Development of the Indicator Amino Acid Oxidation Technique in Chickens: L-[1-^{14}C]Phenylalanine Infusion Dose and Phenylalanine Oxidation

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ABSTRACT Amino acid requirements of broiler breeder chickens are not well known. The indicator amino acid oxidation (IAAO) technique was adapted for use in broiler breeders as a rapid and sensitive method to determine amino acid requirements. During IAAO, phenylalanine oxidation decreases, inversely to the changes in protein synthesis, as the intake of the limiting test amino acid increases from deficient to adequate. Above the adequate level, phenylalanine oxidation remains constant. Before IAAO can be employed, the optimum priming and constant infusion doses of phenylalanine must be determined. Prelaying catheterized birds aged 20 to 24 wk were placed in closed oxidation chambers attached to a breath collection apparatus. A constant L-[1-^{14}C]phenylalanine dose of 3.5 μCi/kg BW/h and priming doses of 4.5, 5.5, and 7.0 μCi/kg BW were used to determine optimal prime:constant dose ratios, minimum time taken for breath $^{14}CO_2$ excretion to become constant (plateau), and adequate percentage of phenylalanine oxidized. At this constant infusion rate, the optimal priming dose of L-[1-^{14}C]phenylalanine was 5.5 μCi/kg BW, resulting in a prime:constant dose ratio of 1.6:1. By using this ratio, the average time taken for breath $^{14}CO_2$ to reach plateau was 60 min. Average phenylalanine oxidation at plateau, corrected for bicarbonate retention, was 5.5 ± 1.4% (mean ± SD), which is adequate for IAAO studies using deficient-to-excess levels of test amino acids. To the authors' knowledge, this study is the first in chickens to establish a primed, constant infusion technique using L-[1-^{14}C]phenylalanine. The IAAO technique will be used in future studies to determine amino acid requirements in chickens.

(*Key words*: amino acid, requirement, infusion dose, indicator amino acid oxidation, broiler breeder chicken)

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INTRODUCTION

The amino acid requirements of broiler breeder hens have not been empirically determined. Little research has been conducted in the last 15 yr on broiler breeder amino acid requirements, although growth potential of these birds has increased dramatically during this time. Currently, the NRC (1994) amino acid requirements for broiler breeders are based on extrapolations from requirements determined for table egg layers and growing broilers. Adjustment to these requirements has often been made based on field experience because scientifically obtained data are lacking for modern broiler breeders. The nutritional goal for broiler production is for increased feed efficiency and quality of meat (Round, 1992). However, in the case of broiler breeders, growth rate and reproduction are inversely related, and their feed intake is restricted to control body weight (Leeson and Summers, 1997). This practice makes exact determination of amino acid requirements of these birds critical. Growth, feed efficiency, and meat yield are the standards for measuring amino acid requirements in broilers; however, these methods are not suitable for broiler breeders where the goals are maintenance plus egg production. Different methods must therefore be explored and developed.

There are several methods for determining amino acid requirements for animals and humans, such as growth rate, feed conversion efficiency (Brake et al., 1998), nitrogen balance (Irwin and Hegsted, 1971), and amino acid oxidation (Zello et al., 1995; Young and Borgonha, 2000). Among these methods, the amino acid oxidation techniques, using either radioactive or stable isotopes, provide more detailed metabolic information and precise requirements and have been used extensively to determine amino acid requirements in humans (Meguid, et al., 1986; Zello et al., 1990, 1993; Wilson et al., 1997; Roberts et al., 2001) and pigs (Ball et al., 1986; House et al., 1997,1998; Bertolo et al., 1998). The two common amino acid oxida-

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Abbreviation Key: IAAO = indicator amino acid oxidation; dpm = decay per minute.

tion methods are direct amino acid oxidation (Meguid, et al., 1986; Zello et al., 1990; House et al., 1997) and indicator amino acid oxidation (IAAO) (Zello et al, 1993; Bertolo et al., 1998; House et al., 1998). A detailed comparison of these two oxidation techniques has been published (Zello et al., 1995).

In the IAAO technique, the oxidation of another amino acid, referred to as the indicator amino acid, is used to determine the requirement of a test amino acid. The IAAO technique is based on the concept that the relative partitioning of any indispensable amino acid between protein synthesis or oxidation is sensitive to the level of the most limiting amino acid. When the test amino acid is limiting protein synthesis, all other amino acids must be in excess and, therefore, must be oxidized.

As the dietary content of the limiting amino acid is increased, the utilization of the other amino acids for protein synthesis increases and their oxidation decreases until the test amino acid requirement is reached. Additional increments of the test amino acid beyond its requirement will have no effect on the utilization of the indicator amino acid for protein synthesis or oxidation. The point at which protein synthesis is maximized and thus the oxidation of the indicator amino acid is minimized is taken as the requirement level. The indicator amino acid is chosen as a sensitive representative of the other essential amino acids. In principle, any indispensable amino acid can be used as the indicator amino acid. However, in most cases phenylalanine has been the choice of indicator amino acid because it has a small and well-regulated body pool and its oxidation, in the presence of excess tyrosine, is irreversible and thus can be quantitatively determined from breath CO_2. An important advantage of the IAAO technique is that requirements of all other amino acids can be determined using one indicator amino acid.

The present study is part of a series of experiments conducted to successfully develop the IAAO technique for use in chickens (Tabiri et al., 2002). In this series, we have successfully developed surgical techniques, a fitted jacket to accommodate indwelling catheters, a breath collection apparatus, a constant intravenous infusion technique, and a protocol to determine the amount of bicarbonate retained in the body. The objective of the present study was to establish a protocol for the continuous infusion of radiolabeled phenylalanine (i.e., the indicator amino acid) in chickens, including the determination of the optimal prime:constant infusion dose ratio.

MATERIALS AND METHODS

Birds and Surgical Protocols

The University of Alberta Animal Care Committee approved all experimental procedures. Female Cobb 500 broiler breeder chickens (n = 6) aged 20 to 24 wk were obtained from the University of Alberta Poultry Research Center. Birds were gradually switched from commercial feed to a purified diet over 3 d, 7 d before surgery. The purified diet (Table 1) was formulated to meet the amino acid requirements of 42- to 56-d-old broiler chickens; the lysine requirement from NRC (1994) for broiler breeder hens at peak production was used with other amino acid requirements determined by ratios recommended by D. H. Baker (2000, University of Illinois, Urbana, IL, personal communication) for 42- to 56-d-old broiler chickens. Tyrosine was provided in excess (i.e., at 200% of recommendations by D. H. Baker, personal communication) so that excess phenylalanine was channeled to oxidation and not to tyrosine synthesis (Zello et al., 1995). The calculated composition of the diet was 3,744 kcal/kg for metabolizable energy (NRC, 1994) and 18.45% for total protein (Table 1).

TABLE 1. Composition of the experimental diet

Ingredient	
Starch (g/kg)	624.8
Corn oil (g/kg)	100.0
Salt mix[1] (g/kg)	53.7
Cellulose[2] (g/kg)	26.0
Vitamin mix[3] (g/kg)	10.0
Ethoxyquin (125 mg/kg) (g/kg)	1.0
Amino acid mix[4] (g/kg)	184.5
L-Arginine (g/kg)	8.2
L-Cystine (g/kg)	2.9
L-Glutamate (g/kg)	120.0
L-Histidine (g/kg)	2.7
L-Isoleucine (g/kg)	5.2
L-Leucine (g/kg)	8.3
L-Lysine·HCl (g/kg)	9.6
DL-Methionine (g/kg)	2.9
L-Phenylalanine (g/kg)	4.0
L-Threonine (g/kg)	5.3
L-Tryptophan (g/kg)	1.3
L-Tyrosine (g/kg)	8.0
L-Valine (g/kg)	6.1
Metabolizable energy (kcal/kg)	3,744

[1]Complete modified Glista chick salts (TD 73007; Harlan Teklad, Madison, WI) provided per kilogram of diet: $CaCO_3$, 3.0 g; $Ca_3(PO_4)_2$, 28.0 g; K_2HPO_4 , 9.0 g; NaCl, 8.8 g; $MgSO_4 \cdot 7H_2O$, 3.5 g; $MnSO_4 \cdot H_2O$, 650 mg; $Fe(C_6H_5O_7)$, 500 mg; $ZnCO_3$, 100 mg; $CuSO_4 \cdot 5H_2O$, 20 mg; H_3BO_3, 9.0 mg; $NaMoO_4 \cdot 2H_2O$, 9.0 mg; KI, 40 mg; $CoSO_4 \cdot 7H_2O$, 1.0 mg; Na_2SeO_3, 0.21 mg.

[2]Solka floc.

[3]AIN-93-VX vitamin mix provided per kilogram of diet: nicotinic acid, 30 mg; D-calcium pantothenate, 16 mg; pyridoxine HCl, 7.0 mg; thiamine HCl, 6.0 mg; riboflavin, 6.0 mg; folic acid, 2.0 mg; D-biotin, 0.2 mg; vitamin B_{12} (0.1% triturated in mannitol), 25 mg; α-tocopherol powder (250 IU/g), 300 mg; vitamin A palmitate (250,000 IU/g), 16 mg; vitamin D_3 (400,000 IU/g), 2.5 mg; phylloquinone, 0.75 mg; powdered sucrose, 9.597 g.

[4]Amino acid mixture (Edwards et al., 1999) extrapolated from requirements for 42- to 56-d-old broiler chicks fed purified diets containing 13.3% protein equivalent and 3,557 kcal ME/kg.

Birds were fitted with custom-made jackets and placed in individual plexiglass oxidation chambers (60 × 40 × 50 cm) fitted with wire mesh flooring, feeders, and nipple drinkers. These birds were adapted to the jackets and chamber environment for 3 d prior to surgery. The room photoschedule was 8 h of light per day.

After adaptation, birds underwent surgery to implant catheters. Custom-made Tygon catheters[2] (70 cm long,

[2]Fisher Scientific Ltd., Nepean, ON, Canada.

0.100 cm i.d., 0.175 cm o.d.) were installed under aseptic conditions. Birds were anesthetized with a mixture of 0.75% isofluorane[3] in oxygen delivered by mask. Feathers were then plucked from the neck region, and the exposed skin was cleaned using ethanol followed by iodine solution.

An incision of approximately 3 cm was made at the neck region; the right jugular vein was exposed, punctured, and implanted with the catheter. The catheter was advanced to a position just anterior to the superior vena cava. Two silastic grommets[4] were used to anchor the catheters to the surrounding tissues and sutured. The free end of catheter was tunneled under the skin to the back between the wings and externalized. The incision area was sutured and covered with a topical antibiotic cream. The jacket was then refitted, and the catheter was passed through a connected tether.[5]

The chicken was returned to the chamber and given tetracycline,[3] 0.44 g/L, through water for 3 d to prevent infection. The catheter was flushed twice daily with 3 mL of heparin[3] solution (200 IU/mL) to prevent blood clotting. Birds recovered from the anesthesia within 3 to 5 min after surgery. Complete recovery from the surgical operation, as indicated by feed intake and behavior, required approximately 2 to 3 d.

Tracer Infusion and $^{14}CO_2$ Collection

On Day 7 post-surgery, birds were placed individually in oxidation chambers, the chambers were closed, and an air pump[6] was used to draw air through each chamber at 20 L/min. After 20 min of air equilibration, a sterile saline solution with L-[1-^{14}C]phenylalanine[7] was infused into the catheter as a priming bolus dose (4.5, 5.5, or 7.0 μCi/kg BW) followed by a 4- to 5-h constant infusion (3.5 μCi/kg BW/h) using a syringe pump.[2] The daily feed allocation of 120 g was divided into four equal portions and fed to chickens hourly beginning immediately after the initiation of the continuous infusion. This feeding protocol was designed to minimize any change in total breath CO_2 excretion due to feed intake over the breath collection period (Hoerr et al., 1989).

Breath collection began simultaneously with the initiation of the continuous infusion. Air was drawn through the chamber and bubbled through three gas collection bottles[2] connected in series. Each of the first two bottles contained 150 mL of CO_2 absorber (monoethanolamine[8] and 2-methoxyethanol,[8] 1:2 vol/vol) with the last bottle containing 100 mL of absorber. The bottles were exchanged, within seconds, with bottles of fresh absorber every 30 min until the constant infusion was stopped. At the end of each collection, the volume of CO_2 absorber in each bottle was measured and 1-mL samples, in triplicate, were pipetted into 6-mL scintillation vials followed by addition of 5 mL of scintillant cocktail.[9] Samples were counted on a liquid scintillation counter[10] for 20 min with background set at 20 decay per minute (dpm).

The rate of $^{14}CO_2$ expiration (dpm/kg per h) from the oxidation of L-[1-^{14}C]phenylalanine was determined for each collection period and plotted against time. The breath $^{14}CO_2$ was considered to have attained plateau when it was unchanging over time as determined by visual inspection of the graphed data and confirmed by linear regression.[11] The rate of $^{14}CO_2$ expiration, corrected for retention of the label in the bicarbonate pool, was calculated as

$$\text{corrected } V^{14}CO_2 \text{ (dpm/kg BW per h)} = V^{14}CO_2 \text{ (dpm/kg BW per h)/BRF}$$

where $V^{14}CO_2$ is the flow rate of the expired $^{14}CO_2$ and BRF is the bicarbonate retention factor (0.86) determined previously for chickens (Tabiri et al., 2002). Percentage of dose oxidized was calculated as

$$\text{corrected } V^{14}CO_2 \text{ (dpm/kg BW per h)/constant infusion dose (dpm/kg BW per h)} \times 100$$

Statistical Analysis

Plateaus in breath $^{14}CO_2$ were confirmed by linear regression analyses,[11] which were not significantly different from zero ($P > 0.05$).

RESULTS

Chickens recovered from surgery within 2 to 3 d and remained healthy throughout the experiment. During the week before surgery, these birds consumed an average of 95 g/d and gained approximately 180 g. Feed intake and weight gain for the week postsurgery were 108 g/d and 210 g, respectively, which compare well with suggested values (Cobb 500 Management Guide, 1995).

Recovery of radioactivity in CO_2 collection Bottles 1, 2, and 3 averaged 79, 18, and 3%, respectively, indicating that all of the expired $^{14}CO_2$ was trapped in the three bottles. Bird 1 was given L-[1-^{14}C]phenylalanine priming and constant infusion doses of 15,400,000 dpm (7 μCi)/kg BW and 7,634,000 dpm (3.5 μCi)/kg BW per h, respectively. In this bird, the breath $^{14}CO_2$ did not reach plateau during the 5 h of breath collection due to overpriming (Figure 1). Overpriming was indicated by a rapid rise of breath $^{14}CO_2$ and a continuous, significant decline during the collection period ($P < 0.05$). Because of this overprime, Bird 2 was infused with a lower priming dose of 9,900,000 dpm (4.5 μCi)/kg BW and the same constant infusion

[3]Wyeth-Ayerst Canada Inc., St-Laurent, PQ, Canada.
[4]Ed-Art, Don Mills, ON, Canada.
[5]Alice King Chatham Medical Arts, Hawthorne, CA.
[6]Model 1028-1010-G608X, Gast Pump Manufacturing Corp., Edmonton, AB, Canada.
[7]ARC 138A, American Radiolabeled Chemicals, Inc., St. Louis, MO.
[8]Caledon Laboratories, Mississauga, ON, Canada.
[9]Atomlight; Dupont Canada, Mississauga, ON, Canada. 1 Beckman Instruments Inc., Irvine, CA.
[10]Beckman Instruments Inc., Irvine, CA.
[11]Version 6.02, Corel Quattro Pro, Ottawa, ON, Canada.

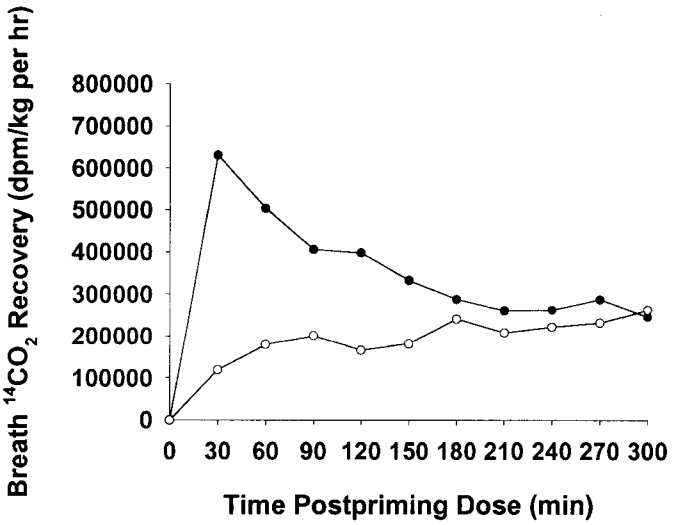

FIGURE 1. Effect of overpriming and underpriming doses of L-[1-^{14}C]phenylalanine on time course recovery of breath $^{14}CO_2$ during constant infusion in chickens. Bird 1 (●) was infused with priming dose of 7.0 μCi/kg BW and constant infusion dose of 3.5 μCi/kg BW per h. Bird 2 (○) was infused with priming dose of 4.5 μCi/kg BW and constant infusion dose of 3.5 μCi/kg BW per h. In both birds, breath $^{14}CO_2$ expiration rate did not attain plateau due to overpriming and underpriming, respectively. DPM = decay per minute.

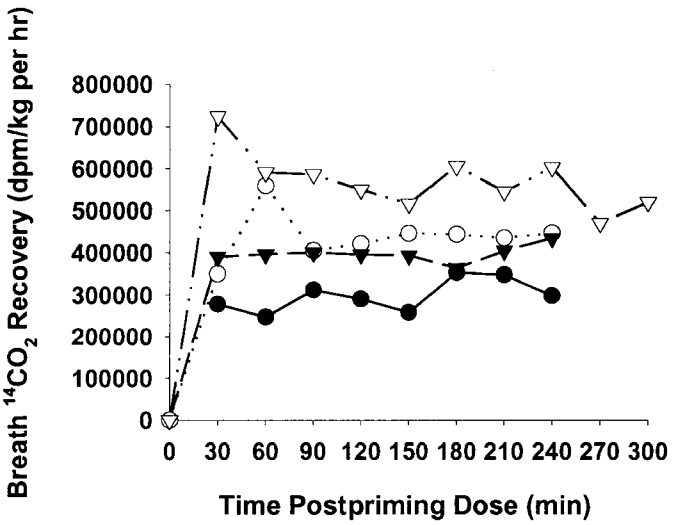

FIGURE 2. Effect of appropriate priming doses of L-[1-^{14}C]phenylalanine on time course recovery of breath $^{14}CO_2$ during constant infusion in chickens. Birds 3 (●), 4 (▼), 5 (▽), and 6 (○) were infused with a priming dose of 5.5 μCi/kg BW and a constant infusion dose of 3.5 μCi/kg BW per h. All birds achieved a plateau rate of breath $^{14}CO_2$ expiration as determined by regression analysis. DPM = decay per minute.

dose of 7,634,000 dpm/kg BW per h for 5 h. In Bird 2, breath $^{14}CO_2$ expiration increased slowly over time and did not achieve a plateau rate during the 5 h of breath collection, indicating an underprime (Figure 1).

In Bird 3, a priming dose of 12,100,000 dpm (5.5 μCi)/kg BW was infused with a constant infusion dose of 7,634,000 dpm/kg BW per h for 5 h, and breath $^{14}CO_2$ reached isotopic plateau by 60 min. Birds 4, 5, and 6 were infused with the same doses as Bird 3 and isotopic plateaus were reached within 30, 30, and 90 min, respectively (Figure 2). The percentage of L-[1-^{14}C]phenylalanine oxidized was 3.90, 5.28, 7.25, and 5.72% for Birds 3, 4, 5, and 6, respectively.

DISCUSSION

In a previous study (Tabiri et al., 2002), we successfully developed a system to quantitatively collect breath $^{14}CO_2$ from catheterized chickens intravenously infused with $NaH^{14}CO_3$. In addition to validating the breath collection apparatus, we also determined the bicarbonate retention factor for chickens. In the present study, we employed the same air collection apparatus, surgical technique, catheter maintenance protocol, and infusion system to determine oxidation rates of L-[1-^{14}C]phenylalanine. However, instead of a commercial diet, we used a synthetic diet (D. H. Baker, 2000, personal communication), which will allow us to extensively manipulate amino acid concentrations in future experiments exploring amino acid requirements with the IAAO technique.

When continuous infusion of radioisotope is initiated, the labeled phenylalanine is diluted by unlabeled phenylalanine in the free amino acid pool of the body. The phenylalanine exiting the pool to protein synthesis and oxidation initially has a low proportion of labeled phenylalanine because the pool has not been adequately enriched. Eventually, the phenylalanine exiting the pool will have the same proportion of radiolabeled phenylalanine as that entering the pool. When the rates of appearance and disappearance are equal, isotopic steady state or plateau has been reached (Wolfe, 1992). Without the optimum priming dose, this process is usually slow and will take several hours before steady state is reached. Priming the pool with an appropriate amount of labeled phenylalanine will rapidly increase the pool enrichment to the equilibrium level; over- or underpriming the pool will delay steady state due to the re-equilibration necessary.

The plateau in expired $^{14}CO_2$ is indicative of a steady state in the body phenylalanine pool because $^{14}CO_2$ enrichment is directly related to the ^{14}C enrichment of the phenylalanine precursor. Labeled breath CO_2 at plateau for each level of test amino acid is used to calculate phenylalanine oxidation, which can then be plotted to determine amino acid requirement (House et al., 1998). Physiologically, when labeled breath CO_2 is at plateau, labeled CO_2 from intracellular phenylalanine oxidation has equilibrated with labeled CO_2 in extracellular fluids; this equilibration results in a constant relationship between labeled CO_2 expiration and labeled phenylalanine infusion. Furthermore, this constant relationship implies that the ratio of labeled to nonlabeled free phenylalanine is also constant. It is important for labeled breath CO_2 to attain plateau because it is only during this constant relationship that phenylalanine oxidation can be accurately determined (Wolfe, 1992).

In a previous attempt to develop the IAAO technique in chickens, Pettit Ewing et al. (2001) employed the approach

originally developed by Bayley and colleagues in pigs (Kim et al., 1983; Ball et al., 1986; Ball and Bayley, 1986). In this approach, labeled amino acid is fed in two bolus meals, and no steady state is achieved, therefore minimizing the amount of kinetic data available. In the more refined IAAO technique later developed by Ball and colleagues (Bertolo et al., 1998; House et al., 1998), labeled indicator amino acid is continuously infused into individuals with labeled breath CO_2 collected over the infusion period. The rate of label expiration eventually becomes constant over time, i.e., a steady state. It is only at steady state that kinetic calculations regarding amino acid oxidation can be made. Constant infusion of labeled amino acid alone requires an extended period of time for the breath CO_2 to reach plateau. To decrease the time required for breath CO_2 to reach plateau, a priming bolus dose was given immediately before the commencement of the constant infusion protocol. The function of the priming dose was to rapidly increase the labeled:unlabeled ratio in the free amino acid pool so that equilibration with pools of metabolic products (i.e., CO_2) also occurs more quickly (Wolfe, 1992). Although the optimum ratio of prime to constant infusion doses has been established for certain indicator amino acids in pigs and humans, there are no similar studies in poultry. Therefore, before the IAAO technique can be successfully employed, the priming and constant infusion doses of phenylalanine must be verified.

The influence of priming dose on time taken for breath $^{14}CO_2$ to reach plateau is evidenced from our studies. At a constant infusion rate of 3.5 μCi/kg BW per h, birds infused with priming doses of 7.0 μCi/kg BW or 4.5 μCi/kg BW were over- and underprimed respectively, resulting in breath $^{14}CO_2$ not reaching plateau in these birds. When the remaining birds were given a priming dose of 5.5 μCi/kg BW and a constant infusion rate of 3.5 μCi/kg BW per h, their breaths $^{14}CO_2$ reached plateau within 60 min postinfusion. Therefore, from our study, the appropriate prime:constant ratio was 1.6:1. These data indicate the importance of the proper priming dose. Breath $^{14}CO_2$ at plateau was 298,000 to 554,000 dpm/kg BW per h and was considered more than adequate for accurate breath analysis in our system.

The CO_2 absorber sampling and analysis protocol employed in our study resulted in scintillation counting error of less than 0.1% with a 5-min counting time. By increasing counting time and accepting a 1% error, less radioactivity per sample would be required, thereby reducing the constant infusion dose and saving costs. Because the prime:constant ratio represents a fixed physiological relationship, the ratio from the present study could still be used in future studies where the constant infusion dose is reduced.

The average percentage phenylalanine oxidized at plateau is dependent on diet formulation and is a critical feature of this method development. First, because phenylalanine oxidation occurs via tyrosine, it is critical that dietary tyrosine concentrations are in excess of its requirements so that phenylalanine is channeled to oxidation and not to tyrosine synthesis (Zello et al., 1995). In the present study, we fed tyrosine at twice the recommendation for these birds (D. H. Baker, 2000, personal communication). Second, dietary phenylalanine concentrations must be carefully chosen. If dietary phenylalanine is fed below requirement, then phenylalanine oxidation will be very low and additions of test amino acid will not affect the oxidation rate. If dietary phenylalanine is fed at too high a concentration, then oxidation will be very high and will not be as sensitive to changes in test amino acid concentrations (Kim et al., 1983). In the present study, 5.5% of the phenylalanine was oxidized, which has previously been shown to be an appropriately sensitive basal oxidation rate in IAAO studies in piglets (Bertolo et al., 1998; House et al., 1998).

The observed variability in phenylalanine oxidized as a percentage of doses among individual birds is not surprising. This oxidation rate represents a basal rate of oxidation when all amino acids are in excess. This basal rate is dependent on the protein synthesis rate, the amount of phenylalanine fed, the level and activity of phenylalanine oxidation enzymes, and the efficiency of utilization of amino acids for protein synthesis. These factors all contribute to variability in percentage of phenylalanine oxidized. For example, a smaller bird depositing protein at a lower rate will oxidize more of its dietary phenylalanine due to lower protein synthesis rates.

The variability observed in this experiment is consistent with that in previous studies in piglets. When all amino acids are in excess of requirements, as in this study, phenylalanine oxidation in piglets ranged from 1.3 to 6.1% of dose (Bertolo et al., 1998; House et al., 1998), proportionately comparable to the 3.90 to 7.25% range in chickens in this study. Furthermore, in future analyses of amino acid requirements using this technique, we intend to determine requirements of individual birds. Therefore, it is the relative phenylalanine oxidation rates within a bird that are important, rather than the absolute oxidation rates.

This is the first study, to the authors' knowledge, to establish primed-continuous infusion methodology in chickens. Through constant intravenous ^{14}C-phenylalanine infusion and collection of breath $^{14}CO_2$, we have demonstrated that amino acid kinetic studies are feasible in chickens. By employing a synthetic diet in the present study, we have established a model that can be used to implement the IAAO technique in chickens to determine amino acid requirements by feeding deficient diets. Because of the short adaptation time in the IAAO technique, such a development would benefit the poultry industry enormously by allowing amino acid determination in individual birds, thereby providing population mean and variability data for the first time. Furthermore, the IAAO technique is particularly suited for nongrowing birds such as breeders for which classic growth assays are unsuitable. Future studies will apply the techniques and data established during the present work to determine amino acid requirements in individual broiler breeder chickens.

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