# Estimating requirements for apparent faecal and standardised ileal digestible amino acids in laying hens by a meta-analysis approach

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LIVESTOCK RESEARCH
WAGENINGEN UR

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Dit rapport geeft een update van de behoefte aan aminozuren (lysine, methionine+cysteine, threonine en tryptofaan) van leghennen, zowel op basis van schijnbaar fecaal verteerbare als gestandaardiseerd ileaal verteerbare aminozuurgehalten van het voer. Hiervoor is een meta-analyse uitgevoerd op peer reviewed dosis-response studies. Studies moesten voldoen aan een vooraf bepaalde set van criteria, voordat ze bij deze analyse betrokken werden.



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# Foreword

Nutrient composition, e.g. concentrations of energy, protein, vitamins and minerals, largely influence the zootechnical performance of laying hens. Nowadays, protein requirements are described in terms of apparent faecal digestible (AFD) or standardised ileal digestible (SID) amino acid requirements, and in amino acid profiles expressing the requirement of each amino acid relative to lysine. Appropriate requirements for amino acids (AA) in poultry diets are essential for optimizing laying hen production and profit of the poultry chain.
CVB, part of the Dutch Product Board Animal Feed (PDV), is responsible for recommending the Dutch poultry chain on AA requirements for various poultry species. The latest review of AA requirements in poultry in the Netherlands was presented almost two decades ago (Schutte, 1996). As a consequence of several recent developments, such as change in genetic predisposition for egg production traits, the increasing trend of formulating low-protein diets and the increasing availability of free AA for diet supplementation, it was recommended that requirement values for AA in laying hens should be updated. The present study was subsidized by the Product Board Animal Feed and the Product Board Poultry and Eggs. The CVB project group 'Standardized Ileal Digestible Amino Acids Poultry', consisting of scientists and representatives of the feed industry, guided this study. We would like to thank the members of this CVB project group for their valuable input to all phases of this project.

Marinus van Krimpen
Project leader WLR

# Samenvatting

In de wetenschappelijke literatuur worden de gehalten van essentiële aminozuren in voeders voor leghennen vaak gebaseerd op de aanbevelingen van de NRC (1994). Deze aanbevelingen zijn echter uitgedrukt op basis van het totale aminozuurgehalte van het voer, terwijl de voerindustrie de voorkeur geeft aan aanbevelingen, die uitgedrukt zijn op basis van schijnbaar fecaal verteerbare of gestandaardiseerd ileaal verteerbare aminozuurgehalten.
Dit rapport geeft een update van de behoefte aan aminozuren (lysine, methionine+cysteine, threonine en tryptofaan) van leghennen, zowel op basis van schijnbaar fecaal verteerbare als gestandaardiseerd ileaal verteerbare aminozuurgehalten van het voer. Hiervoor is een meta-analyse uitgevoerd op peer reviewed dosis-response studies. Studies moesten voldoen aan een vooraf bepaalde set van criteria, voordat ze bij deze analyse betrokken werden.
De nutriëntgehalten van de voeders zijn herberekend op basis van de CVB tabel (2011). Voor het modelleren van de respons van aminozuuropname op de dierprestaties is gebruik gemaakt van de Wood curve. Responscurven zijn berekend voor legpercentage, eigewicht, eimassa en voederconversie. Er zijn aminozuurbehoeften afgeleid voor het realiseren van de maximale respons op legpercentage, eimassa en voederconversie, maar ook voor 95% van deze maximale respons. De op te nemen hoeveelheid aminozuren voor het bereiken van deze responsniveaus, evenals de verhouding van de aminozuren ten opzichte van lysine, op basis van zowel de schijnbaar fecaal verteerbare als gestandaardiseerd ileaal verteerbare aminozuurgehalten zijn weergegeven in de vier tabellen hieronder.

Opnameniveaus van **schijnbaar fecaal verteerbare (AFD) aminozuren** (mg/d) voor het realiseren van 95% van de maximale respons op legpercentage, eimassa en voederconversie, alsmede de bijbehorende aminozuurratio's relatief t.o.v. Lysine

| Basis | AFD behoefte (g/d) | | | AFD behoefte (relatief t.o.v. Lys) | | |
|---|---|---|---|---|---|---|
| Parameter | Leg-% | Eimassa (g/d) | VC | Leg-% | Eimassa (g/d) | VC |
| Lysine | 810 | 820 | 825 | 100 | 100 | 100 |
| Met+Cys | 650 | 670 | 675 | 80 | 82 | 82 |
| Threonine | 485 | 500 | 575 | 60 | 61 | 70 |
| Tryptofaan | 158 | 158 | 186 | 20 | 19 | 23 |

Opnameniveaus van **gestandaardiseerd ileaal verteerbare (SID) aminozuren** (mg/d) voor het realiseren van 95% van de maximale respons op legpercentage, eimassa en voederconversie, alsmede de bijbehorende aminozuurratio's relatief t.o.v. Lysine

| Basis | SID behoefte (g/d) | | | SID behoefte (relatief t.o.v. Lys) | | |
|---|---|---|---|---|---|---|
| Parameter | Leg-% | Eimassa (g/d) | VC | Leg-% | Eimassa (g/d) | VC |
| Lysine | 850 | 855 | 855 | 100 | 100 | 100 |
| Met+Cys | 650 | 670 | 675 | 76 | 78 | 79 |
| Threonine | 520 | 530 | 590 | 61 | 62 | 69 |
| Tryptofaan | 155 | 156 | 180 | 18 | 18 | 21 |

# Summary

NRC requirements (1994) are frequently used in literature as a public reference for limiting amino acid (AA) requirements of laying hens. These values, however, are expressed on the basis of total AA intake, whereas the feed industry prefers to work with requirements expressed on an apparent faecal (AFD) or standardised ileal (SID) digestible basis. Therefore, values for AA requirements (LYS, MET+CYS, THR and TRP) of laying hens were updated, both on a AFD and SID basis, by performing a meta-analysis on peer reviewed dose-response studies. Studies had to meet a set of criteria before including them into evaluation. Nutrient composition of diets were recalculated by using table values of CVB (2011). Responses were fitted by use of the Wood equation, resulting in curves that predicted rate of lay, egg weight, egg mass and FCR in dependence of AA intake. The derived AA requirements are provided for realizing the maximal response on rate of lay, egg mass and FCR, as well as for realizing 95% of these maximal responses. These AA requirements, and the ratio of the limiting amino acids to Lys on AFD and SID basis are summarized in the four tables below.

The derived AA requirements for realizing the maximal response on rate of lay, egg mass and FCR, as well as for realizing 95% of these maximal responses, and the ratio of the limiting amino acids to Lys on AFD and SID basis are summarized in the four tables below.

**Apparent Faecal Digestible** (AFD) amino acid intake levels (mg/d) for realizing 95% of maximal response to rate of lay, egg mass and FCR, as well as the amino acid ratios relative to Lys

| Basis | AFD requirement (g/d) | | | AFD requirement (relative to Lys) | | |
|---|---|---|---|---|---|---|
| Parameter | Rate of lay (%) | Egg mass (g/d) | FCR | Rate of lay (%) | Egg mass (g/d) | FCR |
| Lysine | 810 | 820 | 825 | 100 | 100 | 100 |
| Met+Cys | 650 | 670 | 675 | 80 | 82 | 82 |
| Threonine | 485 | 500 | 575 | 60 | 61 | 70 |
| Tryptophan | 158 | 158 | 186 | 20 | 19 | 23 |

**Standardized Ileal Digestible** (SID) amino acid intake levels (mg/d) for realizing 95% of maximal response to rate of lay, egg mass and FCR, as well as the amino acid ratios relative to Lys

| Basis | SID requirement (g/d) | | | SID requirement (relative to Lys) | | |
|---|---|---|---|---|---|---|
| Parameter | Rate of lay (%) | Egg mass (g/d) | FCR | Rate of lay (%) | Egg mass (g/d) | FCR |
| Lysine | 850 | 855 | 855 | 100 | 100 | 100 |
| Met+Cys | 650 | 670 | 675 | 76 | 78 | 79 |
| Threonine | 520 | 530 | 590 | 61 | 62 | 69 |
| Tryptophan | 155 | 156 | 180 | 18 | 18 | 21 |

# 1 Introduction

NRC requirements are frequently used in literature as a public reference for the amino acid requirements of laying hens (NRC, 1994). The requirement values are expressed on the basis of total amino acid intake, whereas the feed industry has been used to working with requirements expressed on an apparent faecal digestible basis (AFD). Further, the NRC requirements may underestimate the requirements of laying hens, because these requirements are based on the requirements for maximal rate of lay, whereas the amino acid requirements might increase if they are based on minimizing FCR (Schutte, 1996).
The most recent public amino acid requirements for laying hens in The Netherlands dated from 1996 (Schutte, 1996). These requirements are expressed on an AFD basis. In that study the requirements were derived as follows. First, the original data were transformed from absolute to relative responses, where e.g. the response of Met+Cys intake on egg mass ranged from -6 to 0, and the response on FCR from 0.35 to 0. By this approach, random effects between studies were neglected, thereby assuming that all data points originated from one experiment. Secondly, the relative responses of the hens to increased amino acid levels in the feed was fitted by use of an overall exponential model. Thirdly, the amino acid level at which 95% of the maximum response (in case of maximal egg mass), or of the minimum response (in case of FCR) was calculated and used as the basis for the requirements.
Due to an on-going progress in understanding of genetics, laying hens still respond to selection by increasing their performance levels (Tixier-Boichard et al., 2012). Therefore, it is questioned whether the current amino acid requirements meet the requirements of modern types of laying hens.
Further, it is suggested that the basis of expressing amino acid digestibility of dietary ingredients can be improved by using a system of standardised ileal amino acid digestibility (SID). First, from a physiological perspective an ileal system is superior to a faecal system, because there is no quantitative significant post-ileal absorption of amino acids and peptides. Secondly, a system based on standardized amino acid digestibility corrects for basal endogenous amino acids in ileal digesta (Lemme et al., 2004a; Adedokun et al., 2008). So, amino acid contents of ingredients expressed on a SID basis better represent the amino acid value of an ingredient for the bird compared to amino acid contents on a AFD basis, and the use of this system may reduce the amino acid flow into the environment via the bird's excreta (Lemme et al., 2004a). However, when replacing the basis of expressing amino acid digestibility from AFD to SID, the amino acid requirements of laying hens have to expressed on the same basis.

The aim of the present study is to update the requirements for the essential amino acids of laying hens, both on a AFD and SID basis, by performing a meta-analysis on dose-response studies used to derive requirement values for essential amino acids (lysine, methionine+cysteine, threonine and tryptophan) in laying hens as presented in the literature. In this meta-analysis, the data are fitted by use of the Wood equation (see paragraph 2.4). The amino acid intake levels for realizing maximal rate of lay, egg mass and feed efficiency are provided. The amino acid requirements for use in practice are based on the amino acid intake levels at which 95% of these maximum responses were reached.

# 2 Materials and methods

## 2.1 Meta-analysis

A meta-analysis, which combines the results from various experiments at the same time, has more power to detect small differences. For estimating amino acid requirements by use of a meta-analysis approach, formulating criteria for inclusion or exclusion of studies is very important. The main requirement for a proper meta-analysis is a well-executed systematic review. Therefore in the current work, key journals were hand searched and reference lists of papers were checked carefully.

## 2.2 Database

Peer reviewed publications, describing experimental results of feeding diets containing graded levels of amino acids on performance of laying hens were collected. The papers were searched by using the key words 'laying hen' and 'amino acid name' (lysine, methionine, threonine, tryptophan) in the electronic database 'Web of Science'. The papers should be published during the period 1994 - 2013. Relevant search results were found in the journals Poultry Science, Journal of Applied Poultry Research, Revista Brasileira de Zootecnia, Animal and Veterinary Advances, Tecnica pecuaria Meexico, and Arq. Bras. Med. Vet. Zootec.

## 2.3 Criteria for including papers into the database

The studies were reviewed to meet the following criteria:

1) Within the same experiment, feed intake levels should not significantly differ between treatments.
2) Experimental diets should be adequately described in terms of ingredient composition and analysed or calculated contents of at least crude protein and amino acids.
3) Information on the birds used (strain, age) should be provided.
4) Only dose response studies were included, in which besides a basal level at least three levels of supplementation of the amino acid of interest to the same basal diet were tested.
5) Besides the test amino acid, concentration of other amino acids (lysine, methionine, threonine, tryptophan, valine and isoleucine) should be at least 90% of the CVB (2012) requirements. Preferably, levels of non-test essential amino acids should be maintained at a constant non-limiting level of at least 90% of the CVB (2012) requirement among all supplementation levels of the amino acid under study. Studies that gradually increase the level of the non-test amino acids (by adding them in free form) with increasing levels of the test amino acid were also accepted, under the condition that the levels of the other essential amino acids were not limiting (e.g. at least 90% of CVB (2012) requirements) at concentrations of the test amino acid close to the estimated requirement value in that particular study.
6) Only dose response studies were included in which the maximum dose of the test amino acid was at least 10% higher compared to the concentration of the basal diet.
7) Information on basis for expressing amino acid digestibility (total, faecal – ileal, apparent – standardized) should be provided.
8) Performance data (feed intake, rate of lay, egg weight, egg mass, FCR) of laying hens should be provided.
9) The experimental procedure should be adequately provided, meaning a clear description of the experimental units, the number of birds per unit and the duration of the trial.
10) Increasing the amino acid supply should have significant responses to rate of lay, egg weight, egg mass or FCR.

Studies that not met the criteria were excluded from further evaluation. Information of the studies that did meet the criteria was included in an electronic database. Besides the information mentioned above, additional information from the study (if available), comprising the energy content of the diets, including the basis of the value, the calculated and analysed amino acid contents of the diets and its basis (total, apparent or standardized digestible), were added to the database. The recommended requirement values of the authors were included in the database as well.

In all the selected studies regarding methionine + cysteine, threonine and tryptophan, the level of the non-test amino acids were maintained at a constant non-limiting level. As shown in Appendix 8, in 4 out of the 6 selected lysine studies, the AFD methionine + cysteine content proportionally increased with the lysine content, with the aim of maintaining the AFD methionine + cysteine to lysine ratio at a constant level (88 – 90%). The threonine and tryptophan contents in these studies, however, were kept constantly for all lysine dosages. Despite the deviation with respect to the other studies, these 4 lysine studies met criterion 5. Therefore, these lysine studies were accepted for further evaluation.

## 2.4 Recalculation of dietary energy, amino acid and mineral contents

In order to make it possible to compare nutrient composition of the different studies on a similar nutritional basis, energy, amino acid and mineral contents of the diets were recalculated according to the values of the CVB Feeding Table (2011). For some protein sources, e.g. rapeseed meal and soybean meal, the database contains several varieties that differ in protein content. The differences between the published and recalculated crude protein and amino acid contents of the basal diet were minimized by choosing the most appropriate variety of the relevant protein sources.
Subsequently, the contents of apparent faecal digestible (AFD) amino acids of the feeds were calculated, using the AFD coefficients of the individual ingredients as tabulated in the CVB Feed Table (2011). However, for lysine in maize an AFD digestibility coefficient of 77% was used instead of the tabulated value of 61%.
Additionally, standardized ileal digestible (SID) amino acid contents of the basal diets were calculated, based on the amino acid digestibility coefficients of feed ingredients, as published by Dekker and Blok (2012). Recalculated amino acid contents of the diets on i) a total, ii) apparent faecal and iii) standardized ileal basis were included in the database.
All further calculations were based on the recalculated dietary amino acid contents.

## 2.5 Statistical analysis

In poultry, exponential curves were often used to fit the responses to increasing amino acid supply (Schutte, 1996; Sauer et al., 2008). In laying hen studies, however, the responses of the hens to increasing amino acid intake regularly shows an exponential course to a maximum value, followed by a decreasing trend at amino acid intake levels above the level for realizing maximal performance (Peganova et al., 2003; Novak et al., 2004; Filho et al., 2006b; Schmidt et al., 2010). This response pattern corresponds to that of lactation curves of mammals. Wood (1967) developed a model to simulate lactation curves, which is regularly used to modulate milk performance data of dairy cows, horses, sheep and goats (Golebiewski et al., 2011; Centoducati et al., 2012). Because the Wood's model was able to fit the available laying hen data more accurately than an exponential curve, this model was chosen for statistical analysis.

The general Wood equation to model the responses of daily AFD and SID amino acid intake (mg/d) on hen performance within a study is $Y = a.x^{b}.e^{-cx}$ , which can be rewritten as:

$$Y = \beta_0 + \beta_1.Ln(X) + \beta_2.(X) \qquad \text{(equation 1)}$$

whereas Y= the performance parameter, $\beta_0$= the initial level of performance, $\beta_1$= the rate of increase of the performance level. $\beta_2$= the rate of decline, and X= daily AFD or SID amino acid intake (mg/d). The first part of this equation ($\beta_0 + \beta_1.Ln(X)$) contains an natural logarithmic component, resulting in an exponential course of the performance parameter to an asymptotic level as a function of amino acid intake levels. $\beta_2$ is a constant linear negative component that inhibits the response on amino acid intake levels. The combined effects of these parameters in de Wood equation results, in case of e.g. egg mass, in an increased egg mass with increasing amino acid intake levels until the level of maximal response on egg mass is reached. After that point, egg mass showed a decreasing course if the amino acid intake level is further increased.

In case of the responses of Lys intake (mg/d) on hen performance, the following modified equation was used:

$$Y = (\beta_0 + \varepsilon_{\beta o}) + (\beta_1 + \varepsilon_{\beta 1}) + Ln(X/700) + \beta_2.(X-700) + \varepsilon_{residual} \qquad \text{(equation 2).}$$

In equation 2, such a level adjustment was applied that a daily intake level of 700 mg Lys corresponds to the value of $\beta_0$, because at the level of X = 700, $\beta_1$ and $\beta_2$ both are multiplied with 0. Nearly all Lys studies covered an Lys intake level of 700 mg/d, and therefore the factor '700' in equation 2 was added in order to ensure that the value of $\beta_0$ received practical relevance. Inclusion of this factor did not affect the final outcome of the model. Likewise, for Met+Cys, Thr, and Trp the factors '600', '500', and '150', respectively, were used.

The level of X for realizing the maximum production was calculated as: $-\beta1/\beta2$ (equation 3).

A random regression REML procedure in Genstat (2002) was used to estimate the curve parameters ($\beta_0$, $\beta_1$, and $\beta_2$) per experiment and the parameters of the overall response models related to rate of lay, egg weight, egg mass and FCR. The curve parameters of the individual experiment ($\varepsilon_{\beta o}$ and $\varepsilon_{\beta 1}$) and of the overall model were estimated in one procedure, together with the estimation of Covariance ($\varepsilon_{\beta o}$,$\varepsilon_{\beta 1}$). In the results section, the curve parameters of both the individual experiments and of the overall model are presented. Therefore, the random regression procedure could not be used for estimating the curve parameters of these amino acids. Within the set of studies of an individual amino acids, the parameter $\beta_2$ was estimated as one standard value.
Moreover, the daily required amino acid intake levels for realizing maximal rate of lay, egg weight and egg mass, as well as for minimal FCR are provided.
Besides the levels for maximal response, also the amino acid intake levels required for achieving 95% of the response between starting level and maximal responses is calculated. The starting level was defined as the average amino acid intake levels at the lowest doses of the selected experiments.
CV (Coefficient of Variation) is a measure of the goodness of fit and indicates the relative deviation between the treatment average and the model fit.

$$CV = \sqrt{[\text{Variance residual}]} / [\text{mean}] *100) \qquad \text{(equation 4).}$$

## 2.6 Effect of breed and age

The studies that met the criteria were conducted with various breeds of laying hens and at different ages. Because only a limited number of studies met the criteria, the number of selected papers per amino acid were rather low. Therefore, it was not possible to estimate the effects for breed and age in our statistical model.

# 3 Results

## 3.1 Response to Lysine

In total, 19 references comprising 27 Lys dose response trials, were judged. Table 1 provides an overview of the 6 trials that met the criteria for inclusion in the meta-analysis. The number of Lys supplementation levels per experiment ranged from 5 to 7. The AFD Lys contents of the diets ranged from 5.4 to 8.7 g/kg, and the maximal L-Lys inclusion level from 2.0 to 2.8 g/kg. CP contents in the basal diets varied from 14.3 to 17.1%. ME levels used in these studies were almost similar and ranged from 11.7 – 11.9 MJ/kg. Two experiments used white feathered birds, three experiments used brown feathered birds, whereas in one flock white and brown hens were mixed. In most trials, the diets were applied to hens during the starting or the middle phase (24 – 50 wk of age) of the laying period, while in two trials the diets were applied to hens at the end of the laying period (79 – 95 wk of age). Appendix 1 provides an overview of papers that did not meet the inclusion criteria and were not further considered.

Table 1
*List of references used for the meta-analysis and some characteristics of the trials*

| Trial | Reference | Nr. of doses | Range of AFD Lys content (g/kg) | CP[1] | ME[1] | Strain[2] | Age[3] | Average rate of lay (%) | Average egg weight (g) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Filho et al. (2006b) | 7 | 5.7 – 8.7 | 17.1 | 11.7 | 2 | 30-46 | 89.1 | 65.2 |
| 2 | Sa et al. (2007c) exp. 1 | 5 | 5.7 – 7.7 | 15.0 | 11.9 | 4,5 | 34-50 | 90.7 | 60.9 |
| 3 | Sa et al. (2007c) exp. 2 | 5 | 5.7 – 7.7 | 15.0 | 11.9 | 5 | 34-50 | 89.3 | 62.7 |
| 4 | Schmidt et al. (2008) | 5 | 5.4 – 8.0 | 14.3 | 11.7 | 4 | 79-95 | 79.9 | 65.9 |
| 5 | Schmidt et al. (2009b) | 5 | 5.4 – 8.0 | 14.3 | 11.7 | 5 | 79-95 | 76.8 | 66.1 |
| 6 | Schutte and Smink (1998) | 7 | 5.6 – 8.4 | 16.4 | 11.7 | 4 | 24-36 | 96.8 | 58.4 |

[1] Crude protein is in %, whereas ME is in units of megajoules per kg of feed.
[2] Strains: 2 = Hysex Brown, 4 = Lohmann LSL, 5 = Lohmann Brown.
[3] Age is in wk.

In Figure 1, the determined egg mass (g/d) and FCR of the in Table 1 selected studies were plotted as a function of the daily AFD Lys intake. In these studies, egg mass ranged from 45 to 59 g/d, and FCR from 2.4 to 1.8.

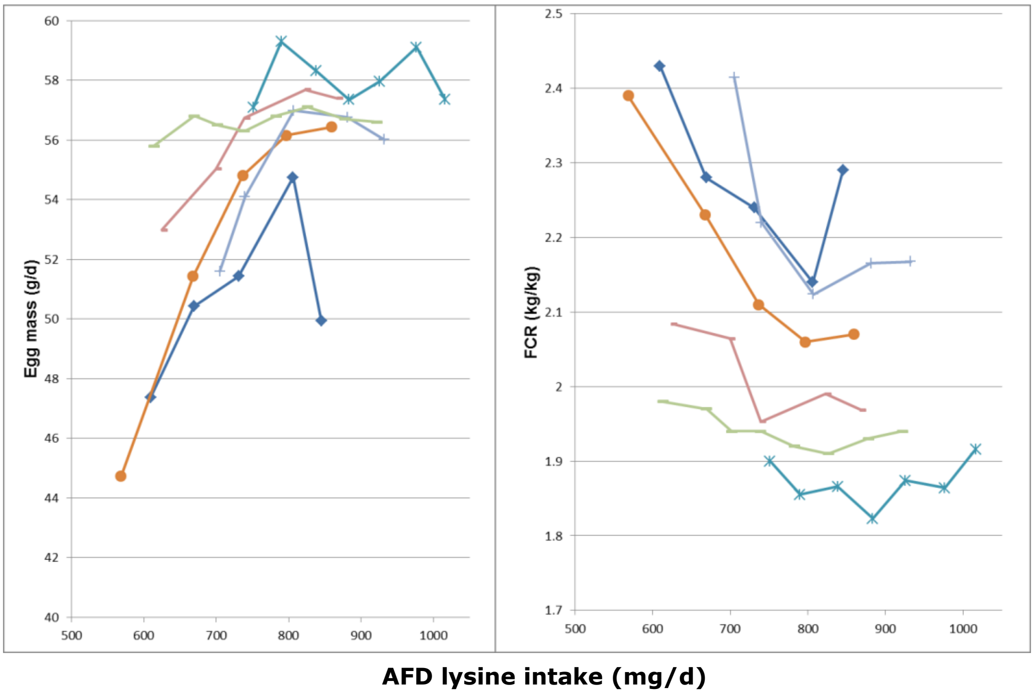



***Figure 1*** *The determined responses of egg mass (g/d) and FCR as a function of the AFD Lys intake of the in Table 1 selected studies.*

The coefficient of variation (CV), the estimates of the parameters $\beta_1$, $\beta_2$, and the AFD Lys intake level (mg/d) for maximal response on egg mass and FCR per individual study are shown in Table 2.

## Table 2

*Coefficient of variation, estimates of the parameters $\beta_1$, $\beta_2$, and the AFD Lys intake level (mg/d) for maximal response on egg mass and FCR per individual study.*

| | | Egg mass | | | | FCR | | | |
|---|---|---|---|---|---|---|---|---|---|
| Trial | Reference | $\beta_1$ | $\beta_2$[1] | Lys intake for max. response | CV (%)[2] | $\beta_1$ | $\beta_2$[1] | Lys intake for max. response | CV (%)[2] |
| 1 | Filho et al. (2006b) | 104.0 | -0.118 | 881 | 0.7 | -2.91 | 0.0035 | 842 | 2.4 |
| 2 | Sa et al. (2007b) Exp. 1 | 110.9 | -0.118 | 940 | 1.9 | -3.42 | 0.0035 | 990 | 3.3 |
| 3 | Sa et al. (2007c) Exp. 2 | 103.3 | -0.118 | 875 | 0.6 | -2.99 | 0.0035 | 867 | 1.6 |
| 4 | Schmidt et al. (2008) | 110.8 | -0.118 | 939 | 1.1 | -3.20 | 0.0035 | 927 | 1.1 |
| 5 | Schmidt et al. (2009b) | 100.6 | -0.118 | 852 | 3.5 | -3.23 | 0.0035 | 935 | 2.9 |
| 6 | Schutte and Smink (1998) | 92.6 | -0.118 | 785 | 0.9 | -2.83 | 0.0035 | 819 | 0.5 |

[1] The estimation of $\beta_2$ differed not significantly between studies and therefore estimated as one standard value for all studies.
[2] CV (Coefficient of Variation) is a measure of the goodness of fit and indicates the relative deviation between the treatment average and the model fit (CV = √[Variance residual] / [mean] *100).

For egg mass, the model fit shows a low CV (<2%) for 5 out of the 6 studies, with an exception for the experiment of Schmidt et al. (2009b) (3.5%). For FCR, the model fit shows a low CV (<2%) for 3 out of the 6 studies, with exceptions for the experiment of Filho et al. (2006b) (2.4%), the first experiment of Sa et al. (2007b) (3.3%), and the experiment of Schmidt et al. (2009b) (2.9%). The predicted daily AFD Lys intake for achieving maximal egg mass production ranged from 785 to 940 mg/d. The predicted daily AFD Lys intake for achieving most efficient feed conversion ranged from 819 to 990 mg/d. In par. 4.2, the AFD Lys intake levels as recommended by the authors themselves, are provided. The effect of the chosen model, as well as the basis of requirement (95% or 100% of maximal response) are discussed in par. 4.2 as well.

The overall fitted response curve, as determined over all selected studies, of daily AFD and SID Lysine intake to the predicted egg mass and FCR are shown in Figure 2 and 3, respectively. The lysine intake level for achieving maximal egg mass and FCR was covered by the tested lysine intake range in 3 out of the 6 experiments.

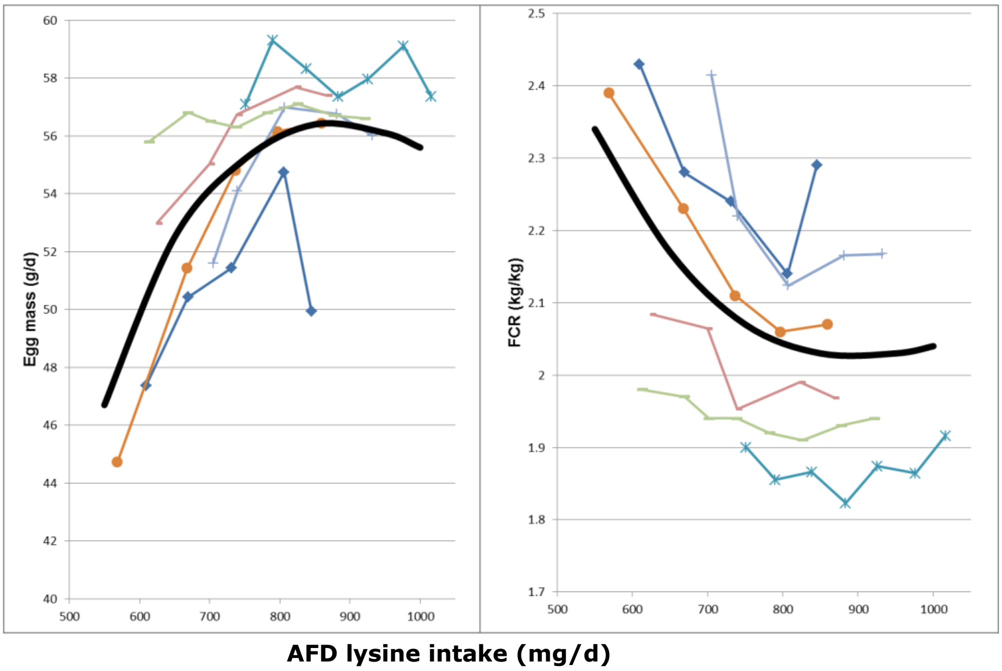



***Figure 2*** *The determined responses of egg mass (g/d) and FCR as a function of the AFD Lys intake of the in Table 1 selected studies, as well as the fitted response (thick solid line).*

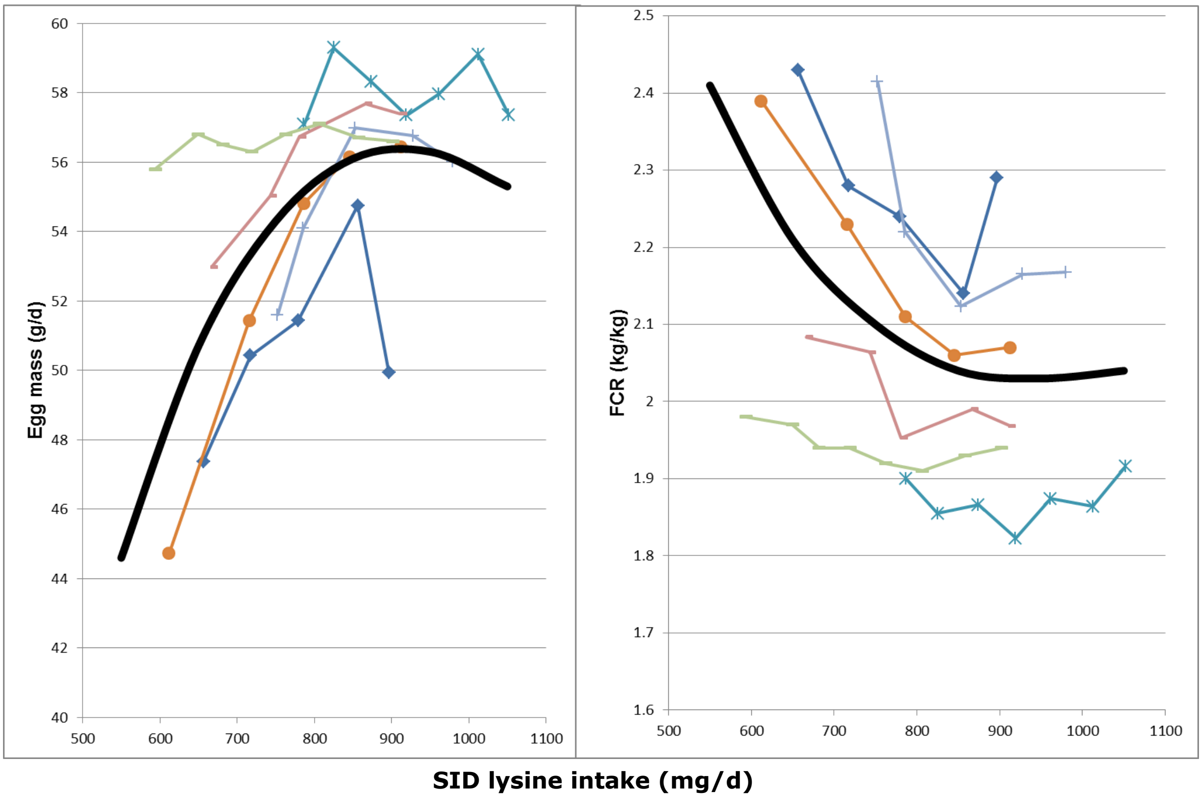



***Figure 3*** *The determined responses of egg mass (g/d) and FCR as a function of the SID Lys intake of the in Table 1 selected studies, as well as the fitted response (thick solid line).*

The estimates of the parameters β0, β1, and β2, as well as their standard error values, for the equations that predict the overall response of AFD or SID Lys intake on rate of lay, egg weight, egg mass, and FCR, are provided in Table 3 and 4, respectively.

Table 3
*Estimates of the parameters β0, β1, and β2, as well as their standard error values, for the equations that predict the response of AFD Lys intake on rate of lay, egg weight, egg mass, and FCR.*

| Parameter | β0 | | β1 | | β2 | |
|---|---|---|---|---|---|---|
| | Value | se | Value | se | Value | se |
| Rate of lay (%) | 86.3 | 3.0 | 105.5 | 30.0 | -0.121 | 0.04 |
| Egg weight (g) | 62.3 | 1.3 | 6.1 | 1.8 | --- | --- |
| Egg mass (g/d) | 54.0 | 1.0 | 103.7 | 24.5 | -0.118 | 0.03 |
| FCR | 2.11 | 0.07 | -3.10 | 0.80 | 0.0035 | 0.001 |

Table 4
*Estimates of the parameters β0, β1, and β2, as well as their se values, for the equations that predict the response of SID Lys intake on rate of lay, egg weight, egg mass, and FCR.*

| Parameter | β0 | | β1 | | β2 | |
|---|---|---|---|---|---|---|
| | Value | Se | Value | se | Value | Se |
| Rate of lay (%) | 85.2 | 3.3 | 106.2 | 32.9 | -0.117 | 0.04 |
| Egg weight (g) | 62.3 | 1.3 | 6.3 | 1.8 | --- | --- |
| Egg mass (g/d) | 52.8 | 1.3 | 106.4 | 27.3 | -0.116 | 0.03 |
| FCR | 2.15 | 0.08 | -3.29 | 0.94 | 0.0035 | 0.001 |

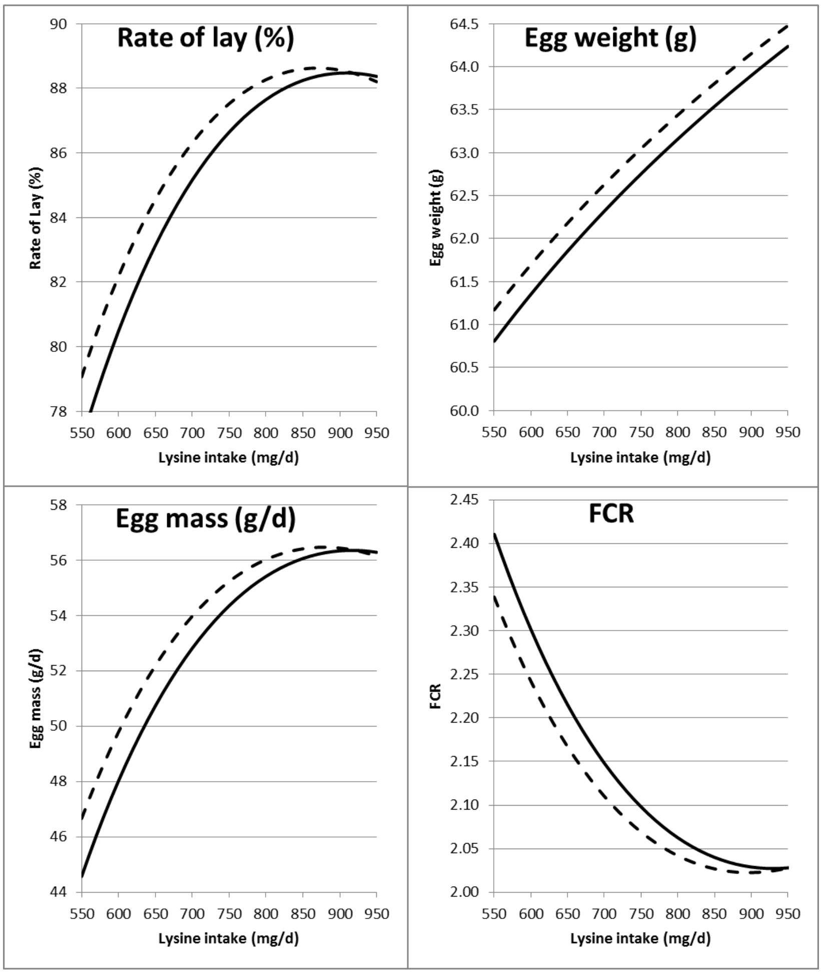
The parameters β0, β1, and β2 had a significant ($P$<0.05) contribution to the prediction of rate of lay, egg mass and FCR in dependence of the AFD and SID Lys intake. β2, however, did not contribute to the prediction of egg weight, resulting in a nearly linear relation between AFD or SID Lys intake and egg weight. Because of the used REML procedure, and because of the relative small Lys dataset, is was not possible to determine the goodness of fit of the general model.
The fitted curves with the relations between the AFD and SID Lys intake on the predicted rate of lay, egg weight, egg mass and FCR are shown in Figure 4. The AFD and SID Lys intake levels for realising maximal performance, as well as for 95% of the maximal response are shown in Table 5. The average starting level of the response amounted 646 mg AFD Lys and 678 mg SID Lys, respectively.

Table 5
*Predicted AFD and SID Lys intake levels (mg/d) for realising maximal hen performance and 95% of the maximal response*

| | AFD Lysine intake (mg/d) | | SID Lysine intake (mg/d) | |
|---|---|---|---|---|
| | Maximal response | 95% of maximal response | Maximal response | 95% of maximal response |
| Rate of lay (%) | 870 | 810 | 908 | 850 |
| Egg weight (g) | - - - | - - - | --- | - - - |
| Egg mass (g/d) | 879 | 820 | 916 | 855 |
| FCR | 897 | 825 | 930 | 860 |

Based on the predicted relationships, laying hens need a daily intake of 879 mg AFD Lys or 916 mg SID Lys intake for realizing maximal daily egg mass production. For realizing the maximal feed efficiency, a daily intake of 897 mg/hen ADF Lys or 930 mg/hen SID Lys is required. For realizing 95% of the maximal egg mass production, laying hens need a daily intake of 820 mg AFD Lys or 855 mg

SID Lys. For realizing 95% of the maximal feed efficiency, a daily intake of 825 mg/hen ADF Lys or 860 mg/hen SID Lys is required.
Some economic considerations of Lys supplementation are shown in appendix 2.



dotted line = AFD Lysine intake; solid line = SID Lysine intake

***Figure 4*** *Curve fitting of the response of AFD and SID Lysine intake on the predicted rate of lay, egg weight, egg mass and FCR.*

## 3.2 Response to Methionine plus Cysteine

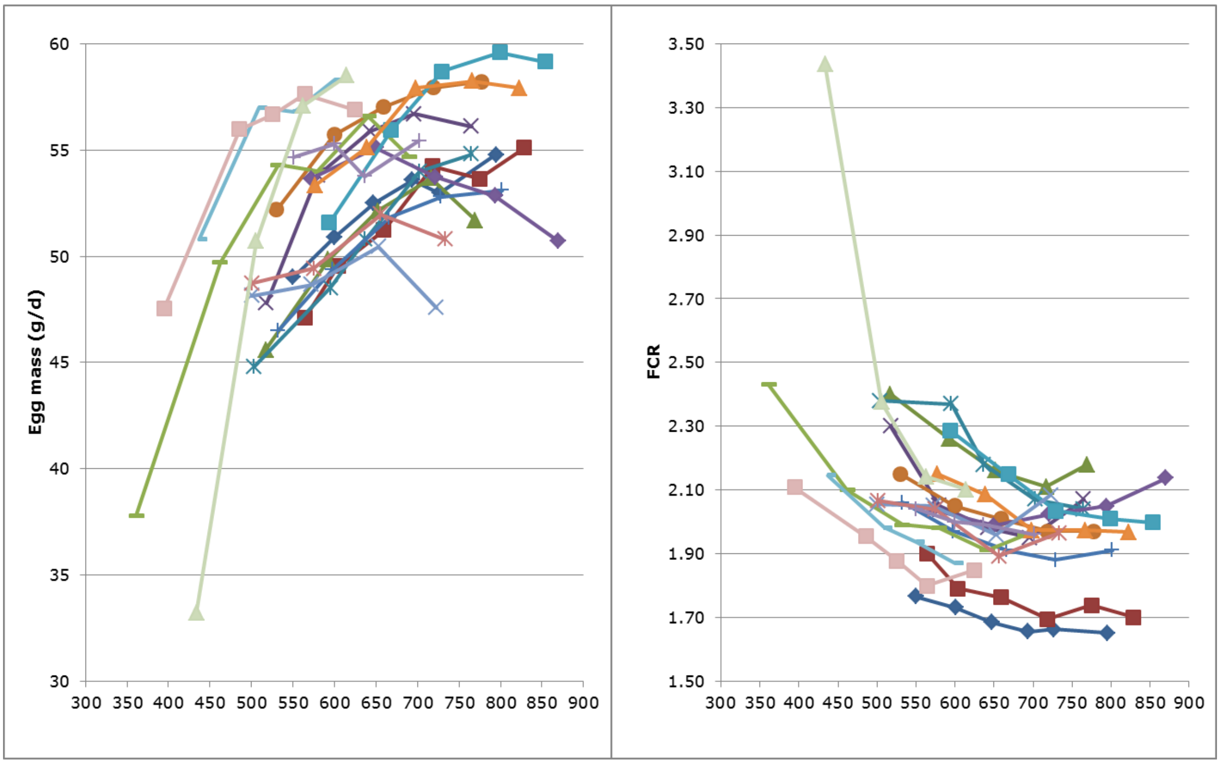
In total, 29 references comprising 40 Met dose response trials, were judged. Table 6 provides an overview of the 17 trials that met the criteria for inclusion in the meta-analysis. The applied Met levels per experiment ranged from 4 to 6. The AFD Met contents of the diets ranged from 1.8 to 6.6 g/kg. CP contents in the basal diets varied from 14.5 to 17.2%, and ME levels from 10.8 – 12.2 MJ/kg. Twelve experiments used white feathered birds, whereas five experiments used brown feathered birds. In twelve trials, the diets were applied to hens during the starting or the middle phase (34 – 50 wk of age) of the laying period, while in five trials the diets were applied to hens at the end of the laying period (50 – 95 wk of age). Appendix 1 provides an overview of papers that did not meet the inclusion criteria and were not further considered.

Table 6
*List of references used for the meta-analysis and some characteristics of the trials*

| Trial | Reference | Nr. of doses | Range of AFD Met content (g/kg) | Range of AFD Met+Cys content (g/kg) | CP[1] | ME[1] | Strain[2] | Age[3] | Average rate of lay(%) | Average egg weight (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Bertram et al. (1995b) Exp. 2 | 5 | 2.0 – 4.0 | 3.9 – 5.9 | 15.7 | 10.8 | 4 | 24-36 | 94.9 | 57.8 |
| 2 | Brumano et al. (2010a) | 6 | 4.1 – 6.6 | 6.3 – 8.8 | 16.9 | 12.1 | 1 | 24-40 | 93.1 | 56.1 |
| 3 | Brumano et al. (2010b) | 6 | 4.1 – 6.6 | 6.3 – 8.8 | 16.9 | 12.1 | 1 | 42-58 | 84.4 | 61.0 |
| 4 | Cupertino et al. (2009b) Exp. 1 | 5 | 2.7 – 4.8 | 4.8 – 6.8 | 15.6 | 11.7 | 4 | 54-70 | 83.2 | 64.9 |
| 5 | Cupertino et al. (2009b) Exp. 2 | 5 | 2.7 – 4.8 | 4.8 – 6.8 | 15.6 | 11.7 | 5 | 54-70 | 77.6 | 65.2 |
| 6 | Dänner and Bessei (2002) | 4 | 2.0 – 3.5 | 4.0 – 5.5 | 14.9 | 11.5 | 4 | 24-48 | 92.6 | 60.1 |
| 7 | Filho et al. (2006a) | 5 | 2.9 – 5.7 | 5.2 – 8.0 | 17.2 | 11.5 | 2 | 20-44 | 88.4 | 60.2 |
| 8 | Geraldo et al. (2010) | 5 | 3.3 – 5.7 | 5.6 – 7.9 | 16.5 | 12.2 | 1 | 25-41 | 90.6 | 56.0 |
| 9 | Lemme et al. (2004b) | 4 | 1.8 – 3.0 | 3.8 – 5.0 | 14.7 | 12.0 | 5 | 22-46 | - - - | - - - |
| 10 | Sa et al. (2007a) Exp. 1 | 5 | 2.9 – 5.1 | 5.0 – 7.2 | 15.7 | 12.0 | 4 | 34-50 | 92.3 | 61.7 |
| 11 | Sa et al. (2007a) Exp. 2 | 5 | 2.9 – 5.1 | 5.0 – 7.2 | 15.7 | 12.0 | 5 | 34-50 | 90.0 | 62.8 |
| 12 | Narváez-Solarte et al. (2005) | 6 | 2.0 – 4.5 | 3.9 – 6.4 | 14.5 | 11.4 | 4 | 22-38 | 87.1 | 58.6 |
| 13 | Novak et al. (2004) Exp. 1 | 4 | 3.0 – 5.4 | 5.1 – 7.6 | 17.2 | 12.2 | 7 | 20-43 | 82.5 | 59.0 |
| 14 | Novak et al. (2004) Exp. 2 | 4 | 3.0 – 5.4 | 5.1 – 7.6 | 17.2 | 12.2 | 7 | 20-43 | 83.4 | 60.2 |
| 15 | Schmidt et al. (2009a) | 5 | 2.7 – 4.8 | 4.8 – 6.8 | 15.6 | 11.7 | 5 | 79-95 | 74.5 | 67.9 |
| 16 | Schmidt et al. (2011b) | 5 | 2.7 – 4.8 | 4.8 – 6.8 | 15.6 | 11.7 | 1 | 79-95 | 81.7 | 67.3 |
| 17 | Schutte et al. (1994) Exp. 2 | 4 | 3.0 – 4.5 | 5.0 – 6.4 | 15.0 | 12.1 | 4 | 25-77 | 89.2 | 61.5 |

[1] Crude protein is in %, whereas ME is in units of megajoules per kg of feed, based on recalculated values.
[2] Strains: 1 = Hy-Line W-36, 2 = Hysex Brown, 4 = Lohmann LSL, 5 = Lohmann Brown, 7 = Dekalb Delta.
[3] Age is in wk.

In Figure 5, the determined  egg mass (g/d) and FCR of the in Table 6 selected studies were plotted as a function of the daily AFD Met+Cys intake. In these studies, egg mass ranged from 33 to 60 g/d, and FCR from 3.44 to 1.65.




**AFD Methionine + Cysteine intake (mg/d)**

***Figure 5*** *The determined responses of egg mass (g/d) and FCR as a function of the AFD Met+Cys intake of the in Table 6 selected studies.*

The coefficient of variation (CV), the estimates of the parameters β₁, β₂, and the AFD Met+Cys intake level (mg/d) for maximal response on egg mass and FCR per individual study are shown in Table 7.

Table 7

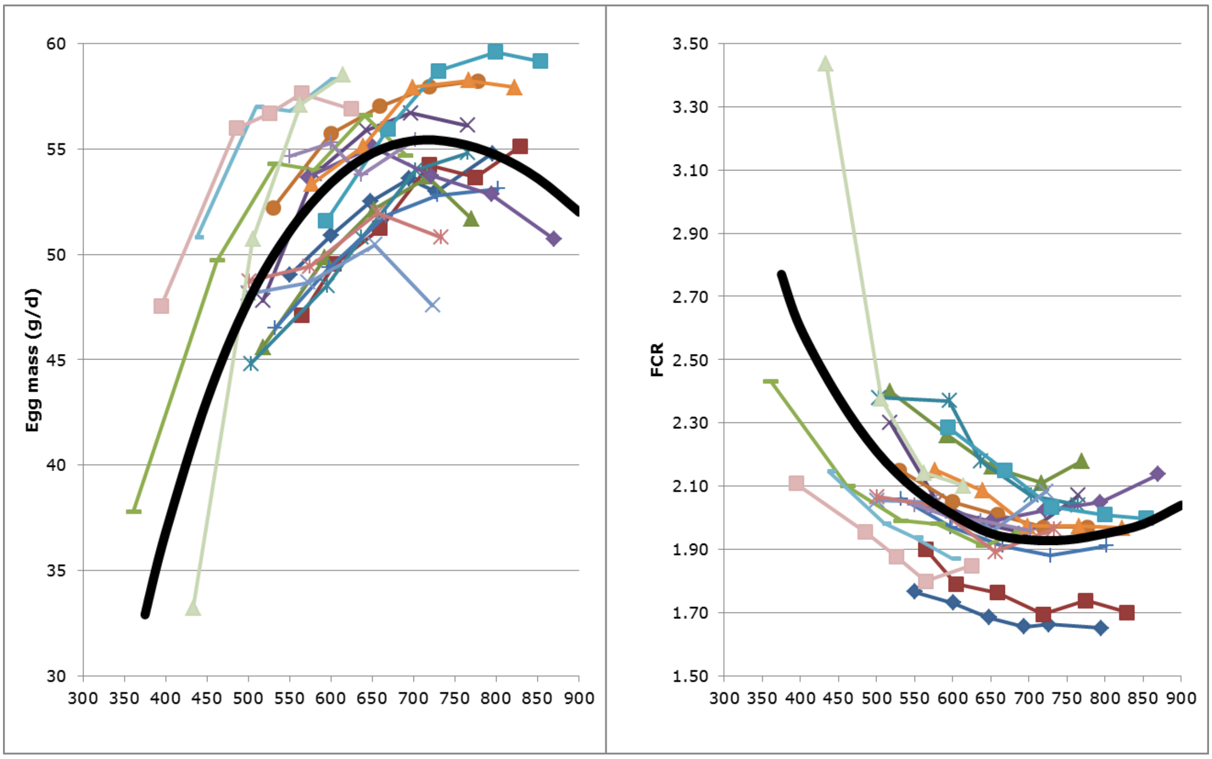
*Coefficient of variation, estimates of the parameters $\beta_1$, $\beta_2$, and the AFD Met+Cys intake level (mg/d) for maximal response on egg mass and FCR per individual study*

| | | Egg mass | | | | FCR | | | |
|---|---|---|---|---|---|---|---|---|---|
| Trial | Reference | $\beta_1$ | $\beta_2$[1] | Met+Cys intake for maximal response | CV (%)[2] | $\beta_1$ | $\beta_2$[1] | Met+Cys intake for maximal response | CV (%)[2] |
| 1 | Bertram et al. (1995b) Exp. 2 | 111.3 | -0.182 | 611 | 1.43% | -3.73 | 0.0062 | 599 | 1.64% |
| 2 | Brumano et al. (2010a) | 134.0 | -0.182 | 736 | 1.41% | -4.48 | 0.0062 | 719 | 1.24% |
| 3 | Brumano et al. (2010b) | 142.9 | -0.182 | 785 | 1.30% | -4.71 | 0.0062 | 756 | 1.40% |
| 4 | Cupertino et al. (2009b) Exp. 1 | 137.4 | -0.182 | 754 | 1.52% | -4.60 | 0.0062 | 738 | 3.20% |
| 5 | Cupertino et al. (2009b) Exp. 2 | 139.9 | -0.182 | 768 | 2.44% | -4.83 | 0.0062 | 775 | 3.91% |
| 6 | Dänner and Bessei (2002) | 124.1 | -0.182 | 681 | 1.41% | -4.34 | 0.0062 | 696 | 0.53% |
| 7 | Filho et al. (2006a) | 120.8 | -0.182 | 663 | 0.96% | -4.16 | 0.0062 | 667 | 0.65% |
| 8 | Geraldo et al. (2010) | 134.7 | -0.182 | 740 | 0.91% | -4.47 | 0.0062 | 717 | 0.54% |
| 9 | Lemme et al. (2004b) | 172.2 | -0.182 | 945 | 5.27% | -7.16 | 0.0062 | 1148 | 8.39% |
| 10 | Sa et al. (2007a) Exp. 1 | 150.3 | -0.182 | 826 | 0.63% | -5.13 | 0.0062 | 823 | 1.11% |
| 11 | Sa et al. (2007a) Exp. 2 | 139.7 | -0.182 | 767 | 0.87% | -4.81 | 0.0062 | 771 | 1.06% |
| 12 | Narváez-Solarte et al. (2005) | 122.7 | -0.182 | 674 | 1.68% | -4.03 | 0.0062 | 646 | 0.92% |

| | | Egg mass | | | | FCR | | | |
|---|---|---|---|---|---|---|---|---|---|
| Trial | Reference | $\beta_1$ | $\beta_2$[1] | Met+Cys intake for maximal response | CV (%)[2] | $\beta_1$ | $\beta_2$[1] | Met+Cys intake for maximal response | CV (%)[2] |
| 13 | Novak et al. (2004) Exp. 1 | 111.4 | -0.182 | 612 | 1.76% | -3.84 | 0.0062 | 616 | 2.11% |
| 14 | Novak et al. (2004) Exp. 2 | 118.1 | -0.182 | 649 | 1.86% | -4.18 | 0.0062 | 670 | 2.28% |
| 15 | Schmidt et al. (2009a) | 134.0 | -0.182 | 736 | 1.35% | -4.61 | 0.0062 | 739 | 1.23% |
| 16 | Schmidt et al. (2011b) | 134.7 | -0.182 | 740 | 0.52% | -4.54 | 0.0062 | 729 | 0.71% |
| 17 | Schutte et al. (1994) Exp. 2 | 117.6 | -0.182 | 646 | 1.93% | -4.27 | 0.0062 | 685 | 0.73% |

[1] The estimation of $\beta_2$ differed not significantly between studies and therefore estimated as one standard value for all studies.
[2] CV (Coefficient of Variation) is a measure of the goodness of fit and indicates the relative deviation between the treatment average and the model fit (CV = √[Variance residual] / [mean] *100).

The model fit for egg mass showed a low CV (<2%) in case of 15 out of the 17 studies. The model fit for FCR showed a low CV (<2%) in case of 12 out of the 17 studies. In particular the data of Cupertino et al. (2009b) and Lemme et al. (2004b) had moderate fits in the used model. The predicted daily AFD Met+Cys intake for achieving maximal egg mass production ranged from 611 to 945 mg/d. The predicted daily AFD Met+Cys intake for achieving most efficient feed conversion ranged from 599 to 1148 mg/d. In appendix 3, the AFD Met+Cys intake levels as recommended by the authors themselves, are provided.
The overall fitted response curve, as determined over all selected studies, of daily AFD and SID Met+Cys intake on the predicted egg mass and FCR are shown in Figure 6 and 7, respectively. The AFD and SID Met+Cys intake levels for achieving maximal response in egg mass and FCR were covered by the tested Met+Cys intake range in 11 out of the 17 experiments.




**AFD Methionine+Cysteine intake (mg/d)**

***Figure 6*** *The determined responses of egg mass (g/d) and FCR as a function of the AFD Methionine+Cysteine intake of the in Table 6 selected studies, as well as the fitted response (thick solid line).*

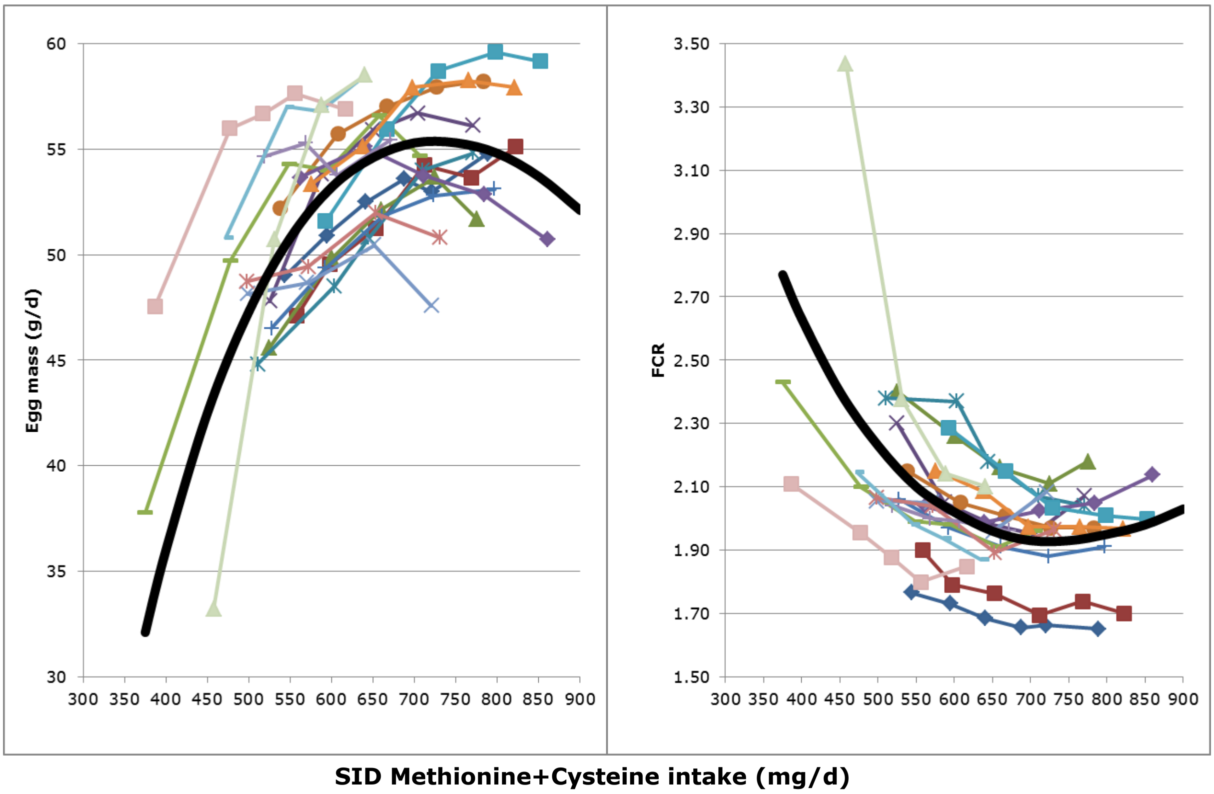



***Figure 7*** *The determined responses of egg mass (g/d) and FCR as a function of the SID Methionine+Cysteine intake of the in Table 6 selected studies, as well as the fitted response (thick solid line).*

The estimates of the parameters β0, β1, and β2, as well as their standard error values, for the equations that predict the overall response of AFD or SID Met+Cys intake on rate of lay, egg weight, egg mass, and FCR, are provided in Table 8 and 9, respectively.

Table 8

*Estimates of the parameters β0, β1, and β2, as well as their standard error values, for the equations that predict the response of AFD Met+Cys intake on rate of lay, egg weight, egg mass, and FCR.*

| Parameter | β0 | | β1 | | β2 | |
|---|---|---|---|---|---|---|
| | Value | se | Value | se | Value | se |
| Rate of lay (%) | 86.9 | 1.6 | 132.2 | 15.2 | -0.191 | 0.02 |
| Egg weight (g) | 61.2 | 0.9 | 39.8 | 7.3 | -0.052 | 0.012 |
| Egg mass (g/d) | 53.4 | 0.8 | 129.8 | 12.6 | -0.180 | 0.019 |
| FCR | 2.01 | 0.04 | -4.55 | 0.74 | 0.006 | 0.001 |

Table 9

*Estimates of the parameters β0, β1, and β2, as well as their se values , for the equations that predict the response of SID Met+Cys intake on rate of lay, egg weight, egg mass, and FCR.*

| Parameter | β0 | | β1 | | β2 | |
|---|---|---|---|---|---|---|
| | Value | Se | Value | se | Value | se |
| Rate of lay (%) | 86.8 | 1.6 | 135.1 | 15.7 | -0.194 | 0.02 |
| Egg weight (g) | 61.2 | 0.9 | 41.2 | 7.6 | -0.054 | 0.012 |
| Egg mass (g/d) | 53.2 | 0.7 | 132.1 | 13.1 | -0.182 | 0.020 |
| FCR | 2.02 | 0.04 | -4.58 | 0.77 | 0.006 | 0.001 |

The parameters β0, β1, and β2 had a significant (*P*<0.05) contribution to the prediction of rate of lay, egg weight, egg mass and FCR in dependence of the AFD and SID Met+Cys intake. Because of the used REML procedure, and because of the relative small dataset, is was not able to determine the goodness of fit of the general model.

The fitted curves with the relations between the AFD and SID Met+Cys intake on the predicted rate of lay, egg weight, egg mass and FCR are shown in Figure 8. The AFD and SID Met+Cys intake levels for realising maximal performance, as well as for 95% of the maximal response are shown in Table 10. The average starting intake level of the response amounted 508 mg/d of AFD Met+Cys and 512 mg/d of SID Met+Cys.

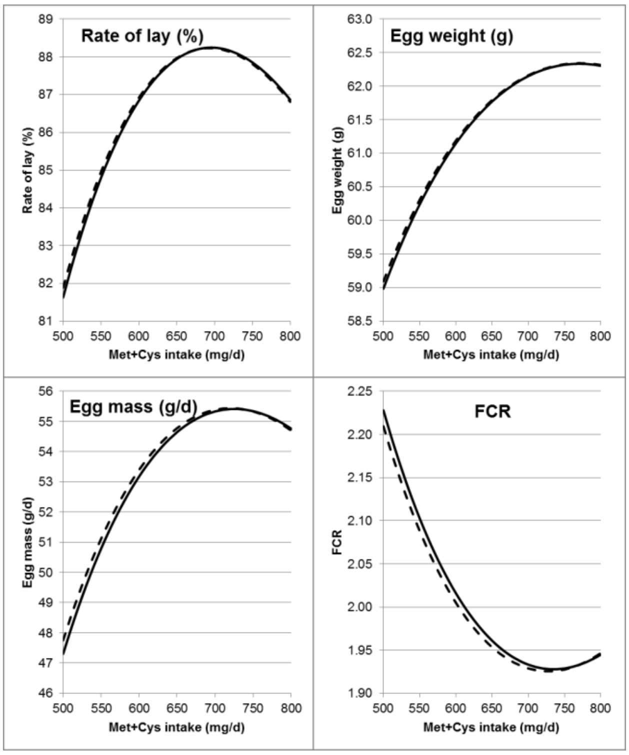
Table 10

*Predicted AFD and SID Methionine+Cysteine intake levels (mg/d) for realising maximal hen performance and 95% of the maximal response*

| | AFD Met+Cys intake (mg/d) | | SID Met+Cys intake (mg/d) | |
|---|---|---|---|---|
| | Maximal response | 95% of maximal response | Maximal response | 95% of maximal response |
| Rate of lay (%) | 693 | 650 | 696 | 650 |
| Egg weight (g) | 771 | 705 | 770 | 705 |
| Egg mass (g/d) | 720 | 670 | 725 | 670 |
| FCR | 728 | 675 | 735 | 675 |

Based on the predicted relationships, laying hens need a daily intake of 720 mg AFD or 725 mg SID Met+Cys intake for realizing maximal daily egg mass production. For realizing the maximal feed efficiency, a daily intake of 728 mg/hen ADF or 735 mg/hen SID Met+Cys is required. For realizing 95% of the maximal egg mass production, laying hens need a daily intake of 670 mg AFD or SID Met+Cys, respectively. For realizing 95% of the maximal feed efficiency, a daily intake of 675 mg/hen ADF or SID Met+Cys is required.

The predicted AFD and SID Met+Cys intake levels for realizing maximal egg weight is considerable higher than for maximal egg mass. Egg mass is the net result of rate of lay and egg weight. As shown in Figure 8, rate of lay significantly decreases at Met+Cys intake levels above the maximal response (± 695 mg/d). In the range of 695 to 770 mg/d Met+Cys intake, egg weight still gradually increases. At the Met+Cys intake level of ± 725 mg/d, the balance between rate of lay and egg weight resulted in maximal egg mass.




dotted line = AFD Met+Cys intake; solid line = SID Met+Cys intake

***Figure 8*** *Curve fitting of the response of AFD and SID Methionine+Cysteine intake on the predicted rate of lay, egg weight, egg mass and FCR.*

## 3.3 Response to Threonine

In total, 12 references comprising 15 Thr dose response trials, were judged. Table 11 provides an overview of the 6 trials that met the criteria for inclusion in the meta-analysis. In each experiment, 5 Thr supplementation levels were applied. The AFD Thr contents of the diets ranged from 3.2 to 5.1 g/kg. CP contents in the basal diets varied from 13.1 to 14.3%, and ME levels from 12.1 – 12.3 MJ/kg. Two experiments used white feathered birds, whereas four experiments used brown feathered birds. In two trials, the diets were applied to hens during the starting or the middle phase (34 – 50 wk of age) of the laying period, while in four trials the diets were applied to hens at the end of the laying period (54 – 95 wk of age). Appendix 1 provides an overview of papers that did not meet the inclusion criteria and were not further considered.

Table 11

*List of references used for the meta-analysis and some characteristics of the trials.*

| Trial | Reference | Nr. Of doses | Range of AFD Thr content (g/kg) | CP[1] | ME[1] | Strain[2] | Age[3] | Average rate of lay (%) | Average egg weight (g) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Cupertino et al. (2010) Exp. 1 | 5 | 3.2 – 4.5 | 13.1 | 12.1 | 4 | 54-70 | 71.8 | 66.5 |
| 2 | Cupertino et al. (2010) Exp. 2 | 5 | 3.2 – 4.5 | 13.1 | 12.1 | 5 | 54-70 | 72.3 | 66.5 |
| 3 | Sa et al. (2007b) | 5 | 3.5 – 5.1 | 14.3 | 12.3 | 5,6 | 34-50 | 83.6 | 63.2 |
| 4 | Sa et al. (2007b) | 5 | 3.5 – 5.1 | 14.3 | 12.3 | 5 | 34-50 | 84.8 | 64.3 |
| 5 | Schmidt et al. (2010) | 5 | 3.2 – 4.5 | 13.3 | 12.1 | 5 | 79-95 | 66.3 | 68.3 |
| 6 | Schmidt et al. (2011a) | 5 | 3.2 – 4.5 | 13.3 | 12.1 | 4 | 79-95 | 68.0 | 67.6 |

[1] Crude protein is in %, whereas ME is in units of megajoules per kg of feed.
[2] Strains: 3 = Isa Babcock B-300, 4 = Lohmann LSL, 5 = Lohmann Brown, 6 = Lohmann.
[3] Age is in wk.

In Figure 9, the determined  egg mass (g/d) and FCR of the in Table 11 selected studies were plotted as a function of the daily AFD Thr intake. In these studies, egg mass ranged from 42.4 to 56.7 g/d, and FCR from 2.55 to 1.96.

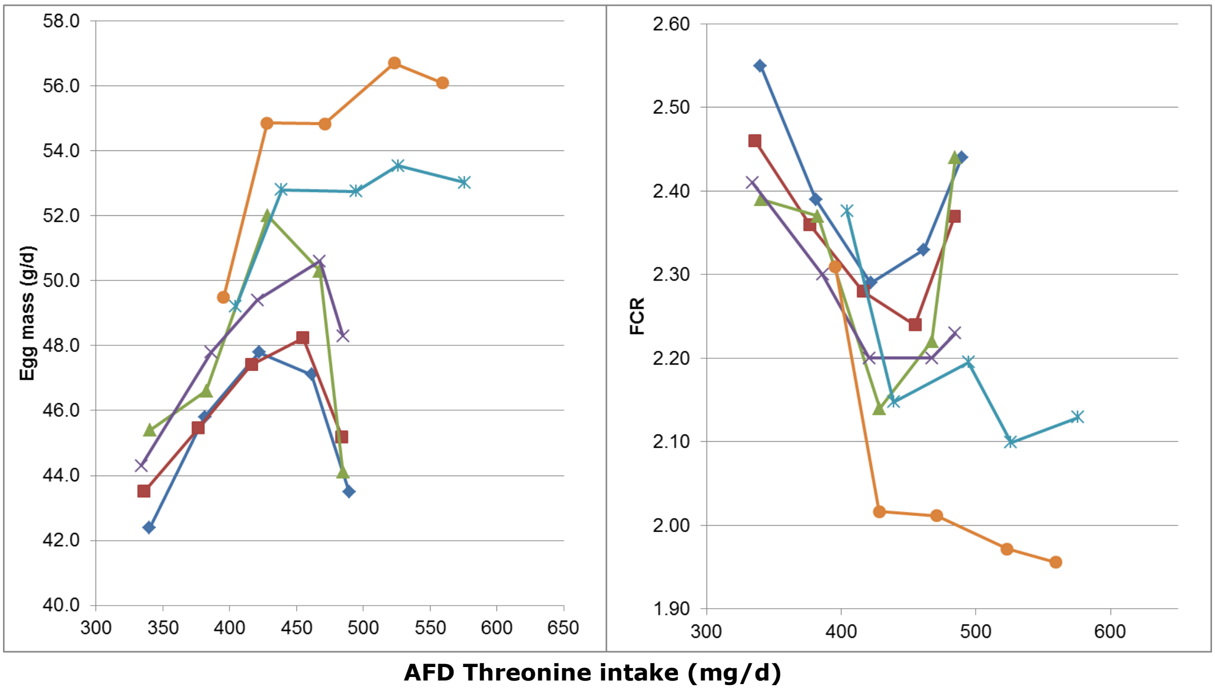



***Figure 9*** *The determined responses of egg mass (g/d) and FCR as a function of the AFD Thr intake of the in Table 11 selected studies.*

The coefficient of variation (CV), the estimates of the parameters $\beta_1$, $\beta_2$, and the AFD Thr intake level (mg/d) for maximal response on egg mass and FCR per individual study are shown in Table 12.

Table 12

*Coefficient of variation, estimates of the parameters $\beta_1$, $\beta_2$, and the AFD Thr intake level (mg/d) for maximal response on egg mass and FCR per individual study*

| | | Egg mass | | | | FCR | | | |
|---|---|---|---|---|---|---|---|---|---|
| Trial | Reference | $\beta_1$ | $\beta_2$[1] | Thr intake for maximal response | CV (%)[2] | $\beta_1$ | $\beta_2$[1] | Thr intake for maximal response | CV (%)[2] |
| 1 | Cupertino et al. (2010) Exp. 1 | 46.2 | -0.09 | 543 | 6.8% | -1.44 | 0.002 | 653 | 5.7% |
| 2 | Cupertino et al. (2010) Exp. 2 | 46.6 | -0.09 | 548 | 2.3% | -1.48 | 0.002 | 671 | 1.5% |
| 3 | Sa et al. (2007b) Exp. 1 | 49.2 | -0.09 | 578 | 2.0% | -1.49 | 0.002 | 673 | 3.3% |
| 4 | Sa et al. (2007b) Exp. 2 | 51.3 | -0.09 | 603 | 3.6% | -1.61 | 0.002 | 728 | 4.8% |
| 5 | Schmidt et al. (2010) | 44.1 | -0.09 | 518 | 4.7% | -1.37 | 0.002 | 621 | 3.5% |
| 6 | Schmidt et al. (2011a) | 44.8 | -0.09 | 527 | 3.2% | -1.43 | 0.002 | 645 | 2.9% |

[1] The estimation of $\beta_2$ differed not significantly between studies, and therefore estimated as one standard value for all studies.

[2] CV (Coefficient of Variation) is a measure of the goodness of fit and indicates the relative deviation between the treatment average and the model fit (CV = √[Variance residual] / [mean] *100).

The model fit for egg mass showed a low CV (<2%) in case of 1 out of the 6 studies. The model fit for FCR showed a low CV (<2%) in case of 1 out of the 6 studies. In particular the data of Cupertino et al. (2010) Exp. 1 and Sa et al. (2007b) had moderate fits in the used model.

The predicted daily AFD Thr intake for achieving maximal egg mass production ranged from 518 to 603 mg/d. The predicted daily AFD Thr intake for achieving most efficient feed conversion ranged from 621 to 728 mg/d. In appendix 3, the AFD Thr intake levels as recommended by the authors themselves, are provided.

The overall fitted response curve, as determined over all selected studies, of daily AFD and SID Thr intake on the predicted egg mass and FCR are shown in Figure 10 and 11, respectively. The Thr intake level for achieving maximal response on egg mass was covered by the tested Thr intake range in 2 out of the 6 experiments.

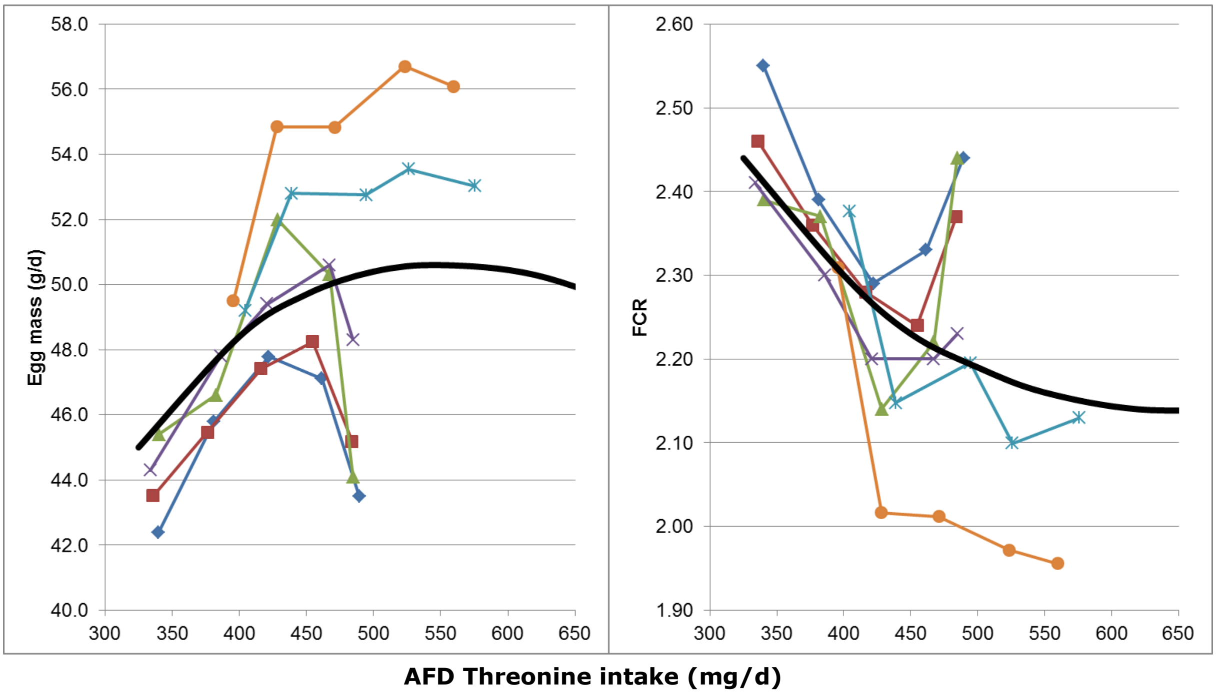



***Figure 10*** *The determined responses of egg mass (g/d) and FCR as a function of the AFD Threonine intake of the in Table 11 selected studies, as well as the fitted response (thick solid line).*

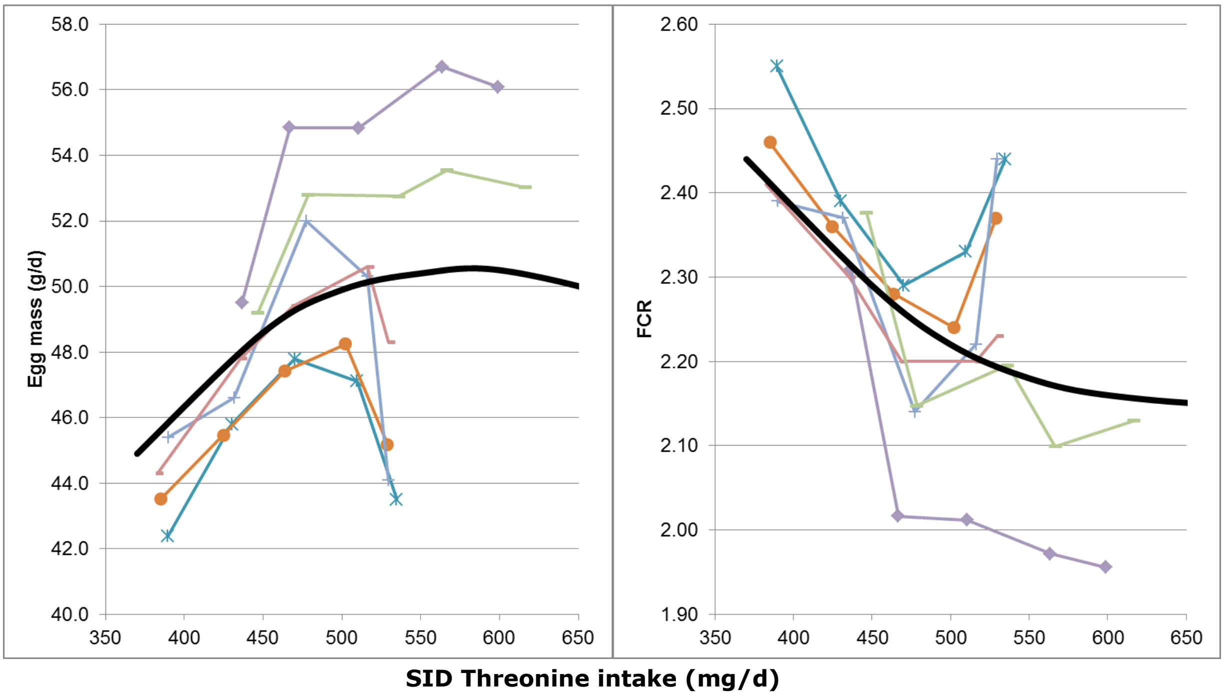



***Figure 11*** *The determined responses of egg mass (g/d) and FCR as a function of the SID Threonine intake of the in Table 11 selected studies, as well as the fitted response (thick solid line).*

The estimates of the parameters β0, β1, and β2, as well as their standard error values, for the equations that predict the overall response of AFD or SID Thr intake on rate of lay, egg weight, egg mass, and FCR, are provided in Table 13 and 14, respectively.

Table 13
*Estimates of the parameters β0, β1, and β2, as well as their standard error values, for the equations that predict the response of AFD Thr intake on rate of lay, egg weight, egg mass, and FCR.*

| Parameter | β0 | | β1 | | β2 | |
|---|---|---|---|---|---|---|
| | Value | se | Value | se | Value | se |
| Rate of lay (%) | 76.7 | 2.9 | 83.4 | 42.5 | -0.156 | 0.10 |
| Egg weight (g) | 65.7 | 0.7 | 6.07 | 15.6 | -0.020 | 0.036 |
| Egg mass (g/d) | 50.4 | 1.3 | 47.0 | 31.5 | -0.085 | 0.073 |
| FCR | 2.19 | 0.1 | -1.47 | 1.37 | 0.002 | 0.003 |

Table 14
*Estimates of the parameters β0, β1, and β2, as well as their se values , for the equations that predict the response of SID Thr intake on rate of lay, egg weight, egg mass, and FCR.*

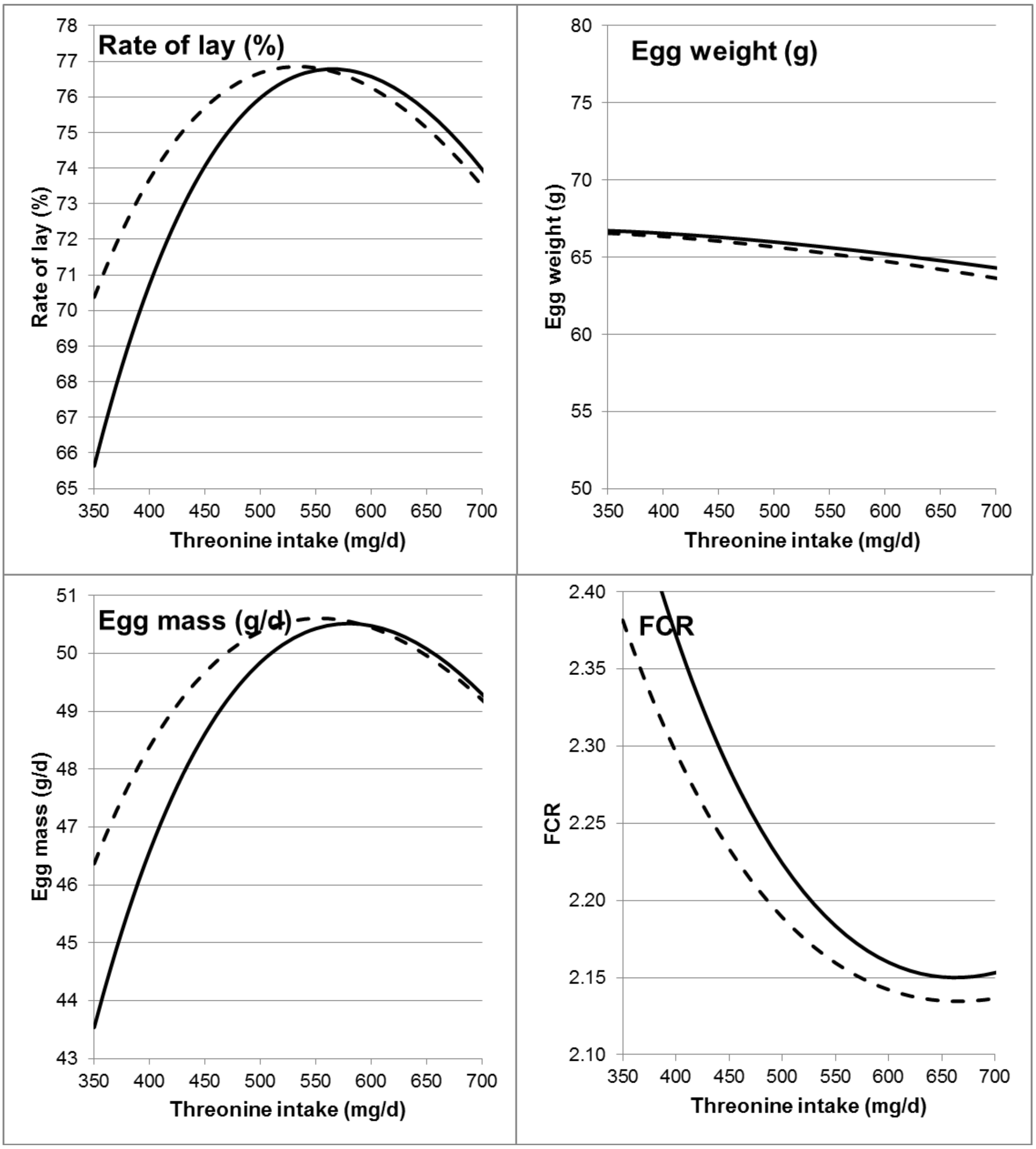
| Parameter | β0 | | β1 | | β2 | |
|---|---|---|---|---|---|---|
| | Value | Se | Value | Se | Value | se |
| Rate of lay (%) | 76.0 | 2.9 | 113.8 | 54.5 | -0.202 | 0.11 |
| Egg weight (g) | 66.0 | 0.7 | 5.05 | 20.3 | -0.017 | 0.043 |
| Egg mass (g/d) | 49.9 | 1.3 | 64.6 | 40.5 | -0.111 | 0.085 |
| FCR | 2.22 | 0.04 | -2.05 | 1.76 | 0.003 | 0.003 |

The parameter β2 did not significantly affect any of the performance traits. β1 had only a significant ($P<0.05$) contribution to the prediction of rate of lay in dependence of the AFD and SID Thr intake. Only the parameter β0 significantly contributed to the estimation of egg weight, egg mass and FCR, both on the AFD and SID basis. Because of the used REML procedure, and because of the relative small Thr dataset, is was not able to determine the goodness of fit of the general model.
The fitted curves with the relations between the AFD and SID Thr intake on the predicted rate of lay, egg weight, egg mass and FCR are shown in Figure 12. The AFD and SID Thr intake levels for realising maximal performance, as well as for 95% of the maximal response are shown in Table 15. The average starting level of the response amounted 358 mg AFD Thr and 403 mg SID Thr, respectively.

Table 15
*Predicted AFD and SID Thr intake levels (mg/d) for realising maximal hen performance and 95% of the maximal response*

| | AFD Threonine intake (mg/d) | | SID Threonine intake (mg/d) | |
|---|---|---|---|---|
| | Maximal response | 95% of maximal response | Maximal response | 95% of maximal response |
| Rate of lay (%) | 553 | 485 | 579 | 530 |
| Egg weight (g) | - - - | - - - | --- | - - - |
| Egg mass | 553 | 500 | 579 | 530 |
| FCR | 665 | 575 | 663 | 590 |

Based on the predicted relationships, laying hens need a daily intake of 553 mg AFD Thr or 579 mg SID Thr intake for realizing maximal daily egg mass production. For realizing the maximal feed efficiency, a daily intake of 665 mg/hen ADF Thr or 663 mg/hen SID Thr is required. For realizing 95% of the maximal egg mass production, laying hens need a daily intake of 485 mg AFD Thr or 530 mg SID Thr. For realizing 95% of the maximal feed efficiency, a daily intake of 575 mg/hen ADF Thr or 590 mg/hen SID Thr is required.




dotted line = AFD Threonine intake; solid line = SID Threonine intake

***Figure 12*** *Curve fitting of the response of AFD and SID Threonine intake on the predicted rate of lay, egg weight, egg mass and FCR.*

## 3.4 Response to Tryptophan

In total, 12 references comprising 29 Trp dose response trials, were judged. Table 16 provides an overview of the 4 trials that met the criteria for inclusion in the meta-analysis. Supplemented Trp levels per experiment ranged from 4 to 5. The AFD Trp contents of the diets ranged from 1.0 to 1.9 g/kg. CP contents in the basal diets varied from 15.0 to 16.4%, and ME levels from 11.4 – 12.7 MJ/kg. Three experiments used white feathered birds, whereas one experiment used brown feathered birds. In one trial, the diets were applied to hens during the starting or the middle phase (24 – 40 wk of age) of the laying period, while in three trials the diets were applied to hens at the middle or end of the laying period (42 – 59 wk of age). Appendix 1 provides an overview of papers that did not meet the inclusion criteria and were not further considered.

Table 16

*List of references used for the meta-analysis and some characteristics of the trials.*

| Trial | Reference | Nr. of doses | Range of AFD Trp content (g/kg) | CP[1] | ME[1] | Strain[2] | Age[3] | Average rate of lay (%) | Average egg weight (g) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Calderano (2011) | 5 | 1.5 – 1.9 | 15.0 | 12.7 | 1 | 42-58 | 77.1 | 54.5 |
| 2 | Calderano et al. (2012) | 5 | 1.5 – 1.9 | 15.0 | 12.6 | 1 | 24-40 | 80.3 | 60.1 |
| 3 | Deponti et al. (2007) | 5 | 1.1 – 1.9 | 16.0 | 11.4 | 10 | 51-58 | 89.1 | 63.9 |
| 4 | Esteve-Garcia et al. (2001) | 4 | 1.0 – 1.6 | 16.4 | 11.8 | 9 | 44-56 | 82.9 | 66.9 |

[1] Crude protein is in %, whereas ME is in units of mega joules per kg of feed.

[2] Strains: 1 = Hy Line W36, 5 = Lohmann Brown, 9 = Hy Line Brown, 10 = Hisex White.

[3] Age is in wk.

In Figure 13, the determined egg mass (g/d) and FCR of the in Table 16 selected studies were plotted as a function of the daily AFD Trp intake. In these studies, egg mass ranged from 33 to 58 g/d, and FCR from 2.55 to 1.8.

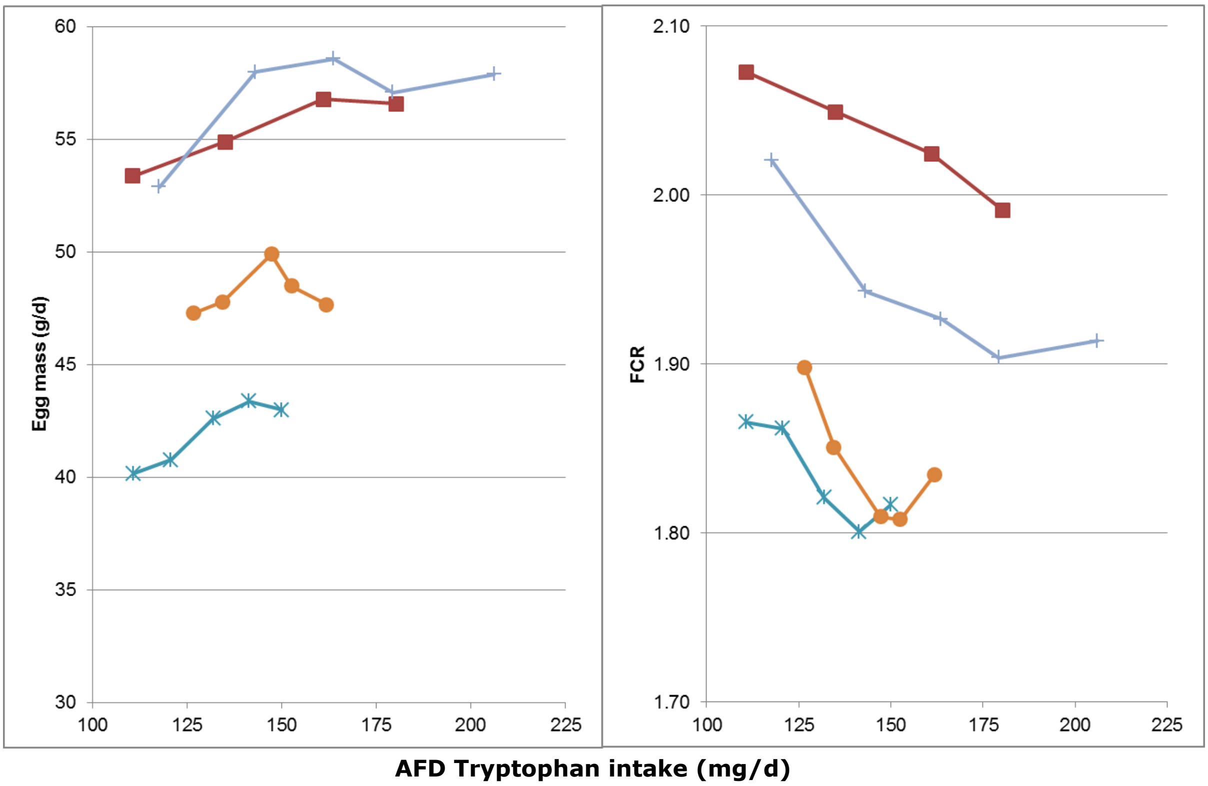



***Figure 13*** *The determined responses of egg mass (g/d) and FCR as a function of the AFD Trp intake of the in Table 16 selected studies.*

The coefficient of variation (CV), the estimates of the parameters $\beta_1$, $\beta_2$, and maximal response of daily AFD Trp intake on egg mass and FCR per individual study are shown in Table 17.

Table 17

*Coefficient of variation, estimates of the parameters $\beta_1$, $\beta_2$, and maximal response of daily AFD Trp intake on egg mass and FCR per individual study.*

| | | Egg mass | | | | FCR | | | |
|---|---|---|---|---|---|---|---|---|---|
| Trial | Reference | $\beta_1$ | $\beta_2$[2] | Maximal response | CV (%)[1] | $\beta_1$ | $\beta_2$[2] | Maximal response | CV (%)[1] |
| 1 | Calderano (2011) | 39.3 | -0.277 | 142 | 1.00% | -0.654 | 0.0031 | 241 | 0.69% |
| 2 | Calderano et al. (2012) | 44.5 | -0.277 | 161 | 2.03% | -0.646 | 0.0031 | 231 | 1.25% |
| 3 | Deponti et al. (2007) | 53.2 | -0.277 | 192 | 1.95% | -0.644 | 0.0031 | 195 | 0.64% |
| 4 | Esteve-Garcia et al. (2001) | 52.0 | -0.277 | 188 | 0.88% | -0.659 | 0.0031 | 171 | 0.78% |

[1] CV (Coefficient of Variation) is a measure of the goodness of fit and indicates the relative deviation between the treatment average and the model fit (CV = √[Variance residual] / [mean] *100).
[2] The estimation of $\beta_2$ differed not significantly between studies, and therefore estimated as one standard value for all studies.

The model fit for egg mass showed a low CV (<2%) in case of 3 out of the 4 studies. The model fit for FCR showed a low CV (<2%) in case of all four studies.
The predicted daily AFD Trp intake for achieving maximal egg mass production ranged from 161 to 192 mg/d. The predicted daily AFD Trp intake for achieving most efficient feed conversion ranged from 207 to 212 mg/d. In appendix 3, the AFD Trp intake levels as recommended by the authors themselves, are provided.
The overall fitted response curve, as determined over all selected studies, of daily AFD and SID Trp intake to the predicted egg mass and FCR are shown in Figure 14 and 15, respectively. The Trp intake level for achieving maximal egg mass and FCR was covered by the tested TRP intake range in 1 out of the 4 experiments.

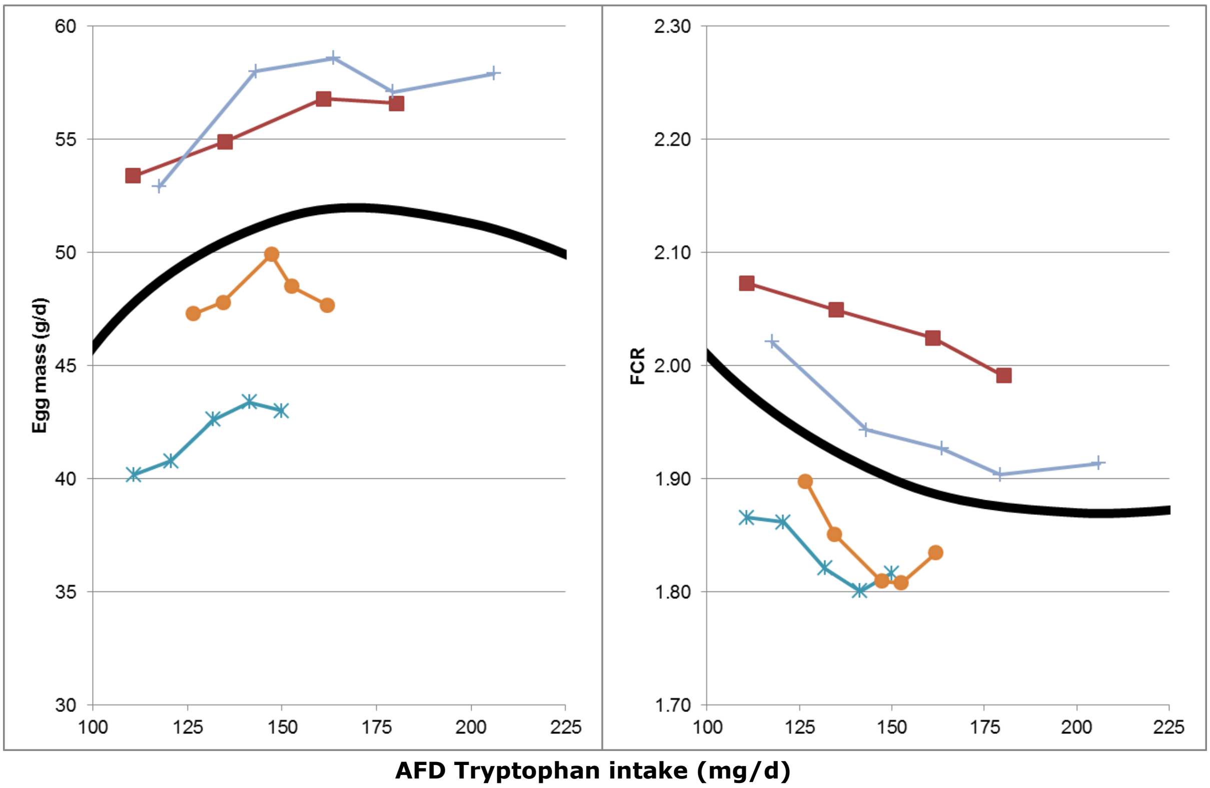



***Figure 14*** *The determined responses of egg mass (g/d) and FCR as a function of the AFD Tryptophan intake of the in Table 16 selected studies, as well as the fitted response (thick solid line).*

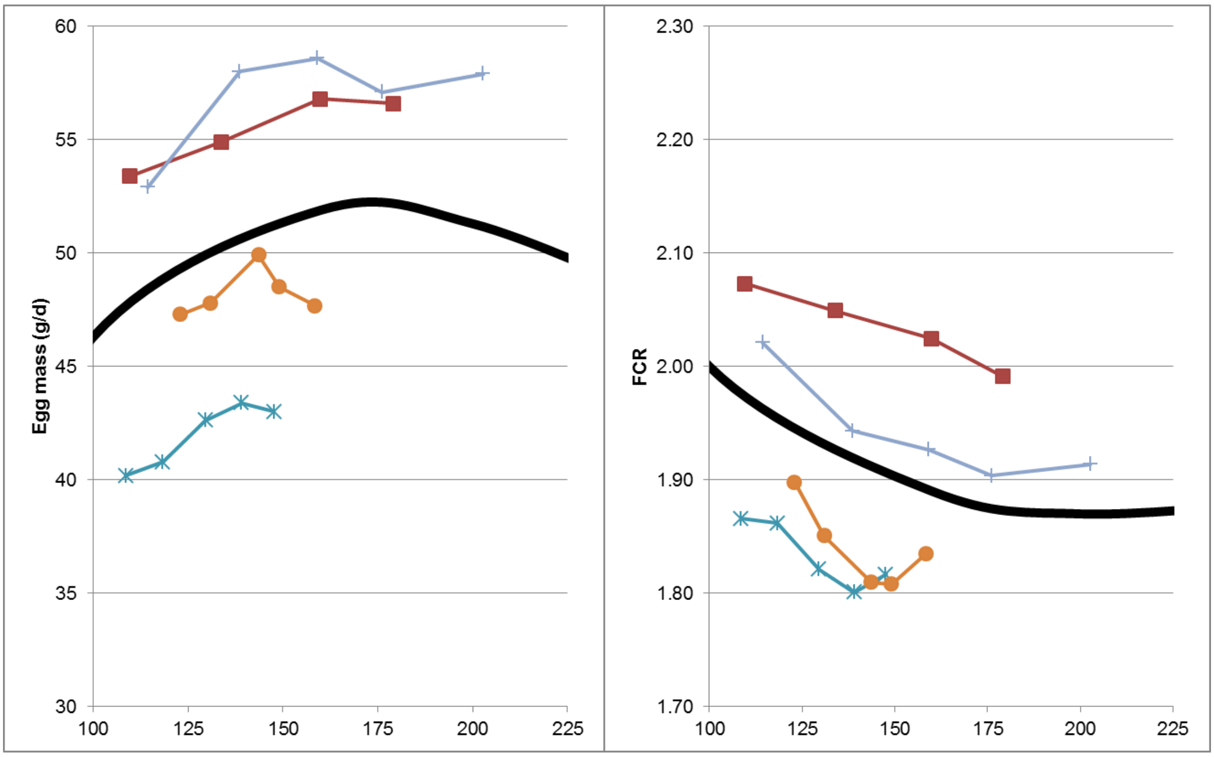



***Figure 15*** *The determined responses of egg mass (g/d) and FCR as a function of the SID Tryptophan intake of the in Table 16 selected studies, as well as the fitted response (thick solid line).*

The estimates of the parameters β0, β1, and β2, as well as their standard error values, for the equations that predict the overall response of AFD or SID Trp intake on rate of lay, egg weight, egg mass, and FCR, are provided in Table 18 and 19, respectively.

Table 18
*Estimates of the parameters β0, β1, and β2, as well as their standard error values, for the equations that predict the response of AFD Trp intake on rate of lay, egg weight, egg mass, and FCR.*

| Parameter | β0 | | β1 | | β2 | |
|---|---|---|---|---|---|---|
| | Value | se | Value | se | Value | se |
| Rate of lay (%) | 83.5 | 2.3 | 70.1 | 20.9 | -0.409 | 0.143 |
| Egg weight (g) | 61.4 | 2.6 | 5.2 | 6.8 | -0.027 | 0.046 |
| Egg mass (g/d) | 51.5 | 3.3 | 49.1 | 14.9 | -0.285 | 0.102 |
| FCR | 1.89 | 0.1 | -0.65 | 0.29 | 0.003 | 0.002 |

Table 19
*Estimates of the parameters β0, β1, and β2, as well as their se values , for the equations that predict the response of SID Trp intake on rate of lay, egg weight, egg mass, and FCR.*

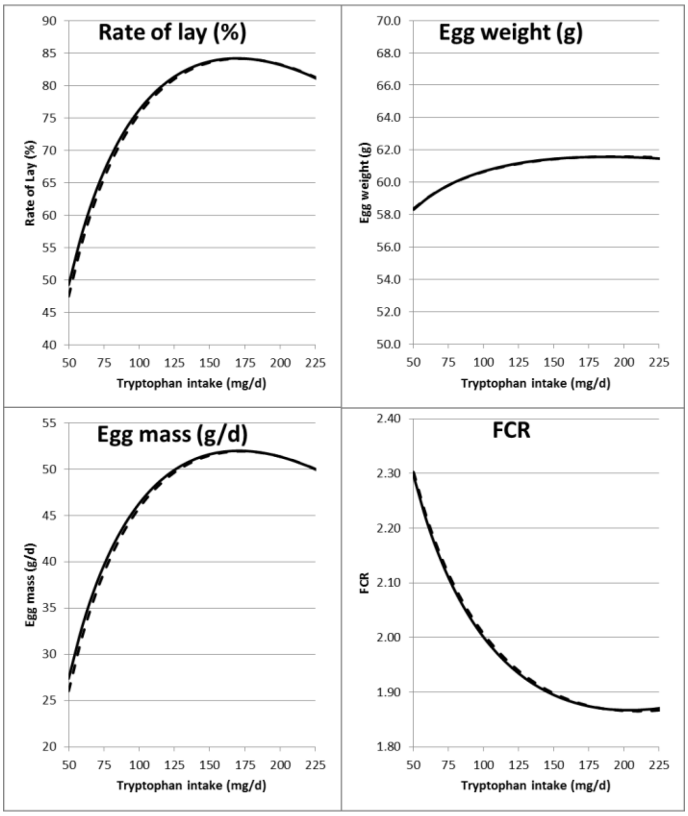
| Parameter | β0 | | β1 | | β2 | |
|---|---|---|---|---|---|---|
| | Value | Se | Value | se | Value | se |
| Rate of lay (%) | 82.7 | 2.4 | 67.9 | 20.4 | -0.401 | 0.141 |
| Egg weight (g) | 61.5 | 2.6 | 5.6 | 6.7 | -0.030 | 0.046 |
| Egg mass (g/d) | 51.6 | 3.3 | 47.3 | 14.5 | -0.277 | 0.100 |
| FCR | 1.90 | 0.1 | -0.66 | 0.28 | 0.003 | 0.002 |

The parameter β0 had a significant ($P<0.05$) contribution to the prediction of rate of lay, egg weight, egg mass and FCR in dependence of the AFD and SID Trp intake. With the exception of egg weight, the same was found for the parameter β1.The parameter β2, however, did not contribute to the prediction of egg weight and FCR. Because of the used REML procedure, and because of the relative small Trp dataset, is was not able to determine the goodness of fit of the general model.
The fitted curves with the relations between the AFD and SID Trp intake on the predicted rate of lay, egg weight, egg mass and FCR are shown in Figure 16. The AFD and SID Trp intake levels for realising maximal performance, as well as for 95% of the maximal response are shown in Table 20. The average starting level of the response amounted 116 mg AFD Trp and 114 mg SID Trp, respectively.

Table 20
*Predicted AFD and SID Trp intake levels (mg/d) for realising maximal hen performance and 95% of the maximal response*

| | AFD Tryptophan intake (mg/d) | | SID Tryptophan intake (mg/d) | |
|---|---|---|---|---|
| | Maximal response | 95% of maximal response | Maximal response | 95% of maximal response |
| Rate of lay (%) | 171 | 158 | 169 | 155 |
| Egg weight (g) | 195 | 173 | 187 | 168 |
| Egg mass | 172 | 158 | 171 | 156 |
| FCR | 210 | 186 | 204 | 180 |

Based on the predicted relationships, laying hens need a daily intake of 172 mg AFD or171 mg SID Trp intake for realizing maximal daily egg mass production. For realizing the maximal feed efficiency, a daily intake of 210 mg ADF or 204 mg SID Trp is required. For realizing 95% of the maximal egg mass production, laying hens need a daily intake of 158 mg AFD Trp or 156 mg SID Trp. For realizing 95% of the maximal feed efficiency, a daily intake of 186 mg ADF or 180 mg SID Trp is required.




dotted line = AFD Tryptophan intake; solid line = SID Tryptophan intake

***Figure 16*** *Curve fitting of the response of AFD and SID Tryptophan intake on the predicted rate of lay, egg weight, egg mass and FCR.*

## 3.5 Responses to Valine, Isoleucine and Arginine

For Val, Ile, and Arg, three, five and one studies, respectively, were collected. None of these studies, however, met the criteria, mainly because the inclusion levels of the non-test amino acids were lower than 90% of the Schutte (1996) requirements.

# 4 Discussion

In par. 4.1 to 4.4, some methodological aspects of this study are discussed. In most cases, Lys is used as an example to demonstrate these aspects, but they are relevant for the other limiting amino acids as well.

## 4.1 Motivation for choosing the Wood’s model

In general, the responses of increasing the intake of limiting amino acids on hen performances within studies showed an exponential course to a maximum value. In some studies, however, amino acid intake levels above the maximum value resulted in a decreasing trend in performance. This typical course of response can be adequately modelled by the Wood’s model, whereas this decreasing trend cannot be simulated by an exponential model. Therefore, the Wood’s model (1967) was chosen for modelling the responses of laying hens on dietary amino acid supply.
In four of the six selected Lys studies (Filho et al., 2006b; Sa et al., 2007b; Sa et al., 2007c; Schmidt et al., 2009b), feed intake level of the highest Lys inclusion level was numerically reduced compared to the penultimate level. Although the highest inclusion level still resulted in an increased absolute Lys intake, the intake of other nutrients, e.g. energy, other amino acids and minerals, decreased. Similarly, a reduced feed intake was observed in at high intake levels of Met+Cys, Thr, and Trp. The reduced feed intake at amino acid intake levels above the requirement may be explained by an imbalance of the dietary amino acid profile (Park, 2006). The current view of amino acid imbalance holds that the
decrease of a limiting amino acid in plasma or an altered ratio of limiting amino acid to total amino acids is detected in the anterior prepyriform cortex of the brain. In the current meta-analysis, dose-response studies were used in which only the amino acid of interest was titrated, whereas the contents of the other amino acids were kept at a constant level. At high inclusion levels of the test amino acids, the ratio between this amino acid and the other ones seriously changed, resulting in the phenomenon of amino acid imbalance. One of the biochemical responses of animals fed amino acid imbalanced diets is a rapid decrease in the plasma concentration of the limiting amino acid, because of an increase in catabolism of the limiting amino acid by the increased activities of enzymes involved in the catabolism of the amino acid (Park, 2006). This results in a competition between the limiting amino acid and the other amino acids in the bloodstream of the anterior prepyriform cortex of the brain. Park (2006) suggested that there is strong evidence that an animal responds to an imbalance in the concentration of a limiting amino acid detected in the bloodstream by decreasing it’s feed intake.

Although the use of the Wood’s model is motivated above, in Appendix 9 for the Lys database a comparison between an exponential model as used by Schutte (1996) and the Wood’s model has been made. Both models are applied on the Lys dataset of Schutte (1996) as well as on the Lys dataset of the current study. It is concluded in Appendix 9 that the differences in recommended Lys levels between the study of Schutte et al. (1996) and the current study can be largely ascribed to differences in datasets between the two studies and to a lesser extent to differences in statistical procedures used. The average recommended AFD lysine level for 95% of maximal response based on the dataset of Van Krimpen is 823 mg/d when using the statistical method used by Van Krimpen. When using the statistical method used by Schutte (1996), the average recommended AFD lysine level based on the dataset of Van Krimpen is 784 (based on all observations) or 801 mg/d (based on a reduced number of observations).

## 4.2 Methods of estimating the requirements

In the present study, the Wood's curve was used to model the response towards increasing intake of a limiting amino acid. In Table 21, for each Lys experiment, the estimated AFD Lys intake according to the Wood's model for achieving maximal egg mass response is compared with the recommended AFD Lys intake values of the authors of the original papers, as well as with the estimated value for achieving 95% of the response based on a logarithmic model.

Table 21
*The estimated AFD Lysine intake according to the Wood's model for achieving maximal egg mass response as compared with the recommended AFD Lys intake values of the authors, as well as with the estimated value for achieving 95% of the response based on a logarithmic model*

| Trial | Reference | Lysine requirement | | |
|---|---|---|---|---|
| | | Maximal response according to the Wood's model | Of the authors | 95% of response according to a logarithmic model |
| 1 | Filho et al. (2006b) | 881 | 910 | 775 |
| 2 | Sa et al. (2007b) Exp. 1 | 940 | 893 | 825 |
| 3 | Sa et al. (2007c) Exp. 2 | 875 | 804 | 975 |
| 4 | Schmidt et al. (2008) | 939 | 885[1] | 970 |
| 5 | Schmidt et al. (2009b) | 852 | 783 | 772 |
| 6 | Schutte and Smink (1998) | 785 | 720 | 660 |

[1] The final Lysine inclusion level still showed a significant effect. Therefore, according to the authors, *at least* this value is recommended.

For some studies (Sa et al., 2007c; Schmidt et al., 2008), the Wood's model and the logarithmic curve estimate a higher AFD Lys intake requirement compared to the requirements of the original authors. This can be explained by the fact that the Lys intake level for maximal response estimated by both models is (far) out of the range of inclusion levels tested in the concerning studies.
As an illustration, in Figures 17 and 18, the differences between the different types of requirements are made transparent by showing the original data and the results of the curve fitting.

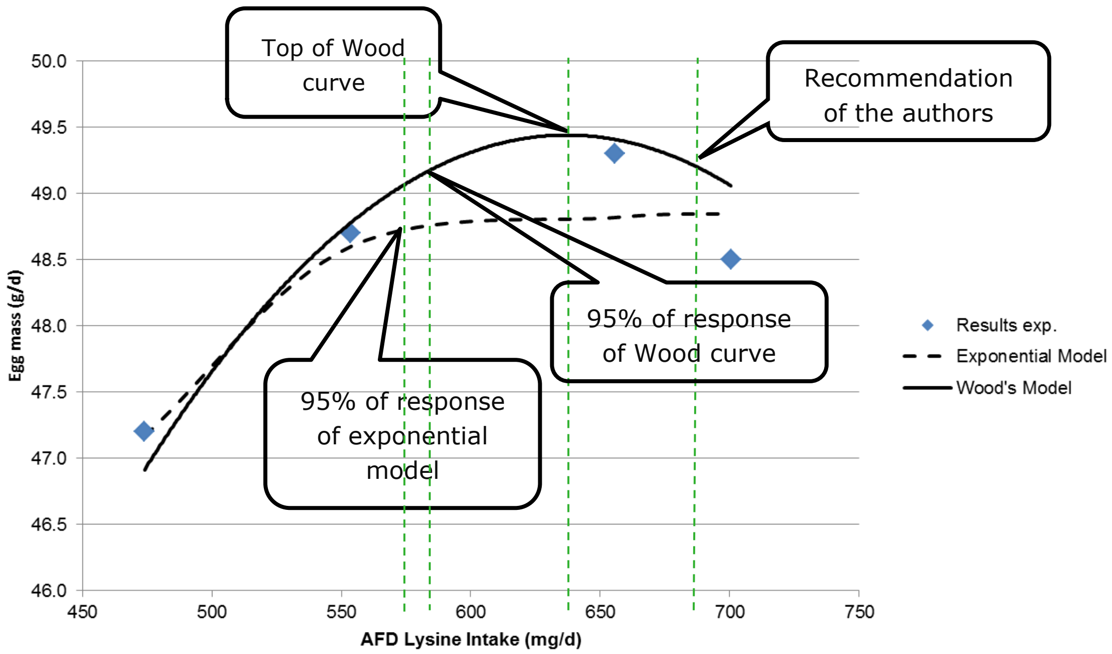



**Figure 17** *Response of hens on AFD Lysine intake in the experiment of De Moraes Jardim Filho et al. (2010), the requirement of the authors and the results of the curve fitting.*

Figure 17 shows that De Moraes Jardim Filho et al. (2010) recommended a daily AFD Lys intake of 684 mg/hen, which is 48 mg/d higher compared to the maximal response according to Wood's model. In this experiment, the FCR further improved at the highest Lys inclusion level and the authors involved this result in their requirement. The level of 95% of the maximal response fitted by an exponential curve was achieved at a AFD Lys intake of 572 mg/d. The Wood's model predicted the results of this study more precisely ($R^2$ = 0.88) than the exponential model ($R^2$ = 0.58).

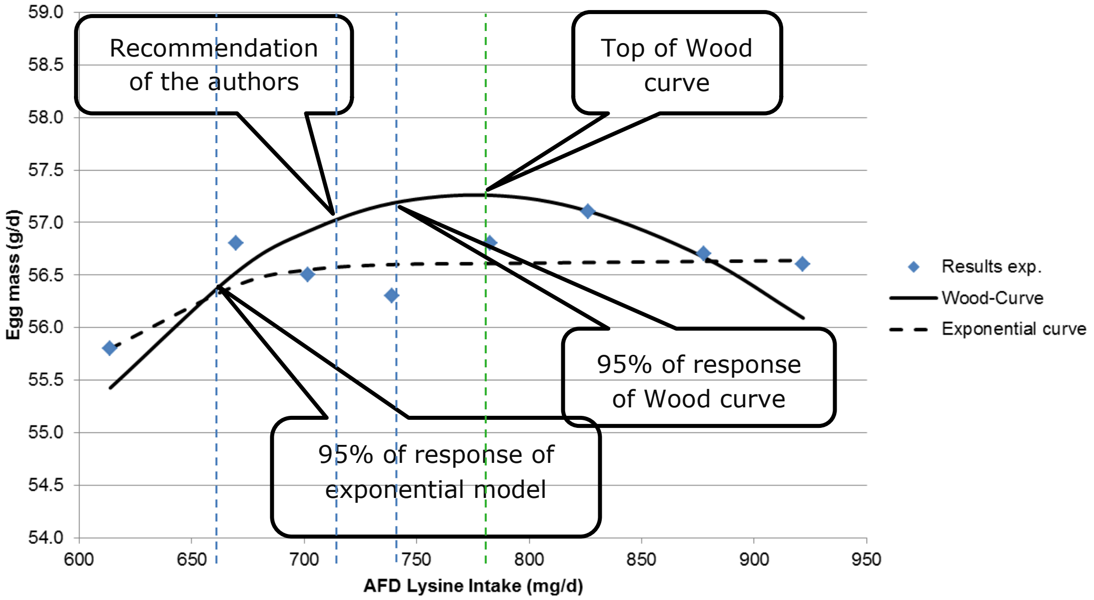



***Figure 18*** *Response of hens on AFD Lysine intake in the experiment of Schutte and Smink (1998), the requirement of the authors and the results of the curve fitting.*

Figure 18 shows that Schutte and Smink (1998) recommended a daily AFD Lys intake of 720 mg/hen, which is 61 mg/d below the maximal response according to Wood's model. The level of 95% of the maximal response fitted by an exponential curve was achieved at a AFD Lys intake of 660 mg/d. The fit for the values of this study was weak for both the exponential model ($R^2$ = 0.49) as well as for the Wood's model ($R^2$ = 0.42). The examples mentioned in Figure 17 and 18 show that the choice for a curve model, as well as the basis for requirement (95% or 100% of response) affect the derived amino acid requirement levels.
Based on the data of the study of Filho et al. (2006b) the estimation of the maximal response to egg mass by use of the Wood curve was lower compared to the requirement of the authors themselves. In this particular study, egg mass was not significantly affected by the AFD Lys intake level. The authors based their requirement on the maximal response to rate of lay and FCR, which was a different basis compared to the basis used for fitting of the Wood curve. In many studies, however, the basis for the requirement of the authors is not clearly described.

# 4.3 The impact of neglecting random terms in curve fitting

In the meta-analysis study of Schutte (1996), which is the basis of the current CVB requirements (CVB, 2012), an exponential model was used to model the response of laying hens on AFD amino acid intake. In the statistical analysis of that study all data were considered as independent observations, thereby neglecting that level and response effects within individual studies (neglecting random terms). To demonstrate this omission, in Figure 19 the response of AFD Lys intake on FCR is shown, thereby both including and excluding the random terms in the overall model.

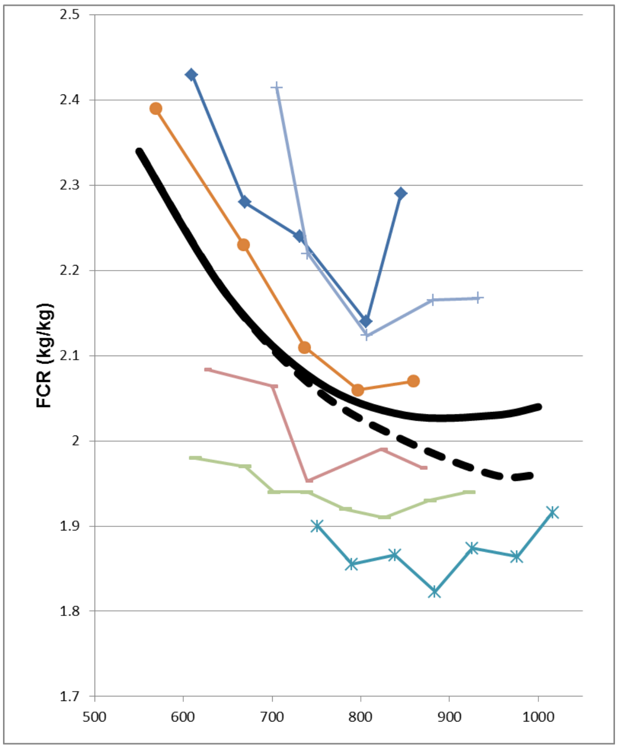



***Figure 19*** *The determined responses of FCR as a function of the AFD Lys intake per individual experiment and the modelled overall curve with (thick solid line) and without (thick dotted line) random terms taken into account.*

In case of neglecting the random term, the daily AFD Lys intake for most efficient FCR was estimated on 1021 mg/d, versus 897 in case of including the random term. Schutte (1996) corrected for ignoring the random terms by transforming the original data. He recalculated the absolute FCR values by subtracting the value of the lowest FCR level from each average per treatment. The applied meta-analysis in the current study, however, eliminates the need of such data transformation. Therefore, to our opinion the approach taken in the current study is more powerful compared to the approach used by Schutte (1996).

## 4.4 Considerations of the dataset with Lys dose response studies

A total of 6 studies met the inclusion criteria, and this number is rather low for a meta-analysis approach. Moreover, although the most recent studies were selected in the current meta-analysis, the performance levels of the acquired studies is below the performance level of modern type laying hens. In the selected lysine studies, average egg mass and FCR at the level of maximal response are predicted to be 56.4 g/d and 2.03 kg/kg, respectively, which is rather low compared to the performance level of modern laying hens. Figure 20 shows the relative response of egg mass, expressed as percentage of increase compared to the performance level at the basal level, as a function of the AFD Lys intake per individual experiment and for the overall model. Surprisingly, all six selected Lys studies reached their maximal egg mass response at an AFD Lys intake level between a rather narrow range of 790 to 860 mg/d, independent of their absolute maximal egg mass level, which ranged from 54.8 to 59.2 g/d. Likewise, within a slightly increased AFD Lys intake range of 740 to 860 mg/d, the maximal relative response of FCR to AFD Lys intake ranged from 1.82 to 2.14, as shown in Figure 21. The absolute performance level of the Lys studies, therefore, seems not the most determining factor for realizing the maximal response.
The rate of lay usually reduces with increasing age, as a consequence of an increase in intra-sequence ovulation and oviposition intervals, as well as due to an increase in the number of pause days (Johnston and Gous, 2003). Although modern strains of hens are used in all selected experiments, the

low egg performance levels in some studies could be the results of the high ages of these hens, consequently resulting in reduced rates of lay due to increased intervals. Nevertheless, these flocks still seem to respond in a comparable way on increasing Lys supplementation levels as high performing flocks. Proof for this phenomena was provided by Wethli and Morris (1978), who showed that the response of a flock of laying hens to Trp at the end of the first production year was different from the response of the same flock at peak production (Morris and Wethli, 1978). Nevertheless, the estimated Trp requirement for maximal response was rather similar (184 vs 174 mg/hen/d) for both ages. The main difference between both ages was the reduced rate of lay of the older hens, but apparently this did not largely affect the Trp requirement. Interestingly, the same flock was moulted at 91 wk of age, where after again the Trp requirement was determined. The response of the moulted flock, as well as the estimated Trp requirement (184 vs 179 mg/hen/d), was similar to those of the young hens. Based on these findings, it is suggested that the Lys responses determined in the current study will also be applicable for nowadays flocks with higher performance levels. This, however, should be validated in studies with high performing laying hens.
As shown in Figure 22 and 23 this conclusion, however, is not valid for the responses to the Met+Cys intake. The Met+Cys intake levels for realizing the maximal response on egg mass and FCR range from 560 to 850 mg/d. This aspect will be discussed in more detail in the Methionine and Cysteine section below.

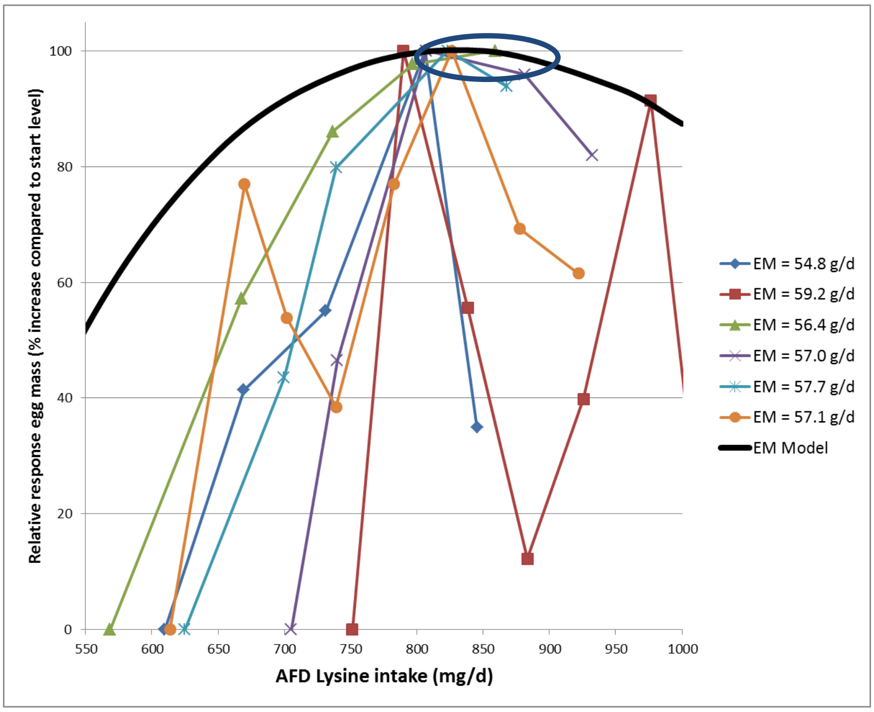



***Figure 20*** *The relative response of egg mass, expressed as percentage of increase compared to the performance level at the basal level, as a function of the AFD Lys intake level per individual experiment and the modelled overall curve (thick solid line); the maximal level of egg mass (EM) of each experiment is mentioned in the legend.*

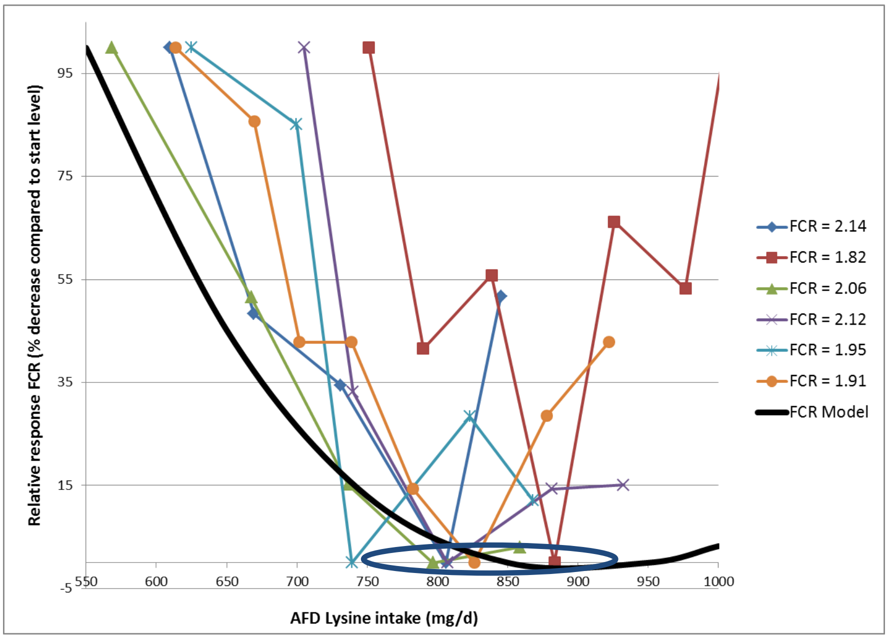



**Figure 21** *The relative response of FCR, expressed as percentage of decrease compared to the performance level at the basal level, as a function of the AFD Lys intake level (mg/d) per individual experiment and the modelled overall curve (thick solid line); the minimal level of FCR of each experiment is mentioned in the legend.*

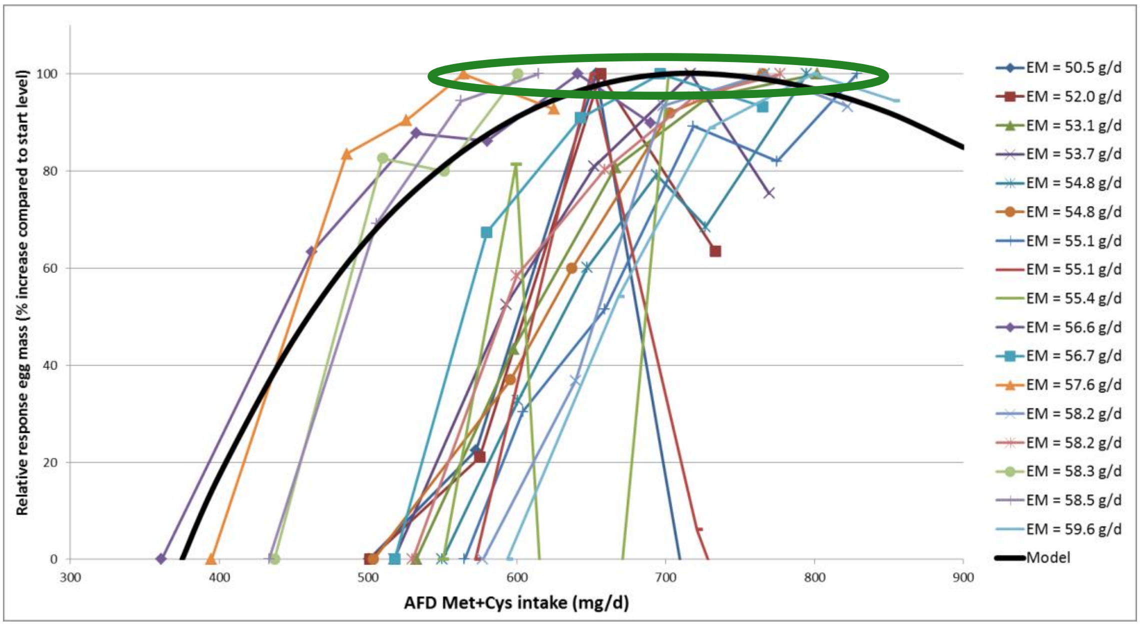



**Figure 22** *The relative response of egg mass, expressed as percentage of increase compared to the performance level at the basal level, as a function of the AFD Met+Cys intake level per individual experiment and the modelled overall curve (thick solid line); the maximal level of egg mass (EM) of each experiment is mentioned in the legend.*

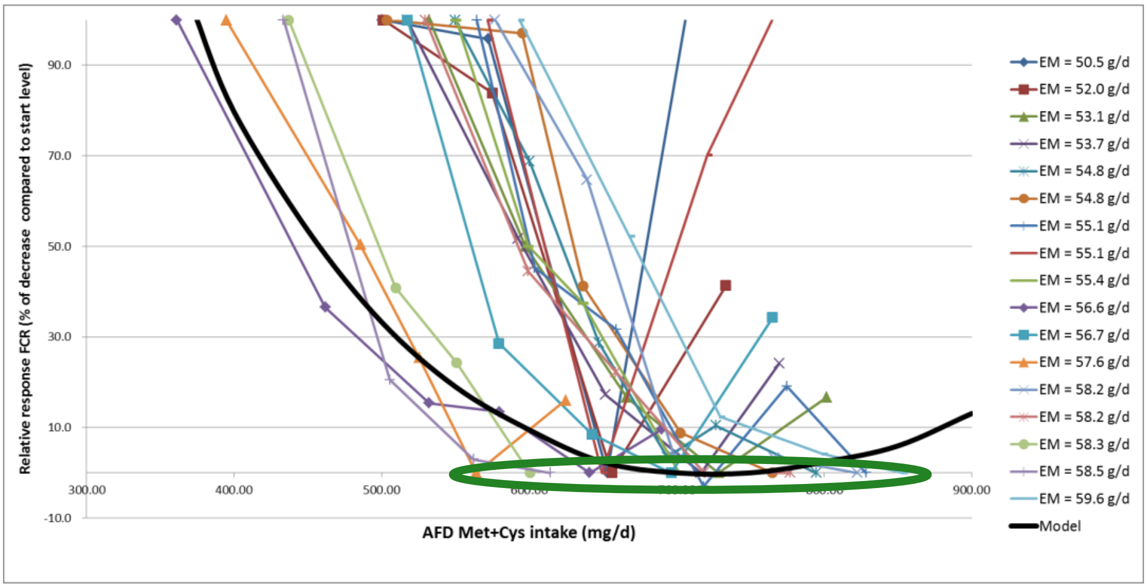



***Figure 23*** *The relative response of FCR, expressed as percentage of decrease compared to the performance level at the basal level, as a function of the AFD Met+Cys intake level (mg/d) per individual experiment and the modelled overall curve (thick solid line); the minimal level of FCR of each experiment is mentioned in the legend.*

## 4.5 Considerations of the amino acid requirement values

*Lysine*

The NRC (1994) requires for white and brown hens a daily total Lys intake of 690 and 760 mg, respectively. Schutte (1996) requires a daily AFD Lys intake of 700 mg, which is based on a performance level of 95% of the maximal response. For realizing 95% of the maximal response, the adjective AFD Lys intake levels for egg mass and FCR in the current study amounted 820 and 825 mg/d, respectively, meaning an increase of 17% and 18% compared to the requirement of Schutte (1996).

These findings are in line with those of Bonekamp *et al.* (2010), who concluded that light and heavy hens need at least a daily intake level of 800 mg true faecal Lys for maximal hen performances. In the study of Bonekamp *et al.* (2010), however, the ratios between Lys and the other essential amino acid were kept on a constant level according to the ratios recommended by Schutte (1996). This was not the case in the current meta-analysis study, and it could be suggested that the levels of the other AA were the limiting factor for further response. Especially in the lysine-database, however, the level of the non-test amino acids were relatively high, and ranged on an AFD basis from 105 to 115% of the level, which is recommended in the current report for egg mass (see Appendix 4). Therefore, the suggestion that the level other amino acids might be the limiting factor for a further Lys response does not seem to be obvious.

In the study of Bonekamp *et al.* (2010), the hens showed still a response at the highest AA dosage. Therefore, an experiment is recommended in which SID Lys intake levels above 800 mg/d are investigated under conditions of ideal protein composition. In such an experiment, the ileal AA digestibility of the basal diet should be determined as well.

*Methionine and Cysteine*

The NRC (1994) recommends for white and brown hens a daily total Met+Cys intake of 580 and 645 mg, respectively. Schutte (1996) recommends a daily AFD Met+Cys intake of 650 mg. For realizing 95% of the maximal response, the adjective AFD Met+Cys intake levels for egg mass and FCR in the current study amounted 670 and 675 mg/d, respectively, meaning an increase of 3% and 4% compared to the requirement of Schutte (1996). The level of Met+Cys increase relative to Schutte (1996), however, is rather low, compared to the increase in Lys, Thr and Trp requirements. This might be explained by the low AFD Lys intake level (88% of the recommended level for egg mass, Appendix 5) in the selected Met+Cys studies. Only 2 out of the 17 selected Met+Cys studies met the Lys requirement of the current study, whereas the AFD Thr (101%) and Trp (103%) intake levels met the

required levels for egg mass. Therefore, it can be concluded that the AFD Lys intake levels of the current study were a limiting factor for further response on AFD Met+Cys intake. The large variation in Lys intake levels might also explain the large variation in relative response of Met+Cys intake on egg mass (Figure 22) and FCR (Figure 23).
The contrasts in Met+Cys intake within experiments were realized by adding free Met to the diet. Thus, Cys intake was constant within experiments and amounted 223 mg/d among the selected experiments. After subtraction of these 224 mg of Cys intake from the Met+Cys intake, the remaining Met intake for maximal egg mass and feed efficiency amounted 447 and 452 mg/d, respectively. These values are both higher than the recommended value of Strathe *et al.* (2011), who concluded after a meta-analysis study that the biological requirement for AFD Met is at least 356 mg/d. The difference between the Met requirements of the current experiment and the study of Strathe *et al.* (2011) might be partly caused by the relative low Cys intake levels of the selected studies in our meta-analysis study. Schutte (1996) recommended a daily AFD Cys intake of 300 mg/d, which is 76 mg/d higher than the average intake level of the selected studies.

*Threonine*
The NRC (1994) recommends for white and brown hens a daily total Thr intake of 470 and 520 mg, respectively. Schutte (1996) recommends a daily AFD Thr intake of 460 mg. For realizing 95% of the maximal response, the adjective AFD Thr intake levels for egg mass and FCR in the current study amounted 500 and 575 mg/d, respectively, meaning an increase of 9% and 25% compared to the requirement of Schutte (1996). In the selected Thr studies, the AFD Lys intake level amounted only 86% of the recommended level for egg mass (Appendix 6). None out of the 6 selected Thr studies met the Lys requirement of the current study, whereas the AFD Met+Cys (97%) and Trp (107%) intake levels were close to or exceeded the required levels for egg mass. Therefore, it can be concluded that the AFD Lys intake levels of the current study were a limiting factor for further response on AFD Thr intake.

*Tryptophan*
The NRC (1994) recommends for white and brown hens a daily total Trp intake of 160 and 175 mg, respectively. Schutte (1996) recommends a daily AFD Trp intake of 130 mg. For realizing 95% of the maximal response, the adjective AFD Trp intake levels for egg mass and FCR in the current study amounted 158 and 186 mg/d, respectively, meaning an increase of 22% and 43% compared to the requirement of Schutte (1996). For 2 of the 4 selected studies, the Trp intake for realizing the maximal FCR response was much higher than the tested Trp intake range, which makes these estimates less reliable.
The results of the current study are based on a limited (n=4) number of useful experiments, which is very low for a meta-analysis. Schutte (1996) had to deal with it as well. He could make use of only two useful studies. There is a need for more studies that determine the Trp requirement of laying hens. In the selected Trp studies, the AFD Lys intake level amounted only 82% of the recommended level for egg mass (Appendix 7). Only 1 out of the 4 selected Trp studies met the Lys requirement of the current study, whereas the AFD Met+Cys (94%) and Thr (98%) intake levels were also (slightly) below the required levels for egg mass. Therefore, it can be concluded that especially the AFD Lys intake levels, and to a lesser extend the Met+Cys intake levels, of the current study were a limiting factor for further response on AFD Trp intake.

*Impact of AA intake on hen performance*
The AA Met+Cys and Lys showed strong effects on egg weight, whereas Thr and Trp had no significant effect on egg weight. Supplementation of Thr and Trp, however, resulted in strong responses to rate of lay, whereas the responses to rate of lay were moderate in case of Lys and Met+Cys supplementation.
The recommended amino acid intake levels for rate of lay were very close or similar to those for egg mass. Observations in practice show that hens realize maximal rate of lay at much lower amino intake levels compared to maximal egg mass. Practical diets, however, show variation in the amino acid content, whereas the ratios between the amino acids usually remain more or less constant. Contrary to this, in the assessed experiments of the current study, all amino acids were kept at a constant level, except for the amino acid of interest. From the determined response curves to Met+Cys intake, for instance, it can be concluded that the rate of lay significantly decreased at a Met+Cys intake level

above the maximal response. The hens, however, need a much higher Met+Cys intake level for realizing maximal egg weight compared to maximal rate of lay. The combination of a strong reduction in rate of lay and a small further increase in egg weight at Met+Cys intake levels above the maximal rate of late, results in a Met+Cys intake level for realizing maximal egg close to that of maximal rate of lay.

## 4.6 Effect of basis of amino acid evaluation on requirements

In Table 22, the AA requirements for 95% of maximal performance are summarized, both on the basis of AFD and SID.

Table 22
*AA requirements (mg/d) for maximal performance, both on the basis of AFD and SID*

| Basis | On AFD basis | | | On SID basis | | |
|---|---|---|---|---|---|---|
| Parameter | Rate of lay (%) | Egg mass (g/d) | FCR | Rate of lay (%) | Egg mass (g/d) | FCR |
| Lysine | 810 | 820 | 825 | 850 | 855 | 855 |
| Met+Cys | 650 | 670 | 675 | 650 | 670 | 675 |
| Threonine | 485 | 500 | 575 | 520 | 530 | 590 |
| Tryptophan | 158 | 158 | 186 | 155 | 156 | 180 |

There is no direct relationship between the amino acid requirements estimated on an AFD or SID basis, and therefore a direct comparison between both systems is not relevant. The background of both AA evaluation systems is explained below.
AFD amino acid contents of feed ingredients are based on studies which determined AA contents in excreta. The excreta of poultry, however, consist not only of faeces but also of urine. Urinary nitrogen is therefore a source of error in determining faecal AA digestibility in poultry. Moreover, in the hindgut, AA could be fermented by the gut flora. In that case, the AA are determined as disappeared, although they are not utilized by the host animal. Hence, faecal digestibility of amino acids will not always represent the amino acids that actually become available to the animal. Therefore, the digestibility at the end of the ileum is considered a better indication of the availability of protein and amino acids than the faecal digestibility (Ravindran and Bryden, 1999; Ravindran et al., 1999; Kadim et al., 2002). Ileal digesta contains not only undigested dietary material, but also endogenous components, such as digestive enzymes, mucus and desquamated epithelial cells (Jansman et al., 2002). At the terminal ileum, the digesta also contains some microbial protein, produced from either feed protein or endogenous protein by bacteria in the small intestine. This bacterial protein is strictly not endogenous, but is usually included in estimations of endogenous protein (Moughan et al., 2005; Miner-Williams et al., 2009).
At the terminal ileum, a distinction can be made in basal endogenous losses (losses due to the passage of dry matter through the digestive tract) and specific endogenous losses (losses induced by diet composition, e.g., caused by presence of anti-nutritional factors and dietary fibre). Basal endogenous losses can be defined as inevitable losses closely associated with general metabolic processes in the animal and are independent of diet composition (Cowieson et al., 2009). The basal endogenous flow of crude protein and amino acid flow at the terminal ileum is independent of the composition of the diet and its ingredients and proportional to dry matter intake (Jansman et al., 2002; Lemme et al., 2004a). The basal endogenous losses may be accounted for in the determination of ileal amino acid digestibility of feed ingredients, yielding the standardized ileal digestibility values for amino acids. As apparent ileal digestibility values for amino acids in feed ingredients are sometimes incorrectly calculated in digestibility studies, determination of standardized ileal amino acid digestibility values (SID) provide a more secure basis for the evaluation of the amino acid value of feedstuffs than apparent ileal digestibility values. Moreover, SID values of the feedstuffs comprising the diet are generally additive of nature (Angkanaporn et al., 1996; Stein et al., 2005; Adedokun et al., 2011).

## 4.7 Amino acid ratios

In Table 23, the ideal amino acid ratios relative to Lysine for realizing 95% of maximal egg mass production of the current study are compared with several other published data. The values of the current study are based on the AA requirements for egg mass, as shown in Table 22.

Table 23
*Amino acid ratios relative to Lysine for realizing egg mass production*

| Source | Current study[1] | | NRC [2] (1994) | Schutte[1] (1996) | Bregendahl [3] *et al.* (2008) | Egg composition (USDA, 2011) |
|---|---|---|---|---|---|---|
| AA basis | AFD | SID | Total | AFD | True dig. | |
| Lysine | 100 | 100 | 100 | 100 | 100 | 100 |
| Met+Cys | 82 | 78 | 84 | 93 | 94 | 72 |
| Threonine | 61 | 62 | 68 | 66 | 77 | 61 |
| Tryptophan | 19 | 18 | 23 | 19 | 22 | 18 |

[1] For 95% of maximal egg mass
[2] For 100% of maximal hen performance
[3] Based on the horizontal plateau value estimated with a broken-line analysis

The ratios of Met+Cys and Thr relative to Lys of the current study, both on an AFD and SID basis, are slightly lower compared to those recommended by NRC (1994), whereas similar values for the Trp ratio were found. Schutte (1996) recommended a higher share of Met+Cys and Thr, whereas Bregendahl *et al.* (2008) suggested a higher ratios of all amino acids.
The ratios of the AA compared to Lys in eggs are lower than the ideal ratios for feed.
The ratios of the amino acids relative to Lys in the current study are based on the derived amino acid intake levels for maximal response in different databases. In each database, the response of titration of one specific amino acid on performance was measured, while the other amino acids were not limiting according to Schutte (1996). The experiments in these databases, however, were not designed for developing the ideal ratio between Lys and the other limiting amino acids. The determined ratio between AFD Met+Cys to Lys of 82 in the current study was rather low compared to the ratio used in practice (about 93). Therefore, the ratios of the amino acids relative to Lys were determined in the individual Met+Cys dose response studies at the Met+Cys intake level for realizing maximal egg mass. The details are provided in Annex 4. The results are summarized in Table 24.

Table 24
*Average amino acid intake levels and ratios relative to Lys in the Met+Cys dose response studies at the Met+Cys intake level for realizing maximal egg mass, as well as the requirements of Schutte (1996) and the current study*

| | AFD amino acid intake level (mg/d) | | | |
|---|---|---|---|---|
| **Basis** | **Lys** | **Met+Cys** | **Thr** | **Trp** |
| Average of 17 Met+Cys studies | 720 | 725 | 506 | 163 |
| Requirement Schutte (1996) | 700 | 650 | 460 | 130 |
| Requirement current study for max. egg mass | 879 | 720 | 553 | 172 |
| | Ratios relative to Lysine | | | |
| Ratio to Lys, based on 17 Met+Cys studies | 100 | 101 | 70 | 23 |
| Ratio to Lys according to Schutte (1996) | 100 | 93 | 66 | 19 |
| Ratio to Lys according to current study for max. egg mass | 100 | 82 | 63 | 20 |

The hens in the Met+Cys experiments realized maximal egg mass with a daily AFD intake of 720 mg Lys and 725 mg Met+Cys, resulting in a ratio relative to Lys of 101. Based on these values, however, it cannot be concluded that this ratio of 101 is ideal for realizing maximal egg mass. There is a need for new experiments to validate the ideal amino acid ratios in laying hen diets.

## 4.8 Comparison with commercial recommendations

Table 25 provides some commercial AA recommendations (mg/d) for laying hens, which are compared with the results of the current study.

Table 25

*Commercial AA recommendations (mg/d) for laying hens, as well as the results of the current study for realizing 95% of maximal egg mass.*

| Source | Current study[1)] | Adisseo | Ajinomoto | ISA | Current Study[1)] | Evonik |
|---|---|---|---|---|---|---|
| AA basis | AFD | AFD | AFD | AFD | SID | SID |
| Lysine | 820 | 838 | 800 | 805 | 855 | 831 |
| Met+Cys | 670 | 714 | 680 | 690 | 670 | 756 |
| Threonine | 500 | 577 | 560 | 560 | 530 | 582 |
| Tryptophan | 158 | 155 | 192 | 178 | 156 | 174 |

[1]) For 95% of maximal response on egg mass

The AFD AA recommendations of Adisseo are in between to the results of the current study for realizing 95% to 100% of the max. response. The AFD AA recommendations of Ajinomoto and ISA (Hendrix Genetics) are also in between the values of the current study for 95% and 100% of maximal response, except for Lys, which recommendation is slightly lower than the value of the current study for realizing 95% of the max. response.

The AA recommendations of Evonik are in between the SID values of the current study for 95% and 100% of maximal response, except for Met+Cys, which exceeds the value for realizing 100% of the max. response by 31 mg/d.

## 4.9 Requirements for valine, isoleucine and arginine

Although several experiments were available for Val (n=3), Ile (n=5) and Arg (n=1), none of these studies met the criteria for inclusion into the final database. Therefore, data was lacking for updating the requirements of these amino acids.

Schutte (1996) estimated an AFD Val requirement of 600 mg/d and an AFD Ile requirement of 550 mg/d. ISA (2009) recommends a daily AFD Val intake of 775 mg and a daily AFD Ile intake of 725 mg. Adisseo (2013) recommends a daily AFD Val intake of 787 mg, a daily AFD Ile intake of 662 mg, and a daily Arg intake of 838 mg. Evonik (2012) recommends a daily SID Val intake of 731 mg, a daily SID Ile intake of 665 mg and a daily Arg intake of 864 mg.

It can be concluded that the current recommendations for Val and Ile in practice are higher compared to the estimated requirements of Schutte (1996). There is a need for further research to underpin these recommendations.

The average AFD intake levels of Ile, Val, and Arg in the different AA databases of the current study are shown in Table 26.

Table 26
*average AFD intake levels (mg/d) of isoleucine, valine, and arginine in the different AA databases of the current study.*

| Database | Isoleucine | Valine | Arginine |
|---|---|---|---|
| Lysine | 634 | 704 | 920 |
| Met+Cys | 595 | 661 | 939 |
| Threonine | 553 | 599 | 722 |
| Tryptophan | 570 | 637 | 756 |

It can be concluded that the average intake levels of Ile, Val, and Arg in the different AA databases met the requirements of Schutte (1996), although they were below the recommendations of some commercial groups. It is unknown whether the intake levels of Ile, Val, and Arg have affected the estimation of the requirement of the essential amino acids.

# 5 Conclusions

The derived AA requirements for realizing the maximal response on rate of lay, egg mass and FCR, as well as for realizing 95% of these maximal responses, and the ratio of the limiting amino acids to Lys on AFD and SID basis are summarized in the four tables below.

Apparent Faecal Digestible (AFD) amino acid intake levels (mg/d) for realizing 95% of maximal response to rate of lay, egg mass and FCR, as well as the amino acid ratios relative to Lys

| Basis | AFD requirement (g/d) | | | AFD requirement (relative to Lys) | | |
|---|---|---|---|---|---|---|
| Parameter | Rate of lay (%) | Egg mass (g/d) | FCR | Rate of lay (%) | Egg mass (g/d) | FCR |
| Lysine | 810 | 820 | 825 | 100 | 100 | 100 |
| Met+Cys | 650 | 670 | 675 | 80 | 82 | 82 |
| Threonine | 485 | 500 | 575 | 60 | 61 | 70 |
| Tryptophan | 158 | 158 | 186 | 20 | 19 | 23 |

Standardized Ileal Digestible (SID) amino acid intake levels (mg/d) for realizing 95% of maximal response to rate of lay, egg mass and FCR, as well as the amino acid ratios relative to Lys

| Basis | SID requirement (g/d) | | | SID requirement (relative to Lys) | | |
|---|---|---|---|---|---|---|
| Parameter | Rate of lay (%) | Egg mass (g/d) | FCR | Rate of lay (%) | Egg mass (g/d) | FCR |
| Lysine | 850 | 855 | 855 | 100 | 100 | 100 |
| Met+Cys | 650 | 670 | 675 | 76 | 78 | 79 |
| Threonine | 520 | 530 | 590 | 61 | 62 | 69 |
| Tryptophan | 155 | 156 | 180 | 18 | 18 | 21 |

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# Appendix 1 Overview of non-selected papers

**Lysine**

| Reference | Reason of exclusion of the reference |
|---|---|
| Bouyeh and Gevorgian (2011) | Other amino acids do not meet CVB requirements; feed intake differs between treatments |
| Cupertino et al. (2009a) | Other amino acids do not meet CVB requirements |
| De Carvalho et al. (2012) | Other amino acids do not meet CVB requirements; too less dose levels |
| De Moraes Jardim Filho (2010) | Feed intake differs between treatments |
| Faria et al. (2003) | Other amino acids do not meet CVB requirements |
| Figueiredo et al. (2012) | Feed intake differs between treatments |
| Gordon (2009) | Other amino acids do not meet CVB requirements |
| Neto et al. (2011) | Other amino acids do not meet CVB requirements; feed intake differs between treatments |
| Pacheco et al. (2010) | Other amino acids do not meet CVB requirements; no performance data available |
| Prochaska et al. (1996) | Feed intake differs between treatments |
| Rao et al. (2011) | Other amino acids do not meet CVB requirements |
| Da Rocha et al. (2009) | Other amino acids do not meet CVB requirements |
| Da Silva et al. (2006) | No dose-response study; too less dose levels |
| Silva et al. (2010) | Other amino acids do not meet CVB requirements |

**Methionine**

| Reference | Reason of exclusion of the reference |
|---|---|
| Bertram et al. (1995a) | Other amino acids do not meet CVB requirements |
| Bertram et al. (1995b) Exp. 1 | Other amino acids do not meet CVB requirements |
| Bouyeh and Gevorgian (2011) | Other amino acids were not kept at a constant level |
| Carvalho et al. (2009) | Performance level extreme low; data not representative for commercial strains |
| Da Silva et al. (2006) | No dose-response study; too less dose levels |
| Da Silva et al. (2009) | Performance data incomplete (dose – strain interactions were not mentioned) |
| Harms and Russel (1998) | Too less dose levels |
| Harms and Russel (2003) | Very low feed intake level; other amino acids were not kept at a constant level |
| Koreleski and Swiatkiewicz (2009) | Too less dose levels |
| Koreleski and Swiatkiewicz (2010) | Too less dose levels |
| Liu et al. (2005) | Performance level extreme low; data not representative for commercial strains |
| Polese et al. (2012a) | No responses of increased amino acid dose on performance |
| Polese et al. (Polese et al., 2012b) | No responses of increased amino acid dose on performance |
| Rao et al. (2011) | Other amino acids do not meet CVB requirements |
| Schutte et al. (1994) Exp. 1 | Other amino acids do not meet CVB requirements |
| Waldroup and Hellwig (1995) | Amino acid contents do not follow a dose-response pattern |
| Zeweil et al. (2011) | Performance level extreme low; data not representative for commercial strains |

**Threonine**

| Reference | Reason of exclusion of the reference |
|---|---|
| Azzam et al. (2011) | Other amino acids do not meet CVB requirements |
| Faria et al. (2002) | Other amino acids do not meet CVB requirements |
| Figueiredo et al. (2012) | No performance data were provided |
| Gomez and Angeles (2009) | Feed intake differs between treatments; too less dose levels |
| Huyghebaert and Butler (1991) | Paper out of the required time range; other amino acids were not kept at a constant level |
| Ishibashi et al. (1998) | Other amino acids do not meet CVB requirements; other amino acids were not kept at a constant level |
| Martinez-Amezcua et al. (1999) | Other amino acids do not meet CVB requirements |

**Tryptophan**

| Reference | Reason of exclusion of the reference |
|---|---|
| Harms and Russell (2000) | Other amino acids do not meet CVB requirements; other amino acids were not kept at a constant level; low performance levels |
| Jensen et al. (1990) | Other amino acids do not meet CVB requirements, except for exp. 4; paper published before 1994. |
| Lelis et al. (2010) | Diet composition is lacking |
| Othani et al. (1989) | Too less dose levels; paper published before 1994. |
| Peganova et al. (2003) Exp. 1 and 2 | Other amino acids do not meet CVB requirements |
| Russell and Harms (1999) | Other amino acids do not meet CVB requirements |

### Valine

| Reference | Reason of exclusion of the reference |
|---|---|
| Peganova and Eder (2003), Exp. 1, 2 and 3 | Other amino acids do not meet CVB requirements |

### Isoleucine

| Reference | Reason of exclusion of the reference |
|---|---|
| Peganova and Eder (2003), Exp. 1, 2 and 3 | Other amino acids do not meet CVB requirements |
| Peganova et al. (2003) | Other amino acids do not meet CVB requirements |
| Shivazad et al. (2002) | Other amino acids do not meet CVB requirements |

### Arginine

| Reference | Reason of exclusion of the reference |
|---|---|
| De Carvalho et al. (2012) | Other amino acids do not meet CVB requirements |

# Appendix 2 Economic evaluation of Lysine supplementation on a SID basis

An economic evaluation was performed to demonstrate the difference between the SID Lys intake level for achieving maximal rate of lay, egg mass, and feed efficiency versus the SID Lys intake level for achieving maximal gross margin.
For this economic evaluation, the price of a laying hen diet was set on € 30,-/100 kg, and the price of L-Lys on € 160,-/kg. Egg price was set on 0.103 € cent/g egg. Gross margin of egg income minus feed costs, excluding and including the costs of L-Lys, was calculated, where after the additional gross margin per 10 mg increase in Lys intake was presented.

Relation between daily SID Lys intake (mg/d) and hen performance, gross margin excluding and including costs of L-Lys (€/100 hens)

| SID Lys intake (mg/d) | Rate of lay (%)[1] | Egg weight (g) | Egg mass (g/d)[1] | FCR[1] | Gross margin excluding costs of L-Lys (€/100 hens)[2] | Additional gross margin excl. costs of L-Lys per 10 mg increase in Lys intake (€/100 hens/) | Additional gross margin incl. costs of L-Lys per 10 mg increase in Lys intake (€/100 hens)[3] |
|---|---|---|---|---|---|---|---|
| 650 | 83.1 | 61.9 | 50.7 | 2.21 | 1.85 | | |
| 660 | 83.6 | 62.0 | 51.2 | 2.20 | 1.89 | 0.040 | 0.019 |
| 670 | 84.0 | 62.0 | 51.6 | 2.19 | 1.93 | 0.038 | 0.018 |
| 680 | 84.4 | 62.1 | 52.0 | 2.17 | 1.97 | 0.036 | 0.016 |
| 690 | 84.8 | 62.2 | 52.4 | 2.16 | 2.00 | 0.035 | 0.014 |
| 700 | 85.2 | 62.3 | 52.8 | 2.15 | 2.04 | 0.033 | 0.013 |
| 710 | 85.5 | 62.4 | 53.2 | 2.14 | 2.07 | 0.031 | 0.011 |
| 720 | 85.8 | 62.5 | 53.5 | 2.13 | 2.10 | 0.030 | 0.009 |
| 730 | 86.1 | 62.6 | 53.8 | 2.12 | 2.13 | 0.028 | 0.008 |
| 740 | 86.4 | 62.7 | 54.1 | 2.11 | 2.15 | 0.027 | 0.006 |
| 750 | 86.6 | 62.8 | 54.3 | 2.10 | 2.18 | 0.025 | 0.005 |
| 760 | 86.9 | 62.8 | 54.6 | 2.09 | 2.20 | 0.023 | 0.003 |
| 770 | 87.1 | 62.9 | 54.8 | 2.08 | 2.22 | 0.022 | 0.001 |
| 780 | 87.3 | 63.0 | 55.0 | 2.08 | 2.24 | 0.020 | 0.000 |
| 790 | 87.5 | 63.1 | 55.2 | 2.07 | 2.26 | 0.019 | -0.002 |
| 800 | 87.7 | 63.2 | 55.4 | 2.06 | 2.28 | 0.017 | -0.003 |
| 810 | 87.8 | 63.2 | 55.6 | 2.06 | 2.29 | 0.016 | -0.005 |
| 820 | 87.9 | 63.3 | 55.7 | 2.05 | 2.31 | 0.014 | -0.006 |
| 830 | 88.1 | 63.4 | 55.8 | 2.05 | 2.32 | 0.013 | -0.008 |
| 840 | 88.2 | 63.5 | 56.0 | 2.04 | 2.33 | 0.011 | -0.009 |
| 850 | 88.2 | 63.5 | 56.1 | 2.04 | 2.34 | 0.010 | -0.010 |
| 860 | 88.3 | 63.6 | 56.1 | 2.04 | 2.35 | 0.009 | -0.012 |
| 870 | 88.4 | 63.7 | 56.2 | 2.03 | 2.36 | 0.007 | -0.013 |
| 880 | 88.4 | 63.8 | 56.3 | 2.03 | 2.36 | 0.006 | -0.014 |
| 890 | 88.5 | 63.8 | 56.3 | 2.03 | 2.37 | 0.005 | -0.016 |
| 900 | 88.5 | 63.9 | 56.3 | 2.03 | 2.37 | 0.003 | -0.017 |
| 910 | 88.5 | 64.0 | 56.3 | 2.03 | 2.38 | 0.002 | -0.018 |
| 920 | 88.5 | 64.0 | 56.3 | 2.03 | 2.38 | 0.001 | -0.019 |
| 930 | 88.4 | 64.1 | 56.3 | 2.03 | 2.38 | 0.000 | -0.021 |
| 940 | 88.4 | 64.2 | 56.3 | 2.03 | 2.38 | -0.001 | -0.022 |

[1]) Bold fields show where the trait reached it maximal (rate of lay, egg mass) or most efficient (FCR) level

[2]) Gross margin = (egg mass (g) * 0.103 eurocent/g) minus (feed intake (g) * 0,030 eurocent/g). Feed intake = egg mass * FCR. Costs of L-Lysine addition are not included here.

[3]) The costs of L-Lysine addition are included here: 10 mg increase in Lys intake requires 12.77 mg L-Lys. Price of L-Lys is assumed to be € 1.60/kg.

Column 2 – 5 show the response of SID Lys intake on rate of lay, egg weight, egg mass and FCR, respectively. Under the described conditions for feed and egg prices, but without taken the costs of additional Lys into account, gross margin improved up to an intake level of 930 mg/d SID Lys, as shown in column 6 and 7. After including the costs of L-Lys in the calculation, gross margin appeared to be maximal at 780 mg/d SID Lys (column 8).
Maximal gross margins, however, largely depend on the actual price levels for feed, egg mass and L-Lys. Therefore, these values have to be considered as an example.

# Appendix 3 Amino acid requirement per experiment on a AFD basis

| Trial | Reference | Methionine+Cysteine | | |
|---|---|---|---|---|
| | | Maximal response according to the Wood's model for egg mass | Maximal response according to the Wood's model for FCR | Requirement of the authors |
| 1 | Bertram et al. (1995b) Exp. 2 | 611 | 599 | 630 |
| 2 | Brumano et al. (2010a) | 736 | 719 | 682 |
| 3 | Brumano et al. (2010b) | 785 | 756 | 708 |
| 4 | Cupertino et al. (2009b) Exp. 1 | 754 | 738 | 712 |
| 5 | Cupertino et al. (2009b) Exp. 2 | 768 | 775 | 723 |
| 6 | Dänner and Bessei (2002) | 681 | 696 | Not provided |
| 7 | Filho et al. (2006a) | 663 | 667 | 697 |
| 8 | Geraldo et al. (2010) | 740 | 717 | 752 |
| 9 | Lemme et al. (2004b) | 945 | 1148 | Not provided |
| 10 | Sa et al. (2007a) Exp. 1 | 826 | 823 | 825 |
| 11 | Sa et al. (2007a) Exp. 2 | 767 | 771 | 793 |
| 12 | Narváez-Solarte et al. (2005) | 674 | 646 | 737 |
| 13 | Novak et al. (2004) Exp. 1 | 612 | 616 | 653 |
| 14 | Novak et al. (2004) Exp. 2 | 649 | 670 | 733 |
| 15 | Schmidt et al. (2009a) | 736 | 739 | 786 |
| 16 | Schmidt et al. (2011b) | 740 | 729 | 796 |
| 17 | Schutte et al. (1994) Exp. 2 | 646 | 685 | 660 |

| Trial | Reference | Threonine | | |
|---|---|---|---|---|
| | | Maximal response according to the Wood's model for egg mass | Maximal response according to the Wood's model for FCR | Requirement of the authors |
| 1 | Cupertino et al. (2010) Exp. 1 | 480 | 560 | 487 |
| 2 | Cupertino et al. (2010) Exp. 2 | 510 | 640 | 505 |
| 3 | Sa et al. (2007b) | 550 | 690 | 583 |
| 4 | Sa et al. (2007b) | 580 | 741 | 575 |
| 5 | Schmidt et al. (2010) | 480 | 624 | 462 |
| 6 | Schmidt et al. (2011a) | 500 | 612 | 459 |

| Trial | Reference | | Tryptophan | |
|---|---|---|---|---|
| | | Maximal response according to the Wood's model for egg mass | Maximal response according to the Wood's model for FCR | Requirement of the authors |
| 1 | Calderano (2011) | 142 | 211 | 142 |
| 2 | Calderano et al. (2012) | 161 | 208 | 158 |
| 3 | Deponti et al. (2007) | 192 | 207 | 175 |
| 4 | Esteve-Garcia et al. (2001) | 188 | 212 | 180 |

# Appendix 4 AFD AA intake levels (mg/d) of the selected Lysine studies at the Lys intake level for realizing maximal performance

| **Trial** | **Reference** | **β1** | **β2** | **FI** | **Lys_intake max. egg mass** | **LYS_intake 95% max. egg mass** | **M+C_ intake** | **Thr_ intake** | **Trp_ intake** |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Filho et al. (2006b) | 104.0 | -0.118 | 108,3 | 881 | | 750 | 581 | 164 |
| 2 | Sa et al. (2007b) (Exp. 1 | 110.9 | -0.118 | 122,0 | 940 | | 787 | 554 | 203 |
| 3 | Sa et al. (2007c) (exp. 2) | 103.3 | -0.118 | 112,5 | 875 | | 730 | 514 | 203 |
| 4 | Schmidt et al. (2008) | 110.8 | -0.118 | 113,5 | 939 | | 717 | 508 | 175 |
| 5 | Schmidt et al. (2009b) | 100.6 | -0.118 | 114,8 | 852 | | 714 | 506 | 187 |
| 6 | Schutte and Smink (1998) | 92.6 | -0.118 | 109,9 | 785 | | 680 | 489 | 155 |
| | | | | | | | | | |
| Average of 6 Lys studies (mg/d) | | | | 114 | 879 | 820 | 730 | 525 | 181 |
| STDEV | | | | 5 | 58 | | 36 | 35 | 20 |
| Ratio to Lys, based on 6 studies | | | | | | **100** | **89** | **64** | **22** |
| ***Percentage of requirement current study (95% max. EM on AFD basis)*** | | | | | | ***100*** | 109 | 105 | 115 |
| | | | | | | | | | |
| Requirement Schutte (1996) (mg/d) | | | | | | 700 | 650 | 460 | 130 |
| Ratio to Lys according to Schutte (1996) | | | | | | **100** | **93** | **66** | **19** |
| | | | | | | | | | |
| Requirement current study for 95% of max. egg mass (mg/d) | | | | | | 820 | 670 | 500 | 158 |
| Ratio to Lys according to the current study | | | | | | **100** | **82** | **61** | **19** |

Conclusion: The intake of the non-test amino acids M+C, Thr en Trp in the selected Lys requirement studies, expressed on an AFD basis, were on average above the required level for egg mass production (95% of maximal response level) as determined in the current study.

# Appendix 5 AFD AA intake levels (mg/d) of the selected Met+Cys studies at the Met+Cys intake level for realizing maximal performance

| | Reference | β1 | β2 | FI | Lys_ intake | M+C_intake max. egg mass | M+C_intake 95%max. egg mass | Thr_ intake | Trp_ intake |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| 1 | Bertram et al. (1995b) Exp. 2 | 111,3 | -0,182 | 105,0 | 714 | 611 | | 472 | 159 |
| 2 | Brumano et al. (2010a) | 134,0 | -0,182 | 88,1 | 625 | 736 | | 475 | 171 |
| 3 | Brumano et al. (2010b) | 142,9 | -0,182 | 93,4 | 663 | 785 | | 504 | 181 |
| 4 | Cupertino et al. (2009b) Exp. 1 | 137,4 | -0,182 | 111,5 | 688 | 754 | | 497 | 168 |
| 5 | Cupertino et al. (2009b) Exp. 2 | 139,9 | -0,182 | 112,0 | 691 | 768 | | 499 | 169 |
| 6 | Dänner and Bessei (2002) | 124,1 | -0,182 | 116,3 | 710 | 681 | | 508 | 168 |
| 7 | Filho et al. (2006a) | 120,8 | -0,182 | 109,3 | 920 | 663 | | 570 | 160 |
| 8 | Geraldo et al. (2010) | 134,7 | -0,182 | 99,7 | 635 | 740 | | 506 | 147 |
| 9 | Lemme et al. (2004b) | 172,2 | -0,182 | 122,9 | 886 | 945 | | 546 | 163 |
| 10 | Sa et al. (2007a) Exp. 1 | 150,3 | -0,182 | 118,9 | 775 | 826 | | 567 | 180 |
| 11 | Sa et al. (2007a) Exp. 2 | 139,7 | -0,182 | 114,9 | 749 | 767 | | 548 | 173 |
| 12 | Narváez-Solarte et al. (2005) | 122,7 | -0,182 | 107,8 | 637 | 674 | | 443 | 146 |
| 13 | Novak et al. (2004) Exp. 1 | 111,4 | -0,182 | 96,8 | 680 | 612 | | 492 | 146 |
| 14 | Novak et al. (2004) Exp. 2 | 118,1 | -0,182 | 97,2 | 781 | 649 | | 495 | 148 |
| 15 | Schmidt et al. (2009a) | 134,0 | -0,182 | 113,1 | 698 | 736 | | 504 | 171 |
| 16 | Schmidt et al. (2011b) | 134,7 | -0,182 | 113,6 | 701 | 740 | | 507 | 172 |
| 17 | Schutte et al. (1994) Exp. 2 | 117,6 | -0,182 | 107,2 | 682 | 646 | | 478 | 154 |
| | | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Average of 17 Met+Cys studies basis (mg/d) | | | 108 | 720 | 725 | 670 | 506 | 163 |
| STDEV | | | 10 | 82 | 84 | | 34 | 12 |
| Ratio to Lys, based on 17 studies | | | | **100** | | **93** | **70** | **23** |
| ***Percentage of requirement current study (95% max. EM on AFD basis)*** | | | | ***88*** | | ***100*** | ***101*** | ***103*** |
| | | | | | | | | |
| Requirement Schutte (1996) (mg/d) | | | | 700 | | 650 | 460 | 130 |
| Ratio to Lys according to Schutte (1996) | | | | **100** | | **93** | **66** | **19** |
| | | | | | | | | |
| Requirement current study for 95% max. egg mass (mg/d) | | | | 820 | | 670 | 500 | 158 |
| Ratio to Lys according to the current study | | | | **100** | | **82** | **61** | **19** |

**<u>Conclusion:</u>** The intake of the non-test amino acid Lys in the selected Met+Cys requirement studies, expressed on an AFD basis, was on average below the required level for egg mass production (95% of maximal response level) as determined in the current study.

# Appendix 6 AFD AA intake levels (mg/d) of the selected Threonine studies at the Thr intake level for realizing maximal performance

| Trial | Reference | β1 | β2 | FI | Lys_ intake | M+C_ intake | Thr_intake max. egg mass | THR_intake 95% max. egg mass | Trp_ intake |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Cupertino et al. (2010) Exp. 1 | 46.2 | -0.09 | 109,2 | 686 | 629 | 543 | | 147 |
| 2 | Cupertino et al. (2010) Exp. 2 | 46.6 | -0.09 | 108,6 | 682 | 626 | 548 | | 147 |
| 3 | Sa et al. (2007b), exp. 1 | 49.2 | -0.09 | 114,3 | 774 | 699 | 578 | | 219 |
| 4 | Sa et al. (2007b), exp. 2 | 51.3 | -0.09 | 111,3 | 754 | 699 | 603 | | 214 |
| 5 | Schmidt et al. (2010) | 44.1 | -0.09 | 108,7 | 683 | 626 | 518 | | 147 |
| 6 | Schmidt et al. (2011a) | 44.8 | -0.09 | 107,3 | 674 | 618 | 527 | | 145 |
| | | | | | | | | | |
| Average of 6 THR studies (mg/d) | | | | 110 | 709 | 650 | 553 | 530 | 170 |
| STDEV | | | | 3 | 43 | 39 | 32 | | 36 |
| Ratio to Lys, based on 6 studies | | | | | **100** | **92** | | **75** | **24** |
| ***Percentage of requirement current study (95% max. EM on AFD basis)*** | | | | | ***86*** | ***97*** | | ***106*** | ***107*** |
| | | | | | | | | | |
| Requirement Schutte (1996) (mg/d) | | | | | 700 | 650 | | 460 | 130 |
| Ratio to Lys according to Schutte (1996) | | | | | **100** | **93** | | **66** | **19** |
| | | | | | | | | | |
| Requirement current study for 95% of max. egg mass (mg/d) | | | | | 820 | 670 | | 500 | 158 |
| Ratio to Lys according to the current study | | | | | **100** | **82** | | **61** | **19** |

**Conclusion:** The intake of the non-test amino acid Lys in the selected Thr requirement studies, expressed on an AFD basis, was in all studies below the required level for egg mass production (95% of maximal response level) as determined in the current study. The intake of the non-test amino acids Met+Cys and Trp were on average slightly below or above the determined required level.

# Appendix 7 AFD AA intake levels (mg/d) of the selected Tryptophan studies at the Trp intake level for realizing maximal performance

| Trial | Reference | β1 | β2 | FI | Lys_ intake | M+C_ intake | Thr_ intake | Trp_intake max. egg mass | TRP intake 95% max. egg mass |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Calderano (2011) | 39.3 | -0.277 | 91,8 | 619 | 635 | 479 | 142 | |
| 2 | Calderano et al. (2012) | 44.5 | -0.277 | 77,0 | 558 | 570 | 435 | 161 | |
| 3 | Deponti et al. (2007) | 53.2 | -0.277 | 110,4 | 819 | 647 | 533 | 192 | |
| 4 | Esteve-Garcia et al. (2001) | 52.0 | -0.277 | 112,7 | 691 | 666 | 513 | 188 | |
| | | | | | | | | | |
| Average of 4 TRP studies (mg/d) | | | | 98 | 672 | 629 | 490 | 171 | 156 |
| STDEV | | | | 17 | 112 | 42 | 43 | 24 | |
| Ratio to Lys, based on 4 studies | | | | | **100** | **94** | **73** | | **23** |
| ***Percentage of requirement current study (95% max. EM on AFD basis)*** | | | | | ***79*** | ***94*** | ***92*** | | ***100*** |
| | | | | | | | | | |
| Requirement Schutte basis(1996) (mg/d) | | | | | 700 | 650 | 460 | | 130 |
| Ratio to Lys according to Schutte (1996) | | | | | **100** | **93** | **66** | | **19** |
| | | | | | | | | | |
| Requirement current study for 95% of max. egg mass (mg/d) | | | | | 820 | 670 | 500 | | 158 |
| Ratio to Lys according to the current study | | | | | **100** | **82** | **61** | | **19** |

**Conclusion:** The intake of the non-test amino acid Lys in the selected Trp requirement studies, expressed on an AFD basis, was on average far below the required level for egg mass production (95% of maximal response level) as determined in the current study. The intake of the non-test amino acids Met+Cys and Thr were on average slightly below the determined required level.

# Appendix 8 Performance and AFD amino acid supply levels (% of Schutte recommendations) of the selected Lysine studies

| Author | Year published | Only change In test AA? | Feed intake (g/kg) | Rate of lay (%) | Egg weight (g) | Egg Mass (g/d) | FCR | Lys req. study for 95% egg mass | Lys supply (% of Schutte recom.) | Met+Cys supply (% of Schutte recom.) | Thr supply (% of Schutte recom.) | Trp supply (% of Schutte recom.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Schmidt et al. | 2009 | No | 113.9 | 74.3 | 63.8 | 47.4 | 2.43 | | 87.1 | 84.7 | 104.0 | 115.7 |
| Schmidt et al. | 2009 | | 114.2 | 76.1 | 66.3 | 50.4 | 2.28 | | 95.6 | 92.8 | 104.3 | 120.4 |
| Schmidt et al. | 2009 | | 114.8 | 76.5 | 67.3 | 51.4 | 2.24 | | 104.4 | 101.4 | 104.8 | 132.4 |
| Schmidt et al. | 2009 | | 117.0 | 80.5 | 68.0 | 54.8 | 2.14 | 791 | 115.2 | 111.4 | 109.4 | 145.8 |
| Schmidt et al. | 2009 | | 114.2 | 76.5 | 65.3 | 49.9 | 2.29 | | 120.8 | 116.9 | 116.0 | 152.9 |
| Fihlho et al. | 2006 | Yes | 108.1 | 86.1 | 65.6 | 57.1 | 1.90 | | 107.3 | 115.1 | 125.9 | 125.5 |
| Fihlho et al. | 2006 | | 107.5 | 89.3 | 65.4 | 59.3 | 1.86 | | 112.8 | 114.4 | 125.2 | 124.8 |
| Fihlho et al. | 2006 | | 108.2 | 90.1 | 64.7 | 58.3 | 1.87 | | 119.8 | 115.2 | 126.1 | 125.7 |
| Fihlho et al. | 2006 | | 108.4 | 91.4 | 64.9 | 57.4 | 1.82 | 857 | 126.2 | 115.4 | 126.3 | 125.9 |
| Fihlho et al. | 2006 | | 108.3 | 88.9 | 65.2 | 58.0 | 1.87 | | 132.3 | 115.3 | 126.2 | 125.8 |
| Fihlho et al. | 2006 | | 109.1 | 91.2 | 64.6 | 59.1 | 1.86 | | 139.5 | 116.1 | 127.1 | 126.7 |
| Fihlho et al. | 2006 | | 108.7 | 86.7 | 66.2 | 57.4 | 1.92 | | 145.2 | 115.7 | 126.6 | 126.2 |
| Schmidt et al. | 2008 | No | 106.3 | 71.2 | 62.8 | 44.7 | 2.39 | | 81.2 | 79.0 | 97.0 | 107.9 |
| Schmidt et al. | 2008 | | 113.9 | 77.4 | 66.5 | 51.4 | 2.23 | | 95.4 | 92.6 | 104.0 | 115.7 |
| Schmidt et al. | 2008 | | 115.6 | 82.5 | 66.6 | 54.8 | 2.11 | | 105.2 | 102.1 | 105.6 | 117.4 |
| Schmidt et al. | 2008 | | 115.7 | 83.3 | 67.5 | 56.2 | 2.06 | | 113.8 | 110.1 | 108.1 | 126.3 |
| Schmidt et al. | 2008 | | 116.1 | 85.3 | 66.2 | 56.4 | 2.07 | 881 | 122.7 | 118.7 | 117.8 | 159.8 |
| Moraes Sa et al. | 2007 | No | 124.6 | 87.5 | 59.1 | 51.6 | 2.41 | | 102.9 | 97.6 | 120.3 | 135.1 |
| Moraes Sa et al. | 2007 | | 120.1 | 90.9 | 59.6 | 54.1 | 2.22 | | 107.9 | 102.7 | 115.9 | 130.3 |
| Moraes Sa et al. | 2007 | | 121.0 | 92.4 | 61.7 | 57.0 | 2.12 | | 117.5 | 111.9 | 116.8 | 143.3 |
| Moraes Sa et al. | 2007 | | 122.9 | 92.1 | 61.7 | 56.8 | 2.17 | 894 | 128.0 | 122.3 | 118.6 | 157.9 |
| Moraes Sa et al. | 2007 | | 121.4 | 90.6 | 62.6 | 56.0 | 2.17 | | 135.3 | 129.2 | 126.2 | 168.1 |

| Author | Year published | Only change In test AA? | Feed intake (g/kg) | Rate of lay (%) | Egg weight (g) | Egg Mass (g/d) | FCR | Lys req. study for 95% egg mass | Lys supply (% of Schutte recom.) | Met+Cys supply (% of Schutte recom.) | Thr supply (% of Schutte recom.) | Trp supply (% of Schutte recom.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Moraes Sa et al. | 2007 | No | 110.4 | 86.2 | 61.5 | 53.0 | 2.08 | | 91.2 | 86.5 | 106.6 | 119.7 |
| Moraes Sa et al. | 2007 | | 113.6 | 89.4 | 61.6 | 55.0 | 2.06 | | 102.1 | 97.2 | 109.6 | 123.2 |
| Moraes Sa et al. | 2007 | | 110.8 | 90.5 | 62.7 | 56.7 | 1.95 | | 107.6 | 102.4 | 106.9 | 131.3 |
| Moraes Sa et al. | 2007 | | 114.8 | 90.7 | 63.6 | 57.7 | 1.99 | 814 | 119.6 | 114.3 | 110.8 | 147.5 |
| Moraes Sa et al. | 2007 | | 113.0 | 89.6 | 64.0 | 57.4 | 1.97 | | 125.9 | 120.3 | 117.4 | 156.5 |
| Schutte and Smink | 1998 | Yes | 110.0 | 97.0 | 57.4 | 55.8 | 1.98 | | 87.7 | 104.9 | 106.7 | 119.3 |
| Schutte and Smink | 1998 | | 112.0 | 97.0 | 58.8 | 56.8 | 1.97 | | 95.7 | 106.8 | 108.6 | 121.5 |
| Schutte and Smink | 1998 | | 110.0 | 97.0 | 58.5 | 56.5 | 1.94 | | 100.3 | 104.9 | 106.7 | 119.3 |
| Schutte and Smink | 1998 | | 109.0 | 96.0 | 58.5 | 56.3 | 1.94 | 741 | 105.6 | 104.0 | 105.7 | 118.2 |
| Schutte and Smink | 1998 | | 109.0 | 97.0 | 58.4 | 56.8 | 1.92 | | 111.8 | 104.0 | 105.7 | 118.2 |
| Schutte and Smink | 1998 | | 109.0 | 97.0 | 58.7 | 57.1 | 1.91 | | 118.0 | 104.0 | 105.7 | 118.2 |
| Schutte and Smink | 1998 | | 110.0 | 96.0 | 58.9 | 56.7 | 1.93 | | 125.4 | 104.9 | 106.7 | 119.3 |
| Schutte and Smink | 1998 | | 110.0 | 97.0 | 58.3 | 56.6 | 1.94 | | 131.7 | 104.9 | 106.7 | 119.3 |

# Appendix 9 Comparison of datasets and models used by Schutte (1996) and the current study

**Introduction**

The recommended AFD lysine level (required to achieve 95% of the maximal possible egg mass and minimal FCR) by the current study of on average 823 mg SID lysine/d is substantially higher than the recommended AFD lysine level by Schutte of 700 mg/d. The question arises whether the difference in recommended lysine level is 1) a result of the difference in datasets between Schutte and the current study, 2) the result of the different statistical procedures used by Schutte and the current study, or 3) a combination of the two. In order to check whether differences in recommended lysine levels could be ascribed to differences in statistical procedures used or to differences between the two datasets it was decided to analyse the dataset of Schutte and the current study using both the statistical procedure used by Schutte and the statistical procedure used by the current study. It was hypothesized that, when differences in recommended lysine levels between the dataset of Schutte and the dataset of the current study disappear when using the same statistical procedure, that differences in recommended lysine levels are the result of the statistical procedure used. However, when differences remain in recommended digestible lysine levels between the dataset of Schutte and the current study remain, despite using the same statistical procedure, it will become clear that the differences in recommended lysine levels are the result of differences between the dataset of the current study and Schutte.

**The statistical procedure used by Schutte**

The following model was used by Schutte (and also used in the present comparison):

Y = a + b[1-e$^{-c(x-d)}$], where:

Y = the difference in in FCR or egg mass (g/hen/dag)
a = intercept,for difference in FCR or egg mass
b = maximum response expressed as difference in egg mass or difference in FCR at increased levels of AFD lysine
c = steepness of the curve

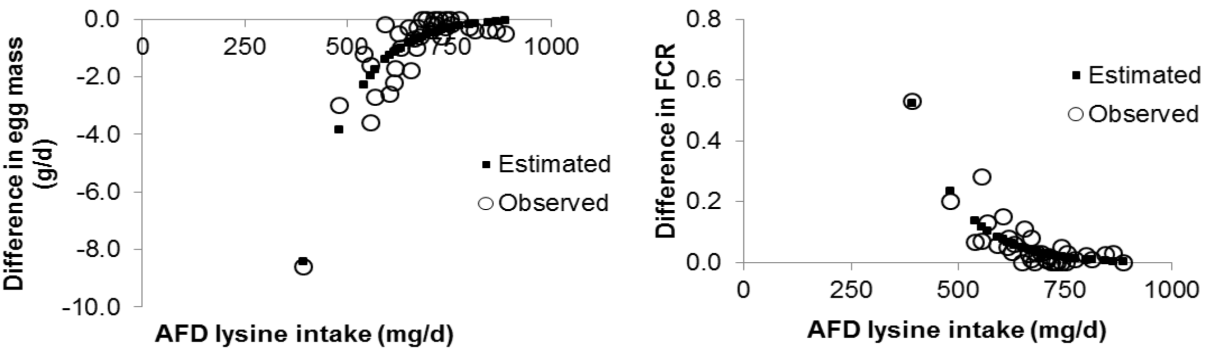


*Figure 1* *Relationship between AFD lysine and difference in egg mass and the relationship between AFD lysine and FCR based on the dataset of Schutte et al. (1996). Model estimates for difference in egg mass: a = -8.45±0.639, b = 8.51±0.639, c = 0.0087±0.00125, R2=0.84. Model estimates for difference in FCR: a = 0.526±0.0415, b = -0.531±0.0414, c = 0.0089±0.00132, R2 = 0.83.*

Figure 2. shows that the fitted curves for differences in egg mass and FCR do not converge to zero as would be expected. The fact that the fitted curves do not converge to zero can be explained from the observation that at increased AFD lysine intake levels somehow the egg mass, after reaching a maximum, declines thereafter and a similar phenomenon is observed for FCR. This phenomenon is likely to result in an incorrect estimation of the maximum response. Therefore it was chosen to remove those observations that follow after the minimum FCR or the maximum egg mass has been reached. Results of the curve fitting are presented in Figure 3 for the dataset of Schutte et al. (1996) and in Figure 4 for the dataset of the current study1.

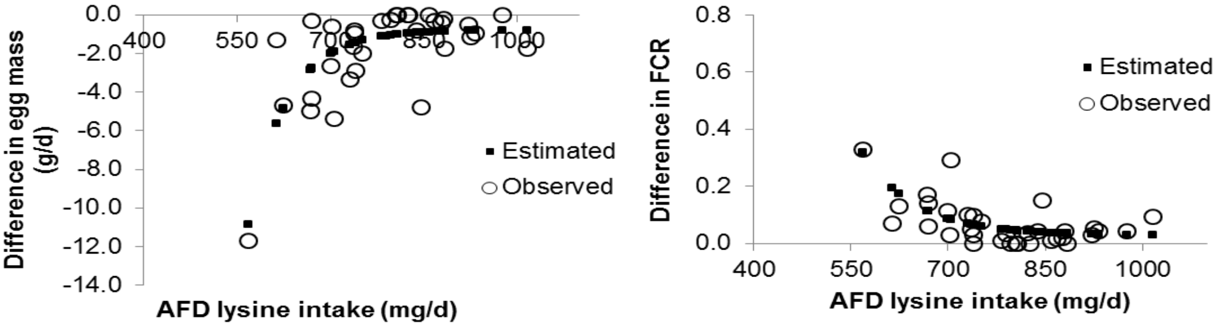


***Figure 2*** *Relationship between AFD lysine intake and difference in FCR and egg masss for the dataset of the current study. Model estimates for difference in egg mass: a = -10.94±1.515, b = 10.14±1.484,* ***c =*** *0.0161±0.00404,* $R^2$*=0.63. Model estimates for differences in FCR: a = 0.318±0.0538, b = -0.290±0.0516, c = 0.0122±0.00413,* $R^2$ *= 0.53.*

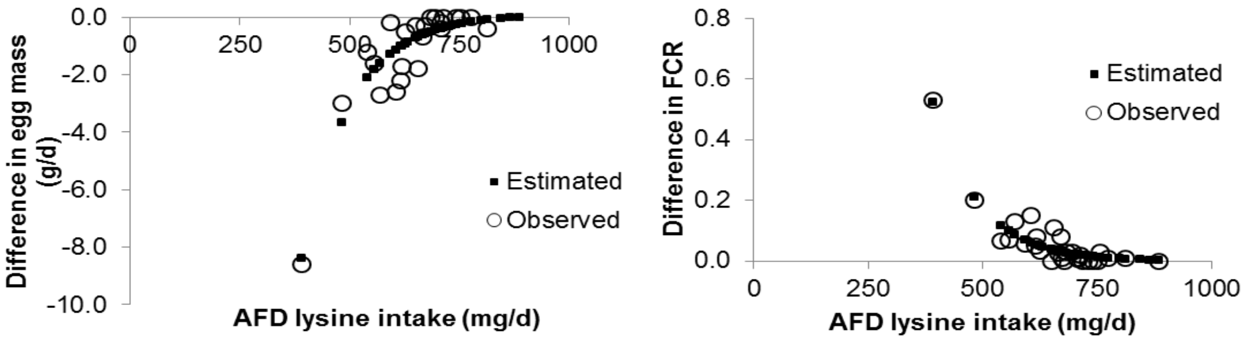


***Figure 3*** *Relationship between ADF lysine intake and difference in FCR and egg mass for the dataset of Schutte et al. (1996). Model estimates for difference in egg mass: a = -8.41±0.709, b = 8.51±0.747, c = 0.0091±0.00181, R2=0.87. Model estimates for difference in FCR: a = 0.525±0.0318, b = -0.526±0.0323, c = 0.0101±0.00130, R2 = 0.91.*

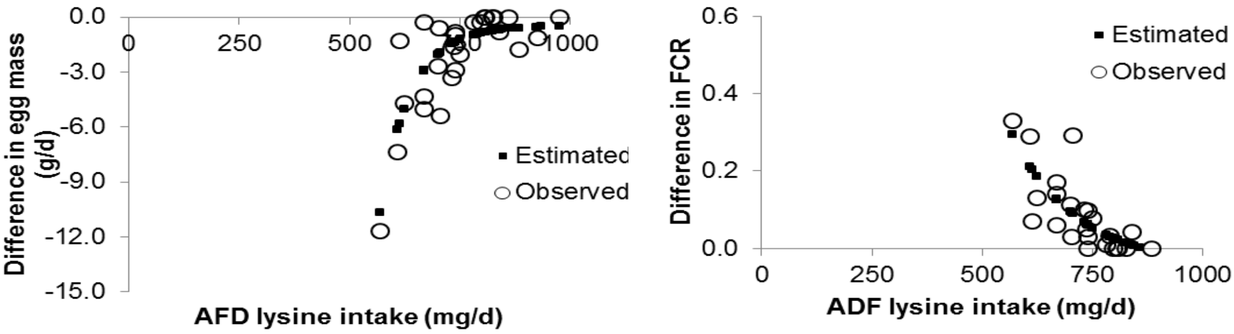


***Figure 4*** *Relationship between ADF lysine intake and difference in FCR and egg mass for the dataset of the current study. Model estimates for difference in egg mass: a = -10.71±1.493, b = 10.25±1.448, c = 0.0142±0.00396, R2=0.69. Model estimates for difference in FCR: a = 0.294±0.0553, b = -0.341±0.0847, c = 0.0067±0.00455, R2 = 0.58.*

In Table 1 the AFD lysine levels are presented required to obtain the maximum and 95% of the maximum egg mass and the lowest FCR when using the statistical procedure of Schutte.

Table 1.
*The AFD lysine intake levels (mg/d) for obtaining the maximum and 95% of the maximum response in egg mass and the lowest value of FCR when using the statistical procedure of Schutte.*

| | | Dataset Schutte | | Dataset the current study | |
|---|---|---|---|---|---|
| | | Maximum | 95% of maximum | Maximum | 95% of Maximum |
| All observations | FCR | 910 | 710 | 1350 | 814 |
| | Egg mass | 960 | 721 | 1350 | 754 |
| Reduced number of observations* | FCR | 1047 | 687 | 863 | 823 |
| | Egg mass | 889 | 701 | 1450 | 779 |

*observations after the maximum egg mass or the lowest FCR was reached were removed.

The results in Table 1 show that by using the same model to evaluate the dataset of Schutte and the dataset of the current study results in substantial differences in recommended (95% of maximum) AFD lysine levels for FCR and egg mass, being on average 705 mg/d for the dataset of Schutte and, on average 793 mg/d for the dataset of the current study.

It can therefore be concluded that the differences in recommended AFD lysine levels between the dataset of Schutte and the dataset of the current study can be largely ascribed to the differences in datasets between Schutte and the current study.

**The statistical procedure used by the current study**
In order to compare the two datasets of the current study and Schutte it was necessary to go to the original data of Schutte and to calculate again AFD and SID lysine intake values and the accompanying levels of FCR and egg mass. However, for one trial used in the meta-analysis study of Schutte it was not possible to obtain all data (trial described in Van Weerden and Schutte (1980)). Furthermore, the AFD and SID lysine intake levels in trials included in the study of Schutte were calculated again using the same nutrient information of feedstuffs as used by the current study. Because 1) some results of the trial described in Van Weerden and Schutte could not be included when using the statistical procedure used by the current study, and 2) calculated AFD lysine intake levels in the dataset van Schutte may differ from the original AFD lysine intake levels reported by Schutte it was not possible to compare the original recommended AFD lysine intake levels in the original study of Schutte with the recommended ADF lysine intake levels in the present study.

In Table 2. The results are given of the required AFD and SID lysine intake levels (mg/d) for obtaining the maximum and 95% of the maximum response in egg mass and the lowest value of FCR when using the statistical procedure used by the current study.

Table 2.

*The AFD and SID lysine intake levels (mg/d) for obtaining the maximum and 95% of the maximum response in egg mass and the lowest value of FCR when using the statistical procedure used by the current study for the dataset of Schutte and the dataset of the current study.*

| | | SID lysine (mg/d) | | AFD lysine (mg/d) | |
|---|---|---|---|---|---|
| | | Maximum | 95% of maximum | Maximum | 95% of Maximum |
| Dataset Schutte | FCR | 806 | 729 | 808 | 727 |
| | Egg mass (g/d) | 811 | 731 | 826 | 737 |
| Dataset the current study | FCR | 930 | 860 | 897 | 825 |
| | Egg mass (g/d) | 916 | 855 | 879 | 820 |

The results in Table 2 showed that by using the statistical procedure of the current study to evaluate the data of Schutte and the current study results in substantial differences in recommended AFD lysine and SID lysine intake levels (lysine levels required to obtain 95% of maximum egg mass or minimal FCR) for FCR and egg mass, being on average 731 mg lysine per day for the dataset of Schutte and on average 840 mg lysine per day for the complete dataset of the current study.

It is concluded that differences in recommended AFD and SID lysine levels between the study of Schutte and the study of the current study (based on the complete dataset of the current study) can be largely ascribed to the difference in datasets between Schutte and the current study.

**A comparison of both statistical procedures**

A comparison of the recommended AFD lysine intake levels (required to achieve 95% of the maximal possible egg mass or minimal FCR) for the dataset of Schutte between the statistical procedure used by Schutte (Table 1) and the statistical procedure used by the current study (Table 2) shows that recommended lysine levels are similar, on average 716 mg AFD lysine per day when using the Schutte procedure (based on all observations) and 732 mg AFD lysine per day when using the current study procedure.

**Conclusions**

It is concluded that the differences in recommended lysine levels between the study of Schutte et al. (1996) and the current study can be largely ascribed to differences in datasets between the two studies and to a lesser extent to differences in statistical procedures used. The average recommended AFD lysine level for egg mass and FCR, based on the dataset of the current study, is 823 mg/d when using the statistical method used by the current study, whereas when using the statistical method used by Schutte the average recommended AFD lysine level based on the dataset of the current study is 784 (based on all observations) or 801 mg/d (based on a reduced number of observations).

To explore
the potential
of nature to
improve the
quality of life

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