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Preface

Diet composition, e.g. concentrations of energy, protein, vitamins and minerals, largely influence the zootechnical performance of poultry. Nowadays, protein requirements are more precisely described in terms of apparent faecal digestible amino acid (AFD) or standardised ileal digestible amino acid (SID) requirement values and amino acid profiles expressing the requirement of each amino acid relative to lysine. Appropriate requirement values for amino acids (AA) in poultry diets are essential for optimizing poultry production and profit of the poultry chain.
CVB, formerly part of the Dutch Product Board Animal Feed (PDV) and now part of the Federatie Nederlandse Diervoedingsketen (FND; Federation Dutch Animal Feed Chain), is responsible for recommending the Dutch poultry chain on AA requirements for various poultry species. The latest public review of AA requirements in poultry in the Netherlands was presented two decades ago (Schutte et al., 1996). As a consequence of several recent developments such as change in genetic predisposition for growth, the increasing trend of formulating low-protein diets and the increasing availability of free AA for supplementation in broilers diets, it was recommended that requirement values for AA in broiler diets should be updated. The present study was subsidized by the (former) Product Board Animal Feed and the (former) Product Board Poultry and Eggs.

Summary

Requirement values for apparent faecal digestible and standardised ileal digestible lysine of broilers at different ages were estimated by a meta-analysis approach. This study was part of a project to estimate the apparent faecal (AFD) and standardized ileal digestible (SID) amino acid requirement values of the first limiting amino acids in both broilers and laying hens.

Peer reviewed papers were selected, describing experimental results of dose response studies in which the effect of graded levels of free amino acids supplemented to a basal diet on body weight gain and feed conversion ratio in broilers was studied. The papers searched for were published during the period 1994 - 2012. Subsequently, a stepwise process was applied for the selection of the research data to be used in the meta-analysis. In total 11 criteria were set. Two of these criteria are: at least three graded levels of supplementation of the amino acid of interest to the same basal diet; maximal supplementation of the amino acid of interest was at least 10% higher compared to the concentration of the amino acid of interest in the basal (non-supplemented) diet. Feed ingredient composition of the experimental diets should be present in each paper; this information was included in a separate database and nutrient composition of the experimental diets was recalculated by using data on the nutritional composition of the individual feed ingredients according to the CVB Feed Table (2007). When the determined level of the amino acid of interest (and – when presented – of other amino acids) on an AFD basis was published, this information was used. In case this information was not presented, the level of the amino acid of interest (and of other amino acids) was calculated by using either the total amino acid levels in the basal diets as analysed by the authors or as calculated by using the CVB Feed Table (2007) in combination with the digestibility on an AFD basis as published by CVB (CVB Table, 2007) In addition, the concentrations of standardized ileal digestible (SID) amino acids in the diets of each study were also calculated using the digestibility on an SID basis as tabulated by CVB (Dekker and Blok, in press) and were included in the database. These (calculated) dietary concentration of AFD and SID amino acids were used in the present study for the regression analyses to derive requirement values for AFD and SID amino acids in broilers.

The responses of body weight gain (BWG) and feed conversion ratio (FCR) to supplementation of the free amino acid of interest to a basal diet were analysed for each individual experiment included in the database by regression analysis. Mean data for BWG and FCR per experimental group as provided in the original paper were used as response parameters. Response of BWG and FCR to supplementation of the free amino acid of interest was determined by use of an exponential model. For each individual experiment the estimated requirement (Req) for the amino acid of interest was calculated as the amino acid concentration at which 95% of the response (BWG and FCR) between intercept and asymptotic value was reached. Data of studies that could not be fitted with the exponential model were excluded from further evaluation. Also studies, where the estimated requirement value was over 110% of the maximum concentration of the amino acid of interest in the diet with the highest supplementation level, were excluded from further evaluation. Studies in which a non-test amino acid might have been co-limiting (< 90% of CVB 1996) at higher supplementation levels of the amino acid of interest were also excluded from the dataset. After estimation and evaluation of the amino acid requirement values for the individual studies, an overall regression model was used to fit the requirement values of the amino acid of interest on an AFD and SID basis for BWG and FCR as a function of age of the broilers. For this purpose the mean age of broilers in each experiment was calculated as (age at start of the experimental period + age at the end of the experimental period)/2.

In total, 26 studies each containing one or more experiments, were judged. In total, 28 experiments from 11 papers that met the criteria were included into the database. Requirement values of AFD and SID lysine concentrations in the diet were estimated at different ages. A summary is presented in the Table.

Day	AFD lysine (% in diet)	AFD lysine (% in diet)		SID lysine (% in diet)	
	CVB (2007)	Body weight gain	Feed conversion ratio	Body weight gain	Feed conversion ratio
7	1.05	1.19	1.26	1.20	1.28
14	1.02	1.11	1.19	1.13	1.21
21	1.02	1.07	1.15	1.09	1.17
28	0.99	1.04	1.12	1.06	1.15
35	0.99	1.02	1.10	1.04	1.12
42	0.99	1.00	1.08	1.02	1.11

Estimated AFD and SID lysine requirements for BWG are lower than for FCR. Until four weeks of age the AFD and SID lysine requirement for BWG are significantly higher than the CVB (2007) recommendations for AFD lysine requirement. AFD and SID lysine requirement for FCR are over the entire period significantly higher than the CVB (2007) recommendations for AFD lysine requirement.

1 Introduction

Diet composition, e.g. concentrations of energy, protein, vitamins and minerals, largely influence the zootechnical performance of poultry. Nowadays, protein requirements are more precisely described in terms of digestible amino acid requirements and amino acid profiles expressing the requirement of each amino acid relative to lysine. Appropriate digestible amino acid requirements in poultry diets are essential for optimizing poultry production and profit of the poultry chain.
CVB, formerly part of the Dutch Product Board Animal Feed (PDV) and now part of the Federatie Nederlandse Diervoedingsketen (FND; Federation Dutch Animal Feed Chain), is responsible for advising the Dutch feed industry on nutrient requirements for various poultry species. The latest public review on amino acid requirements in poultry in the Netherlands, however, was conducted two decades ago (CVB, 1996). The requirement values of amino acids (CVB, 1996) were expressed on an apparent faecal digestible (AFD) basis. As a consequence of several recent developments, it was recommended that amino acid requirements should be updated:

- The genetic predisposition for body weight gain of broilers has increased substantially during the last decades;
- The increasing trend of formulating low-protein diets;
- The increasing availability of free amino acids for diet supplementation;
- Different feeding strategies are developed to improve animal welfare and (intestinal) health.

Nutrient requirements have been determined in many experiments. A general method for integrating quantitative knowledge from multiple experiments has been proposed and is referred to as meta-analysis (St-Pierre, 2001). The technique is based on collecting data from multiple published studies that fulfil a number of criteria and formulating a statistical model that best explains the observations (van Houwelingen et al., 2002). Moreover, the meta-analytical approach is highly suited for establishing requirements values because it focuses on estimating on a population level from multiple studies, while accounting for the heterogeneity between studies. The statistical model used in meta-analytical studies should be based on a hierarchical or a mixed model, which has at least two stages (van Houwelingen et al., 2002). The first-stage hierarchy models the within-study variability as a function of the primary covariate (e.g., Lys content). The second-stage hierarchy models the between-study variability through individual random effects and study-related covariates (e.g. strain, gender, year of publication etc.), identifying systematic trends among studies.

Meta-analysis, which combines the results from various experiments at the same time, has more power to detect small differences. For estimating amino acid requirement values 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 searched and reference lists of papers were checked carefully.

The current requirement values for amino acids in broilers (CVB, 1996) are expressed on an apparent faecal digestible (AFD) basis. For the present study it was recommended by CVB to estimate requirement values for amino acids in broilers on a standardized ileal digestible basis. According to Lemme et al. (2004) and Adedokun et al. (2008) standardised ileal amino acid digestibility (SID) coefficients are corrected for the contribution of amino acids of basal endogenous origin to the total ileal digesta pool. Changing the system of expressing amino acid requirement values based on AFD into SID amino acid concentration of dietary ingredients affect the amino acid requirement values of broilers. It is important that amino acid requirement values and the dietary supply of amino acids are expressed identically.

The present study was part of a project conducted to estimate requirement values for the first limiting apparent faecal digestible (AFD) and standardized ileal digestible (SID) amino acids in broilers and laying hens at different ages using a meta-analysis approach. In this report the requirement values for lysine in broilers are described.

2 Material and Methods

2.1 Database

Peer reviewed papers were selected, describing experimental results of dose response studies in which the effect of graded levels of free amino acids of interest supplemented to a basal diet on body weight gain (BWG) and feed conversion ratio (FCR) in broilers was studied. The papers were searched by using the key words ‘broiler’ and ‘name of relevant amino acid’ in the electronic database ‘Web of Science’. The papers searched for were published during the period 1994 - 2012. Search results in which the requirement of lysine was studied were found in Asian-Australian Journal of Animal Science, Brazilian Journal of Veterinary and Animal Science , British Poultry Science, Italian Journal of Animal Science, Journal of Applied Poultry Research, Journal of Animal and Veterinary Advances, Poultry Science, Revista Brasileira de Zootecnia and Statistics in Medicine . A stepwise process was applied for the selection of research data to be used.

2.2 Criteria for inclusion of papers into the database

The studies were reviewed according to the following inclusion criteria:

1. The experimental procedure should be adequately provided, meaning a clear description of the experimental units, the number of broilers per unit, the age of the broilers and the duration of the experiment;
2. Provision of information on the broilers used (strain, age);
3. Provision of information on the (metabolizable) energy content of the diets (for adult cockerels);
4. Provision of information on how amino acid levels in the basal diet(s) in the paper are expressed (total, faecal, ileal, on an apparent or standardized basis);
5. Only dose response studies were included in which besides a basal level of the amino acid of interest at least three graded levels of supplementation of the amino acid of interest to the same basal diet were tested;
6. Only dose response studies were included in which the maximal supplementation of the amino acid of interest was at least 10% higher compared to the concentration of the amino acid of interest in the basal (non-supplemented) diet;
7. With the exception of the concentration of the amino acid of interest (that should be – far – below the CVB requirement), the concentration of the following amino acids in the basal diet should be at least 90% of the CVB (1996) requirement (on AFD basis) for methionine+cysteine, threonine and tryptophan. For isoleucine, arginine and valine the concentration in the basal diet should be at least 85% of the CVB (1996) requirement (on AFD basis), because the requirements of these amino acids were documented less accurately;
8. Experimental diets should be adequately described in terms of ingredient composition and should contain analysed or calculated contents for at least crude protein and essential amino acids;
9. Feed intake levels of experimental groups (receiving the diets with supplemented free amino acid) within the same experiment should be less than 150% relative to the feed intake level of the group fed the basal, non-supplemented basal diet;
10. Provision of data on feed intake, BWG and FCR in dose response studies with broilers in which the effects of increasing levels of the dietary amino acid of interest was evaluated by supplementing a basal diet with different levels of the free amino acid of interest;
11. Supplementation of the free amino acid of interest to the basal diet should have a statistical significant effect on BWG and/or FCR according to the original author.

Information of the papers that met these inclusion criteria was included in a database. Besides the information on the inclusion criteria as mentioned above, additional information from the study (if available) was added also to the database (e.g. strain, sex, etc.). Further, the amino acid requirement value as derived by the original author(s) of the study was included in the database as well and also the statistical method to estimate the amino acid requirement under study was included. Studies not meeting the inclusion criteria as mentioned above, were excluded from the database and the reason for exclusion was recorded (See Appendix 1).

2.3 Calculations

Feed ingredients in the basal diet composition used in each experiment of studies that met the criteria in Paragraph 2.2 were included in a separate database. Subsequently, nutrient composition of these experimental diets was recalculated by using data on the nutritional composition of the individual feed ingredients according to the CVB Feed Table (2007). Regarding the levels of digestible amino acids the following procedure was used:

a. When the paper presents the level of lysine in the basal diets expressed on a (apparent faecal) digestible basis, it was decided to use this figure. As far as the levels of one or more other amino acids (see criterion 7 for the other amino acids that were considered to be relevant), were also expressed on this basis, this information was used in the further processing of the study. For those amino acids for which this information was lacking, the level of digestible amino acid was calculated according to option b. or c.;
b. When no information was presented in the paper on the level of (apparent) faecal digestible lysine and/or other amino acids, the next option was to use the total level of lysine and/or of the other amino acids as analysed in the basal diets. Using the faecal amino acid digestibility of the feed ingredients in the CVB Feed Table (2007), the faecal digestibility of the amino acids in the basal diet and, subsequently, the level of apparent faecal digestible amino acids was calculated;
c. When no information as described in the options a. and b. was available, the total levels of the amino acids needed were calculated using the ingredient composition of the experimental diets (see criterion 8) as presented in the paper. In these cases the starting point was the ingredient composition (Weende analysis, ME value and amino acid pattern) as published in the CVB Feed Table 2007. To reproduce satisfactory the level of crude protein and – when given – the metabolizable energy level as given in the paper, in a number of cases (slight) adjustment of the protein level and – as a consequence – the amino acid levels of – preferably – the protein rich ingredients was necessary. Subsequently, the digestible amino acid levels on an AFD basis were calculated using the digestibility in the CVB Feed Table (2007).

In addition, the concentrations of standardized ileal digestible (SID) amino acids in the basal diets of each study were also calculated using option b. or c. and were included in the database. The standardized ileal amino acid digestibility coefficients of feed ingredients required for the calculation of SID amino acid contents were derived from Dekker and Blok (in press).
The supplemented free amino acids were considered to be 100% digestible, both on an AFD and SID basis.
The calculated dietary concentrations of AFD and of SID lysine were used in the present study for the regression analyses to derive requirement values for lysine on an AFD and SID basis for Body Weight Gain (BWG) and Feed Conversion Ratio (FCR) in broilers.

2.4 Statistical analysis

2.4.1 Regression analysis per experiment

The responses of BWG and FCR to supplementation of the free amino acid of interest to a basal diet were analysed by regression analysis. Mean data for BWG and FCR per experimental group as provided in the original paper were used as response parameters. The response of BWG and FCR to supplementation of free amino acid of interest was determined by use of an exponential model as is described by the following mathematical equation:

$Y_{ij} = a_i + b_i * (1 - e^{(-C_i * dx)}) + \varepsilon ij$ **(1)**

Where: Y_{ij} = response value of BWG or FCR for experiment i and treatment j;
a_i = estimated basal level (for dx=0) of the amino acid of interest for experiment i;
b_i = difference between basal level and estimated asymptotic level for BWG and FCR response for experiment i;
C_i = rate parameter (for speed of curving) for experiment i;
dx = difference in amino acid concentration of interest (AFD or SID based) compared to basal (non-supplemented diet) in experiment i ; (X_i – MIN(X_i)); X_i= amino acid concentration of interest in experimental diets, MIN(X_i)= amino acid concentration of interest in basal (non-supplemented) diet;
εij = error ij.

For each individual experiment the estimated requirement (Req) for the amino acid of interest was calculated as the amino acid concentration where 95% of the response (BWG and FCR) between intercept and asymptotic value was reached. The estimated amino acid requirement was calculated by the following mathematical equation:

$Req_i = \frac{\ln(0.05)}{-Ci} + MIN(Xi)$ **(2)**

Where: Req_i = Estimated amino acid requirement (%) of the individual experiment i;
Ln(0.05) = $^e\log$ (0.05);
C_i = rate parameter (for speed of curving) for experiment i;
MIN(X_i) = amino acid (%) in basal (non-supplemented) diet.

2.4.2 Overall regression (Regression analysis over experiments)

After estimation of the amino acid requirement values for individual studies by using the exponential model according to equation 2, the amino acid requirement as a function of age was studied. However, before doing this the results from the previous step were evaluated according to the following criteria:

- Mean age of the animals.
  The mean age of broilers was determined in each experiment as (days of age at start of the experimental period + days of age at the end of the experimental period)/2. Experiments in which the mean age was >42 days were excluded from the database for the overall regression.
- Calculated requirement (as the AFD or SID amino acid level at which 95% of the plateau level was reached) was compared to the highest dietary amino acid level in the experiment.
  When the calculated requirement was >110% of the amino acid level in the treatment with the highest supplemented amino acid level, the study was excluded from the database for the overall regression
- Lack of fit.
  Studies in which no requirement could be estimated according to equation 2 were excluded from the database for the overall regression.
- Co-limitation of other amino acids

In the first review (see paragraph 2.2, criterion 7) only studies were included in the database if, besides the concentration of the amino acid of interest, the concentration of several other essential amino acids in the basal diet was at least 90% or 85% (depending on the amino acid) of the requirement (on AFD basis) according to CVB (1996). In this second review it was evaluated if the ratios of these amino acids relative to the amino acid of interest on an AFD basis were at least 0.90 of the ratio of the requirement of the same amino acids on an AFD basis according to CVB (1996). In formula:
(level non test amino acid X basal diet in $study_i$)/(calculated requirement test amino acid in $study_i$) $\geq$ 0.90 * (requirement non test amino acid X, CVB 1996)/(requirement test amino acid, CVB 1996).

The regression model for the requirement of SID amino acid content and AFD amino acid content is described by the following mathematical equation:

$Req_i = ß_0 + ß_1 * \ln(Age_i) + \underline{\varepsilon i}$ **(3)**

Where: Req_i = amino acid requirement (content (% in diet))
$ß_0$ = estimated amino acid requirement at hatch
$ß_1$ = estimated linear effect of ln(Age)
$\ln(Age_i)$ = $^e\log$ (Age i)
Age = average age of broilers in experiment (d)

Selection of candidate models with more factors included such as strain, gender, year, length of the experimental period, was not possible because of the restricted number of experiments that were accepted for overall regression analysis.

3 Lysine requirement values

3.1 Lysine background information on meta-analysis

3.1.1 Study details individual studies

In total, 26 studies each containing one or more experiments, were judged. Table 1 provides an overview of the 28 experiments from 11 papers that met the criteria described in Paragraph 2.2 for inclusion into the database. The procedure used to calculate the level of apparent faecal digestible lysine (see Par. 2.3) is also mentioned. The number of lysine supplementation levels per experiment ranged from 4 to 10. The data in the database covered various age periods of birds. In 15 experiments males were used , in 11 experiments females were used and in 2 experiments broilers were as hatched (mixed). Different strains were used in the experiments.

Papers that were not included in the database because studies did not meet the inclusion criteria are mentioned in Appendix 1.

Table 1

List of references that met the inclusion criteria for further evaluation of the lysine requirement of broilers in the present study.

Experiment number	Reference	Nr. of dose levels	Range of AFD Lys content (g/kg)	Age of broilers (d)	Gender	Strain
11 [a)]	Mahdavi et al. (2012)	6	7.7-11.2	35-49	male	-
12 [a)]	Mahdavi et al. (2012)	6	7.7-11.2	35-49	female	-
21 [a)]	Garcia et al. (2006)	5	7.0 – 11.0	7 – 21	male	Cobb
22 [a)]	Garcia et al. (2006)	4	7.0 – 10.0	7 - 21	male	Cobb
23 [a)]	Garcia et al. (2006)	5	7.0 – 11.0	7 - 21	female	Cobb
24 [a)]	Garcia et al. (2006)	4	7.0 – 10.0	7 - 21	female	Cobb
25 [a)]	Garcia et al. (2006)	5	8.0 – 11.2	7 - 21	male	Cobb
26 [a)]	Garcia et al. (2006)	5	8.0 – 11.2	7 - 21	male	Cobb
27 [a)]	Garcia et al. (2006)	5	8.0 – 11.2	7 - 21	female	Cobb
28 [a)]	Garcia et al. (2006)	5	8.0 – 11.2	7 - 21	female	Cobb
31 [a)]	Dozier et al. (2009)	6	9.0 – 12.5	14-28	male	Ross
41 [a)]	Dozier et al. (2009)	9	8.5 – 12.5	14-28	male	Ross
42 [a)]	Dozier et al. (2009)	9	8.5 – 12.5	14-28	female	Ross
51 [a)]	Dozier and Payne exp. 1 (2012)	7	9.2 – 13.9	7-15	female	Ross
52 [a)]	Dozier and Payne exp. 2 (2012)	7	9.1 – 14.0	7-15	female	HubbardxCobb
53 [a)]	Dozier and Payne exp. 1 (2012)	7	9.2 – 13.9	1-7	female	Ross
54 [a)]	Dozier and Payne exp. 2 (2012)	7	9.1 – 14.0	1-7	female	HubbardxCobb
81 [c)]	Mehri et al. (2010)	6	6.2 – 12.2	15-28	mixed	Ross
111 [a)]	Aftab et al. (2007) exp. 1	5	7.2 – 9.7	4-21	mixed	Hubbard
121 [b)]	Fatufe et al. (2004)	10	3.1 – 15.3	8-21	male	Ross
131 [a)]	Berri et al. (2008)	4	8.3 – 11.3	21-42	male	Ross
132 [a)]	Berri et al. (2008)	4	8.3 – 11.3	21-42	male	Ross
141 [a)]	Dozier et al. (2010)	9	6.4 – 12.0	28-42	male	Ross
142 [a)]	Dozier et al. (2010)	9	6.4 – 12.0	28-42	male	Cobb
151 [a)]	Mack et al (1999)	6	6.6 – 11.1	20-40	male	Ross
152 [a)]	Mack et al (1999)	6	6.6 – 11.1	20-40	male	ISA
210 [a)]	Garcia et al. (2006)	5	8.9 – 12.1	7 - 21	male	Cobb
212 [a)]	Garcia et al. (2006)	5	8.9 – 12.1	7 - 21	female	Cobb

[a)] Digestible Lys level (on AFD basis) in basal diet analysed by authors and used in present study.

[b)] Total Lys level in basal diet analysed by authors and used in present study; calculation of digestible Lys level (on AFD basis) in basal diet was done by using these data in combination with the AFD coefficients in the CVB Feed Table (2007).

[c)] Total Lys level calculated using the ingredient composition as published and the amino acid profile of CVB Feed Table (2007); calculation of digestible Lys level (on AFD basis) was done as described in [b)].

3.1.2 Results of curve fitting and lysine requirements for individual studies

In general, the response of BWG and FCR to AFD and SID lysine content in the experimental diet showed an exponential relationship, when using as input the mean data for BWG and FCR per experimental group as provided in the original paper. The response of BWG and FCR to graded supplementation levels of dietary lysine was determined for all individual experiments according to the exponential model (**1**) described in paragraph 2.4.1.

The requirement for AFD and SID lysine was determined for each study and defined as the dietary lysine concentration at which 95% of the response (difference between performance at no additional lysine supplementation and the performance at the asymptotic value) was met according to equation **(2)** in the paragraph 2.4.1.

The results of fitting the individual studies according to equation **(2)** in the paragraph 2.4.1, together with details of the study and estimated AFD lysine requirement and SID lysine requirement for BWG and FCR are presented in Appendix 2 and 3, respectively. In these Appendices only the results of studies are presented that also met the criteria for inclusion for the overall analysis in paragraph 2.4.2.

3.1.3 Results of overall curve fitting and lysine requirements as a function of age

For estimating the AFD and SID lysine requirement as a function of age, an overall regression analysis was conducted on AFD and SID lysine requirement values derived from the individual experiments and the mean age in these experiments according to the general model **(3)** described in paragraph 2.4.2. An overview of experiments that were included in the overall regression analysis of the experiments is presented in Appendix 4 and 5. Details of the individual experiments and estimated AFD lysine requirement and SID lysine requirement for BWG and FCR are presented in these appendixes as well. Some studies had to be excluded from the overall fitting for both BWG and FCR because of co-limitation of other amino acids or lack of fit (see Appendix 6 and 7).

The results of the overall fitting are presented Paragraph 3.2 (figures 1 – 4) and in Paragraph 3.3 (Table 3)

3.2 Lysine requirement values

In paragraph 3.2.1 up to paragraph 3.2.4 the results of the overall regression analysis on requirement values derived from individual experiments are presented in graphs. Lysine requirement values in these paragraphs are expressed on AFD or SID lysine content in the diet and are expressed for BWG as well as FCR.

3.2.1 Requirement of AFD lysine expressed on dietary content for body weight gain

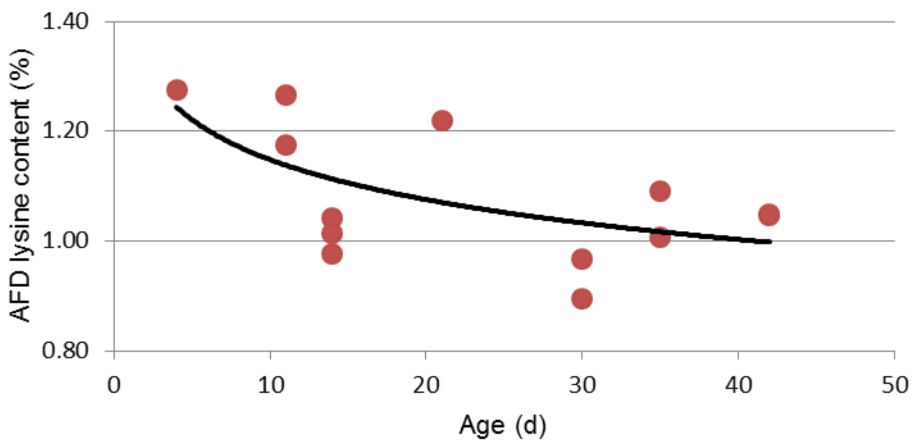

Figure 1 *Requirement of AFD lysine content (% in diet) for body weight gain at different ages (based on Exp.no. 11, 12, 22, 28, 212, 41, 51, 52, 54, 141, 142, 151, 152). The point at day 42 covers the results of two experiments.*

The fitted requirement of AFD lysine, expressed as a percentage of diet, for BWG was based on thirteen experiments.

3.2.2 Requirement of AFD lysine expressed on dietary content for feed conversion ratio

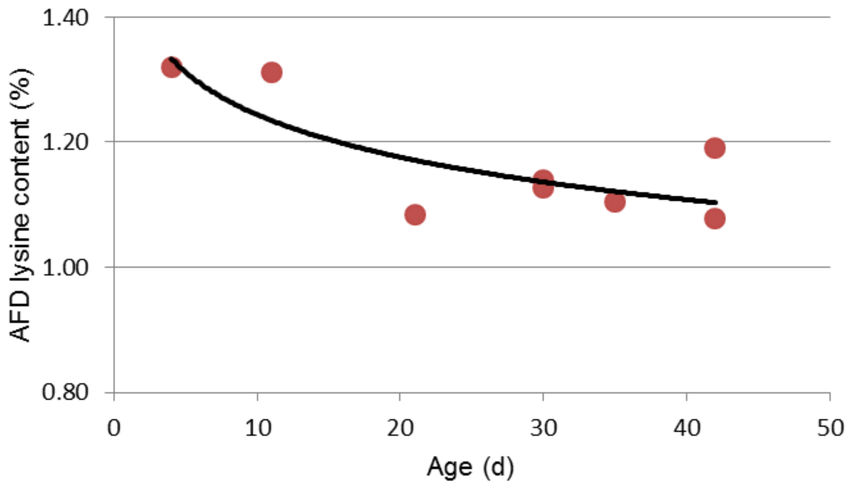

Figure 2 *Requirement of AFD lysine content (% in diet) for feed conversion ratio at different ages (based on Exp.no.11, 12, 42, 52, 54, 142, 151 and 152). The point at day 30 covers the results of two experiments.*

The fitted requirement of AFD lysine content expressed as a percentage of diet for FCR at different ages was based on eight experiments.

3.2.3 Requirement of SID lysine expressed on dietary content for body weight gain

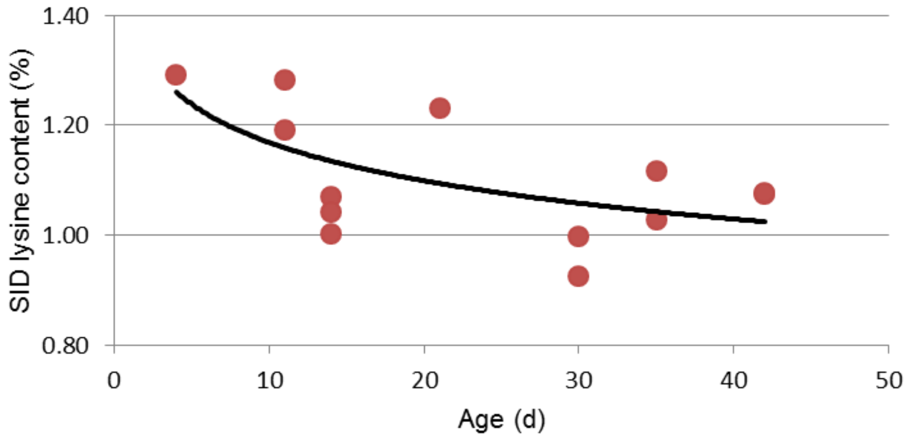

Figure 3 *Requirement of SID lysine content (% in diet) for body weight gain at different ages (based on Exp.no. 11, 12, 22, 28, 212, 41, 51, 52, 54, 141, 142, 151 and 152). The points at day 42 covers the results of two experiments.*

The fitted requirement of SID lysine content, expressed as a percentage of diet for BWG at different ages was based on thirteen experiments.

3.2.4 Requirement of SID lysine expressed on dietary content for feed conversion ratio

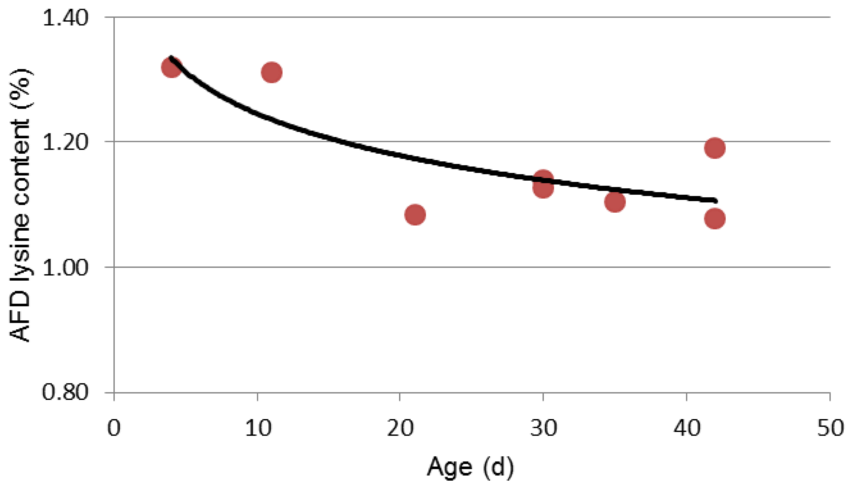

Figure 4 *Requirement of SID lysine content (% in diet) for feed conversion ratio at different ages (based on Exp.no 11, 12, 42, 52, 54, 142, 151 and 152). The point at day 30 covers the results of two experiments.*

The fitted requirement of SID lysine content for FCR expressed as a percentage of diet at different ages was based on eight experiments.

3.3 Requirement values of lysine on AFD and SID basis, expressed as content in the diet, at different ages for BWG and FCR

The overall regression analyses for the requirement values of AFD and SID lysine content for BWG and FCR as a function of age, resulted in the formulas presented in Table 2. In all cases, lysine requirement decreased with age significantly.

Table 2
Mathematical description of the AFD and SID lysine requirement, expressed as content in the diet, for BWG and FCR as a function of age based on the overall regression analysis (standard errors in brackets)

	Overall regression analysis [1]	P-value for age
AFD / BWG	Y=1.390 *(0.1259)* - 0.1050*(0.04145)**LN(age)	0.028
AFD/FCR	Y=1.461 *(0.0867)* - 0.1020 *(0.02730)**LN(age)	0.010
SID/BWG	Y=1.400 *(0.1200)* - 0.1006 *(0.03952)**LN(age)	0.027
SID/FCR	Y=1.468 *(0.0898)* - 0.0968 *(0.02829)**LN(age)	0.014

[1]): Y = lysine content in the diet (%).

The requirement values of AFD and SID lysine content at different ages are presented for BWG and FCR in Table 3.

Estimated AFD and SID lysine requirements for BWG are lower than for FCR. Until three weeks of age the AFD and SID lysine requirement for BWG are significantly higher than the CVB (2007) recommendations for AFD lysine requirement. AFD and SID lysine requirement for FCR are over the entire period significantly higher than the CVB (2007) recommendations for AFD lysine requirement.

Table 3

Requirement of lysine content (% of diets) according to the regression models for body weight gain and feed conversion ratio

Day	AFD lysine (% in diet)	AFD lysine (% in diet)		SID lysine (% in diet)	
	CVB (2007)	Body weight gain	Feed conversion ratio	Body weight gain	Feed conversion ratio
5	1.05	1.22	1.30	1.24	1.31
6	1.05	1.20	1.28	1.22	1.29
7	1.05	1.19	1.26	1.20	1.28
8	1.05	1.17	1.25	1.19	1.27
9	1.05	1.16	1.24	1.18	1.26
10	1.05	1.15	1.23	1.17	1.25
11	1.05	1.14	1.22	1.16	1.24
12	1.05	1.13	1.21	1.15	1.23
13	1.05	1.12	1.20	1.14	1.22
14	1.02	1.11	1.19	1.13	1.21
15	1.02	1.11	1.18	1.13	1.21
16	1.02	1.10	1.18	1.12	1.20
17	1.02	1.09	1.17	1.11	1.19
18	1.02	1.09	1.17	1.11	1.19
19	1.02	1.08	1.16	1.10	1.18
20	1.02	1.08	1.16	1.10	1.18
21	1.02	1.07	1.15	1.09	1.17
22	1.02	1.07	1.15	1.09	1.17
23	1.02	1.06	1.14	1.08	1.16
24	1.02	1.06	1.14	1.08	1.16
25	1.02	1.05	1.13	1.08	1.16
26	1.02	1.05	1.13	1.07	1.15
27	1.02	1.04	1.12	1.07	1.15
28	0.99	1.04	1.12	1.06	1.15
29	0.99	1.04	1.12	1.06	1.14
30	0.99	1.03	1.11	1.06	1.14
31	0.99	1.03	1.11	1.05	1.14
32	0.99	1.03	1.11	1.05	1.13
33	0.99	1.02	1.10	1.05	1.13
34	0.99	1.02	1.10	1.05	1.13
35	0.99	1.02	1.10	1.04	1.12
36	0.99	1.01	1.10	1.04	1.12
37	0.99	1.01	1.09	1.04	1.12
38	0.99	1.01	1.09	1.03	1.12
39	0.99	1.01	1.09	1.03	1.11
40	0.99	1.00	1.08	1.03	1.11
41	0.99	1.00	1.08	1.03	1.11
42	0.99	1.00	1.08	1.02	1.11

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The CVB project group ‘Digestible Amino Acids Poultry’, consisting of scientists and representatives of the Dutch feed industry, guided this study. The authors would like to acknowledge with much appreciation the members of this project group for their valuable input to all phases of this project. The members of the CVB project group ‘Digestible Amino Acids Poultry’ were:

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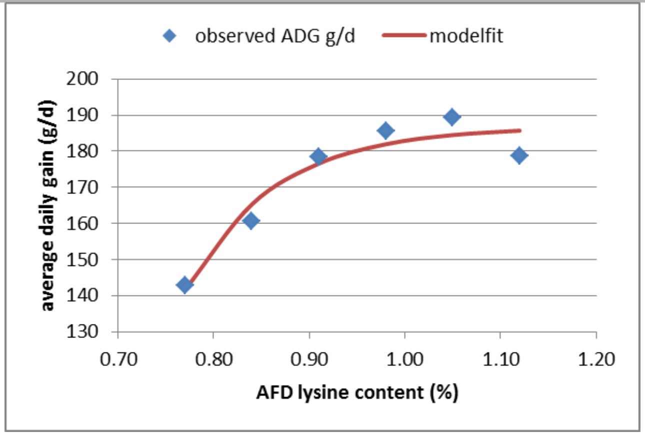
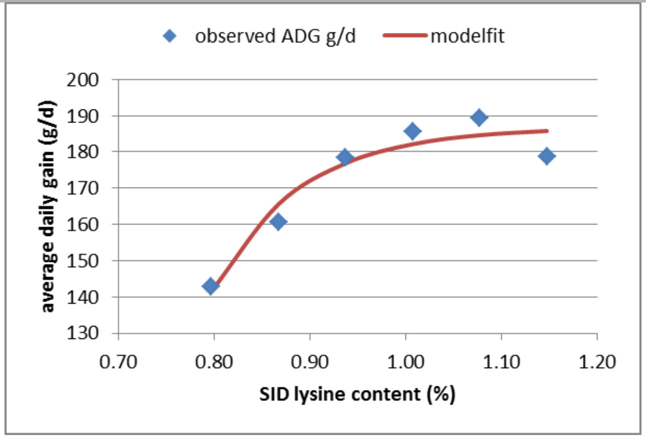
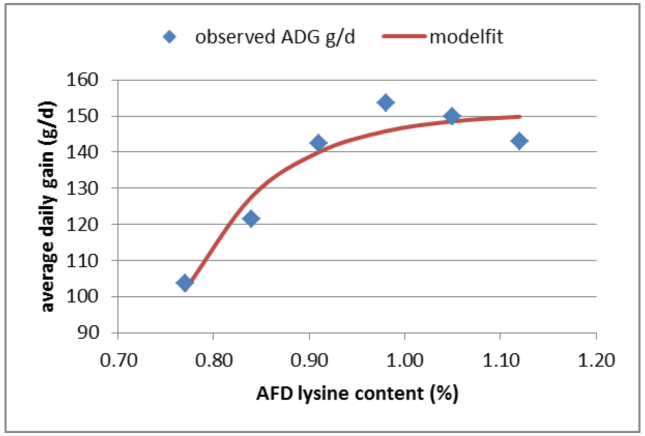
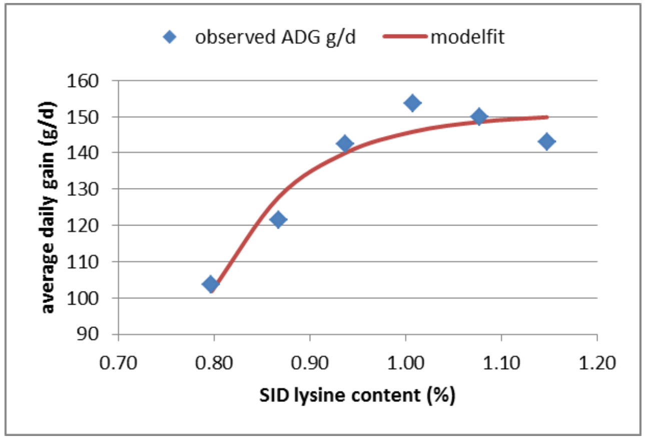
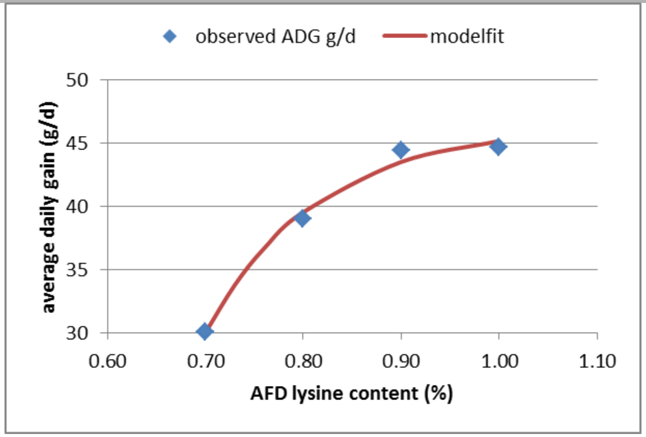
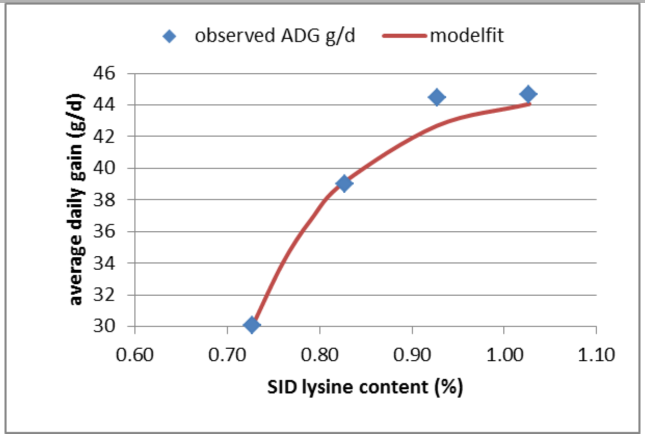
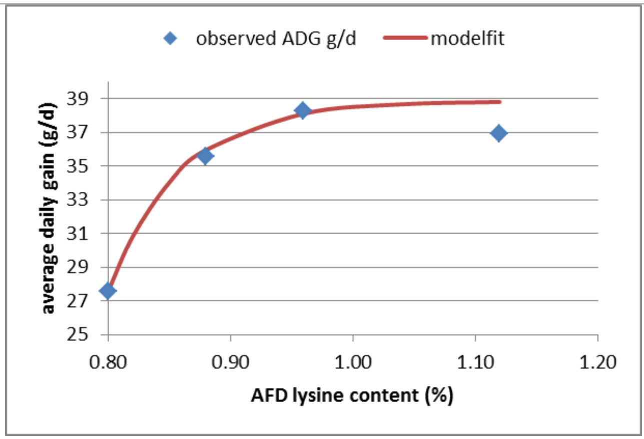
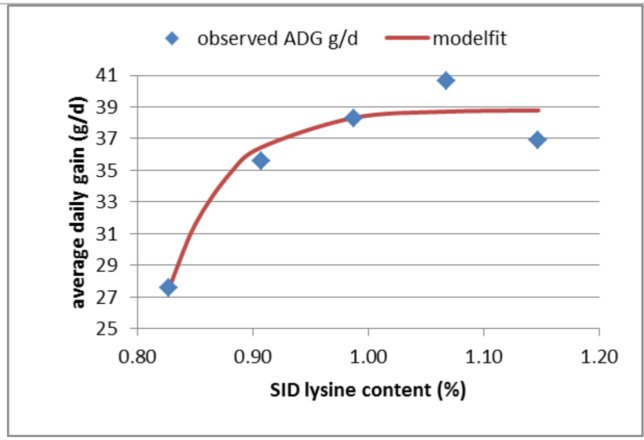
Appendices

Appendix 1 List of references which have not been included in the database with reason

Reference	Journal	Reason
Knowles & Southern (1998)	Poultry Sci: 77:564-569	Experiment was done in a factorial arrangement (lys×TSAA) and there were no data for performance (weight gain & feed intake) per factor because of interactions.
Wijtten et al. (2004)	British poultry Sci: 45:504-511	There were only 2 levels of lysine
Wijtten et al. (2004)	Poultry Sci: 83:2005-2015	There were only 2 levels of lysine
Lemme et al. (2006)	Poultry Sci: 85:721-730	Experiment was done in a factorial arrangement and there were no data for performance (weight gain & feed intake) per factor because of interactions.
Garcia et al. (2006)	Poultry Science 85:498–504	Study 9 and 11: no statistical significant effect on BWG and/or FCR
Garcia et al. (2006)	Poultry Science 85:498–504	Study 13 and 14: sum of ingredient composition <100%
Usama Aftab et al., 2007	Asian-Aust. J. Anim. Sci	Second study: M+C and THR <-5% and ILE and VAL <-10% of limited level
Takeara et al., 2010	R. Bras. Zootec.	LYS >-10% of limited level
Campestrini et al., 2010	R. Bras. Zootec.	LYS >-10% of limited level
Dozier et al. (2010)	Poultry Sci. 89: 2173-2182	Diet dilution technique was used for creating the graded levels of diets and final diets were made by blending a high-lysine diet with a low-Lysine diet, therefore recalculation of diets according to CVB was not possible
Coca-sinova et al. (2010)	Poultry Sci. 89:1440-1450	Experiment was done in a factorial arrangement (lys×type of soybean meal) and there were no data for performance (weight gain & feed intake) per factor because of interactions.
Trindade Neto et al. (2011)	R. Bras. Zootec: 40:602-608	Data for feed intake were not given.
Cuevas A et al. (2011)	Rev. Mex. Cienc: 2:259-266	Diets were formulated in a wrong way and data were not valid
Rodrigo Santana Toledo et al. 2011	Revista Brasileira de Zootecnia	LYS >-10% of limiting level
Trindade Neto et al, 2011	Revista Brasileira de Zootecnia	LYS >-10% of limiting level
Hassan et al., 2011	www.epsaegypt.com/pdf	M+C <-10% of limited level
Neto et al., 2011	R. Bras. Zootec.	M+C <-10% of limited level
Panda et al., 2011	Asian-Aust. J. Anim. Sci.	THR and VAL <-10% of limited level
Corzo et al. (2012)	J. appl. Poultry Sci: 21:70-78	Experiment was done in a factorial arrangement (lys×Diet texture) and there were no data for performance (weight gain & feed intake) per factor because of interactions.

Appendix 2 The determined responses of body weight gain (g/d) as a function of the AFD and SID lysine content for each individual experiment (% CV=coefficient of variation; in brackets)

Experiment Number	AFD LYS	SID LYS	Exponential Curve for AFD	Exponential Curve for SID
11	1.05 (2.9)	1.07 (2.9)		
12	1.05 (4.2)	1.08 (4.2)		

Experiment Number	AFD LYS	SID LYS	Exponential Curve for AFD	Exponential Curve for SID
22	1.02 (2.2)	1.04 (2.2)		
28	0.95 (4.4)	0.98 (4.4)		

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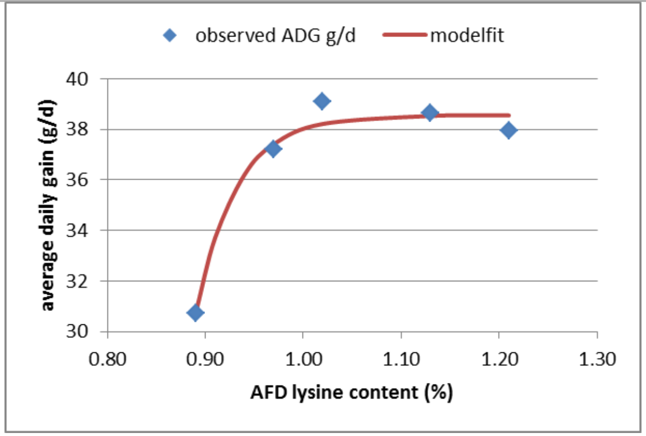
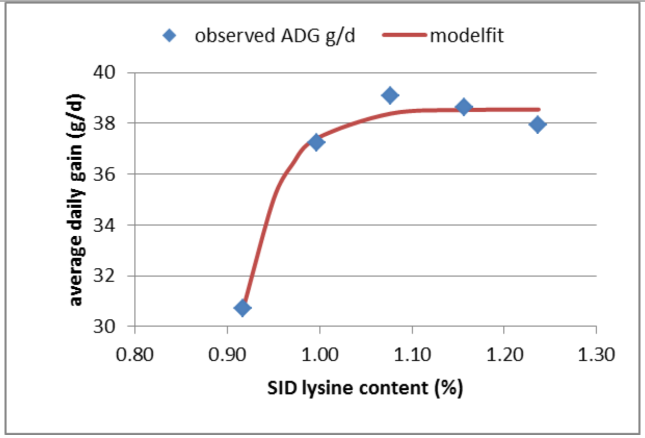
<tr><td>212</td><td>1.01 (1.6)</td><td>1.04 (1.6)</td><td>
</td><td>
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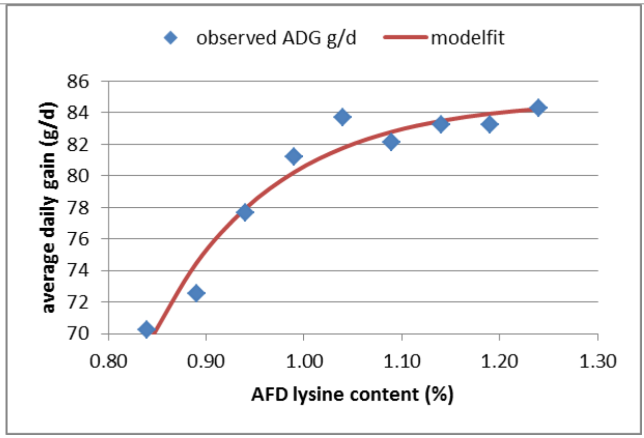
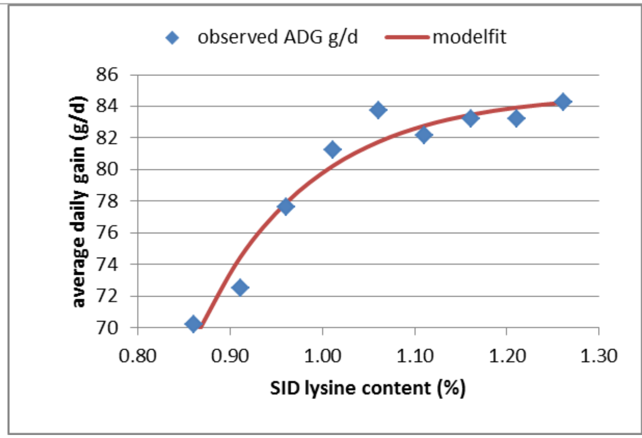
<tr><td>41</td><td>1.22 (1.4)</td><td>1.23 (1.4)</td><td>
</td><td>
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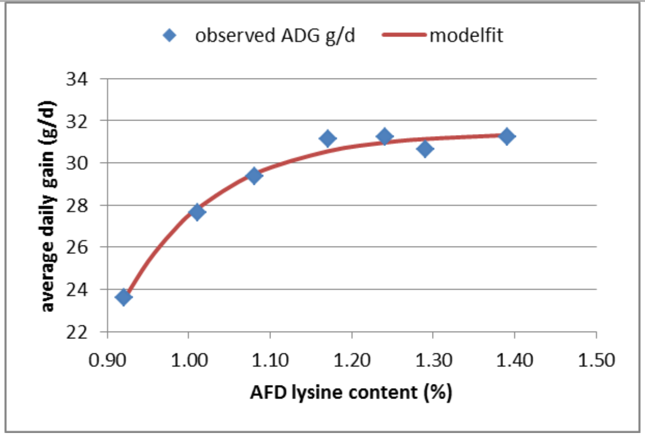
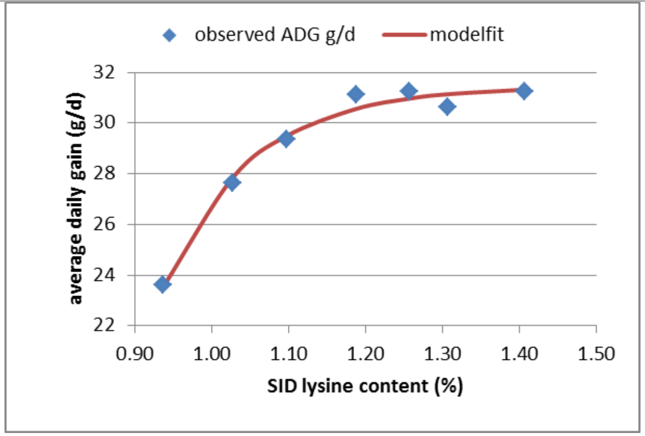
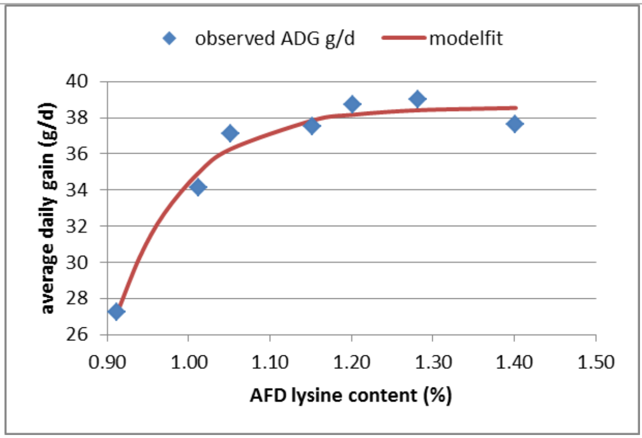
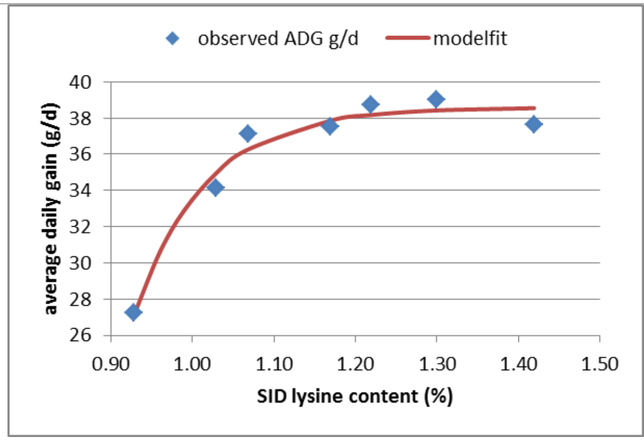
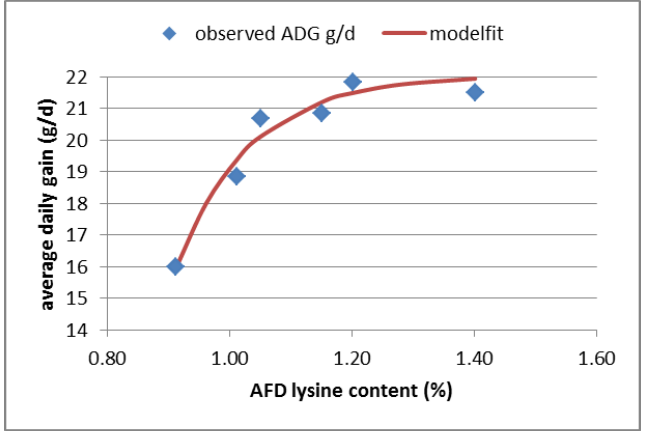
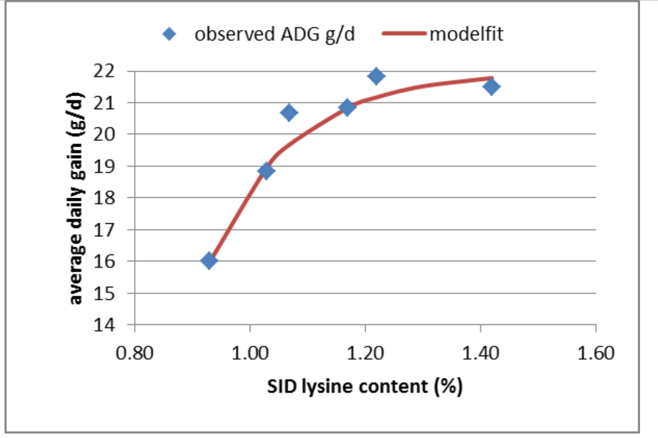
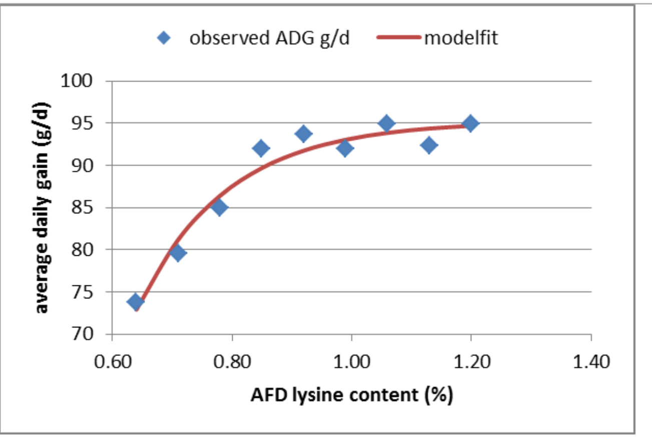
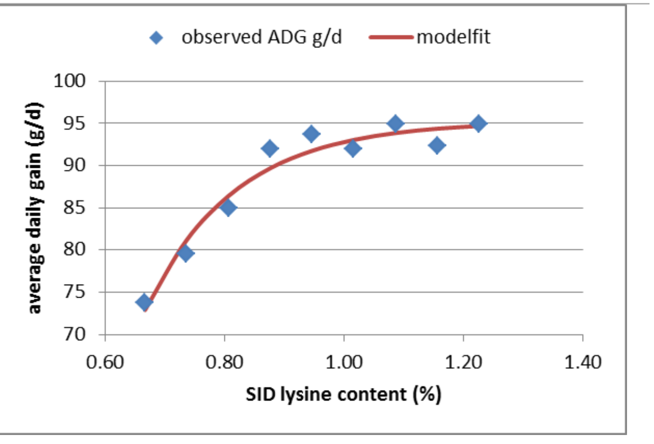
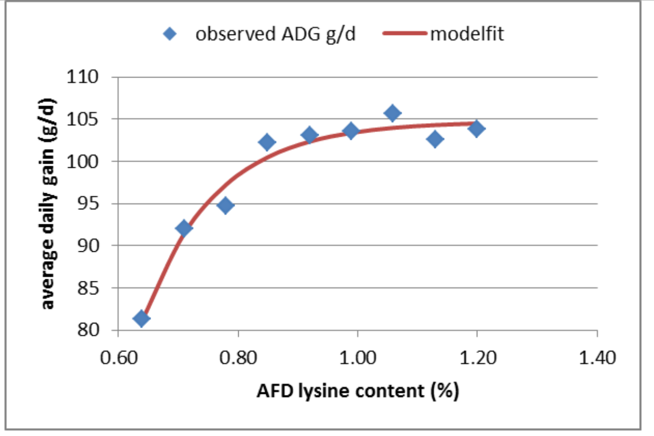
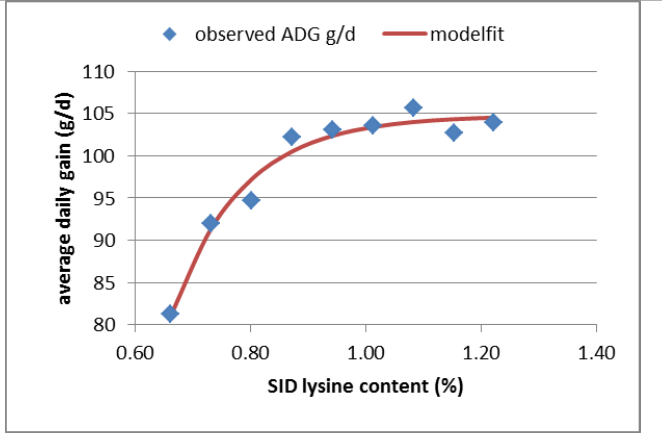
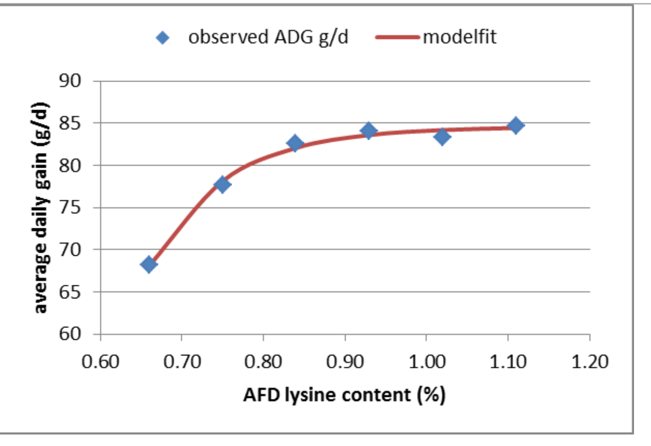
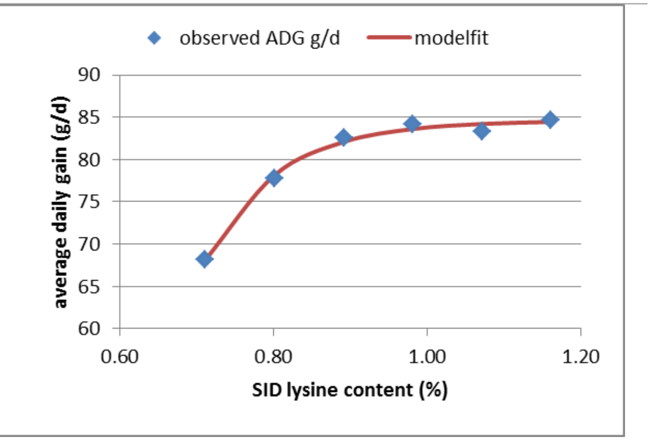
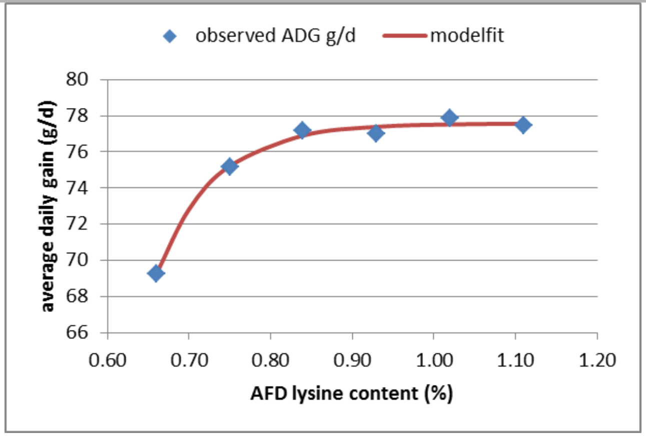
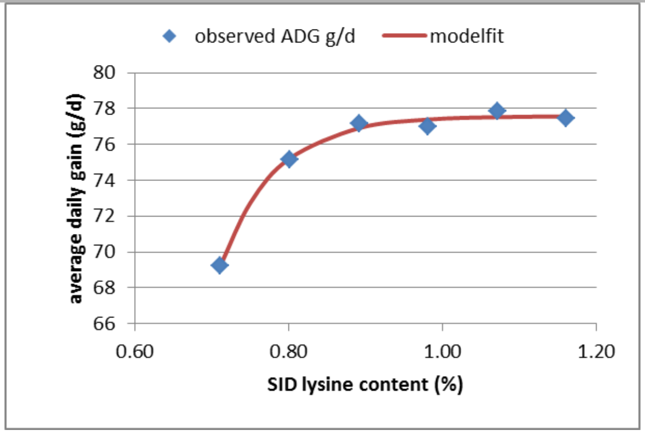
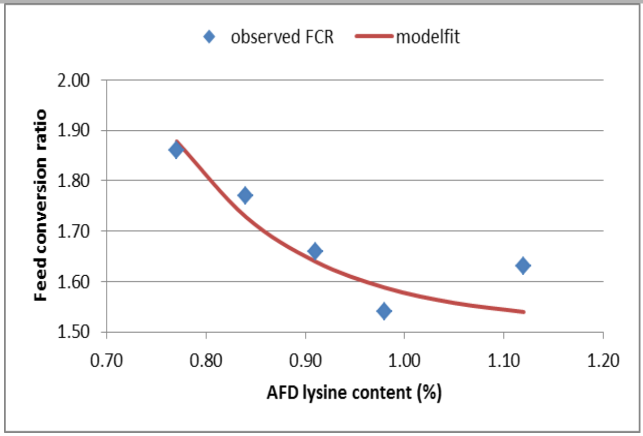
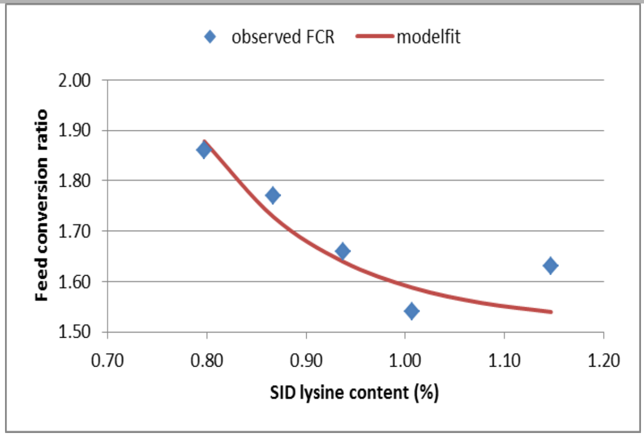
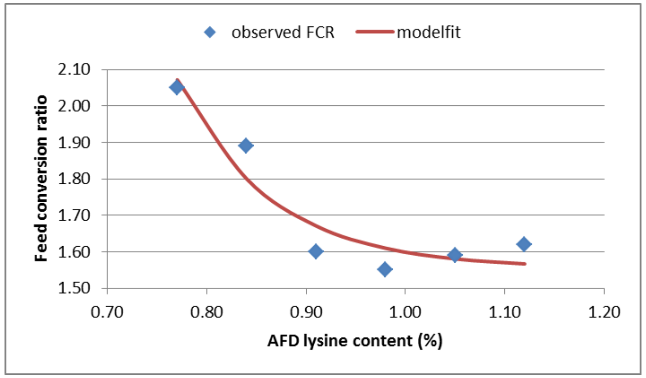
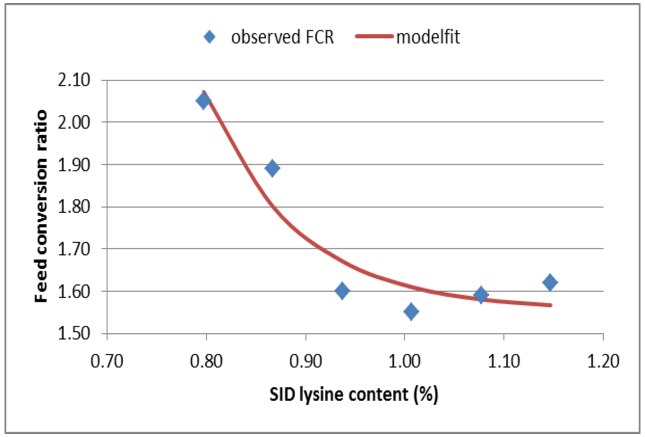
Experiment Number	AFD LYS	SID LYS	Exponential Curve for AFD	Exponential Curve for SID
51	**1.27 (1.2)**	**1.28 (1.2)**		
52	**1.17 (2.0)**	**1.19 (2.0)**		

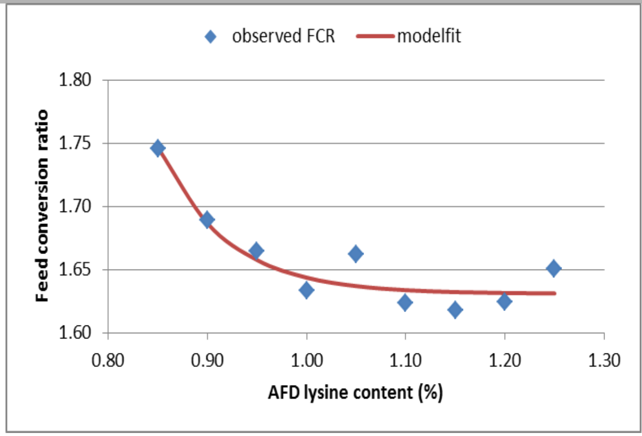
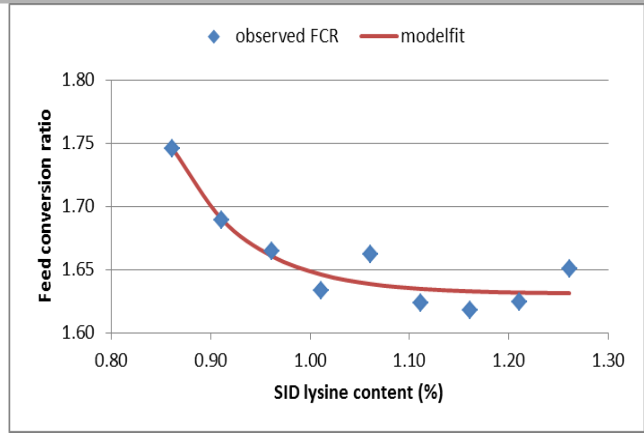
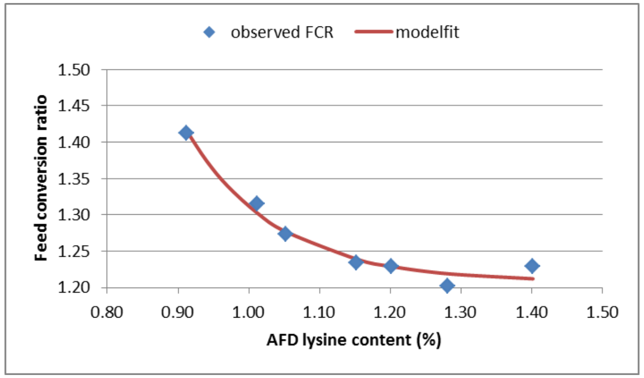
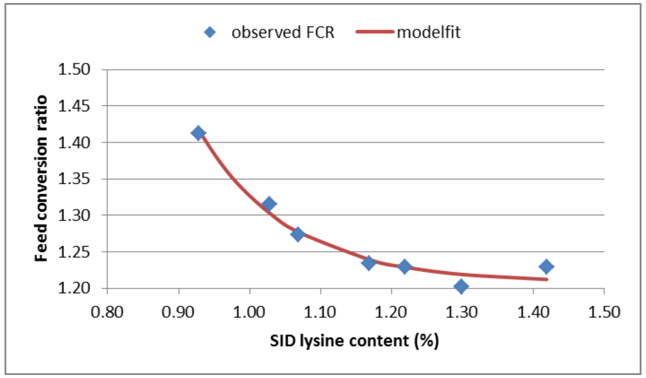
Experiment Number	AFD LYS	SID LYS	Exponential Curve for AFD	Exponential Curve for SID
54	1.35 (2.2)	1.36 (2.2)		
141	1.09 (1.8)	1.12 (1.8)		

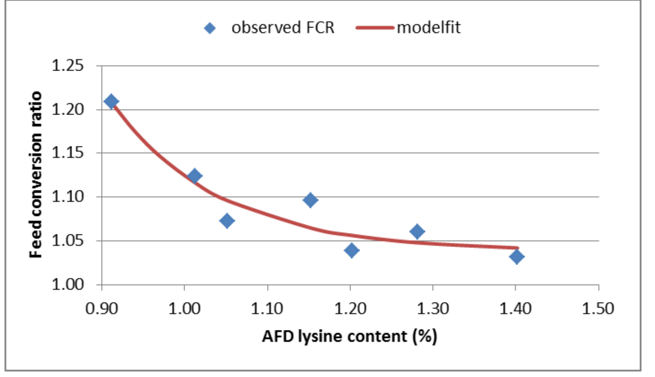
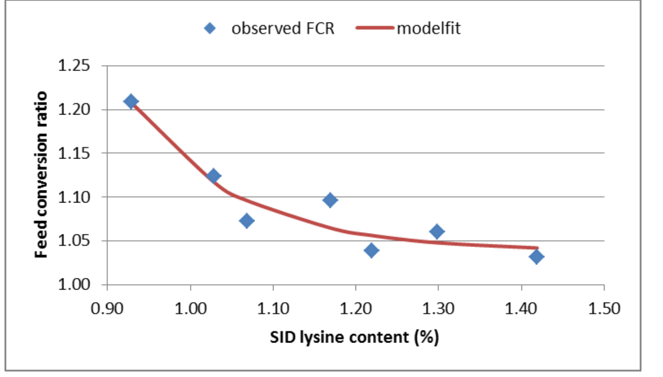
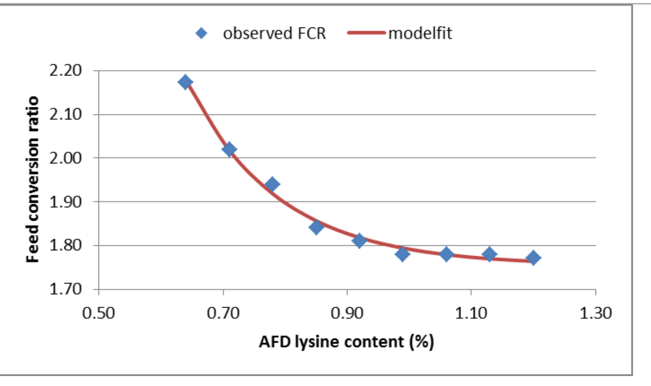
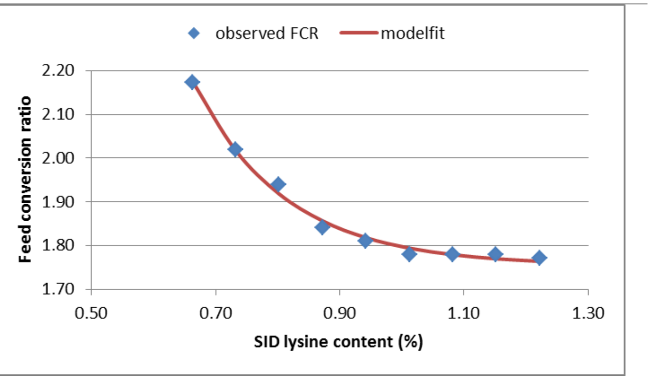
Experiment Number	AFD LYS	SID LYS	Exponential Curve for AFD	Exponential Curve for SID
142	**1.01 (1.4)**	**1.03 (1.4)**		
151	**0.97 (0.8)**	**1.00 (0.8)**		

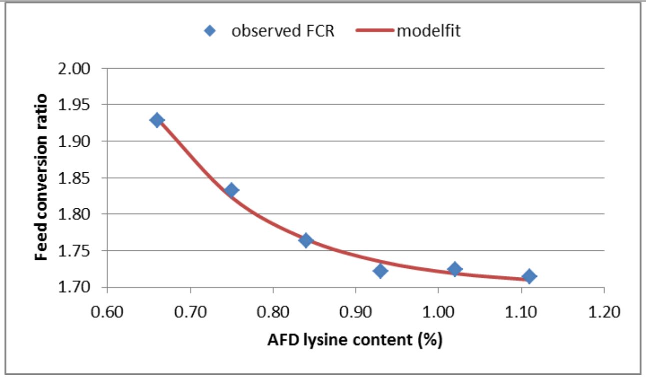
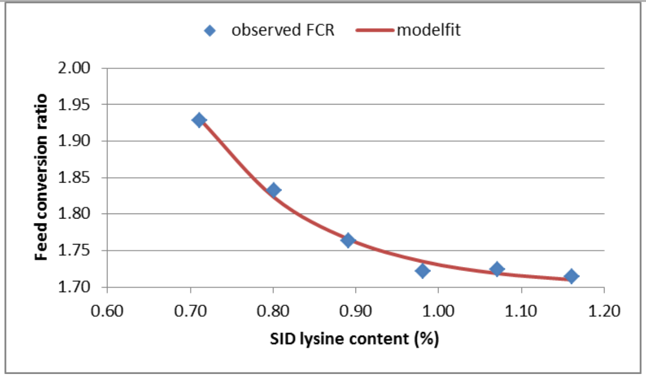
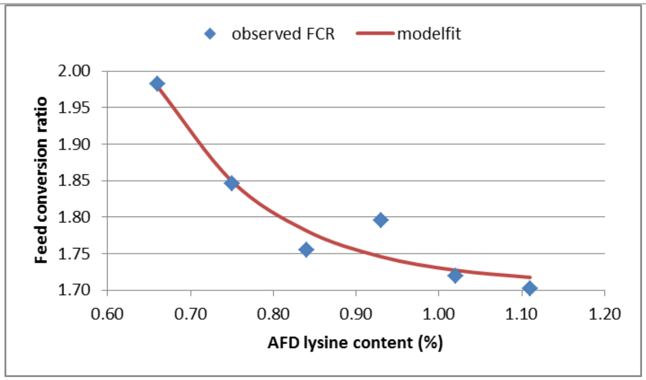
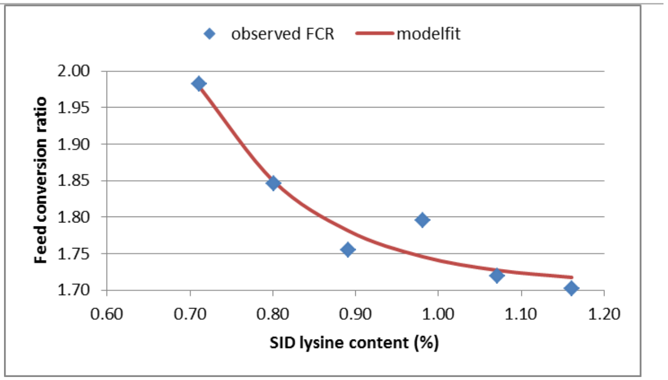
Experiment Number	AFD LYS	SID LYS	Exponential Curve for AFD	Exponential Curve for SID
152	0.89 (0.4)	0.92 (0.4)		

Appendix 3 The determined responses of feed conversion ratio as a function of the AFD and SID lysine content for each individual experiment (%CV=coefficient of variation; in brackets)

Experiment Number	AFD LYS	SID LYS	Exponential Curve for AFD	Exponential Curve for SID
11	1.16 (6.3)	1.19 (6.3)		
12	1.05 (6.3)	1.08 (6.3)		

Experiment Number	AFD LYS	SID LYS	Exponential Curve for AFD	Exponential Curve for SID
42	1.07 (1.0)	1.08 (1.0)		
52	1.29 (1.3)	1.31 (1.3)		

Experiment Number	AFD LYS	SID LYS	Exponential Curve for AFD	Exponential Curve for SID
54	**1.30 (2.4)**	**1.32 (2.4)**		
142	**1.08 (0.9)**	**1.10 (0.9)**		

Experiment Number	AFD LYS	SID LYS	Exponential Curve for AFD	Exponential Curve for SID
151	**1.11 (0.6)**	**1.14 (0.6)**		
152	**1.10 (3.7)**	**1.13 (3.7)**		

Appendix 4 Experiments for which the requirement on the AFD and SID lysine concentration for BWG was estimated according to equation 2 and that also met the criteria for the overall regression to estimate the relationship of the AFD and SID lysine requirement for BWG with age

Exp No.	Reference	AFD lysine concentration in the diet (%)	Gender	Strain	Age of birds	Published Requirement (%)	Models used by the reference	Re-calculated requirement using exponential models (%)	
								AFD lysine content for BWG	SID lysine content for BWG
11	Mahdavi et al (2012)	0.77, 0.84, 0.91, 0.98, 1.05, 1.12	Male	-	35-49	0.98	Broken-line, exponential, quadratic	1.05	1.07
12	Mahdavi et al (2012)	0.77, 0.84, 0.91, 0.98, 1.05, 1.12	Female	-	35-49	0.93	Broken-line, exponential, quadratic	1.05	1.08
22	Garcia et al (2006)	0.70, 0.80, 0.90, 1.00	Male	Cobb	7-21	0.93	Lineair and Quadratic	1.04	1.07
28	Garcia et al (2006)	0.80, 0.88, 0.96, 1.04, 1.12	Female	Cobb	7-21	0.97	Lineair and Quadratic	0.98	1.00
212	Garcia et al (2006)	0.89, 0.97, 1.02, 1.13, 1.21	Female	Cobb	7-21	0.99	Lineair and Quadratic	1.01	1.04
41	Dozier et al (2009)	0.85, 0.90, 0.95, 1.00, 1.05, 1.10, 1.15, 1.20, 1.25	Male	Ross	14-28	1.15	Broken-line	1.22	1.23
51	Dozier & Payne (2012)	0.91, 1.01, 1.05, 1.15, 1.20, 1.28, 1.40	Female	Ross	7-15	-	Quadratic broken line	1.27	1.28
52	Dozier & Payne (2012)	0.91, 1.01, 1.05, 1.15, 1.20, 1.28, 1.40	Female	Hubbard×Cobb	7-15	-	Quadratic broken line	1.17	1.19
54	Dozier & Payne (2012)	0.91, 1.01, 1.05, 1.15, 1.20, 1.28, 1.40	Female	Hubbard×Cobb	1-7	1.35	Quadratic broken line	1.27	1.29

Exp No.	Reference	AFD lysine concentration in the diet (%)	Gender	Strain	Age of birds	Published Requirement (%)	Models used by the reference	Re-calculated requirement using exponential models (%)	
								AFD lysine content for BWG	SID lysine content for BWG
141	Dozier et al (2010)	0.64, 0.71, 0.78, 0.85, 0.92, 0.99, 1.06, 1.13, 1.20	Male	Ross	28-42	1.05	Quadratic broken line	1.09	1.12
142	Dozier et al (2010)	0.64, 0.71, 0.78, 0.85, 0.92, 0.99, 1.06, 1.13, 1.20	Male	Cobb	28-42	1.01	Quadratic broken line	1.01	1.03
151	Mack et al (1999)	0.66, 0.75, 0.84, 0.93, 1.02, 1.11	Male	Ross	20-40	1.01	Broken-line, exponential	0.97	1.00
152	Mack et al (1999)	0.66, 0.75, 0.84, 0.93, 1.02, 1.11	Male	ISA	20-40	1.10	Broken-line, exponential	0.89	0.92

Appendix 5 Experiments for which the requirement on the AFD and SID lysine concentration for FCR was estimated according to equation 2 and that also met the criteria for the overall regression to estimate the relationship of the AFD and SID lysine requirement for FCR with age

Exp No.	Reference	SID lysine concentration in the diet (%)	Gender	Strain	Age of birds	Published Require-ment (%)	Models used by the reference	Re-calculated requirement using exponential models (%)	
								AFD lysine content for FCR	SID lysine content for FCR
11	Mahdavi et al (2012)	0.80, 0.87, 0.94, 1.01, 1.08, 1.15	Male	-	35-49	0.98	Broken-line, exponential, quadratic	1.16	1.19
12	Mahdavi et al (2012)	0.80, 0.87, 0.94, 1.01, 1.08, 1.15	Female	-	35-49	0.93	Broken-line, exponential, quadratic	1.05	1.08
42	Dozier et al (2009)	0.86, 0.91, 0.96, 1.01, 1.06, 1.11, 1.16, 1.21, 1.26	Male	Ross	14-28	1.15	Broken-line	1.07	1.08
52	Dozier & Payne (2012)	0.93, 1.03, 1.07, 1.17, 1.22, 1.30, 1.42	Female	Hubbard×Cobb	7-15	1.26	Quadratic broken line	1.29	1.31
54	Dozier & Payne (2012	0.93, 1.03, 1.07, 1.17, 1.22, 1.30, 1.42	Female	Hubbard×Cobb	1-7	-	Quadratic broken line	1.30	1.32
142	Dozier et al (2010)	0.66, 0.73, 0.80, 0.87, 0.94, 1.01, 1.08, 1.15, 1.22	Male	Cobb	28-42	1.01	Quadratic broken line	1.08	1.10
151	Mack et al (1999)	0.71, 0.80, 0.89, 0.98, 1.07, 1.16	Male	Ross	20-40	1.23	Broken-line, exponential	1.11	1.14
152	Mack et al (1999)	0.71, 0.80, 0.89, 0.98, 1.07, 1.16	Male	ISA	20-40	1.22	Broken-line, exponential	1.10	1.13

Appendix 6 References omitted in the overall regression of BWG response to AFD and SID lysine content

Exp No.	Reference	Lysine concentration in the diet (%)		Gender	Strain	Age of birds	Published Requirement (%)	Models used by the reference	Re-calculated requirement for BWG using exponential models (lysine content in %)		Reason for exclusion*
		On AFD basis	On SID basis						On AFD basis	On SID basis	
1	Garcia et al (2006)	0.7, 0.8, 0.9, 1.0, 1.1	0.73, 0.83, 0.93, 1.03, 1.13	Male	Cobb	7-21	0.91	Linear and Quadratic	-	-	2
23	Garcia et al (2006)	0.7, 0.8, 0.9, 1.0, 1.1	0.73, 0.83, 0.93, 1.03, 1.13	Female	Cobb	7-21	0.90	Linear and Quadratic	-	-	2
24	Garcia et al (2006)	0.7, 0.8, 0.9, 1.0, 1.1	0.73, 0.83, 0.93, 1.03	Female	Cobb	7-21	0.82	Linear and Quadratic	-	-	2
25	Garcia et al (2006)	0.80, 0.88, 0.96, 1.04, 1.12	0.83, 0.91, 0.99, 1.07, 1.15	Male	Cobb	7-21	1.01	Linear and Quadratic	1.14	1.17	3
26	Garcia et al (2006)	0.80, 0.88, 0.96, 1.04, 1.12	0.83, 0.91, 0.99, 1.07, 1.15	Male	Cobb	7-21	0.97	Linear and Quadratic	1.10	1.13	3
27	Garcia et al (2006)	0.80, 0.88, 0.96, 1.04, 1.12	0.83, 0.91, 0.99, 1.07, 1.15	Female	Cobb	7-21	0.97	Linear and Quadratic	1.07	1.10	3
210	Garcia et al (2006)	0.89, 0.97, 1.02, 1.13, 1.21	0.92, 1.00, 1.08, 1.16, 1.24	Male	Cobb	7-21	1.03	Linear and Quadratic	1.15	1.18	3
31	Dozier et al. (2009)	0.9, 0.97, 1.04, 1.11, 1.18, 1.25	0.92, 0.99, 1.06, 1.13, 1.20, 1.27	male	Ross	14-28	0.99	Broken-line	-	-	2
42	Dozier et al (2009)	0.85, 0.90, 0.95, 1.00, 1.05, 1.10, 1.15, 1.20, 1.25	0.86, 0.91, 0.96, 1.01, 1.06, 1.11, 1.16, 1.21, 1.26	Male	Ross	14-28	1.15	Broken-line	1.42	1.43	1
53	Dozier & Payne (2012)	0.92, 1.01, 1.08, 1.17, 1.24, 1.29, 1.39	0.94, 1.03, 1.10, 1.19, 1.26, 1.31, 1.42	Female	Ross	1-7	1.38	Quadratic broken line	1.72	1.74	1

Exp No.	Reference	Lysine concentration in the diet (%)		Gender	Strain	Age of birds	Published Requirement (%)	Models used by the reference	Re-calculated requirement for BWG using exponential models (lysine content in %)		Reason for exclusion*
		On AFD basis	On SID basis						On AFD basis	On SID basis	
81	Mehri et al (2010)	0.62, 0.74, 0.86, 0.98, 1.10, 1.22	0.61, 0.73, 0.85, 0.97, 1.09, 1.21	M+F	Ross	15-28	1.08	Linear and Quadratic broken line	-	-	2
121	Fatufe et al (2004)	0.31, 0.36, 0.43, 0.60, 0.68, 0.73, 0.93, 1.12, 1.29, 1.53	0.32, 0.38, 0.44, 0.61, 0.69, 0.74, 0.94, 1.13, 1.30, 1.54	Male	Ross	8-21	0.99	Logistic models	3.22	3.23	1
131	Berri et al (2008)	0.83, 0.93, 1.03, 1.13	0.82, 0.92, 1.02, 1.12	Male	Ross	21-42	-	Mean comparison test	-	-	2
132	Berri et al (2008)	0.83, 0.93, 1.03, 1.13	0.82, 0.92, 1.02, 1.12	Male	Ross	21-42	-	Mean comparison test	-	-	2

*Explanation of codes for exclusion:

1 = Calculated requirement >110% of highest inclusion level of test amino acid; 2 = Lack of fit; 3 = Co-limitation of non-test amino acid(s).

Appendix 7 References omitted in the overall regression of FCR response to AFD and SID lysine content

Exp No.	Reference	Lysine concentration in the diet (%)		Gender	Strain	Age of birds	Published Requirement (%)	Models used by the reference	Re-calculated requirement for FCR using exponential models (lysine content in %)		Reason for exclusion*
		On AFD basis	On SID basis						On AFD basis	On SID basis	
21	Garcia et al (2006)	0.7, 0.8, 0.9, 1.0, 1.1	0.73, 0.83, 0.93, 1.03, 1.13	Male	Cobb	7-21	0.96	Lineair and Quadratic	-	-	2
22	Garcia et al (2006)	0.7, 0.8, 0.9, 1.0, 1.1	0.73, 0.83, 0.93, 1.03, 1.13	Male	Cobb	7-21	0.96	Lineair and Quadratic	-	-	2
23	Garcia et al (2006)	0.7, 0.8, 0.9, 1.0, 1.1	0.73, 0.83, 0.93, 1.03, 1.13	Female	Cobb	7-21	0.90	Linear and Quadratic	-	-	2
24	Garcia et al (2006)	0.7, 0.8, 0.9, 1.0, 1.1	0.73, 0.83, 0.93, 1.03	Female	Cobb	7-21	0.82	Linear and Quadratic	-	-	2
25	Garcia et al (2006)	0.80, 0.88, 0.96, 1.04, 1.12	0.83, 0.91, 0.99, 1.07, 1.15	Male	Cobb	7-21	1.01	Linear and Quadratic	-	-	2
26	Garcia et al (2006)	0.80, 0.88, 0.96, 1.04, 1.12	0.83, 0.91, 0.99, 1.07, 1.15	Male	Cobb	7-21	0.97	Linear and Quadratic	-	-	2
27	Garcia et al (2006)	0.80, 0.88, 0.96, 1.04, 1.12	0.83, 0.91, 0.99, 1.07, 1.15	Female	Cobb	7-21	0.97	Linear and Quadratic	-	-	2
28	Garcia et al (2006)	0.80, 0.88, 0.96, 1.04, 1.12	0.83, 0.91, 0.99, 1.07, 1.15	Female	Cobb	7-21	0.97	Linear and Quadratic	-	-	2
210	Garcia et al (2006)	0.89, 0.97, 1.02, 1.13, 1.21	0.92, 1.00, 1.08, 1.16, 1.24	Male	Cobb	7-21	1.03	Linear and Quadratic	-	-	2
212	Garcia et al (2006)	0.89, 0.97, 1.02, 1.13, 1.21	0.92, 1.00, 1.08, 1.16, 1.24	Female	Cobb	7-21	1.03	Linear and Quadratic	-	-	2
31	Dozier et al. (2009)	0.9, 0.97, 1.04, 1.11, 1.18, 1.25	0.92, 0.99, 1.06, 1.13, 1.20, 1.27	Male	Ross	14-28	0.99	Broken-line	2.09	2.09	1

Exp No.	Reference	Lysine concentration in the diet (%)		Gender	Strain	Age of birds	Published Requirement (%)	Models used by the reference	Re-calculated requirement for FCR using exponential models (lysine content in %)		Reason for exclusion*
		On AFD basis	On SID basis						On AFD basis	On SID basis	
41	Dozier et al (2009	0.85, 0.90, 0.95, 1.00, 1.05, 1.10, 1.15, 1.20, 1.25	0.86, 0.91, 0.96, 1.01, 1.06, 1.11, 1.16, 1.21, 1.26	Male	Ross	14-28	1.15	Broken-line	1.35	1.36	3
51	Dozier & Payne (2012)	0.92, 1.01, 1.08, 1.17, 1.24, 1.29, 1.39	0.94, 1.03, 1.10, 1.19, 1.26, 1.31, 1.42	Male	Ross	7-15	1.38	Quadratic broken line			3
53	Dozier & Payne (2012)	0.92, 1.01, 1.08, 1.17, 1.24, 1.29, 1.39	0.94, 1.03, 1.10, 1.19, 1.26, 1.31, 1.42	Female	Ross	1-7	1.38	Quadratic broken line	2.19	2.21	1
81	Mehri et al (2010)	0.62, 0.74, 0.86, 0.98, 1.10, 1.22	0.61, 0.73, 0.85, 0.97, 1.09, 1.21	M+F	Ross	15-28	1.08	Linear and Quadratic broken line	3.08	3.07	1
121	Fatufe et al (2004)	0.31, 0.36, 0.43, 0.60, 0.68, 0.73, 0.93, 1.12, 1.29, 1.53	0.32, 0.38, 0.44, 0.61, 0.69, 0.74, 0.94, 1.13, 1.30, 1.54	Male	Ross	8-21	0.99	Logistic models	1.37	1.39	3
131	Berri et al (2008)	0.83, 0.93, 1.03, 1.13	0.82, 0.92, 1.02, 1.12	Male	Ross	21-42	-	Mean comparison test	-	-	2
132	Berri et al (2008)	0.83, 0.93, 1.03, 1.13	0.82, 0.92, 1.02, 1.12	Male	Ross	21-42	-	Mean comparison test	-	-	2
141	Dozier et al (2010)	0.64, 0.71, 0.78, 0.85, 0.92, 0.99, 1.06, 1.13, 1.20	0.67, 0.74, 0.81, 0.88, 0.95, 1.02, 1.09, 1.16, 1.23	Male	Ross	28-42	1.05	Quadratic broken line	1.11	1.14	3

*Explanation of codes for exclusion:

1 = Calculated requirement >110% of highest inclusion level of test amino acid; 2 = Lack of fit; 3 = Co-limitation of non-test amino acid(s).

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

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