Lysine Requirements of Pre-lay Broiler Breeder Pullets: Determination by Indicator Amino Acid Oxidation\(^1,2\)

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ABSTRACT The indicator amino acid oxidation (IAAO) method allows the determination of amino acid requirements under conditions of low growth rate as found in pre-laying broiler breeder pullets. Cobb 500 breeder pullets (20 wk old; 2290 ± 280 g, n = 4) were adapted (6 d) to a pelleted, purified control diet containing all nutrients at ≥110% of NRC recommendations. After recovery from surgery for implantation of a jugular catheter, each bird was fed, in random order, test diets containing one of nine levels of lysine (0.48, 0.96, 1.92, 2.88, 3.84, 4.80, 7.68, 9.60 and 14.40 g/kg of diet). Indicator oxidation was determined during 4-h primed (74 kBq/kg body, constant infusions (44 kBq · h\(^{-1}· kg\)body\(^{-1}\)) of L-[\(1\)\(^{14}\)C]phenylalanine. Using the breakpoint of a one-slope broken-line model, the lysine requirement was determined to be 4.88 ± 0.96 g/kg of diet or 366 ± 72 mg · hen\(^{-1}· d\(^{-1}\) with an upper 95% CI of 6.40 g/kg of diet or 480 mg · hen\(^{-1}· d\(^{-1}\). IAAO allows determination of individual bird amino acid requirements for specific ages and types of birds over short periods of time and enables more accurate broiler breeder pullet diet formulation. J. Nutr. 133: 2826–2829, 2003.

KEY WORDS: individual requirement, maintenance requirement, broiler breeder pullet, indicator amino acid oxidation, continuous infusion.

The indicator amino acid oxidation (IAAO)\(^5\) technique for determining amino acid (AA) requirements has been developed and validated in pigs (1–8), humans (9–11) and chickens (12,13). Several reviews based on the human studies that describe the benefits and disadvantages of the IAAO method have been published (14,15). The technique is based on the concept that a deficiency of one indispensable AA will restrict protein synthesis. Therefore, all other indispensable AA will be in relative excess and will be oxidized. As the dietary intake of the test amino acid (AA\(_{\text{test}}\)) increases, the oxidation of all other indispensable AA decreases, corresponding to the increase in protein synthesis. If the intake of the AA\(_{\text{test}}\) increases beyond the requirement, no further change in indicator oxidation will occur (14,15). The point at which the oxidation of the indicator AA reaches a plateau is taken as the requirement, provided no other nutrient is limiting. The indicator AA must have an oxidative pathway distinct from and unrelated to the AA\(_{\text{test}}\) (14,15), so that a change in dietary AA\(_{\text{test}}\) will not affect the pool size of the indicator AA. Phenylalanine and lysine have been shown to be suitable indicator AA for IAAO studies in humans (16). The oxidation pattern of L-[\(1\)\(^{14}\)C]phenylalanine (the indicator AA) after changes in the dietary levels of lysine (1,7), histidine (1), threonine (6), tryptophan (5), arginine (3), proline (3) and total protein (4,8) was demonstrated in pigs.

Amino acid requirements for broiler breeder pullets are not well described. Broiler breeders are the parents of meat-type (broiler) chickens, and therefore have been genetically selected for rapid growth rate. To maintain optimum egg production, broiler breeders are feed restricted throughout life to prevent obesity and related reproductive problems (17). Currently, the NRC does not publish any AA requirements for broiler breeder pullets (18). They concluded that there is insufficient research data on which to base suggested requirements for growing and developing broiler breeder meat-type pullets (18). Industry standards suggest formulating diets on the basis of interpolations from requirements determined for table egg layers and growing broilers. Traditionally, AA requirements have been determined using growth assays (19,20) or nitrogen balance (21), which involved feeding graded levels of the AA\(_{\text{test}}\) to the subject and looking for a clearly definable change in a relevant biological parameter (14). However, the limited growth rate of broiler-breeder pullets due to feed restriction prevents the accurate determination of requirements using these conventional methods.

Broiler breeder pullets, 20–25 wk of age, were chosen as the experimental animal because of the dearth of information on their AA requirements. In addition, the birds were near their mature body weight (BW), were not sexually mature and were therefore metabolically stable over the course of the experiment. The objective of this study was to determine lysine requirements of individual prelay broiler breeder pullets, based on the oxidation of an indicator AA, L-[\(1\)\(^{14}\)C]phenylalanine.

MATERIALS AND METHODS

The University of Alberta Faculty Animal Policy and Welfare Committee approved all experimental procedures. Female Cobb 500 broiler breeder pullets (17 wk of age, n = 4) were selected from a

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commercial farm and transferred to the Metabolic Research Unit at the University of Alberta. The pullets were caged individually; they were given 2 wk to adapt to their new environment and 1 wk to recover from surgery in which a catheter was implanted into the left jugular vein. An 8-h:16-h light:dark schedule was used. The lights were automatically turned on at 0800 h each day. The birds were fed 75 g/d of test diets based on management of BW according to the Cobb 500 breeder management guide (22) during the 3-wk trial. For the first 14 d of the study, the birds were fed a standard mash broiler breeder pullet diet formulated to meet NRC (18) recommendations. The diet was then changed to a control (9.6 g lysine/kg diet) pelleted, purified diet, which was formulated to meet the estimated lysine requirement of broiler breeder hens at peak production (18). The diet was formulated to ensure that all indispensable AA were in excess of the NRC requirement for broiler breeder layers (18) by at least 10% except for the AA_{test} (lysine) to ensure that lysine was the only limiting AA during the experiment. This diet was fed to the pullets 3 d before surgery and continued until the first oxidation study at 20 wk of age.

The composition of pelleted, purified test diets (g/kg diet) was: cornstarch, 624.8; corn oil, 100.0; salt mix, 53.7; cellulose (Solka Floc; International Fiber, North Tonawanda, NY), 20.9; vitamin mix (AIN-93-VX, 960402, ICN Biomedicals, Aurora, OH), 10.0; all diets contained 189.4 g of AA mixture. In addition, three basal diets were formulated, each having different levels of l-lysine-HCl (0.48, 1.92, 2.88, 3.84, 4.80, 7.68, and 14.4 g lysine/kg diet) balanced with L-glutamate at 134.4, 129.6 and 120.0 g/kg diet, respectively, to keep each of the diets isonitrogenous. Each of the nine A_{test} diets was obtained by mixing together the correct ratio of a basal diet above and below the A_{test} level.

Twelve hours before surgery at 19 wk of age, feed and water were removed from the birds. The surgical insertion of a catheter into the left jugular vein was described in detail (12,13). Observation from previous experiments (13) indicated that a minimum of 3 d was required for birds to recover from surgery, as indicated by daily consumption of a quantity of diet equal to the presurgery amount, activity level and responsiveness to the environment. Beginning at 20 wk of age, at ~9000 h on each oxidation day, the birds were weighed and placed into individual metabolic chambers for determination of oxidation rate. Initially, each bird was fed the control diet (9.6 g lysine/kg diet) and oxidation of the indicator amino acid was determined. Subsequently, each bird received one of the eight additional test levels of lysine (0.48, 0.96, 1.92, 2.88, 3.84, 4.80, 7.68 and 14.4 g lysine/kg diet) for an adaptation period of 43 h before each oxidation study. Assignment of dietary treatments to each bird was in random order until each bird had been fed each test level of lysine. Basal diets were mixed proportionally to obtain the required lysine concentration.

Tracer infusion and 14CO2 recovery. Each of the test diets was fed for 43 h; the oxidation rate over a 4-h period was determined using the IAAO method. l-[1-14C]phenylalanine (American Radiochemicals, St. Louis, MO), prepared in a sterile saline solution with a concentration of 37 kBq/mL, was used as the indicator AA. Each bird received a priming dose of 133 g/kg body weight followed by a 4-h constant infusion of 44 kBq/kg/h of amino acid labeled with 14C. Recovery of 14CO2 was measured by gas chromatography and radioactivity was determined. The typical response pattern (Fig. 1) seen in IAAO of 14CO2 recovered over time with bird 1 fed nine levels of lysine/kg of diet. The response pattern follows the A_{test} intake is increased until the requirement is reached. At that stage, there is no further response to additional A_{test} intake.

The mean peak CV for all oxidations was 6.73 ± 3.28% for each oxidation was taken at a level of P < 0.05; differences between least-square means were obtained by pair-wise comparisons using Tukey’s test as an option within the general linear model procedure. Lysine requirements were established using a linear-plateau model adapted from SAS (24), regressing oxidation rate on dietary lysine concentration. The breakpoint between the slope and plateau phases of the model was changed iteratively until the residual mean-square error was minimized. The individual requirements were used to determine the population mean and its SD. An upper 95% CI for the breakpoint was calculated from the mean requirements.

RESULTS

The birds were in good physical condition, with no signs of stress (inspected at least twice per day) during the oxidation period. Mean BW gain (±SD) during the 17-d experimental period was 202 ± 26 g. The Cobb 500 management guide (22) recommends 340 g BW gains over the same period. The restricted but steady growth of birds was assumed to be due to the limited lysine in some of the test diets fed to the birds during portions of the experimental period. Two observations (Bird 2, 7.68 g lysine/kg diet and Bird 4, 0.96 g lysine/kg diet) were removed from the data set due to technical difficulties during the infusion period.

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The individual lysine requirements (breakpoint) for Birds 1, 2, 3 and 4 were determined to be 4.6, 3.9, 4.8 and 6.2 g lysine/kg diet or 345, 293, 360, 465 mg g lysine · hen^{-1} · d^{-1}, respectively. The mean requirement was 4.88 ± 0.96 g lysine/kg diet or 365.6 ± 62.6 mg g lysine · hen^{-1} · d^{-1}. The lysine intake necessary to meet the requirement of 95% of the population (upper 95% CI) 20–23 wk of age was calculated as 6.4 g lysine/kg diet or 480 mg g lysine · hen^{-1} · d^{-1}.
95% CI of 480 mg lysine

The NRC (18), [765 mg lysine to the lysine requirement for broiler-breeder hens according to

lysine functions (i.e., growth and maintenance) would be 447 mg egg production, so that the lysine requirement for the other 50% of the lysine requirement at peak production was due to the requirement for egg production. Fisher (25) estimated that our birds had not been induced to lay through photostimulation. Therefore, it is reasonable to assume that the lysine requirement of our birds would be equivalent to the estimate by Fisher of 893 mg lysine · hen⁻¹ · d⁻¹; (25) minus the requirement for egg production. Fisher (25) estimated that 50% of the lysine requirement at peak production was due to egg production, so that the lysine requirement for the other functions (i.e., growth and maintenance) would be 447 mg lysine · hen⁻¹ · d⁻¹. Applying the same estimated partitioning to the lysine requirement for broiler-breeder hens according to the NRC (18), [765 mg lysine · hen⁻¹ · d⁻¹], the requirement minus egg production would be 383 mg lysine · hen⁻¹ · d⁻¹. In our study, we determined mean lysine requirement of 365 mg lysine · hen⁻¹ · d⁻¹, with an upper 95% CI of 480 mg lysine · hen⁻¹ · d⁻¹. Our mean requirement agrees with the predicted nonlaying lysine requirement extrapolated from the values published by the NRC (18). Moreover,

our calculated “safe intake” or upper 95% CI just exceeds the requirements calculated from Fisher (25). It is important to note that this nonlaying requirement includes the lysine needed for growth as well as maintenance. Although our birds grew during the 3 wk of experimentation (increasing their BW by ~10%), the frequent periods of lysine deficiency and the necessary feed restriction make the lysine requirement for growth and for maintenance impossible to calculate. However, IAAO allows the calculation of total metabolic requirement for lysine. In addition, previous estimates for the lysine requirement for maintenance alone are highly variable and have been estimated at 13–215 mg lysine · hen⁻¹ · d⁻¹ (23,25). In conclusion, our results for the lysine requirement of prelaying broiler breeder pullets are confirmed by similar estimates calculated from existing literature data.

The IAAO method is sensitive to rapid changes in the AA pool (intracellular protein synthesis) within the test animal and does not rely on indirect responses such as growth and egg production to determine AA requirements. This principle makes the IAAO an excellent method with which to determine essential or conditionally indispensable AA requirements in broiler breeder pullets (14).

The results generated using this method are ideally suited to inclusion in mathematical models of poultry AA metabolism and requirements for maintenance, egg production and growth, which Fisher (25) suggested to obtain more accurate determinations of requirements in poultry. The IAAO technique also allows for the determination of the amino acid requirements of individual birds over a very short period of time. This capability allows, for the first time, an accurate assessment of the variability of requirements within a population, which would allow the development of stochastic models for growth and requirement.

In addition, these flock variability data will allow nutritionists to determine accurately the cost of meeting the lysine requirements of a greater proportion of individual birds. To account for population variance in requirement, feeding at the 95% CI (6.4 g lysine/kg diet) will meet the requirement of 95% of the birds. This safety margin for feed formulations will have considerable economic effect with respect to efficiency of production. Furthermore, these results provide poultry nutritionists with a starting point from which to calculate the ideal AA requirements for broiler breeder pullets (20–23 wk of age) using lysine as the reference AA. Swine nutritionists have used ideal AA requirements for a number of years (26); this approach has gained acceptance in the poultry industry as well.
(27,28). Factorial modeling has been used to determine energy and AA requirements in poultry (25). Data collected using the IAAO method have the potential to make calculations based on factorial modeling more accurate.

Until now there has not been a suitable method with which to determine AA requirements in individual birds. The IAAO method was adapted to determine individual breeder pullet requirements because it allows multiple measurements to be performed within the same animal, without a change in physiologic state during the experiment. The determination of the lysine requirement of individual breeder pullets allows nutritionists to make an accurate assessment of the cost of meeting the lysine requirements of an increased proportion of the population. The AA requirements of broiler breeders would be expected to change substantially as they increase in frame size early in life, as they approach sexual maturity (as in the present study), as they reach sexual maturity and peak egg production and as egg production declines with age. The IAAO methodology will enable the determination of individual bird requirements at each of these stages, thus allowing a much more precise formulation of broiler breeder diets.

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LITERATURE CITED