SKELETAL MUSCLE PROTEIN TURNOVER IN BROILER AND LAYER CHICKS

S. J. Jones2, E. D. Aberle2 and M. D. Judge

Purdue University, West Lafayette, IN 47907

ABSTRACT

Dietary infusion of L-[U-14C]tyrosine was used to estimate the fractional protein synthesis rates (FSR) in broiler and layer chickens. Six 2-wk-old birds of each strain were placed in individual metabolism cages and given a purified diet in agar-gel containing 2 μCi L-[U-14C] tyrosine for 6 h. The birds were sacrificed and the pectoralis major (PM) and two combined leg (LM) muscles (gastrocnemius and peroneous longus) were removed for analysis. Subgroups of chickens were sacrificed 3 d before and 3 d after infusion to observe changes in muscle composition to calculate fractional protein accretion rates (FAR). Fractional protein breakdown rates (FBR) were calculated by difference (FBR = FSR − FAR). Protein, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) concentrations were determined to observe relationships between these cellular constituents and FSR. Fractional whole body growth rate and FSR in PM was greater (P<.05) in broiler than layer birds. The FSR in LM of the layer was not different (P>.05) from that of broilers, and from the FSR of the PM in each bird-type. The calculated FBR in the layer PM was at least 17% higher than that of the other muscles. Ratios of FSR to FBR indicated that 16% of the protein synthesized in the layer PM was retained, compared with 45% in the broiler PM. The RNA activity of the layer PM was less (P<.05) than that of the other muscles investigated. Deoxyribonucleic acid activity was lower (P<.05) in the PM than LM of either bird-type. These results indicate that differences in FAR between the two strains of birds were caused by higher FSR in the broiler PM and lower FDR in both muscles of broiler as compared with layer chicks.

(Key Words: Muscles, Growth, Protein Turnover, Chickens.)

Introduction

Skeletal muscle proteins, like all proteins in the body, are in a dynamic state (Schoenheimer et al., 1939). Protein accretion occurs when total protein synthesis exceeds total protein breakdown. Age (McDonald and Swick, 1981), nutritional status (Garlick et al., 1973, 1975) and several hormones (Waterlow et al., 1978) affect protein turnover rate. However, there have been few reports of the effects of genetic selection for muscle mass and growth rate on protein turnover.

Chickens are useful models for studying muscle protein turnover and growth; while broiler-type chickens have been selected for rapid growth and large muscle mass, layer-type chickens have been selected for egg production. As a result of many generations of selection pressure, broilers exhibit approximately 50% more muscle mass (Mizuno and Hikami, 1971), larger diameter muscle fibers (Smith, 1963; Aberle et al., 1978) and more muscle fibers per muscle (Mizuno and Hikami, 1971) than layers. The fact that broilers have larger-diameter muscle fibers than layers suggests that muscle protein turnover rate is different between bird-types. The objective of this investigation was to compare muscle protein synthesis and degradation in broiler and layer chicks. This information may provide insight into the consequences of selection for muscle mass in meat-producing animals, and thereby contribute to the enhancement of efficiency of muscle protein deposition.

Experimental Procedures

Hubbard broiler cockerels3, representing a rapidly growing line of broiler chickens, and Shaver Starcross Leghorn cockerels4, representing a slow-growing line of egg producing chickens, were obtained at 1 d of age. All birds

2 Present address: Dept. of Anim. Sci., Univ. of Nebraska, Lincoln 68583-0821.
3 Fairview Farms, Remington, IN.
4 Commercial Chicks Inc., Thorntown, IN.
Received August 21, 1985.
Accepted December 19, 1985.

were wing-banded, weighed and placed in conventional electrically heated starting-batteries located in a room that was lighted 24 h/d.

Water and commercial chick starter diet containing 18% crude protein, 3% fat and 6.5% crude fiber was given ad libitum until d 7. On d 8, the commercial diet was replaced with a crystalline amino acid diet (Baker et al., 1979). On d 10, an agar-gel diet was introduced, so the birds would be acclimated to such a diet when they were placed on the radiotracer study. The agar-gel diet was composed of one part of the crystalline amino acid diet and two parts of a 1.8% (w/v) agar solution (Maruyama et al., 1978).

Groups of four to five chicks given the agar-gel diet were killed 3 d before and 3 d after the tracer experiments to measure muscle weight and protein content. Rate of muscle protein accumulation for each muscle studied was estimated from regressions of total muscle protein on age.

On d 14 ± 1, fed chicks (n=6) were placed in metabolism cages and given 15 g (dry weight) of the agar-gel diet containing 2 μCi of L-[U-14C]tyrosine, specific activity 450 mCi/mmol. During feeding of the radioactive diet, expired air was trapped in 20 ml of a solution containing one part (v/v) ethanolamine and two parts methyl cellosolve. Tubes were changed every .5 h for the first 2 h and every hour thereafter until 6 h. Radioactivity in the expired CO2 was sampled by combining 3 ml of the trapping solution with 15 ml of a toluene/methyl cellosolve scintillation cocktail (Jeffay and Alvaraz, 1961). Samples were counted for 10 min or 10,000 counts in a liquid scintillation spectrophotometer. Correction for quenching was made by automatic external standardization.

Chicks that failed to maintain a plateau in 14CO2 expiration were discarded from the experiment, i.e., about 10% of the birds.

At 6 h, birds were removed from metabolism cages and blood was obtained from each bird via cardiac puncture using a syringe. Serum fractions were prepared by centrifugation in a refrigerated centrifuge and then stored at −20 C until analyzed. Birds were sacrificed by cervical dislocation; the pectoralis major (PM) and two leg muscles (LM), the gastrocnemius and peronious longus, were removed, weighed, frozen in liquid nitrogen and stored at −20 C until analyzed.

To measure the amount of radioactive tyrosine absorbed by the bird (i.e., tyrosine flux), the uneaten labeled agar and excreta were washed from the metabolism cage, and gastrointestinal contents from infused bird were recovered and combined. Radioactivity was measured in homogenates of the combined feed, excreta and gastrointestinal contents after diluting to a constant volume of 500 ml with distilled water. Duplicate 1-ml samples of homogenates were solubilized at 37 C in 4 ml of Protosol0. After solubilization, 15 ml of Aquasol0 scintillation cocktail was added, and samples counted from 20 min or 10,000 counts.

Tyrosine concentration in deproteinized serum was determined using methods of Undenfriend and Cooper (1952). Protein-bound and free tyrosine in muscle tissue were separated as described by Laurent et al. (1978). One gram of muscle was homogenized in 5 ml of cold distilled water in a siliconized centrifuge tube with a Polytron homogenizer for 30 s at about 1,500 rpm. The homogenizer was washed three times with 5 ml of cold 5% trichloroacetic acid (TCA); washings were combined with the homogenate. Homogenates were placed in ice for .5 h to allow proteins to precipitate, and then centrifuged for 15 min at 4,000 x g. Supernatant fractions containing the free tyrosine were decanted, pellets were washed with 5 ml of cold TCA and the washings were combined with the supernatants. Trichloroacetic acid supernatants were extracted three times with 10 ml of diethyl ether, and the ether fraction was aspirated. Aqueous (bottom) layers were evaporated to dryness using a rotary evaporator. Dried residues were dissolved in 5 ml of .5 M citrate buffer, pH 5.5.

Dried protein pellets were extracted with 15 ml of hot 5% TCA, 15 ml of 3:1 ethanol/diethyl ether (v/v) and, finally, with 20 ml of diethyl ether. Pellets were dried, weighed and 50 mg were placed in a vial containing 15 ml of 6 N hydrochloric acid (HCl). These samples were hydrolyzed for 8 h at 125 C, evaporated to dryness using a rotary evaporator, and finally dissolved in 5 ml of .5 M citrate buffer, pH 5.5.

Specific activity of tyrosine in supernatants and hydrolysates (free and bound fractions, 5 Amersham/Searle, Des Plaines, IL.
6 New England Nuclear Corp., Boston, MA.
7 Polytron Ltd., Lucerne, Switzerland.
respectively) were analyzed using the method described by Garlick and Marshall (1972) as modified by Schaefer and Krishnamurti (1982). L-tyrosine decarboxylase (EC 4.1.1.25)\(^8\) was used to convert L-tyrosine to tyramine. Tyramine was extracted repeatedly with ethyl acetate, followed by extraction into acidified water. Tyramine was assayed using the method of Underfriend and Cooper (1952). Samples of the acidified layer, which contains extracted tyramine, were added to 15 ml of Aquasol and counted for radioactivity for 50 min, or until 10,000 counts were obtained.

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) were separated using the method of Shibko et al. (1976). Deoxyribonucleic acid was measured by the diphenylamine method (Burton, 1956) using calf thymus DNA as a standard. Ribonucleic acid was measured with the orcinol reaction (Lin and Schjeide, 1969) using purified yeast RNA as a standard. Protein was measured using the biuret method (Gornall et al., 1949) with bovine serum albumin as a standard.

Fractional protein synthesis rates (FSR, %/d) were calculated from the specific activities of the tissue free and bound tyrosine with the equation of Garlick et al. (1973) solved by repeated approximation. Fractional breakdown rates (FBR, %/d) were calculated as the difference between the FSR fractional accretion rates (FAR, %/d).

Data were analyzed by a randomized factorial analysis of variance using a split-plot design. The main effect, bird-type, was tested by the bird within bird-type interaction. The main effect, muscle, and the muscle x bird-type interaction were tested by the residual. When significant interactions were observed, means were separated using the Student Newman-Kuels' test at the .05 level (Anderson and McLean, 1974).

Results and Discussion

Estimation of muscle protein synthesis rate using continuous dietary infusion requires that the specific activity of the precursor amino acid in the free amino acid pool reach a plateau or equilibrium during infusion. To determine if a plateau could be reached and maintained, radioactivity in serum, muscle of three birds of each bird-type and expired CO\(_2\) was monitored during dietary infusion to determine the rise in specific activity of the precursor amino acid.

Specific radioactivities of tyrosine in serum during dietary infusion are illustrated in figure 1. Equilibrium of tyrosine-specific activity in serum was attained by 2 h after starting infusion, and was maintained through 6 h of dietary infusion.

Levels of radioactivity in muscle reached a plateau by 2 h (figure 2) after initiation of infusion, and were maintained throughout the infusion period. The time required to reach an equilibrium in serum and muscle were similar to data reported by Maruyama et al. (1978), who also used the dietary infusion method.

Radioactivity present in expired \(^{14}\)CO\(_2\) was measured to verify that tyrosine-specific

\[\text{Sigma Chemical, St. Louis, MO.}\]
activity reached equilibrium in the free amino acid pool of individual animals. Labeled carbon in expired CO\textsubscript{2} was measured because of the difficulty in taking serial blood samples from the small veins of 2-wk-old birds. Plateau levels of expired \textsuperscript{14}CO\textsubscript{2} were achieved in all birds used in the study between 2 and 3 h after they were given access to the diet.

These data confirm that isotopic equilibrium in serum, muscle and expired CO\textsubscript{2} occurred during the dietary infusion, and indicate specific radioactivity of the free amino acid pool achieves an equilibrium, which is maintained for much of the experimental period. Thus, muscle protein synthesis can be determined using the calculations of Garlick et al. (1973).

Broilers have been selected specifically for rapid growth rate and large muscle size. Several researchers have shown that broilers grow faster and yield a higher percentage of meat and lower percentage of bone than do layers (Dawson et al., 1958; Smith, 1963). The difference in growth curves between broilers and layers in this study (figure 3) was very similar to that reported by Smith (1963) and Hentges et al. (1983). In the present study, rate of body weight gains for broilers were twofold greater compared with layers (avg daily gain of 18.1 compared with 7.7 g). Differences in body weight became apparent early in life. Both bird-types averaged approximately 40 g at 2 d of age, but by wk 2 broilers weighed twice that of layers. Broiler PM muscle weights were also twice that of layers at 2 wk of age (table 1). Muscle size differences in layers and broilers are caused by differences in muscle fiber number (Mizuno and Hikami, 1971) and diameter (Smith, 1963; Aberle et al., 1978). The fact that broiler muscle fibers are larger in diameter indicates differences in capacity of muscle fibers to accumulate muscle proteins. Because age has an effect on protein turnover (McDonald and Swick, 1981), the differences observed may ultimately be due to differences in the physiological age between broilers and layers.

Amino acid flux, in this case tyrosine flux (Q; Garlick et al., 1973), was estimated from the amount of radioactive tyrosine absorbed (l), which amounted to 35 to 60% of the amount offered, and specific radioactivity (sp)

<table>
<thead>
<tr>
<th>Item</th>
<th>Layer</th>
<th>Broiler</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>139\textsuperscript{d}</td>
<td>274\textsuperscript{e}</td>
<td>3.56</td>
</tr>
<tr>
<td>Muscle wt, g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pectoralis major</td>
<td>4.0\textsuperscript{d}</td>
<td>8.2\textsuperscript{e}</td>
<td>1.44</td>
</tr>
<tr>
<td>Leg muscle\textsuperscript{b}</td>
<td>2.9\textsuperscript{d}</td>
<td>5.2\textsuperscript{e}</td>
<td>.65</td>
</tr>
<tr>
<td>Fractional whole-body growth rate, %/d</td>
<td>5.0\textsuperscript{d}</td>
<td>6.0\textsuperscript{e}</td>
<td>.10</td>
</tr>
<tr>
<td>Tyrosine flux, mol·h\textsuperscript{-1}·100 g body wt\textsuperscript{-1}</td>
<td>37.0</td>
<td>39.4</td>
<td>.53</td>
</tr>
<tr>
<td>Whole-body protein synthesis\textsuperscript{c}, g/d</td>
<td>7.0\textsuperscript{d}</td>
<td>14.7\textsuperscript{e}</td>
<td>.62</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Each value is the mean of six observations.
\textsuperscript{b}Leg muscle consists of the gastrocnemius and peroneus longus.
\textsuperscript{c}Values calculated using 3.2% as tyrosine content in whole-body proteins.
\textsuperscript{d,e}Values on the same line with different superscripts differ (P<.05).
of free tyrosine, i.e., $Q = 1/sp$. The amount of tyrosine oxidized, represented by $^{14}$CO$_2$ in the expired air, was subtracted from 1 to give a minimal estimate of the rate of incorporation of tyrosine in total body protein. Tyrosine flux between broilers and layers did not differ (table 1). The rate of whole body protein synthesis (table 1) was calculated using the values for tyrosine flux and whole body tyrosine content (3.2 ± 4% as determined by amino acid analysis). In this study, broilers synthesized more protein on a per bird basis because of their greater body weight (table 1), but were similar to layers on a weight-constant basis (5.35 g d$^{-1}$ 100 g body wt$^{-1}$ for the broiler and 500 g d$^{-1}$ body wt$^{-1}$ for layers).

Table 2 contains values for fractional synthesis (FSR) and breakdown (FBR) rates of protein in muscles of layers and broiler strains. The interaction between muscle location and bird-type for FSR was significant, with the broiler PM having a higher FSR ($P<.05$) than the PM of the layer. Fractional synthesis rate in leg muscles did not differ between the two types of birds and was intermediate to the FSR of broiler and layer PM.

Higher FSR in the PM of broilers may reflect inherent differences in genetic programs responsible for growth rate and stage of development of the birds. Age affects protein synthesis such that as the animal matures, FSR declines (McDonald and Swick, 1981). It is possible that through genetic selection, the timing for the decline of FSR in broilers has been shifted by extending the accelerating portion of the growth curve.

Differences in FBR were noted between muscles and between broilers and layers, with the calculated value for FBR in layer PM being 1.2-fold greater than in layer LM and 1.3-fold greater than in broiler PM (table 2). Calculated values from subgroups of birds revealed that FAR were 2.7%/d in the layer PM compared with 8.3%/d in the broiler PM. The ratios of FSR to FBR were higher in the PM muscle of the broiler than the layer (1.8 compared to 1.2, respectively). This means that approximately 45% of the protein synthesized in the PM of broiler birds was retained compared with only 16% in the PM of layers. Orcutt and Young (1982) observed that protein accumulation was greater in primary muscle cell cultures from broilers than in those from layers. These authors reported that synthesis of myosin heavy chain was 30% greater in muscle cell cultures established from layer-type birds. They concluded that increased accumulation of muscle protein by cultured muscle cells from broilers was caused primarily by a slower protein FBR ($P<.05$) than in layer PM, but differences in synthesis rates also accentuated differences in protein accumulation between layers and broilers.

Protein, RNA and DNA concentrations and the protein:RNA and protein:DNA ratios of PM and LM for broilers and layers are given in

| TABLE 2. FRACTIONAL SYNTHESIS AND DEGRADATION RATES OF MUSCLE PROTEIN IN THE PECTORALIS MAJOR AND MIXED LEG MUSCLES FROM 2-WK-OLD LAYER AND BROILER CHICKS$^a$ |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Item                           | Layer                           | Broiler                         |                                |                                |
|                                | Pectoralis major                | Leg muscle                       | Pectoralis major                | Leg muscle                       |
|                                |                                 |                                 |                                 |                                 |
| Fractional synthesis rate (FSR), %/d | 15.9e                           | 17.6ef                          | 18.5f                           | 17.2ef                          |
| Fractional breakdown rate (FBR)$^c$, %/d | 13.2                            | 11.0                            | 10.2                            | 10.0                            |
| Fractional accretion rate (FAR)$^d$, %/d | 2.7                             | 6.6                             | 8.3                             | 7.2                             |

$^a$Each value is the mean of six observations.

$^b$Leg muscle consists of the gastrocnemius and peroneous longus.

$^c$FBR was determined by difference (FBR = FSR - FAR).

$^d$Calculated by linear regression from muscle data of subgroups contemporary to those infused of birds sacrificed 3 d before and 3 d after dietary infusion studies.

$^e,f$Values on the same line that do not have a common superscript differ ($P<.05$).
Protein concentration did not differ between muscle or bird types.

Ribonucleic acid concentrations were higher \((P<.05)\) and protein:DNA ratios lower in the PM than in the LM of both broilers and layers (table 3). Concentration of RNA indicates ribosome number, and is recognized as an index of the intensity of protein synthesis (Goldberg, 1967). The relationship of RNA to protein synthesis can be described in terms of the capacity for protein synthesis (RNA concentration) and the activity of RNA, defined as the rate of protein synthesis per unit RNA (Millward et al., 1973, 1976). Protein:RNA ratio is the most appropriate expression for comparison of RNA concentration to FSR. Millward and Waterlow (1978) reported the protein:RNA ratio is inversely related to FSR. In the present study, the PM of the broilers had lower \((P<.05)\) protein:RNA ratios (table 3) and higher FSR (table 2) than LM of either strain of bird. However, PM of layers had the lowest FSR, and the RNA ratio was equal to the broiler PM, which contradicts the statement of Millward and Waterlow (1978). The PM of layer birds had a lower \((P<.05)\) RNA activity (table 4) than the PM of broilers. These data indicate there is RNA in the layer PM that is not active in the synthesis of protein.

Deoxyribonucleic acid concentration was significantly higher in the LM than in the PM of both bird types (table 3). Enesco and Puddy (1964) and Cheek et al. (1971) reported that as much as 30% of the DNA present in muscle tissue is from non-muscle cells. Differences between muscles may relate to differing populations of non-muscle cells in the PM and LM. Assays used to measure DNA are not specific for muscle DNA; therefore, smaller non-muscle cells such as adipocytes, fibroblasts, vascular cells and satellite cells would cause an overestimation of muscle DNA in muscles such as LM, which may contain a larger percentage of non-muscle cells. However, Enesco and Puddy (1964) have reported that the relative proportion of non-muscle cells is relatively constant among animals, as long as sampling is within the same muscle.

Because muscle fibers are multinucleated, Cheek et al. (1971) defined the term “muscle DNA unit” (i.e., protein:DNA ratio) as a conceptualized volume of cytoplasm in a muscle fiber managed by a single nucleus. An inverse relationship between muscle DNA unit size and FSR has been reported such that the smaller the DNA unit, the more rapid the FSR (Laurent et al., 1978). Data from the present study support this inverse relationship because PM of layers had a greater \((P<.10)\) protein:DNA ratio and a lower \((P<.05)\) FSR than the PM of broilers.

If a relationship between FSR and DNA unit size exists, a knowledge of the factors that control DNA unit sizes is important in understanding muscle protein turnover. DNA unit size can be determined by rate of protein synthesis or breakdown, if either of these two processes is kinetically first-order (Millward et al., 1977). The accepted viewpoint is that protein synthesis is zero-order [i.e., a fixed rate of synthesis per unit of DNA (DNA activity)] and breakdown is first-order (a fractional rate, \(k_d\); Swick, 1977).
TABLE 4. RATES OF PROTEIN SYNTHESIS PER UNIT OF RNA (RNA ACTIVITY) AND DNA (DNA ACTIVITY) IN THE PECTORALIS MAJOR AND MIXED LEG MUSCLES FROM 2-WK-OLD LAYERS AND BROILERS

<table>
<thead>
<tr>
<th>Item</th>
<th>Layer</th>
<th>Broiler</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pectoralis major</td>
<td>Leg muscle</td>
</tr>
<tr>
<td>RNA activity, g of protein synthesized · g of RNA · d⁻¹</td>
<td>9.7c</td>
<td>13.0d</td>
</tr>
<tr>
<td>DNA activity, g of protein synthesized · g of DNA · d⁻¹</td>
<td>57.9d</td>
<td>49.6c</td>
</tr>
</tbody>
</table>

SE .61 1.71

aEach value is the mean of six observations.
bLeg muscle consists of the gastrocnemius and peroneous longus.
c,dValues on the same line that do not have a common superscript differ (P < .05).

1982). Laurent et al. (1978), in their study of the kinetics of protein turnover, reported that DNA unit size is determined by the expression:

\[
\frac{\text{protein}}{\text{DNA}} = \frac{\text{DNA activity}}{k_d}.
\]

This expression is often used to define the steady-state concentration of an enzyme (Schimke, 1970). Because DNA activity reported is the same within muscle type for broilers and layers in this study (table 4), differences in DNA unit size may reflect the differences in FBR, which were also observed (table 2). However, the hypothesis that breakdown rate of muscle protein determines DNA unit size depends on the assumption that protein breakdown is a first-order process. Although it is assumed that muscle protein breakdown is first-order, there is little evidence of that at the whole-tissue level (Millward, 1978). If protein breakdown is zero-order, then there would be no relationship between size of DNA unit and rate of protein breakdown. The true reaction order of synthesis and breakdown of muscle proteins must be known in order to determine the real relationship between FBR and DNA unit size.

The RNA:DNA ratio was 1.6-fold higher (P < .05) in the PM than in the LM for layers, but did not differ in broilers. A high ratio suggests that DNA is more active in RNA synthesis in all cells present in muscle tissue. However, differences in RNA:DNA ratio could be the result of differing populations of non-muscle cells present in muscle tissue. This would cause difficulty in comparing FSR of muscle protein and RNA:DNA ratio.

In summary, genetic selection may have an effect on protein turnover, as demonstrated by an increased FSR in the broiler PM and an increased FBR in the layer PM. Differences in FSR may be caused by differences in amounts and activities of RNA, which were observed between the breast muscle of the two bird types. Within muscle, differences of the protein:DNA ratio may be a reflection of the differences observed in FBR because DNA activities were the same within muscle-type and between bird-types.

Literature Cited

Enesco, M. and D. Puddy. 1964. Increase in the num-


