relation to expenditure results in storage of excess fat. The partition of energy balance in a controlled system as an animal is regulated by a series of enzymes which are responsive to feed-back elements Leveille et al., (1975). Leveille et al. (1975) suggested that, the regulatory enzymes could become unbalanced through an abnormality in the feed-back of control factors, which in turn may cause acceleration of lipogenesis. Broilers fed amino acid deficient diets produced excess fat deposition, as a result of overconsumption of energy in a compensatory attempt to overcome the limiting amino acids required for optiomal growth (Lipstein et al., 1975). Conversely, a reduction in the rate of hepatic lipogenesis was observed in chicks fed diets containing increased protein concentrations (Yeh and Leveille, 1969;1971). High dietary protein concentrations elevate uric acid concentration in

blood, liver and kidney of chicken (Okumura and Tasaki 1969). Some energy is required for synthesis of this compound (Buttery and Boorman, 1976). Thus, the effect of a high protein diet to decrease carcass fat may be due to more energy spent to eliminate excess nitrogen from the body. Excess dietary isoleucine or lysine decreased carcass fat in birds purified diets (Velu et al., 1971). Donaldson et al. (1965), Rand et al. (1957), Davidson et al. (1964), Summers et al. (1965), Yoshida and Morimoto (1970), Thomas and Twining (1971), and Kubena et al. (1972) demonstrated that, as the calorie:protein (cal/p) ratio of diet increased, energy intake and carcass fat increased. The inclusion of lipid in the diet of the bird resulted in a reduced rate of lipogenesis from carbohydrate sources (Pearce, 1968); Pearce, 1971a) and overcoming a degree of liver fatness (Haghighi-Rad and Polin, 1982) in the bird, because, the dietary lipid can be a direct source of body fat and through a feed-back mechanism partially inhibits enzymes of the liver involved in lipogenesis (Yeh and Leveille, 1969, 1971).

The responsiveness of regulatory enzymes to produce fat deposits in the chicken is probably specific at different ages (Allee et al., 1971). Our results demonstrated that, the percentage of fat in whole body linearly increased as the chicken aged. This is in agreement with Combs (1968), Edwards (1971), and Kubena et al. (1972). There is little information about the enzymatic activities of fatty acid biosynthesis in the chicken at different ages. In the bird, the liver is the main site of lipogenesis (Goodridge, 1968b; Leveille et al., 1975; Shapira et al., 1978). The activities of several enzymes in liver and adipose tissue which are involved in glucose metabolism and

the conversion of glucose to lipids were studied by Goodridge (1968a;c). The lipogenesis in the chick embryo is very low (Kilsheimer et al., 1960); Goodridge, 1968c). Soon after hatching the activity of citrate cleavage enzyme, which converts the citrate (derived from glucose) to acetyl-coA, and other enzyme activities at one week of age (Goodridge, 1968c). However, in the adipose tissue, the enzyme activities remained low from lage embryonic stage until 4 weeks after hatching. Our data showed that, the fat deposition relative to the body growth of male-type chickens sharply increased at about 4 weeks and continued to 6 weeks of age. This is also in agreement with Tzeng and Becker (1981). Thus, the enzymatic activities of the lipogenesis pathway in the adipose tissue may have increased at about 4 weeks of age, and should be studied. The total fat content of chickens also increased as they aged from 10 to 45 days. Therefore, both increased in activities of enzymes for fatty acid synthesis and high volume of andipose tissue may be a problem of enhanced fat accumulation after 4 weeks of age in the chicken.

Protein Turnover as Influenced by a Chicken's Age

Many investigators agree that, in the growing animal, the protein content of the carcass increases in the animal ages (Kubena (et al., 1972; Kubena et al., 1974; and Bergen, 1974). Our results indicated an increase in the protein content of the two muscles and whole chickens of both sex and strainas they aged from 26 to 45 days. In the mature animal (slow or no growth state) protein accretion almost ceases (Zucker and Zucker, 1963; Bailey and Zobrisky, 1968; Searle and Mc

Grahm, 1972). In other words, the rate of protein synthesis and degradation in the muscles of young animals are quite rapid (Waterlow and Stephen, 1968; Soltes et al., 1973; Maruyama et al., 1978; and Reads et al., 1980). The animal growth is the difference between the rates of protein synthesis and breakdown (Millward et al., 1975: Maruyama et al., 1978). Maruyama et al., (1978) postulated that, a high rate of growth may be achieved by increasing this difference, either by increasing the rate of synthesis, or by decreasing the rate of degradation. Millward et al., (1975) showed that, as the rat aged, rates of protein synthesis and degradation declined and the growth rate decreased. Mulvaney, (1981) demonstrated a similar trend in the rate of protein synthesis and degradation in the muscles of pigs as they aged. Our results for leg and breast muscles indicated that the difference between protein synthesis and degradation declined as the chicken aged from 28 to 43 days. This was concomitant with a decrease in the fractional accretion rate (FAR) for both msucles or whole chicken. However, the rate of protein synthesis measured in the muscles did not change as the chicken aged from 28 to 43 days. Similarly, Maruyama et al., (1978) reported no change in the fractional synthesis rate (FSR) of leg muscles of chicken aged from 1 to 2 weeks. This may suggest that, the decline in growth rate could be a result of an increase in the activity of degrading enzymes as the chicken ages. Maruyama et al., (1978) showed that the FSR in pectoralis muscles was 38.0%/day at one week and declined to 21.5%/day for chickens at 2 weeks old. The FSR in the Rock Cornish Chicks (fast-growing) was about 25%/day in the leg and breast muscles at 2 weeks of age (Maruyama et

al., 1978). Our results indicated FSR to be 17.0 and 27.0%/day in the leg and breast muscles of slow-growing (SCWL) birds, respectively, over the period of 28-43 days of age. The FSR of breast muscles at 43 days of age was higher than that in the meat-type chick, 2 weeks old (Maruyama et al., 1978). Apparently, conflicting conclusions arise because different strains of birds, different raidoisotopes and different experimental approaches were used to obtain FSR. The FSR for the breast muscles of our experiment was very high (53-61%/day) for meat-type chickens 28 to 43 days of age. This value was higher than that previously reported in the chicken of even a younger age (Table 41). The FSR in the leg and breast muscles of our meat-type chickens were higher than in the Rock Cornish chickens 2 weeks old, as measured by ^{14}C-Tyrosine and a dietary infusion method (Maruyama et al., 1978). In contrast to Maruyama et al., (1978) experiments, the FSR for protein in the breast muscles was higher than in the leg muscles of both meat and egg type chickens. Our results also indicated that, the FSRs of leg and breast muscle proteins were higher in the meat type chicke than egg type at all ages studied. Thus, one may postulate that protein synthesis is more productive (more efficient and/or more polyribosomes) in the meat type than slow growing chicken.

Protein Turnover in Over-Fed Chickens

Several reports have assessed the responses of tissues to nutrient deprivation. A decline in rate of protein syntheis in muscle was reported in response to starvation (Waterlow and Stephen, 1968; Garlick et al., 1975; Goldbert and Chang, 1978). Glick et al., (1982) studied

Table 41. Comparison of Fractional Synthesis Rate (FSR) of Chicken at Different Age

Type of Chicken	Age (day)	Age of Muscle	FSR (%/day)	Reference
Rock Cornish	14	Leg	25.0	Maruyama et al., 1978
	7	Breast	25.0	Maruyama et al., 1978
Cross-bred	7	Leg	25.0	Maruyama et al., 1978
	7	Breast	38.0	Maruyama et al., 1978
Cross-bred	14	Leg	23.0	Maruyama et al., 1978
	14	Breast	21.0	Maruyama et al., 1978
White Leghorn Cockerels	245	Anterior Latissimus dorsi	17.0	Laurent et al., 1978
SCWL	28	Leg	17.0	Our results
		Breast	29.0	Our results
Meat-type (Heavy-breed)	28	Leg	35.0	Our results
		Breast	61.0	Our results

the effect of over-feeding on protein turnover in muscle,and found that protein synthesis on rat muscle was reduced. They concluded that, protein degradation must also decline in force-fed rats, because, no changes in protein was observed. Several invesitgators studied the effect of force-feeding in fat deposition of chickens. The activities of malic and citrate cleavage enzymes in liver and carcass adipose tissue increased in force-fed than in ad-libitum fed chicks of 26 days of age (Shapiro et al., 1978). This excess feed intake by force-fed chicks resulted in an increase in % of fat in both liver and carcass. Polin and Chee (unpublished data) noted an absolute increase in protein content of SCWL chickens, 2 weeks of age, which were force-fed for 4 days. Our results are in agreement with work of Polin and Chee (unpublished data). Our data also indicated that, the protein accretion in the leg or breast msucles from 4 days of over-feeding was higher than in ad-libitum fed birds. The FSR was depressed in the protein of leg muscles from force-fed chicks. This depression of FSR in the leg muscle of chicken was similar to that in rats observed by Glick et al., (1982). The FAR value in the leg muscle protein was higher in the force-fed chickens. Therefore, the decline in protein synthesis in the protein of leg muscle for force-fed chickens was concomitant with an even greater suppression of protein degradation, which resulted in a higher net protein deposition. The response of protein turnover in breast muscles to over-feeding was different from that in the leg muscles. The FSR was enhanced in the protein of breast muscles from chicks force-fed for 4 days. This enhanced FSR was accompanied by a lesser increase in the rate of protein degradation,

which produced a higher protein accumulation in the breast muscles of force-fed chickens. Thus, the machinery for protein synthesis in breast muscle was somehow stimulated while it was depressed in the leg muscles of force-fed chickens.

SUMMARY AND CONCLUSIONS

This study was designed to provide a technique for measuring the rate of protein synthesis in muscles of chicken. The results obtained revealed that the specific radioactivity (S.A.) of ^{3}H-leucine in the plasma and free pool of amino acid of leg and breast mucles was almost constant throughout the experimental period of 30 minutes. Thus, the calculation of the n moles of leucine incorporated into protein molecules during the exerimental period is theoretically permissible according to the criteria of Henshaw et al., (1971); Garlick and Millward, (1972).

The percentage of water declined, while the % fat and % protein increased in the whole body as the meat-and egg-type chickens aged from 4 to 6 weeks. The weight of abdominal fat and total body fat increased more rapidly than whole body growth during the meat-and egg-type chickens 5th and 6th week's of age. The protein content of breast and leg muscles were 22.0 and 19.0%, respectively, in both egg-and meat-type chickens 4-6 weeks of age. The percentage of lipid was about 1.0% in breast muscles of both strains studied. The fat content of leg muscles was 4.0 and 2.2% in meat-and egg-type birds, respectively, 4-6 weeks of age.

The protein turnover study was conducted when the chickens were 4-6 weeks of age at the time the nutrient energy appeared to have shifted toward production of fat rather than protein. The fractional

accretion rates or FAR (%/day) were 7.7, 6.3 and 4.4 %/day in the whole body of meat-type chickens at 4, 5 and 6 weeks of age, respectively. The whole body FAR for egg-type chickens were 6.1, 5.4 and 3.4 %/day for 4,5 and 6 weeks of age, respectively. The FAR in the breast (pectoralis superficial + pectoralis subclavis) muscles decreased from 7.4 to 4.7 in meat-type and 6.0 to 3.1 %/day in the egg-type chickens, respectively, as they aged from 4 to 6 weeks. The FAR in the leg (gastrocnemius + peroneous longus) muscles declined from 9.9 to 3.9 and 6.1 to 4.4 %/day in the meat-type and egg-type birds, respectively as they aged from 4 to 6 weeks. The fractional synthesis rate or FSR (%/day) in the leg or breast muscles were similar at 4, 5 and 6 weeks of age in either strains of chicken examined. But the meat-type chicken had a much higher FSR than egg-type chicken (approximately 33.0 vs 17.0 or 57.0 vs 28 %/day) in both leg and breast muscles, respectively. In conclusion, the catabolic activity of protein of leg and breast muscles increased as the egg-and meat-type chicken aged from 4 to 6 weeks.

Experiment #6 was conducted for investigation about the effect of over-feeding on FAR, FSR and fractional breakdown rate (FBR) of breast and leg muscles of egg-type chickens. The FAR in the leg or breast muscles were 6.6 and 6.3 or 8.1 and 5.7 %/day for force-fed and ad-libitum fed chicken, respectively. The FSR was 25.2 %/day in the breast muscles of ad-libitum fed birds and enhanced to 29.6 %/day in a 4-day force-fed chicken. The FSR in the leg muscles was 20.3 %/day in control and declined to 14.1 %/day in the over-fed chickens. The results substantiated that, over nutrition may be associated with a

decline in rate of protein synthesis in the leg muscles of chicken. This is similar to results obtained using the rat (Glick et al., 1982). But over-feeding enhanced the rate of protein synthesis in the breast muscles of chicken.

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CHAPTER VII

BIBLIOGRAPHY

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APPENDICES

VIII
APPENDICES

Appendix A

Table 1. Preparation of infused radioactive solution (Experiment #3).

Ingredients	μCi	μg	Volume (Ml)
^{3}H-leucine	1250	0.55	0.75
"cold" leucine	-	3850	-
Saline	-	-	34.25
Total	1250	3850.55	35

Appendix A

Table 2. Preparation of infused radioactive solution for chickens, 28 days old. (Experiment #4)

Ingredients	μCi	μg	Volume (Ml)
^{3}H-leucine	1279.2	0.563	0.65
"cold" leucine	-	4446.4	-
Saline	-	-	15.35
Total	1279.2	4446.96	16.0

Appendix A

Table 3. Preparation of infused solution for chickens, 36 days of age. (Experiment #4).

Ingredients	μCi	μg	Volume (ml)
^{3}H-leucine	2071.7	0.911	0.85
"cold" leucine	-	6034.	-
Saline	-	-	16.
Total	2071.7	6034.91	16.85

Appendix A

Table 4. Preparation of infused solution for chickens, 43 days of age. (Experiment #4).

Ingredients	μCi	μg	Volume (ml)
^{3}H-leucine	2832.2	1.25	1.42
"cold" leucine	-	8901.0	-
Saline	-	-	12.58
Total	2832.2	8902.25	14.0

Appendix A

Table 5. Preparation of infused radioactive solution for chickens 28 days of age. (Experiment #5).

Ingredients	μCi	μg	Volume (ml)
^{3}H-leucine	468	0.21	0.5
"cold" leucine	-	1620.	-
Saline	-	-	19.5
Total	468	1620.21	20.0

Appendix A

Table 6. Preparation of infused radioactive solution for chickens 36 days of age. (Experiment #5).

Ingredients	μCi	μg	Volume (ml)
^{3}H-leucine	615	0.27	0.6
"cold" leucine	-	1725	-
Saline	-	-	15.0
Total	615	1725.27	15.6

Appendix A

Table 7. Preparation of infused radioactive solution for chickens 43 days of age. (Experiment #5).

Ingredients	μCi	μg	Volume (ml)
^{3}H-leucine	928.2	0.41	0.9
"cold" leucine	-	2608.	-
Saline	-	-	16.1
Total	928.2	2608.41	17.0

Appendix A

Table 8. Preparation of infused radioactive solution (Experiment #6)

Ingredients	μCi	μg	Volume (ml)
^{14}C-leucine	207.7013	91.39	1.8
"cold" leucine	-	4400.	-
Saline	-	-	38.2
Total	207.7013	4491.39	40.0

Appendix A

Table 9. Preparation of dansyl chloride which was used for determination of leucine specific activity in the gastrocnemius + proneous longus in muscle protein. (Experiment #3).

Ingredients	dpm	μg	Volume (ml)
^{14}C-dansyl chloride	$3X10^6$	3.5	40.
"cold" dansyl chloride	-	22663.1	225.
Acetone	-	-	300.
Total	$3X10^6$	22666.6	565.

Appendix A

Table 10. Preparation of dansyl chloride which was used for determination of leucine specific activity in pectoralis (superficial + subclavis) muscles. (Experiment #3).

Ingredients	dpm	μg	Volume (ml)
^{14}C-dansyl chloride	3886528.	4.5	50.0
"cold" dansyl chloride	-	11005.5	110.
Acetone	-	-	350.
Total	3886528	11010.	510.

Appendix A

Table 11. Preparation of dansyl chloride which was used for determination of leucine specific activity in plasma. (Experiment #3).

Ingredients	dpm	µg	Volume (ml)
^{14}C-dansyl chloride	4000298	4.7	55.0
"cold" dansyl chloride	-	17540.	175.
Acetone	-	-	300.
Total	4000298	17545.	530.

Appendix A

Table 12. Preparation of dansyl chloride (Experiment #4 and #5).

Ingredients	dpm	µg	Volume (ml)
^{14}C-dansyl chloride	6574230	7.7	90.
"cold" dansyl chloride	-	18000.	180.
Acetone	-	-	900.
Total	6574230	18007.7	1170.

Appendix A

Table 13. Preparation of dansyl chloride (Experiment #6).

Ingredients	dpm	µg	Volume (ml)
^{3}H-dansyl chloride	8345607.0	0.84	160
"cold" dansyl chloride	-	10000.	100
Acetone	-	-	740
Total	8345607.0	10000.84	1000

Appendix B

Table 1. Individual weights of live body, breast muscles, leg muscles and abdominal fat of meat-type chickens at 10, 17, 24, 31, 38 and 45 days of age (Experiment #1).

	Tissue Weights - g																							
	Live body Wt. (g)						Breast Muscle[1]						Leg Muscle[2]						Abdominal Fat[3]					
	Age (day)						Age (day)						Age (day)						Age (day)					
	10	17	24	31	38	45	10	17	24	31	38	45	10	17	24	31	38	45	10	17	24	31	38	45
Male	94	235	449	725	1270	1640	3,04	9.27	21.2	40.3	78.6	89.0	2.27	5.54	14.35	23.8	44.9	51.3	0.201	1.83	2.57	8.08	10.74	19.35
	129	260	499	760	1420	1630	3.65	11.64	23.1	47.4	82.7	84.7	4.50	6.45	13.85	26.6	51.7	56.6	0.119	2.56	4.36	5.48	19.74	24.9
	93	190	539	830	1180	1685	2.86	7.67	26.7	44.8	63.7	96.2	2.00	4.87	17.74	30.9	40.4	60.5	0.078	0.83	3.55	7.14	7.70	37.5
	99	282	472	920	1500	1540	2.15	12.78	21.9	48.7	65.4	85.9	1.95	7.03	13.23	29.9	38.8	59.0	0.221	0.68	5.15	9.11	23.9	16.6
	89	235	395	845	1000	1800	2.7	11.47	22.6	50.0	62.3	107.3	1.99	6.45	11.35	27.5	33.1	65.8	0.102	1.81	1.77	8.70	2.8	15.9
Female	107	228	464	725	1095	1360	4.24	9.48	23.11	40,3	60.3	75.7	2.76	5.65	14.7	22.5	32.0	45.8	0.40	1.30	2.83	4.87	13.9	21.6
	100	211	532	760	955	1630	3.07	11.12	28.9	43.4	56.1	109.5	2.21	5.36	14.6	26.1	30.8	57.0	0.26	1.15	9.48	7.84	14.9	27.7
	78	201	477	790	1000	1095	1.91	8.96	22.9	41.0	60.6	61.5	1.39	4.36	14.6	25.0	34.8	33.0	0.10	1.61	4.50	8.88	9.6	27.0
	128	267	416	815	664	1360	4.19	13.02	20.9	42.5	33.1	89.1	3.20	7.48	11.8	23.1	20.5	45.5	0.21	1.20	3.91	5.26	9.0	14.8
	100	254	504	670	800	1255	2.21	12.44	27.7	40.2	42.1	85.3	1.84	7.15	15.9	24.0	25.6	44.8	0.54	2.04	2.35	9.75	10.3	10.5

[1]Pectoralis superficial + pectoralis subclavis from right side.

[2]Gastrocnemius + peroneous longus muscles from right leg.

[3]Abdominal fat excised from after gizzard.

Appendix B

Table 2. Individual weights of live body, breast muscles, leg muscles and abdominal fat of Single Comb White Leghorn (SCWL) chickens at 12, 19, 26, 33, 47, 61 and 82 day of age. (Experiment #2).

	Tissue Weights - g																												
	Live body Wt. (g)							Breast Muscle[1]							Leg Muscle[2]							Abdominal Fat[3]						
	Age (day)							Age (day)							Age (day)							Age (day)						
	12	19	26	33	47	61	82	12	19	26	33	47	61	82	12	19	26	33	47	61	82	12	19	26	33	47	61	82
Male	77	119	137	281	580	795	1295	2.22	3.92	4.73	11.6	28.1	41.54	74.8	1.72	2.90	3.61	8.1	20.6	28.5	53.8	0.03	0.37	0.39	1.25	1.19	4.42	9.23
	63	138	215	154	540	705	1185	1.70	5.65	8.86	4.91	28.41	33.5	68.0	1.18	3.52	5.87	3.6	18.8	21.6	47.1	0.004	0.07	0.49	0.60	1.51	3.93	9.15
	74	166	206	272	515	940	1120	1.74	7.08	8.73	7.76	26.3	48.1	56.94	1.37	4.44	5.71	7.78	16.8	30.9	41.0	0.04	0.32	0.48	1.24	3.0	3.45	10.62
	69	95	-	315	430	648	-	1.88	3.13	-	14.9	21.0	26.7	-	1.57	2.74	-	8.92	13.8	21.3	-	0.012	0.16	-	1.87	1.12	8.8	-
	-	-	-	278	560	-	-	-	-	-	11.6	26.3	-	-	-	-	-	8.39	18.4	-	-	-	-	-	0.87	0.34	-	-
	-	-	-	251	495	-	-	-	-	-	7.03	20.1	-	-	-	-	-	6.63	14.91	-	-	-	-	-	1.26	3.31	-	-
Female	73	83	168	285	420	680	1030	2.43	1.87	7.22	14.0	18.59	38.2	63.8	1.55	1.51	4.9	8.6	13.9	23.4	34.4	0.02	0.11	0.17	0.70	2.53	4.30	18.4
	82	91	197	-	-	600	920	2.83	3.31	8.41	-	-	31.3	47.9	1.79	2.14	5.2	-	-	19.9	31.7	0.03	0.42	0.37	-	-	6.21	6.00
	78	125	118	-	-	520	915	2.10	5.54	3.80	-	-	25.4	46.1	1.42	3.98	2.7	-	-	15.6	28.6	0.004	0.08	0.33	-	-	7.28	13.24
	-	-	153	-	-	-	780	-	-	5.39	-	-	-	39.14	-	-	3.9	-	-	-	25.0	-	-	1.61	-	-	-	11.86

[1]Pectoralis superficial + pectoralis subclavis muscles from right side.

[2]Gastrocnemius + peroneous longus muscles from right leg.

Appendix B

Table 3. Percentage of Leucine in Muscle Protein (Experiment #3)

Bird's Code#	Type of Muscle	%Leucine
1	Gastrocnemius + peronedus longus	7.3
2	Gastrocnemius + peronedus longus	7.2
3	Gastrocnemius + peronedus longus	7.0
1	Pectoralis	7.2
2	Pectoralis	7.3
		7.2 ± 0.14[1]

[1]Mean (+S.D.) is used for Calculation of fractional synthesis rate in experiments 4, 5 and 6.

Appendix B

Table 4 . Individual leucine specific radioactivity in the precursor pool of breast muscles, leg muscles and plasma of chickens at 3,10, 20 and 30 minutes of infusion. (Experiment # 3).

Infusion Time (Min.)	Bird's Code	Specific activity of leucine (dpm/nmol)		
		Breast Muscles	Leg Muscles[2]	Plasma
0	1	0.0	0.0	0.0
	2	0.0	0.0	0.0
	3	0.0	0.0	0.0
3	4	42.0	124.0	579.0
	5	73.0	356.0	520.0
	6	169.0	232.0	533.0
10	7	60.0	219.0	469.0
	8	110.0	295.0	467.0
	9	54.0	145.0	501.0
20	10	72.0	345.0	632.0
	11	171.0	413.0	653.0
	12	130.0	313.0	641.0
30	13	73.	297.0	462.0
	14	82.	263.0	461.0
	15	88.	254.0	555.0

[1]Pectoralis superficial + pectoralis subclavis muscles from right side.

[2]Gastrocnemius + peroneous longus muscles from right leg.

Appendix B

Table 5. Individual empty crop body weight, weights of breast or leg muscles and proximate analysis for these 3 tissues in male meat-type chickens at 26,30,34,38,41 and 45 days of age. (Experiment #4)

Age (day)	Band #	E.C.B.wt[1](g)	Breast[2](g)	Leg[3](g)	Proximate Analysis								
					E.C. Body			Breast Muscle			Leg Muscle		
					$\%H_2O$	%Lipid	%Prot.	$\%H_2O$	%Lipid	%Prot.	$\%H_2O$	% Lipid	%Prot.
26	20081	763.	33.4	22.5	68.0	9.8	17.8	73.0	0.9	23.4	74.1	4.7	17.8
	20034	778.	40.7	23.7	70.9	6.8	16.7	74.0	0.6	23.0	75.0	4.0	17.8
	20113	633.	28.0	18.3	70.9	6.5	17.1	74.4	0.7	22.7	75.2	4.0	18.4
	20029	773	45.7	22.5	69.0	8.1	17.1	74.4	0.8	23.2	75.3	3.5	18.2
	20037	770	41.1	23.9	70.4	6.9	17.1	74.4	0.3	23.1	75.8	2.8	18.5
30	20070	1118.	57.9	39.0	70.6	6.9	17.1	74.6	0.8	22.4	75.9	3.7	18.9
	20005	1054.	57.0	35.7	68.3	9.5	17.1	73.9	1.2	23.0	75.6	3.6	18.3
	20057	936.	50.4	32.3	68.8	8.3	18.1	74.8	0.7	22.8	75.4	4.3	18.0
	20087	1000.	50.1	31.6	69.8	7.6	18.0	74.2	0.6	23.7	75.6	3.5	19.3
	20118	850.	39.4	22.3	68.9	8.3	17.9	74.2	0.9	23.7	75.9	3.4	19.1
34	20045	1238.0	59.8	40.1	67.5	9.1	17.4	74.9	0.7	21.6	74.4	4.7	18.6
	20086	1066	50.3	36.3	66.6	10.2	17.6	74.0	1.5	22.3	74.3	4.9	18.7
	20023	1154.	59.	37.7	70.2	6.8	17.8	74.7	0.5	21.7	76.1	3.5	18.6
	20068	1142.	77.	38.9	69.6	7.1	18.2	73.9	0.7	22.5	74.8	3.6	20.1
	20042	1102.	70.2	36.9	69.3	7.0	18.4	74.0	0.8	21.5	74.6	4.1	19.6
38	20038	1376.	76.0	44.0	66.9	10.3	18.0	74.1	0.9	22.4	74.9	3.6	18.9
	20028	1380	70.7	44.5	67.6	8.8	18.9	73.8	1.0	22.6	75.0	3.4	19.7
	20103	1428.	85.0	48.4	69.4	7.0	19.1	73.8	0.8	22.8	75.3	3.2	19.6
	20027	1446.	89.8	50.4	68.4	7.9	18.6	74.0	0.9	21.2	74.9	4.0	19.4
	20020	1414.	77.6	46.6	68.	8.5	18.7	74.4	0.9	21.6	75.1	3.6	19.3
41	20065.	1546.	90.0	53.7	67.8	8.5	18.5	74.1	1.2	21.5	75.1	3.3	19.8
	20116.	1454.	84.6	51.5	66.9	8.9	19.0	72.9	1.7	22.3	74.9	3.2	20.0
	20097.	1422.	69.5	46.2	65.7	10.7	18.4	74/2	0.8	22.2	75.2	4.1	18.6
	20085	1638.	102.1	58.1	67.9	8.6	18.6	73.9	0.6	22.3	74.6	4.9	18.7
	20083	1564.	82.2	51.4	64.9	12.1	18.4	74.2	0.8	22.4	75.6	4.0	18.7
45	20041	1888.	108.3	66.2	68.1	8.6	19.0	74.4	1.2	21.8	74.8	4.3	19.1
	20036	1922.	106.5	66.6	67.1	9.4	18.3	73.6	0.7	22.7	74.4	4.7	18.9
	20066	1832.	110.3	56.4	64.1	12.7	18.0	73.9	1.1	22.0	75.0	4.4	19.0
	20060	1724.	92.5	56.9	67.5	9.1	18.5	73.9	1.2	21.4	75.2	3.6	19.2
	20009	1720	102.3	59.4	68.6	7.9	19.2	73.7	0.9	22.0	75.6	3.3	19.4

[1]Empty crop body weight (g).

[2]Pectoralis superficial + pectoralis subclavis muscles from right side.

[3]Gastrocnemius + peroneous longus muscles from right leg.

Appendix B

Table 6. Individual leucine specific radioactivity in the precursor pool of breast or leg muscles or leucine incorporation into protein of either muscles or plasma radioactivity at 3, 10, 20, and 30 minutes of infusion period of male meat-type chickens at 28, 36 and 43 days of age. (Experiment #4).

Age (day)	Band #	^{3}H-leu. inco.[1] dpm/g/30 min.		S.A. of leu.[2] (dpm/nm 1)		Plasma radioactivity (dpm/ml) Infusion Time (min.)			
		Breast[3] Muscle	Leg[4] Muscle	Breast Muscles	Leg Muscles	3	10	20	30
28	20021	214640.	205641.	157.2	303.5	440128	530686.	673176.	626180.
	20092	218396.	159546.	103.5	300.0	410515.	3987.2.	470171.	471030.
	20064	241294.	222058.	163.5	301.0	407081.	360515.	441201.	444420.
	20700	233089.	282508.	156.0	290.0	-	-	-	-
36	20078	172419.	100316.	141.5	213.5	-	-	-	-
	20119	176656.	175264.	123.0	232.0	33.250.	287280.	401535.	-
	20073	195437.	218602.	140.1	226.5	412390.	297368.	3444736.	335745.
	20114	174730.	151013.	112.5	334.5	352476.	330701.	396820.	387061.
43	20053	177467.	141442.	125.5	259.5	-	-	-	-
	20072	173777.	147064.	139.2	277.5	-	-	-	-
	20062	223989.	176816.	184.2	329.0	-	-	-	-
	20040	176388	235379.	82.2	223.0	-	-	-	-

Every datum is the average of two replicates.

[1] ^{3}H-leucine incorporation into the muscle T.C.A. precipitate (protein), dpm/g/30 minutes of infusion.

[2] Specific activity of leucine in precursor pool of muscle, dpm/nmol.

[3] Pectoralis of superficial + pectoralis subclavis muscles from right side.

[4] Gastrocnemius + peroneous longus muscles from right leg.

Appendix B

Table 7. Individual empty crop body weight (E.C.B.wt.), breast muscles and leg muscle's weight of Single Comb White Leghorn (SCWL) ch: at 26, 30, 34, 38, 41 and 45 day of age. (Experiment #5).

	Tissues																	
	E.C.B. wt. (g)[a]						Breast muscles (g)[b]						Leg muscles (g)[c]					
	Age (day)						Age (day)						Age (day)					
	26	30	34	38	41	45	26	30	34	38	41	45	26	30	34	38	41	45
	211	280	399	376	580	637	9.4	10.7	16.6	19.3	25.9	26.6	5.1	7.5	12.1	10.8	18.7	19.2
	224	266	276	309	394	459	8.4	105	11.4	13.9	18.3	21.7	5.6	6.7	7.8	8.7	11.6	15.0
	226	228	320	426	460	583	9.8	7.1	13.0	18.9	24.0	28.9	6.3	5.9	9.7	12.2	13.3	17.5
	220	237	309	404	-	-	9.0	9.5	13.5	16.6	-	-	5.8	6.3	8.6	11.7	-	-
	214	333	-	-	-	-	8.3	15.4	-	-	-	-	5.7	9.7	-	-	-	-
$\bar{x}$	219^{1}	269^{5}	326^{9}	379^{13}	478^{17}	560^{21}	9.0^{1}	10.7^{5}	13.6^{9}	17.2^{13}	22.7^{17}	25.7^{21}	5.7^{1}	7.2^{5}	9.6^{9}	10.9^{18}	14.5^{17}	17.2^{21}
	225	259	342	417	546	495	11.3	10.6	16.1	18.0	22.7	20.9	5.6	7.2	9.6	12.5	16.9	14.6
	247	295	287	342	425	655	8.5	12.1	10.6	14.5	18.6	31.6	7.5	7.9	7.3	10.4	13.3	21.9
	189	291	360	406	490	495	7.0	13.2	15.7	20.1	23.5	24.9	4.8	8.0	10.0	12.9	15.1	15.7
	215	248	301	383	-	-	8.3	10.0	14.9	19.4	-	-	5.7	6.8	8.4	11.5	-	-
	214	282	-	-	-	-	8.7	13.7	-	-	-	-	6.0	8.4	-	-	-	-
$\bar{x}$	218^{2}	275^{6}	322^{10}	387^{14}	487^{18}	548^{22}	8.8^{2}	11.9^{6}	14.3^{10}	18.0^{14}	21.6^{18}	25.8^{22}	5.9^{2}	7.7^{6}	8.8^{10}	11.8^{14}	15.1^{18}	17.4^{22}
	218	248	387	463	533	679	9.3	9.5	17.0	21.7	22.5	32.9	5.8	6.2	11.7	13.8	15.4	20.6
	234	264	317	319	439	537	9.4	10.1	14.0	13.3	22.3	25.7	6.7	7.3	8.9	9.2	13.6	15.6
	233	293	316	372	465	455	9.2	12.3	13.3	14.9	20.1	23.5	6.2	9.0	8.8	10.9	13.9	14.6
	190	259	265	358	-	-	7.2	9.2	11.5	17.0	-	-	4.3	7.2	7.2	9.7	-	-
	203	278	-	-	-	-	8.5	12.3	-	-	-	-	5.6	7.3	-	-	-	-
$\bar{x}$	216^{3}	268^{7}	32.1^{11}	378^{15}	479^{19}	557^{23}	8.7^{1}	10.7^{7}	14.0^{11}	16.7^{15}	21.6^{19}	27.4^{23}	5.7^{3}	7.4^{7}	9.2^{11}	10.9^{15}	14.3^{19}	16.9^{23}
	195	273	338	354	518	629	7.7	12.1	13.7	14.9	23.4	30.1	4.7	7.4	10.3	10.0	15.5	21.5
	247	246	382	347	513	511	10.8	10.0	11.9	14.2	23.4	20.3	7.1	6.5	7.7	10.8	16.8	16.6
	221	244	353	369	403	530	8.1	8.7	15.8	18.5	19.8	28.2	5.4	5.7	9.2	11.2	11.7	15.0
	220	289	301	484	-	-	9.4	12.2	13.3	22.0	-	-	6.0	7.8	8.8	14.7	-	-
	202	294	-	-	-	-	7.9	12.3	-	-	-	-	5.4	7.8	-	-	-	-
$\bar{x}$	217^{4}	269^{8}	319^{12}	388^{6}	478^{20}	557^{24}	8.8^{4}	11.1^{8}	13.7^{12}	17.4^{16}	22.4^{24}	26.2^{24}	5.7^{4}	7.1^{8}	9.0^{12}	11.7^{16}	14.7^{20}	17.7^{24}
Overall Mean	217.5	270	322	388	480	556	8.8	11.1	13.9	17.3	221	26.3	5.8	7.3	9.1	11.3	14.6	17.3

All the means with the same superscripts are derived from the same group of birds and it is the group number.

[a]Empty crop body weight(g).

[b]Pectoralis superficial + pectoralis subclavis muscles from right side.

[c]Gastrocnemius + Peroneous longus muscles from right leg.

[1-24]These superscripts are group numbers.

Appendix B

Table 8. Group empty crop body weight, weight of breast and leg and proximate analysis for these 3 tissues in mixed sex Single Comb White Leghorn (SCWL) chickens at 26, 30, 34, 38, 41 and 45 days of age. (Experiment # 5).

Age (day)	Group[1] #	E.C.B.wt.[2] (g)	Breast[3](g)	Leg[4](g)	Proximate Analysis								
					E.C. Body[2]			Breast[3]			Leg[4]		
					%H_2O	%Lipid	%Prot.	%H_2O	%Lipid	%Prot.	%H_2O	%Lipid	%Prot.
26	1	219	9.0	5.7	69.8	6.3	18.3	74.8	0.9	21.6	76.3	3.0	19.1
	2	218	8.8	5.9	70.6	5.0	19.0	75.4	0.6	22.1	77.1	2.4	18.9
	3	216	8.7	5.7	69.3	6.8	17.8	75.1	0.9	21.7	76.7	2.8	18.3
	4	2.7	8.8	5.7	69.2	7.5	17.1	75.4	1.1	21.6	76.4	3.1	18.2
30	5	269	10.6	7.2	69.0	7.2	18.8	75.4	0.8	21.9	77.0	2.4	18.6
	6	275	11.9	7.7	69.9	6.2	18.7	76.0	0.7	22.0	77.1	2.4	18.7
	7	268	10.9	7.4	69.5	6.3	18.4	75.1	0.8	22.0	76.3	2.7	19.1
	8	269	11.1	7.1	70.3	5.5	18.4	75.5	0.6	21.7	77.4	2.3	18.4
34	9	326	13.6	9.5	70.1	5.4	18.8	74.9	0.6	21.9	77.5	2.4	18.5
	10	322	14.3	8.8	68.5	7.0	18.9	75.5	0.5	21.5	76.4	2.4	19.4
	11	321	14.0	9.1	69.7	5.6	18.7	75.3	0.5	21.6	77.3	2.1	18.7
	12	319	13.7	9.0	69.2	5.8	18.7	75.1	0.4	22.2	77.4	1.7	19.1
38	13	379	17.2	10.8	68.1	6.4	20.3	74.5	0.8	22.2	75.9	2.6	20.4
	14	387	18.0	11.8	69.2	5.9	19.9	74.2	0.7	22.6	77.0	2.2	19.4
	15	378	16.7	10.9	69.3	5.9	18.9	75.1	0.6	22.3	76.8	2.3	19.1
	16	388	17.3	11.7	70.0	5.2	19.4	75.1	0.6	22.5	77.2	1.8	19.2
41	17	478	22.7	14.5	69.4	5.3	20.3	74.5	1.1	22.7	76.3	2.1	20.1
	18	487	21.6	15.1	68.5	6.1	20.1	74.6	0.8	22.7	76.9	2.1	19.6
	19	479	21.6	14.3	69.6	5.5	19.6	74.9	0.9	21.8	76.9	1.9	19.6
	20	478	22.4	14.7	68.1	6.5	19.9	74.7	0.8	22.0	76.3	2.2	19.8
45	21	560	25.7	17.2	67.8	6.2	20.3	74.2	1.0	22.5	76.3	2.1	19.7
	22	548	25.8	17.4	67.9	6.9	19.7	74.5	1.1	22.0	76.7	2.1	19.2
	23	557	27.4	16.9	68.4	6.1	19.5	73.8	1.2	22.5	76.2	2.3	20.0
	24	557	26.2	17.7	68.3	6.5	19.6	74.7	1.0	22.5	77.3	1.7	19.1

[1]Every group is the average of 3-5 birds. Two replicates from pooled of each group was used for proximate analysis.

[2]Empty Crop body weight.

[3]Pectoralis superficial + Pectoralis subclavis muscles from right side.

[4]Gastrocnemius + Peroneous longus muscles from right leg.

Appendix B

Table 9. Individual leucine specific radioactivity in the precursor pool of breast or leg muscles or leucine incorporation into protein of either muscles or plasma radioactivity at 3, 10, 20 and 30 minutes of infusion period (SCWL) chickens at 28, 36 and 43 day of age. (Experiment #5).

Age (day)	Sex[1]	Bird's #	^{3}H-leucine inco.[2] dpm/g/30 min. of		S.A. of leu.[3] dpm/nmol)		Plasma radioactivity (dpm/ml) Infusion Time (min.)			
			Breast[4] Muscle	Leg[5] Muscle	Breast Muscle	Leg Muscle	3	10	20	30
28	M	21767	124103.	194726.	166.	486.0	-	-	-	-
	M	21673	157888.	172515.	163.	435.0	384330	266322	408739	389590.
	F	21695	122068.	136132.	229.	358.0	298845.	280134	-	310388.
	F	21686	142655.	124272.	178.	425.0	-	-	-	-
36	M	21660	137953.	186291.	186.	354.0	233384.	258575.	335766.	319499.
	M	21744	116870.	148358.	180.	407.0	361849.	364265.	367990.	373660.
	M	21612	150146.	145192.	229.	452.0	265078.	204442.	295614.	311169.
	M	21745	128117.	112512.	182.	452.0	245967.	239489.	284323.	306462.
43	M	21726	171778.	176267.	244.	476.	353728.	317673.	365899.	377994.
	M	21759	140855.	154516.	233.	448.	343351.	327629.	386417.	406108.
	M	21762	152980.	165420.	183..	472.	325505.	280686.	348870.	327205.
	M	21756	187286.	168677	215.	414	-	-	-	-

Every datum is the average of 2 replicates.

[1]Male (M), Female (F).

[2]^{3}H-leucine incorporation into the muscles T.C.A. precipitate, dpm/g/30 min. of infusion.

[3]Specific activity of leucine in precursor pool of muscles dpm/nmol.

[4]Pectoralis superficial + Pectoralis subclavis muscles from right side.

[5]Gastrocnemius + Peroneous longus muscles from right leg.

Appendix B

Table 10. Individual empty crop body weight (E.C.B.wt.), weight of breast or leg muscles and nitrogen X 6.25 determination for these 3 tissues in control and force-fed SCWL chickens at 33 and 37 days of age. (Experiment #6).

Treatment	Age (day)	Bird's #	E.C.B.wt.[1] (g)	Breast[2] Muscles (g)	Leg[3] Muscles (g)	% Protein[4] in Breast Muscle	% Protein[4] in Leg Muscles
Control	33	21001	346.0	15.2	10.4	23.3	20.1
		21074	288.	11.1	7.8	23.5	19.4
		21051	288.	11.2	7.8	23.5	19.9
		21019	305.	11.5	8.2	22.5	19.1
		21013	299	12.1	8.0	22.3	19.0
	37	21018	391.0	16.9	11.9	23.8	19.0
		21027	378.	13.3	9.6	23.2	20.6
		21059	408.	18.7	11.6	22.7	19.8
		21056	416.	16.8	11.4	23.1	20.4
		21061	357.	11.9	8.5	23.1	21.3
Force-Fed	33	21003	355	14.0	11.5	23.1	20.6
		21014	309	11.7	8.7	22.7	20.2
		21052	324	12.7	8.6	21.7	20.0
		21062	289	9.1	8.0	21.7	19.9
		21080	331	13.2	10.7	22.3	19.3
	37	21012	419.	18.3	11.1	22.8	20.3
		21055	487.	16.1	11.9	23.2	20.4
		21067	506.	17.4	14.2	23.1	20.1
		21077	416.	12.8	10.8	23.1	21.2
		21090	454.	17.1	13.8	23.5	20.5

[1]Empty crop body weight.

[2]Pctoralis superficial + pectoralis subclavis muscles from right side.

[3]Gastrocnemius + Peroneous longus muscles from left side.

[4]Percent protein on wet basis.

Appendx B

Table 11. Individual leucine specific radioactivity (S.A.) in the precursor pool of breast or leg muscles or leucine incorporation into protein of either muscles or plasma radioactivity at 3,10, 20 and 30 minutes of infusion in male SCWL at 35 day of age. (Experiment #6).

Treatment	Bird's #	^{14}C-leucine. inco.[1] dpm/g/30 min.		S.A.of leu.[2] dpm/n mol.)		Plasma radioactivity (dpm/ml) Infusion time (min.)		
		Breast[3] Muscles	Leg[4] Muscles	Breast Muscles	Leg Muscles	3	15	30
Control	21030	18746.0	11152.0	2.2	30.9	67100.0	604700.0	-
	21087	16110.0	11415.0	23.1	35.9	-	-	-
	21025	16930.0	12894.0	28.5	29.5	-	-	-
	21026	15184.0	17671.0	28.1	29.4	-	-	-
	21058	15999.0	13252.0	25.0	30.5	48900.	55770.	59170.
Force-fed	21007	13108.0	8867.0	23.8	49.1	50850.	65700.	-
	21050	16348.0	9625.0	19.5	33.0	60360.	61000.	51800.0
	21054	16944.0	12948.0	28.7	37.5	-	75360.	62920.0
	21005	19116.0	13775.0	24.5	40.6	60380.	56890.0	59700.0
	21009	21520.0	14474.0	18.9	43.4			

Every datum is the average of two replicates.

[1] ^{14}C-leucine incorporation into the muscle's T.C.A. precipitate (protein), dpm/g/30 min. of infusion.

[2] Specific activity of leucine in precursor pool of muscles dpm/nmol.

[3] Pectoralis superficial + Pectoralis subclavis muscles from right side.

[4] Gastrocnemius + Peroneous longus muscles from right leg.

Appendix B

Calculation of Fractional Accretion Rate (FAR)

The protein accretion is measured by starting with two groups of chickens of equal weight. Both groups are weighed and one group (group 1) is killed. Then a few days later the other group (group 2) is weighed and killed. At each time the chickens were killed, the desired tissue(s) is excised and weighed and then analyzed for protein. The FAR is calculated as follows:

$$\frac{\text{wt. of protein from tissues of group 2} - \text{wt. of protein from tissues of group 1} \div \text{no. of days between weighing}}{\text{wt. protein from tissues of group 2} + \text{wt. of total protein in tissues of group 1} \div 2} \times 100$$

= FAR (%/day).

Appendix B

CALCULATION OF PROTEIN SYNTHESIS RATE

The specific radioactivity of leucine was calculated in the free pool of amino acids in two types of muscles. The labeled leucine is reacted with dansyl chloride which is labeled with another isotopic species. For example : if the leucine is ^{14}C labeled, the dansyl chloride is ^{3}H labeled. Since leucine and dansyl chloride are reacted on a mol/mol basis, then the specific activity(S.A) of leucine is obtained by the following formulas.

(1) $$\frac{\text{dpm of dansyl in the dansyl-leucine complex}}{\text{S.A of dansyl chloride used in the original reaction}}$$

= nmols of dansyl which is equal to the nmols of leucine

(2) $$\frac{\text{dpm of leucine in the complex with dansyl}}{\text{nmols leucine from equation(1)}}$$

=S.A of leucine (dpm/nmol)

The rate of protein synthesis (%/day) was calculated as follows;

(3) $$\frac{\text{dpm of leucine incorporated into protein/30 min./g. of muscle}}{\text{S.A of leucine (dpm/nmol) from equation (2)}}$$

= nmols of leucine incorporated into protein/30 min./g. of muscle

(4) $$\frac{\text{nmols from (3) x 48 (48-1/2 periods to a day) x 131(M.W. of leucine)}}{10^6}$$

= mg. leucine incorporated into protein per day

(5) $$\frac{\text{mg. leucine}}{\text{day into protein}} \times \frac{1}{0.072 \text{ (amount of leucine per unit of protein)}} \times \frac{1}{185^{*} \text{ (mg. of protein per gram of leg muscle)}} \times 100$$

= Fractional synthesis rate (%/day)

* Value equals 230 for breast muscle.