Effects of amino acids on egg number and egg mass of brown (heavy breed) and white (light breed) laying hens

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ABSTRACT Different types (light to heavy) of laying hens are used in practice. There are questions about the optimum level of balanced protein (BP) supply in feed for different types of hens. Therefore, a broad range of amino acids intake levels [550 to 800 mg of true fecal digestible (TFD) Lys/hen per d] was tested on heavy (Lohmann Brown Classic) and light (Lohmann LSL Classic) laying hens from 24 to 60 wk of age. The other indispensable amino acids were fed in fixed ratios to TFD Lys in all treatments. A total of 282 Lohmann Brown Classic and 282 Lohmann LSL Classic hens (24 wk of age) were divided into 12 experimental groups (individually housed) based on daily egg mass production and BW. Replicates of the heavy strain started with a similar average daily egg mass production (51.1 g/hen per d), laying percentage (95.9%), and hen weight (1,860 g). Replicates of the light strain started with a similar average daily egg mass production (52.0 g/hen per d), laying percentage (97.3%), and hen weight (1,478 g). Diets were fed restrictively with an aimed feed intake of 110 g/hen per day [308 kcal/hen per d of AMEn (layers)] and 100 g/hen per day [280 kcal/hen per d of AMEn (layers)] for heavy and light hens, respectively, to achieve the required BP intake levels. For light hens, a BP intake with 600 mg of TFD Lys was sufficient for optimal laying percentage, whereas maximum laying percentage was not achieved with the highest TFD Lys in heavy hens. For egg weight, daily egg mass production as well as feed conversion regression analysis revealed that asymptotes were not achieved with the highest amino acid levels in both layer strains.

Key words: laying hen, amino acid, balanced protein, egg production, egg mass

INTRODUCTION

There is very little research that describes the effects of balanced protein (BP) levels on layer performance. Many of the BP requirements were established before the 1990s and were for laying hens that were genetically inferior to the hens used in today’s production systems. More recent references (Parsons et al., 1993; Novak et al., 2004) suggest that the current recommendations for BP intake (NRC, 1994; CVB, 2001) should be increased. Hence, there is a demand for trial data that quantify the daily requirements of BP intake for optimum performance of modern-type laying hens. Literature that is available in that respect describes mainly the influence of single amino acid (AA) levels on the performance of laying hens. For instance, in Antar et al. (2004), 3 different levels of digestible Trp were tested. However, the level of this single essential AA that is either deficient or in excess of the requirement may result in a diet that does not optimize the economic efficiency of a laying hen production system. For that purpose, the whole package of AA or BP should be taken into account as done in trials by other authors (Jais and Kirchgessner, 1995; Coon and Zhang, 1999; Locatelli, 2004; Wijtten et al., 2004).

In literature and often in practice, BP requirements for white (light) hens are assumed to be 10% lower than for brown (heavy) hens (Schutte, 1996). These assumptions are based on rather old literature. For instance, Leeson and Caston (1996) tested 2 different BP levels with fixed ratio between all AA with 4 different strains. The results showed a strain × protein interaction (P < 0.05) for egg mass production, which indicates different requirements per breed. Currently, different BP recommendations for optimum laying percentage and optimum daily egg mass production are used. This requires a better understanding of different BP requirements for laying percentage versus daily egg mass production per strain.

The present study was therefore conducted to test the effects of a broad range of BP levels on egg produc-
tion and egg mass in light (Lohmann LSL Classic) and heavy (Lohmann Brown Classic) laying hens (24 to 60 wk of age).

MATERIALS AND METHODS

Experiment Design

The experiment was arranged in a 2 × 6 factorial. True fecal digestible (TFD) BP intake levels of 550, 600, 650, 700, 750, and 800 mg of TFD Lys/hen per day and fixed ratios [AA profile: Lys (100), Met (50), Met + Cys (93), Thr (66), Trp (19), Ile (79), Val (86); Schutte, 1996] to other AA were anticipated in a heavy strain (Lohmann Brown Classic) and a light strain (Lohmann LSL Classic) laying hen. The heavy hens were fed restrictively at 110 g/hen per day [308 kcal/hen per d of AMEn (layers)] and the light hens were fed restrictively at 100 g/hen per day [280 kcal/hen per d of AMEn (layers)] to ensure the aimed daily intake of BP. Each of the 12 treatments consisted of 47 layers, divided over 4 replicates equally divided over the layer house. Each replicate consisted of 11 or 12 individually housed hens.

Birds and Housing

In total, 600 seventeen-week-old laying hens (300 Lohmann Brown Classic and 300 Lohmann LSL Classic) were purchased from a commercial rearing company. During the rearing period, hens were subjected to a regular vaccination program. At arrival, the birds were randomly divided over 600 double-deck battery cages (individually housed hens, cage size: 0.2 × 0.5 m). In wk 23 (3 d before the start of the experiment), all birds were weighed individually. At the start of the experiment (wk 24), 282 heavy and 282 light selected hens were divided into 12 experimental groups and assigned to the treatment replicates based on daily egg mass production during the 2 previous weeks and BW. Replicates of the heavy strain started with a similar average daily egg mass production (51.1 g/hen per d), laying percentage (95.9%), and hen weight (1,478 g). Treatments were evenly distributed in blocks in the house by deck.

Bulb lights were on for 10 h/d at arrival, which was gradually increased to 16 h/d in wk 23 and remained at 16 h/d until the end of the experiment. Temperature and ventilation were computer-controlled. Temperature was set at 23°C throughout the experiment. During the preexperimental period, the hens had unlimited access to feed and during the experiment, the hens were fed restrictively to ensure aimed BP intake. All hens had unlimited access to drinking water before and during the experiment through 1 or 2 drinking nipples per hen in the back of the cage.

Diets

All experimental diets were prepared in a plant specialized in the production of experimental diets. In advance of diet formulation, batches of maize (Zea mays), wheat, wheat middlings, barley, sunflower meal, and soybean meal were reserved and analyzed for nitrogen (Dumas), crude fat (6-h extraction with petroleum ether), crude fiber content, crude ash (incineration at 550°C for 4 h), DM (103°C for 4 h), calcium (ISO 6490.2), and phosphorous (ISO 6491). In addition, wheat middlings, sunflower meal, and soybean meal were analyzed for AA content (Llames and Fontaine, 1994). Based on the analyses of the raw materials, the experimental diets with the highest and lowest BP levels were formulated based on apparent fecal digestible AA and calculated to TFD with the subscribed endogenous losses by CVB (2001) (Table 1).

Diets were formulated based on BW and expected energy requirement with the calculated feed intake of 110 and 100 g/hen per day for the heavy and light strain, respectively, assuming a growth of approximately 10 g per month. Balanced protein levels were increased by increasing the soybean meal content at the expense of maize while balancing with synthetic AA. According to the Dutch ME_Lay system, diets were kept isocaloric by adjusting the soybean oil and animal fat content (Table 1). The linoleic acid level of the diets was kept constant by varying the animal fat:soybean oil ratio. Potassium levels were kept within an acceptable range by varying the potassium bicarbonate content at the expense of maize. The remaining nutrient levels were achieved with the minerals and microingredients. The diets with the highest and lowest BP levels were produced first. Accordingly, intermediate diets were obtained by diluting the high-BP diet with the low-BP diet. During the whole experimental period, 4 additional batches of diets were produced similarly as described above for the first batch. In the last period (8 wk) of the whole experimental period, BP levels were more concentrated in the feeds because of lower feed intakes (because of high ambient temperature in the summer) of the hens of both breeds in the lowest BP groups. Moreover, this adjustment was also done for the other BP level groups of the heavy strain. With the more concentrated BP in the diets, the changed feed intake secured the required BP intake per experimental group.

Diets were fed in mash form. Diets were calculated to be adequate in all nutrients except protein and AA. All diets were analyzed for nitrogen, crude fat, crude ash, DM, calcium, phosphorous, and AA content, according to the same methods as described for the analyses of the ingredients.

Data Collection

Egg data and hen weights were collected per hen. All hens were individually weighed on the fourth day of every 4-wk period of the experiment. To exclude varia-
tion in stomach-gut filling and consequently biased results of weight gain, weighing was done before the hens were fed. Laying percentage was calculated as number of eggs produced per hen divided by the number of days of the experimental period. Egg mass production per hen per day was calculated as laying percentage multiplied by average egg weight of the hen. Average egg weight was determined per hen once a week. Feed intake was determined per replicate. Feed conversion ratio (FCR) was calculated as grams of feed consumed per gram of egg mass.

Statistical Analysis

Statistical analyses were performed with Genstat (ninth edition, VSN International, Hemel Hempstead, UK) statistical package. The average data per replicate (11 or 12 hens) were treated as the experimental unit. Performance data were subjected to ANOVA per strain using the following statistical linear models:

$$Y_{ijk} = \mu + T_i + L_j + e_{ijk}. $$
where $Y$ = a specific trait per experimental unit (replicate of 11 or 12 hens); $\mu$ = overall mean; $T$ = BP level diet; $L$ = block effect (deck, n = 2); and $e$ = residual error term.

Differences between the 6 BP intake levels per strain were analyzed for significance ($P \leq 0.05$) using the Student-Newman-Keuls test.

Linear and exponential regression analyses were performed on the treatment means per strain for the following variables: laying percentage, egg weight, egg mass production, FCR, and weight gain, with the following models:

**Linear**

$$Y = a + bx,$$

where $Y$ = dependent variable; $a$ = intercept (= performance at lowest TFD BP intake/hen per d per strain; heavy = 541 mg/hen per d; light = 544 mg/hen per d); $b$ = slope; and $x$ = daily realized TFD BP intake (mg/hen per d) and

**Exponential**

$$Y = a + b \left[1 - e^{-c(x - d)}\right]$$

where $a + b$ = asymptote; $c$ = slope; $d$ = lowest realized TFD BP intake (mg/h per d) per strain (heavy = 541 mg/hen per d; light = 544 mg/hen per d).

**Figure 1.** Realized feed intake during the experimental period 24 to 60 wk of hen age for the different treatment groups. TFD = true fecal digestible.
RESULTS AND DISCUSSION

Aimed and Realized Feed Intake

Analyses of the diets were in reasonable accordance with calculated values. Health status of the birds was good and mortality during the experiment was acceptable (12 birds, 2.1%). Although temperature in the room was close to the set temperature (23°C), during the summer, it surpassed this value due to high outside temperatures. The high temperatures resulted in a lower feed intake in that period. To maintain the aimed BP intake, dietary BP levels were adjusted in this period (Figure 1).

Egg and Feed Efficiency

Enhanced TFD BP intake levels significantly ($P < 0.05$) improved laying percentage for the heavy and light strain hens (Table 2 and 3). Laying percentage for the heavy hens was significantly increased between 600 (90.8%) and 650 (93.5%) mg of TFD Lys intake; moreover, laying percentage still seemed to increase from 650 to 700 with no further change from 700 to 850 mg of TFD Lys intake (Table 2). For the light hens, the laying percentage reached a plateau at 600 mg of TFD Lys intake (95.0% of asymptotic response) (Figure 2). For laying percentage, the observed effects suggest different BP requirements for the heavy and light hens. Such a difference between strains regarding the effect of AA intake and balance on laying percentage is supported by literature (Gous and Kleyn, 1988; Kiiskinen and Helander, 1998; Harms and Russel, 1999; Harms et al., 2000).

Egg weight improved significantly ($P < 0.05$) with increased TFD BP intake in both heavy and light hens (Table 2 and 3). Figure 3 suggests that the maximum performance was not achieved because responses on egg weight were linear. Also, daily egg mass production increased significantly ($P < 0.05$) with increasing BP intake, but the responses were of rather nonlinear nature (Figure 4). However, regression analysis (95% of as-

| Table 2. Effect of different daily amino acid intake levels on performance of heavy (Lohmann Brown Classic) layers (wk 24 to 60 of age)$^1$ |
|------------------|------------------|------------------|------------------|------------------|------------------|
|                | I              | II             | III            | IV              | V               | VI               |
| Item            | 550/308$^2$   | 600/308        | 650/308        | 700/308         | 750/308         | 800/308          |
| Laying$^{3,4}$ (%) | 88.6$^b$      | 90.8$^b$        | 93.5$^a$      | 95.2$^a$        | 95.7$^a$        | 95.9$^a$         | 0.68             |
| Egg weight$^{3,4}$ (g)       | 57.5$^b$      | 59.1$^b$        | 60.6$^b$      | 61.7$^b$        | 62.6$^b$        | 63.1$^b$        | 0.42             |
| Egg mass$^{3,4}$ (g/hen per d) | 50.9$^b$      | 53.6$^b$        | 56.0$^b$      | 58.6$^b$        | 59.9$^b$        | 60.5$^b$        | 0.49             |
| Feed intake$^{3,4}$ (g/hen per d) | 108.0$^b$      | 110.0$^b$       | 110.2$^b$     | 110.8$^b$       | 109.5$^b$       | 109.7$^b$       | 0.57             |
| Feed conversion ratio$^{3,4}$ | 2.120$^b$      | 2.051$^b$       | 1.946$^b$     | 1.891$^b$       | 1.830$^b$       | 1.813$^b$       | 0.014            |
| TFD Lys intake$^2$ (mg/hen per d) | 541.2        | 599.7           | 650.5          | 704.8           | 746.7           | 798.4           |                  |
| Hen weight wk 24$^{3,4}$ (kg) | 1.860        | 1.860           | 1.860          | 1.860           | 1.861           | 1.860           |                  |
| Hen weight wk 60$^{3,4}$ (kg) | 1.893        | 1.943           | 2.040          | 2.079           | 2.070           | 2.056           |                  |
| Weight gain wk 24 to 60$^{3,4}$ (g) | 33$^a$         | 83$^b$           | 180$^b$        | 219$^b$         | 210$^b$         | 196$^b$         | 30                |

$^*$Values in the same row with no common superscripts are significantly different ($P \leq 0.05$).

1n = 4.

$^2$True fecal digestible (TFD) Lys intake (mg/hen per d)/AME$^c$ intake (kcal/hen per d). Both values are aimed intakes.

$^3$Means of 47 birds per treatment.

$^4$Degrees of freedom: 23 for all treatments.

Table 3. Effect of different daily amino acid intake levels on performance of light (Lohmann LSL Classic) layers (wk 24 to 60 of age)$^1$

<table>
<thead>
<tr>
<th>Item</th>
<th>VII</th>
<th>VIII</th>
<th>IX</th>
<th>X</th>
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<th>XII</th>
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<td>Laying$^{3,4}$ (%)</td>
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<td>56.8$^b$</td>
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<td>53.9$^b$</td>
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<td>Feed intake$^{3,4}$ (g/hen per d)</td>
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<td>100.7</td>
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<td>1.872$^b$</td>
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<td>1.773$^c$</td>
<td>1.717$^d$</td>
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<td>TFD Lys intake$^2$ (mg/hen per d)</td>
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<td>654.0</td>
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<td>Hen weight wk 24$^{3,4}$ (kg)</td>
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<td>Hen weight wk 60$^{3,4}$ (kg)</td>
<td>1.426</td>
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<td>1.556</td>
<td>1.610</td>
<td>1.604</td>
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<td>Weight gain wk 24 to 60$^{3,4}$ (g)</td>
<td>−52$^b$</td>
<td>76$^a$</td>
<td>77$^a$</td>
<td>132$^a$</td>
<td>127$^a$</td>
<td>164$^a$</td>
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$^a$–$d$Values in the same row with no common superscripts are significantly different ($P \leq 0.05$).

1n = 4.

$^2$True fecal digestible (TFD) Lys intake (mg/hen per d)/AME$^c$ intake (kcal/hen per d). Both values are aimed intakes.

$^3$Means of 47 birds per treatment.

$^4$Degrees of freedom: 23 for all treatments.
ymptotic response) suggests that particularly the heavy hens but also the light hens did not achieve maximum performance with the highest daily BP intake (Table 4). Feed conversion ratio was improved by increasing dietary BP levels ($P < 0.05$). The shape of the curves indicated that the maximum effect on FCR would be reached at a lower dietary BP intake for light hens compared with heavy hens. However, also for the light hens, minimum FCR was not achieved with the highest tested BP intake (95% of asymptotic response). Overall, the experimental data indicate that optimal TFD BP intake for maximum egg weight and egg mass performance is above 800 mg of Lys/hen per day. These observations are supported by earlier research (Prochaska et al., 1996; Scheideler et al., 1996; Novak et al., 2004; Eits et al., 2005; Halle et al., 2005). Increases in egg production were even observed at TFD Lys levels as high as 1,000 mg of Lys intake per hen per day. However, this was done with titration of only Lys. A more comparable observation with current research was done by Balnave et al. (2000), who observed increased egg weights with increased protein levels in the diets. This also underlines the importance of balance of all AA in laying hen diets.

From the BP levels, 550 to 800 mg of TFD Lys intake (heavy) and 550 to 750 mg of TFD Lys intake (light) each step of increased TFD Lys intake of 50 mg improved FCR (Figure 5). This indicates that for both strains feed efficiency can be optimized with TFD BP intake above 800 mg of Lys/hen per day.

Daily egg mass production and FCR slightly indicate higher optima for the heavy hens compared with the light hens. However, for egg weight, no maximum response was found.

Figure 2. Effects of daily balanced protein intake on laying percentage for heavy (♦) and light (□) hens (wk 24 to 60 of age). TFD = true fecal digestible.

Figure 3. Effects of daily balanced protein intake on egg weight for heavy (♦) and light (□) hens (wk 24 to 60 of age). TFD = true fecal digestible.

Figure 4. Effect of daily balanced protein intake on egg mass production for heavy (♦) and light (□) hens (wk 24 to 60 of age). TFD = true fecal digestible.

Figure 5. Effect of daily balanced protein intake on feed conversion ratio for heavy (♦) and light (□) hens (wk 24 to 60 of age). TFD = true fecal digestible.
During the start of the experiment, hens were allocated with similar average start weights per treatment (heavy hens = 1,860 g; light hens = 1,478 g). After the experimental period of 12 wk (36 wk of age), most of the treatment groups gained weight (heavy hens = 82 g; light hens = 40 g) (Tables 2 and 3). The aim was to allow a minimum weight gain of 10 g/hen per month. Weight gain was thus expected, because the hens were still in the development stage of life. At the end of the experimental period of 36 wk (60 wk of age), the heavy hens had all gained weight on average per treatment. This was also the case for the light hens, except for the lowest TFD BP intake level treatment (550 mg of Lys/hen per day, treatment VII), which showed a decreased weight (52 g) (Figure 6). At all BP levels, weight gain was lower for the light hens than for the heavy hens. This demonstrates that energy intake of the light hens was closer to the requirement (less energy surplus) than for the heavy hens. This in combination with a decrease in feed intake (Figure 1) and the low AA intake must have caused the weight loss of the lowest TFD BP intake level treatment (550 mg of Lys/hen per d, treatment VII) of the light hens. Hen weight increased in a nonlinear pattern with increased TFD BP intake for both strains (Figure 6). This increase showed a maximum for the heavy hens at 700 mg of TFD Lys intake; for the light hens, the maximum was less consistent. Both strains showed decreased weights for the groups fed 550 mg of TFD Lys intake and 600 mg of TFD Lys intake of the heavy hens over the entire period (Figure 6). In combination with the lower feed intake after 45 wk for these groups (Figure 1), this indicates that this level of AA intake is too low and risky for maximum protein accretion and therefore optimal egg production of the hen.

### Conclusions

The present experiment showed that for light hens, the maximum laying percentage was reached at a BP level with 600 mg of TFD Lys/hen per day and for heavy hens, the maximum level was not reached, but seemed to be close to the maximum tested level (800 mg of TFD Lys/hen per d). For the light hens, an intake of 600 mg of TFD Lys/hen per day was sufficient to maximize laying percentage. However, the observed laying percentage and the decrease in weight within the lower TFD BP intake treatments showed that this level is risky. Performance can drop considerably when circumstances (i.e., feed intake) change. Egg weight, daily egg mass production, and FCR improved up to the highest tested TFD BP intake with 800 mg of Lys/hen per day. Results of regression analyses per performance factor for light (Lohmann LSL Classic) and heavy (Lohmann Brown Classic) layers (wk 24 to 60 of age)

#### Table 4

<table>
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<tr>
<th>Item</th>
<th>Type of regression</th>
<th>Model(^1)</th>
<th>(R^2)</th>
<th>(P)-value</th>
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<td>Laying (%)</td>
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<td>Heavy</td>
<td>Exponential</td>
<td>(Y = 24.0 + 209 \left[1 - e^{-0.01(x - 541)}\right])</td>
<td>0.85</td>
<td>0.027</td>
<td>29.7</td>
</tr>
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\(^1\)Linear: \(Y = a + bx\), where \(Y = \) dependent variable; \(a = \) intercept \(=\) performance at lowest true fecal digestible (TFD) balanced protein (BP) intake per hen per d per strain; heavy = 541 mg/hen per d; light = 544 mg/hen per d; \(b = \) slope; \(x = \) daily realized TFD BP intake (mg/hen per d).

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<td>29.7</td>
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Figure 6. Effect of daily balanced protein intake on weight gain for heavy (♦) and light (□) hens (wk 24 to 60 of age). TFD = true fecal digestible.

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\(Y = a + bx\), where \(Y = \) dependent variable; \(a = \) intercept \(=\) performance at lowest true fecal digestible (TFD) balanced protein (BP) intake per hen per d per strain; heavy = 541 mg/hen per d; light = 544 mg/hen per d; \(b = \) slope; \(x = \) daily realized TFD BP intake (mg/hen per d).

Exponential: \(Y = a + b\left[1 - e^{-c(x - d)}\right]\), where \(a + b = \) asymptote; \(c = \) slope; \(d = \) lowest realized TFD BP intake (mg/h per d) per strain (heavy = 541 mg/hen per d; light = 544 mg/hen per d); \(x = \) daily realized TFD BP intake (mg/hen per d).
hen per day for both strains, suggesting that the optimal BP intake was higher. The slopes of the curves might indicate that for those measurement factors, the required TFD BP level for maximum performance is lower for light than for heavy hens.

This research shows differences in BP requirements between heavy and light strain hens; whether similar differences can be found between other light and heavy strains should be determined in the future.

ACKNOWLEDGMENTS

We gratefully acknowledge the staff of the Provimi Research Centre (Velddriel, the Netherlands) for the accuracy and dedication to their work during the course of the experiment.

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