ABSTRACT

Three hundred and twenty Hy-Line W36 commercial laying hens, 39 weeks of age, were used to determine the sequence and the length of time needed for hens to recover performance characteristics after an eight-week period under graded levels of threonine deficiency. Eight experimental diets with Thr levels ranging from 0.35 to 0.53% were randomly fed with eight replicates of five hens each. After the previous experiment, the hens were fed a control diet (0.53% Thr) for a four-week period. Feed consumption (FC), energy intake (EI), egg production (EP), egg weight (EW), egg mass (EM), and body weight (BW) were evaluated. All performance characteristics were impaired on Thr deficient diets. The recovery sequence order was FC and EI; EP, EW and EM, and finally BW, with the length of time of two, three, and four weeks, respectively. The data indicated that an amino acid deficiency does not cause permanent damage to the reproductive system of the hens.

RESUMO

Trezentas e vinte poedeiras comerciais Hy-Line W36 com 39 semanas de idade foram utilizadas com o objetivo de determinar a sequência e o tempo necessário para as galinhas recuperarem as características de desempenho após um período de oito semanas de alimentação, com dietas contendo diferentes níveis de treonina. Oito dietas experimentais, com níveis de treonina variando de 0,35 a 0,53%, foram distribuídas ao acaso, com oito repetições de cinco aves cada. Após esse prévio experimento, as aves foram alimentadas com a dieta controle (0,53% Thr) por quatro semanas em que as características: consumo de ração (CR), de energia (CE), produção de ovos (PO), peso de ovos (PE), massa de ovos (MO) e peso corporal (PC) foram avaliadas. Todas as características foram prejudicadas com dietas deficientes em Thr e a sequência da recuperação ocorreu na seguinte ordem: CR e CE; PO, PE e MO e, por último, PC, nos prazos de duas, três e quatro semanas, respectivamente. Os dados obtidos indicaram que uma deficiência em aminoácidos não causa um dano permanente ao sistema reprodutivo das galinhas.
INTRODUCTION

Nutritionists usually formulate diets for commercial laying hens to achieve maximum performance and maximum economic return. Feeding programs should meet the nutrient requirements according to the production phase or age of hens. Most of these feeding programs are based on the daily feed intake concept, which was first suggested by Harms et al. (1978) and later modified by Harms (1981).

Dietary manipulation can have different objectives such as increasing early egg weight (Bohnsack & Harms, 2000), reducing egg weight and improving eggshell quality in old and young hens (Roland, 1980a,b), improving egg size classification (Zimmerman, 1997; Harms, 2000), and for diets used just before hens are marketed or molted (Kuchinski & Harms, 1993). However, if the margin of safety is too small and variation of feed ingredients results in considerably less amino acids in the feed, performance of the hen will be reduced (Harms & Russell, 1998).

Harms & Ivey (1993) evaluated the performance of layers fed various supplemental amino acids in a corn-soybean meal diet and concluded that methionine and lysine were the first two limiting amino acids, with tryptophan, arginine or threonine being third. On the other hand, NRC (1994) suggested a daily intake of 470 mg/day of threonine for maximum performance of laying hens.

Therefore, this experiment was conducted to determine the sequence and the length of time needed for hens to recover various performance characteristics after receiving diets deficient in amino acids with threonine being the most limiting.

MATERIALS AND METHODS

Three hundred and twenty Hy-Line W36® commercial laying hens, 39 weeks of age, were used in this experiment. They were randomly housed one bird per wire cage (25.6 x 42.6 cm) in a windowless, fan-ventilated house. The temperature was controlled to get a uniform feed intake. It was not allowed to fall below 26.7°C and was almost constant because the experiment was conducted in February and March. They were given artificial light (16h light:8h dark). Feed and water were provided ad libitum.

Previously, the hens were fed eight experimental diets with various deficient amino acids levels (Table 1) from 31 to 38 weeks of age. In this previous study (Faria et al., 2002), Diet 1 was a positive control diet containing 0.53% Thr and other amino acids previously found to support maximum performance (Harms & Russell, 1996). Diet 2 contained 0.50% Thr (95% of Diet 1) and other supplemental amino acids (AA), at levels equal to Diet 1 and expected to support maximum performance. Diet 3 contained 0.48% Thr (90% of Diet 1), and other AA were included at 95% of the levels in Diet 2. Diet 4 contained 0.45% Thr (85% of Diet 1), and other AA were included at 90% of the levels in Diet 2. Diet 5 contained 0.42% Thr (80% of Diet 1), and other AA were included at 85% of the levels in Diet 2. Diet 6 contained 0.40% Thr (75% of Diet 1), and other AA were included at 80% of the levels in Diet 2. Diet 7 contained 0.37% Thr (70% of Diet 1), and other AA were included at 75% of the levels in Diet 2. Diet 8 contained 0.35% Thr (65% of Diet 1), and other AA were included at 70% of the levels in Diet 2. At the termination of the previous experiment the hens receiving Diets 2 to 8 were placed on Diet 1 for four weeks (Table 1). The eight replicates of five hens, which were fed these diets for eight weeks, were used to evaluate the performance recovery from each diet.

Performance of the last week of the previous experiment is indicated as week -1. Feed consumption (FC), energy intake (EI), egg production (EP), egg weight (EW), and the final body weight were used to indicate the severity of the amino acid deficiency. Egg mass was obtained for multiplying EP by EW. Hens were individually weighed at the termination of the previous experiment and bi-weekly during the next four weeks. Body weight (BW) change was calculated biweekly from initial BW during the four-week period. Weekly FC, EI, EP, EW, and EM were obtained and replicate values were used for statistical analyses. The data were subject to a one-way ANOVA using the general linear model procedure of SAS® (SAS Institute, 1996). Duncan’s multiple-range test (1955) was used to determine significant differences among treatment means.

RESULTS

FEED CONSUMPTION AND ENERGY INTAKE

Feed consumption and energy intake were significantly reduced when hens were fed the three diets containing the lowest levels of amino acids, as indicated at week -1 (Table 2). There was a small recovery of FC and EI when hens were fed the control diet for one
week. However, FC and EI were equal to control after hens had received the control diet for two weeks.

There were no differences in FC and EI among treatments during weeks 3 and 4. This indicated that the severity of the amino acid deficiencies did not affect the recovery for these measurements. This disagrees with a previous report (Harms & Russell, 1998) that hens fed a diet with a very low level of Trp significantly increased FC during the first two weeks after they were fed the control diet. Possibly, it indicates that the hen responds differently to deficiencies of various amino acids.

**EGG PRODUCTION**

Egg production was significantly reduced for hens fed the three diets containing the lowest levels of amino acids in week -1 (Table 3). Egg production gradually increased during the first two weeks after returning the hens to the control diet. During weeks 3 and 4 there were no significant differences in EP among the treatments. The recovery in EP when the hens were previously fed a diet extremely low in amino acids is the same response as previously found for Trp (Harms & Russell, 1998). The lack of an increase during the first two weeks was expected because the number of ova that had started to mature when the hens were returned to the control diet determined the number of eggs during this period.

**EGG WEIGHT**

Egg weight was significantly reduced for hens fed the three diets containing the lowest levels of amino acids in week -1 (Table 3). There was an increase in EW after the hens received the control diet for one week. Egg weight continued to increase during the second week. During the third week there was no differences in EW among treatments. The recovery in EW from hens previously fed a diet extremely low in amino acids agrees with a previous report (Harms & Russell, 1998) that hens fed a diet extremely low in Trp restored EW in three weeks.

**EGG MASS**

Egg mass was significantly reduced for hens fed the three diets containing the lowest levels of amino acids in week -1 (Table 3). Egg mass from hens previously fed diets deficient in amino acids was increased at the end of one week. The EM continued to increase during week 2. There were no significant differences in EM among all treatments during weeks 3 and 4.

**BODY WEIGHT AND BODY WEIGHT CHANGE**

Body weight was significantly reduced when the hens previously received diets deficient in amino acids (Table 4). Hens previously fed amino acids deficient diets gained significantly more weight during the first two weeks after receiving the control diet. They also continued to gain more weight during the second two weeks. Hens previously fed the diet containing the lowest level of amino acids weighed significantly less at the end of fourth week than control hens.

**DISCUSSION**

Hens previously fed the three diets containing the lowest levels of amino acids during eight weeks (Faria et al., 2002) showed a reduction in feed consumption and energy intake about 21 and 16%, respectively, in comparison to control diet. Also, egg production, egg weight, egg mass, and body weight were reduced about 27, 7, 30, and 13%, respectively. The findings about reductions in EP and EW are in agreement with findings of Morris & Gous (1988). They stated that the egg size does not decrease below 90% of its maximum value until amino acid intake is well below 50% of the optimum value, whereas; rate of lay is only 70% of its potential value when amino acid intake is half the optimum.

The results of this experiment indicated that hens fed a diet deficient in amino acids will return to normal performance in three weeks when fed a nutritionally adequate diet. Figure 1 shows the length of time needed for hens to recover FC, EP, EW, and BW, and the recovery percentage each week after hens have received the control diet.

Egg production and EW gradually increased, and by the end of week 3 was equal to the EP and EW of control hens. The small increase in EP during the first two weeks was a result of the growth of ova, which had started during the previous experiment when hens were fed deficient diets. A more rapid increase in EW was observed as FC increased, which furnished more amino acids for formation of yolks. The increase in EM during the first two weeks was due to the increase of EP and EW.

The hens previously fed amino acid deficient diets increased FC during the first two weeks after receiving the control diet. The increased energy and amino acid intakes resulted in increase BW, EP, EW, and EM. These increases resulted in no significant difference among
treatments after four weeks, except in BW. This indicates no permanent damage was done to the reproductive system from feeding an amino acid deficient diet for eight weeks. This is important when reducing the margin of safety for amino acids.

A new program has recently been developed to more precisely calculate the needed amino acids (Harms & Faria, 2001). This program also allows for the early detection of an amino acid deficiency, which can be rapidly corrected.

**CONCLUSIONS**

Feeding of diets deficient in amino acids reduces egg production, egg weight, egg mass, and body weight about 27, 7, 30, and 13%, respectively. Hens returned to a complete diet rapidly return to normal production parameters. Feed consumption was recovered within two weeks; egg production, egg weight, and egg mass were recovered within three weeks. Body weight was recovered within four weeks, except for hens fed the diet with extremely low level of amino acids. Therefore, there is no permanent damage to the reproductive system when hens were fed amino acid deficient diets for eight weeks.

**Table 1 - Composition of experimental diets.**

| Ingredient (%) | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   |
|----------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Yellow corn    | 71.39 | 72.88 | 74.61 | 76.27 | 78.00 | 79.67 | 81.39 | 83.06 |
| Soybean meal (48%) | 18.63 | 16.97 | 15.22 | 13.54 | 11.79 | 10.11 | 8.36 | 6.67 |
| Limestone      | 7.51 | 7.51 | 7.50 | 7.49 | 7.48 | 7.48 | 7.47 | 7.46 |
| Dicalcium phosphate | 1.27 | 1.30 | 1.33 | 1.36 | 1.40 | 1.43 | 1.46 | 1.49 |
| Salt           | 0.38 | 0.38 | 0.38 | 0.38 | 0.38 | 0.38 | 0.38 | 0.38 |
| Vitamin mix²   | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Mineral mix³   | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| DL-Methionine  | 0.08 | 0.09 | 0.08 | 0.08 | 0.07 | 0.06 | 0.05 | 0.04 |
| Lys-HCl        | 0.12 | 0.18 | 0.18 | 0.19 | 0.20 | 0.20 | 0.21 | 0.22 |
| Trp            | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.03 | 0.03 |
| Ile            | 0.07 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 |
| Val            | 0.03 | 0.07 | 0.06 | 0.06 | 0.06 | 0.05 | 0.05 | 0.04 |
| **Calculated analysis** | **%** | | | | | | | |
| Protein        | 14.44 | 13.87 | 13.16 | 12.48 | 11.77 | 11.09 | 10.38 | 9.69 |
| Calcium        | 3.20 | 3.20 | 3.20 | 3.20 | 3.20 | 3.20 | 3.20 | 3.20 |
| Phosphorus     | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 |
| Thr            | 0.53 | 0.50 | 0.48 | 0.45 | 0.42 | 0.40 | 0.37 | 0.35 |
| Met            | 0.34 | 0.34 | 0.32 | 0.31 | 0.29 | 0.27 | 0.26 | 0.24 |
| Lys            | 0.82 | 0.82 | 0.78 | 0.74 | 0.70 | 0.66 | 0.62 | 0.57 |
| Trp            | 0.19 | 0.19 | 0.18 | 0.17 | 0.16 | 0.15 | 0.14 | 0.13 |
| Ile            | 0.65 | 0.65 | 0.62 | 0.59 | 0.55 | 0.52 | 0.49 | 0.46 |
| Val            | 0.70 | 0.70 | 0.67 | 0.63 | 0.59 | 0.56 | 0.53 | 0.49 |
| ME, kcal/kg    | 2,860 | 2,876 | 2,891 | 2,905 | 2,920 | 2,935 | 2,950 | 2,964 |

1 - Contains 18.5% P and 21% Ca.
2, 3 - See Harms & Russell (2000).
4 - Based on analysis of corn and soybean meal.
Table 2 - Feed consumption and energy intake of commercial laying hens.

| Dietary Thr | Week -1  | Week 1  | Week 2  | Week 3  | Week 4  |
|-------------|----------|---------|---------|---------|---------|
|             | Feed consumption (g/hen/day) |         |         |         |         |
| 0.53        | 97.77a   | 100.12a | 93.31a  | 97.68a  | 93.63a  |
| 0.50        | 100.12a  | 100.60a | 92.99a  | 101.09a | 94.18a  |
| 0.48        | 95.33a   | 97.68ab | 91.85a  | 94.93a  | 93.31a  |
| 0.45        | 96.21a   | 99.39a  | 93.80a  | 96.35a  | 93.07a  |
| 0.42        | 94.44a   | 99.30a  | 94.12a  | 97.68a  | 96.09a  |
| 0.40        | 85.62b   | 91.69c  | 93.31a  | 97.68a  | 95.42a  |
| 0.37        | 83.02b   | 92.70bc | 89.91a  | 95.13a  | 93.96a  |
| 0.35        | 69.74c   | 86.18d  | 92.66a  | 97.20a  | 94.61a  |
| CV (%)      | 5.80     | 5.54    | 5.53    | 6.55    | 4.76    |

|             | Energy intake (kcal ME/hen/day) |         |         |         |         |
|-------------|---------------------------------|---------|---------|---------|---------|
| 0.53        | 280a                            | 286a    | 267a    | 279a    | 268a    |
| 0.50        | 288a                            | 289a    | 267a    | 291a    | 271a    |
| 0.48        | 276a                            | 282ab   | 266a    | 274a    | 270a    |
| 0.45        | 280a                            | 289a    | 273a    | 280a    | 270a    |
| 0.42        | 276a                            | 290a    | 275a    | 285a    | 281a    |
| 0.40        | 251b                            | 269bc   | 274a    | 287a    | 280a    |
| 0.37        | 245b                            | 273ab   | 265a    | 281a    | 277a    |
| 0.35        | 207c                            | 255c    | 275a    | 288a    | 280a    |
| CV (%)      | 5.82                            | 5.56    | 5.55    | 6.54    | 4.75    |

a-d: Means with the same superscript within a column do not differ significantly (p<0.05).
Table 3 - Egg Production, egg weight, and egg mass from commercial laying hens.

| Dietary Thr | Week -1 | Week 1 | Week 2 | Week 3 | Week 4 |
|-------------|---------|--------|--------|--------|--------|
|             | Egg production (% egg/hen/day) |        |        |        |        |
| 0.53        | 88.93a  | 90.00a | 90.54a | 87.86a | 87.86ab|
| 0.50        | 88.30a  | 89.02a | 88.21ab| 87.50a | 85.83b |
| 0.48        | 86.88a  | 88.21ab| 88.75ab| 88.13a | 87.14ab|
| 0.45        | 90.27a  | 89.46a | 91.34a | 87.23a | 92.02* |
| 0.42        | 85.00a  | 83.57bc| 91.43a | 89.64a | 87.14ab|
| 0.40        | 73.93b  | 79.11c | 85.71bc| 87.86a | 87.50ab|
| 0.37        | 70.09b  | 72.68d | 83.48c | 86.43a | 87.23ab|
| 0.35        | 53.93c  | 65.36e | 85.00bc| 88.57a | 89.80a |
| CV (%)      | 6.08    | 5.78   | 4.18   | 4.04   | 4.76   |
|             | Egg weight (g) |        |        |        |        |
| 0.53        | 58.62ab | 59.39ab| 59.30ab| 59.54a | 60.36ab|
| 0.50        | 59.80a  | 59.99a | 60.24a | 59.97a | 61.28a |
| 0.48        | 58.03b  | 58.35ab| 58.75ab| 59.33a | 59.70ab|
| 0.45        | 57.12bc | 57.86bcd| 58.22b | 59.38a | 58.73b |
| 0.42        | 57.66bc | 58.21bc| 59.00ab| 58.97a | 60.43ab|
| 0.40        | 56.13c  | 58.34abc| 57.81b | 59.31a | 58.96b |
| 0.37        | 56.26c  | 57.14cd| 58.68ab| 60.36a | 61.10a |
| 0.35        | 54.29d  | 56.26d | 57.84b | 58.61a | 58.51b |
| CV (%)      | 2.85    | 2.64   | 2.74   | 2.86   | 3.03   |
|             | Egg mass (g/hen/day) |        |        |        |        |
| 0.53        | 52.11a  | 53.47a | 53.69a | 52.32a | 53.01a |
| 0.50        | 52.78a  | 53.40a | 53.12a | 52.51a | 52.58a |
| 0.48        | 50.42ab | 52.53a | 52.13ab| 52.26a | 52.02a |
| 0.45        | 51.56ab | 51.75a | 53.20a | 51.78a | 54.02a |
| 0.42        | 49.00b  | 48.61b | 53.98a | 52.86a | 52.66a |
| 0.40        | 41.46c  | 46.13b | 49.56bc| 52.10a | 51.53a |
| 0.37        | 39.46c  | 41.57c | 48.95c | 52.17a | 53.25a |
| 0.35        | 29.25d  | 36.80d | 49.20c | 51.90a | 52.52a |
| CV (%)      | 6.18    | 6.23   | 5.14   | 4.83   | 4.53   |

a-d: Means with the same superscript within a column do not differ significantly (p<0.05).
Table 4 - Body weight and body weight change of commercial laying hens.

| Dietary Thr | Initial body weight | Week 2  | Week 4  |
|-------------|---------------------|---------|---------|
|             | Body weight (g)     |         |         |
| 0.53        | 1,521ab             | 1,550ab | 1,556b  |
| 0.50        | 1,589a              | 1,613a  | 1,650a  |
| 0.48        | 1,485bc             | 1,530ab | 1,507b  |
| 0.45        | 1,492bc             | 1,539ab | 1,537b  |
| 0.42        | 1,498bc             | 1,554ab | 1,564b  |
| 0.40        | 1,419cd             | 1,486b  | 1,492b  |
| 0.37        | 1,385d              | 1,468b  | 1,493b  |
| 0.35        | 1,258e              | 1,384c  | 1,391c  |
| CV (%)      | 5.44                | 5.46    | 5.44    |

|             | Body weight change (g) |         |         |
|-------------|------------------------|---------|---------|
| 0.53        | 1,521ab                | 29.0ed  | 7.6bc   |
| 0.50        | 1,589a                 | 25.0e   | 25.3abc |
| 0.48        | 1,485bc                | 45.0cde | 3.6c    |
| 0.45        | 1,492bc                | 47.0cde | 13.5bc  |
| 0.42        | 1,498bc                | 56.0bcd | 14.8bc  |
| 0.40        | 1,419cd                | 67.0bc  | 33.5ab  |
| 0.37        | 1,385d                 | 82.0b   | 25.8abc |
| 0.35        | 1,258e                 | 126.0a  | 43.3a   |
| CV (%)      | 5.44                   | 42.94   | 120.61  |

a-e: Means with the same superscript within a column do not differ significantly (p<0.05).

Figure 1 - Recovery percentage of feed consumption, egg production, egg weight and body weight of hens fed amino acid-deficient diets.
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