



Estimating requirement values for apparent faecal digestible and standardised ileal digestible threonine in broilers by a meta-analysis approach

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This study provides an update of the requirement values for apparent faecal digestible and standardised ileal digestible threonine in broilers by a meta-analysis approach.

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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 threonine. 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 broiler 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 threonine 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, 2015) and included in the database. These (calculated) dietary concentrations 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, 31 studies each containing one or more experiments, were judged. In total, 18 experiments from 10 papers met the criteria for inclusion into the database. The relation between the threonine requirement for BWG and FCR on AFD and on SID basis and age was not significant, which means that this desk study does not result in reliable information to estimate the levels of required threonine for broilers at different ages.

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, part of the former 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 of 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 growth 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 requirement 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 affects 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 threonine 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 threonine was studied were found in British Journal of Poultry Science, International Journal of Poultry Science, International Journal of Poultry Science, Journal of Animal Feed Science and Technology, Journal of Applied Poultry Research, Journal of Animal and Veterinary Advances Poultry Science, Poultry Science en and Revista Brasileira de Zootecnia. 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 lysine, methionine+cysteine 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 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 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 they used 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 threonine 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 threonine and/or other amino acids, the next option was to use the total level of threonine 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 threonine were used in the present study for the regression analyses to derive requirement values for threonine 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:

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

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 ; $(X_i - \text{MIN}(X_i))$; X_i = amino acid concentration of interest in experimental diets, $\text{MIN}(X_i)$ = amino acid concentration of interest in basal (non-supplemented) diet;
 ε_{ij} = 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:

$$\text{Req}_i = \frac{\ln(0.05)}{-C_i} + \text{MIN}(X_i) \quad (2)$$

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;
 $\text{MIN}(X_i)$ = 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 when, 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:

$$\frac{(\text{level non test amino acid X basal diet in study}_i)/(\text{calculated requirement test amino acid in study}_i)}{0.90 * (\text{requirement non test amino acid X, CVB 1996})/(\text{requirement test amino acid, CVB 1996})} \geq$$

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

$$\text{Req}_i = \beta_0 + \beta_1 * \ln(\text{Age}_i) + \varepsilon_i \quad (3)$$

Where: Req_i = amino acid requirement (content (% in diet))
 β_0 = estimated amino acid requirement at hatch
 β_1 = estimated linear effect of $\ln(\text{Age})$
 $\ln(\text{Age}_i)$ = \ln (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 Threonine requirement values

3.1 Threonine background information on meta-analysis

3.1.1 Study details individual studies

In total, 31 studies each containing one or more experiments, were judged. Table 1 provides an overview of the 18 experiments from 10 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 threonine (see Par. 2.3) is also mentioned. The number of threonine supplementation levels per experiment ranged from 4 to 6. The data in the database covered various age periods of birds. In 9 experiments males were used, in 3 experiments females were used and in 6 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 threonine requirement of broilers in the present study.

Experiment number	Reference	Nr. of dose levels	Range of AFD Thr content (g/kg)	Age of broilers (d)	Gender	Strain
51 ^{a)}	Mack et al. 1999	6	0.44-0.74	20-40	Male	ISA
52 ^{a)}	Mack et al. 1999	6	0.44-0.74	20-40	Male	Ross 208
71 ^{b)}	Ayasan et al. 2009	5	0.60-0.80	22-42	Male	Ross 308
161 ^{b)}	Kidd et al. 1996	6	0.53-0.69	1-21	Male+Female	Cobb
181 ^{c)}	Corzo et al. 2003	6	0.43-0.68	30-42	Female	Ross
231 ^{c)}	Corzo et al. 2007	6	0.38-0.74	21-42	Male	Ross 708
232 ^{c)}	Corzo et al. 2007	6	0.38-0.74	21-42	Male	Ross 708
241 ^{b)}	Ciftci and Ceylan 2004	4	0.50-0.68	1-22	Male+Female	Ross PM3
242 ^{b)}	Ciftci and Ceylan 2004	4	0.51-0.69	1-22	Male+Female	Ross PM3
243 ^{b)}	Ciftci and Ceylan 2004	4	0.46-0.64	21-42	Male+Female	Ross PM3
244 ^{b)}	Ciftci and Ceylan 2004	4	0.45-0.63	21-42	Male+Female	Ross PM3
711 ^{b)}	Rosa et al. 2001	6	0.48-0.68	1-18	Male	Classic Arbor Acres
712 ^{b)}	Rosa et al. 2001	6	0.48-0.68	1-18	Male	High yield broiler chicken
713 ^{b)}	Rosa et al. 2001	6	0.48-0.68	1-18	Male	High yield broiler chicken
714 ^{b)}	Rosa et al. 2001	6	0.48-0.68	1-18	Female	High yield broiler chicken
721 ^{a)}	Mehri et al. 2010	6	0.46-0.81	15-28	Male+Female	Ross 308
731 ^{a)}	Duarte et al. 2012	6	0.56-0.83	22-42	Male	Cobb
741 ^{a)}	Corza et al. 2009	6	0.43-0.83	14-28	Female	Ross TP16

^{a)} Digestible threonine level (on AFD basis) in basal diet analysed by authors and used in present study.

^{b)} Total threonine level in basal diet analysed by authors and used in present study; calculation of digestible Thr 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 threonine level calculated using the ingredient composition as published and amino acid profile of CVB Feed Table (2007); calculation of digestible Thr level (on AFD basis) was done as described in ^{b)}.

3.1.2 Results of curve fitting and threonine requirements for individual studies

In general, the response of BWG and FCR to AFD and SID threonine content in the experimental diet showed an exponential relationship, when using as input data 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 threonine was determined for all individual experiments according to exponential model (1) described in paragraph 2.4.1.

The requirement for AFD and SID threonine was determined for each study and defined as the dietary threonine concentration at which 95% of the response (difference between performance at no additional threonine 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 threonine requirement and SID threonine 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 threonine requirements as a function of age

For estimating the AFD and SID threonine requirement as a function of age, an overall regression analysis was conducted on AFD and SID threonine 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 threonine requirement and SID threonine 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 Threonine 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. Threonine requirement values in these paragraphs are expressed on AFD or SID basis on content in the diet and are expressed for BWG as well as FCR.

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

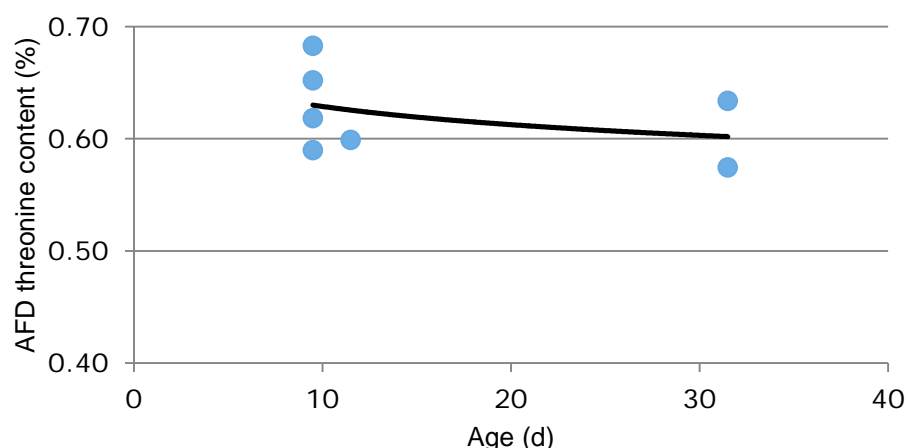


Figure 1 Requirement of AFD threonine content (% in diet) for body weight gain at different ages (based on Exp.no.231, 241, 243, 711, 712, 713 and 714).

The fitted requirement of AFD threonine, expressed as a percentage of diet, for BWG at different ages was based on 7 experiments. The mean ages in the different experiments were not well distributed over the entire production period. The variation in threonine requirement values, especially at young ages, was large. The relation between the threonine requirement and age for BWG on AFD basis was not significant.

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

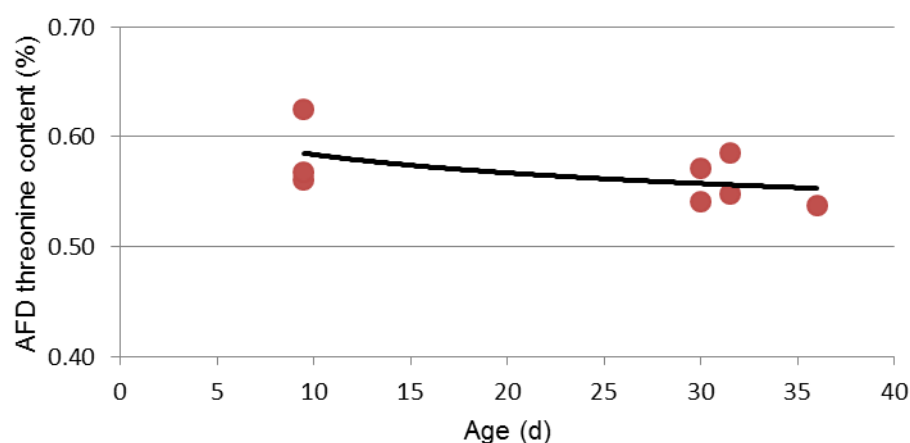


Figure 2 Requirement of AFD threonine content (% in diet) for feed conversion ratio at different ages (based on Exp.no. 51, 52, 181, 231, 232, 711, 712 and 713).

The fitted requirement of AFD threonine content, expressed as a percentage of diet, for FCR at different ages was based on 8 experiments. The mean ages in the different experiments were not well distributed over the entire production period. The variation in threonine requirement values, especially at young

ages, was large. The relation between the threonine requirement and age for BWG on AFD basis was not significant.

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

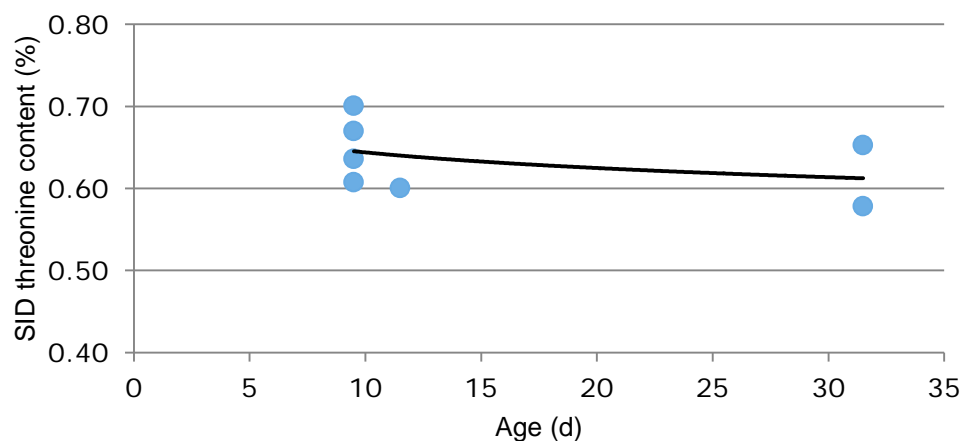


Figure 3 Requirement of SID threonine content (% in diet) for body weight gain at different ages (based on Exp.no. 231, 241, 243, 711, 712, 713 and 714).

The fitted requirement of SID threonine, expressed as a percentage of diet, for BWG at different ages was based on 7 experiments. The mean ages in the different experiments were not well distributed over the entire production period. The variation in threonine requirement values, especially at young ages, was large. The relation between the threonine requirement and age for BWG on SID basis was not significant.

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

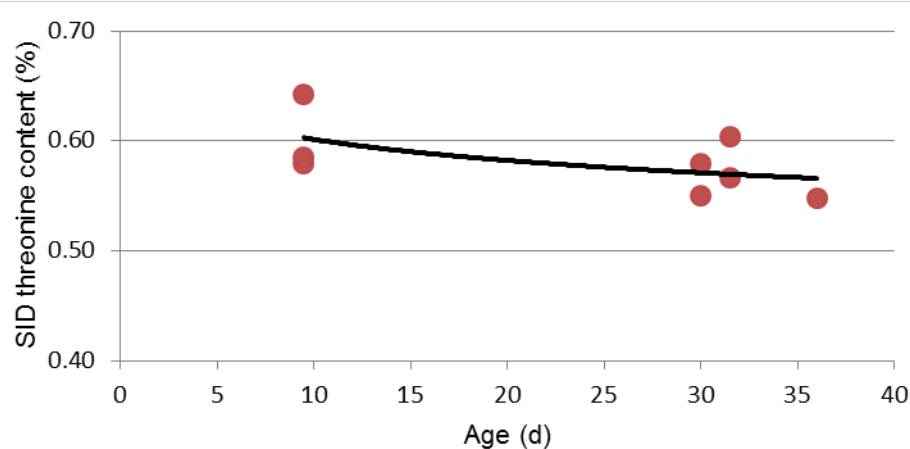


Figure 4 Requirement of SID threonine content (% in diet) for feed conversion ratio at different ages (based on Exp.no 51, 52, 181, 231, 232, 711, 712 and 713)

The fitted requirement of SID threonine, expressed as a percentage of diet, for FCR at different ages was based on 8 experiments. The mean ages in the different experiments were not well distributed over the entire production period. The variation in threonine requirement values, especially at young ages, was large. The relation between the threonine requirement and age for BWG on SID basis was not significant.

3.3 Requirement values of threonine 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 threonine content for BWG and FCR as a function of age, resulted in the formulas presented in Table 2. The relation between the threonine requirement and age for BWG and FCR on AFD and SID basis was not significant.

Table 2

Mathematical description of the AFD and SID threonine 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	$Y=0.683 (0.0742) - 0.0235 (0.02775)*\text{LN}(\text{age})$	0.44
AFD/FCR	$Y=0.639 (0.0481) - 0.0240 (0.01570)*\text{LN}(\text{age})$	0.18
SID/BWG	$Y=0.707 (0.0835) - 0.0275 (0.03121)*\text{LN}(\text{age})$	0.42
SID/FCR	$Y=0.665 (0.0505) - 0.0278 (0.01648)*\text{LN}(\text{age})$	0.14

¹⁾ Y = threonine content in the diet (%).

The relation between the threonine requirement for BWG and FCR on AFD and on SID basis and age was not significant, which means that this desk study does not result in reliable information to estimate the levels of required threonine for broilers at different ages.

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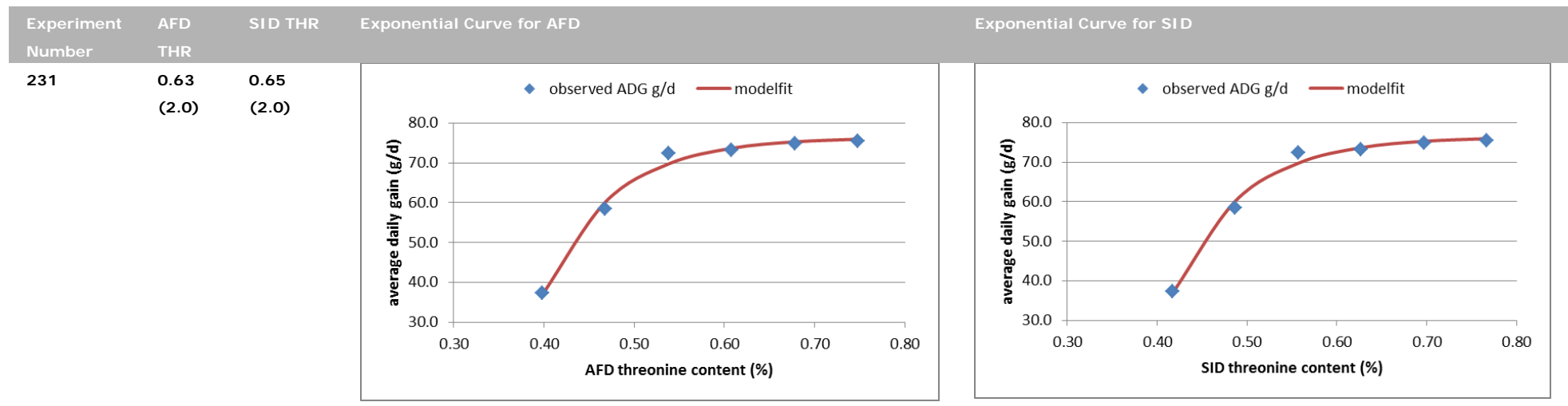
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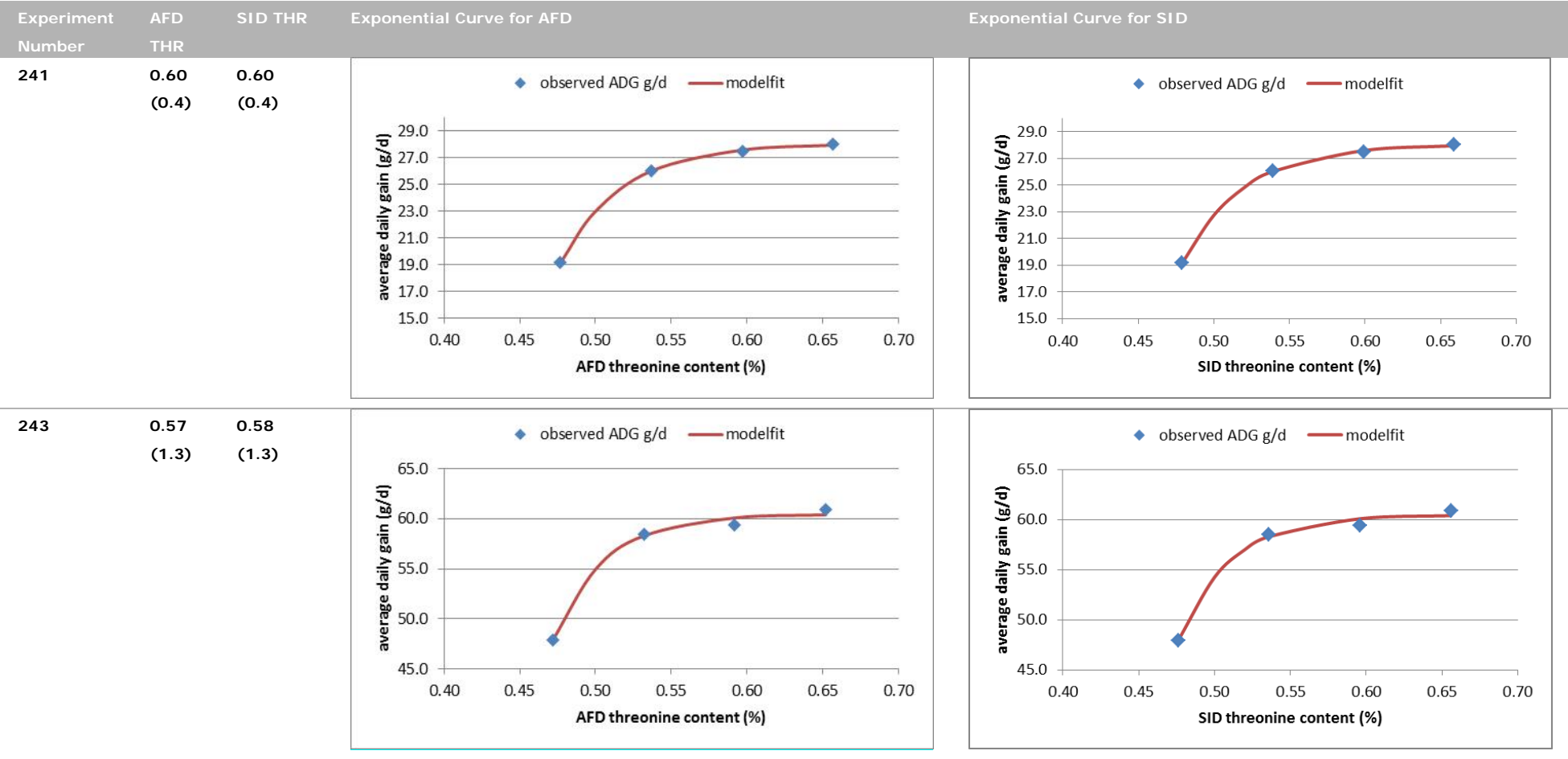
Appendices

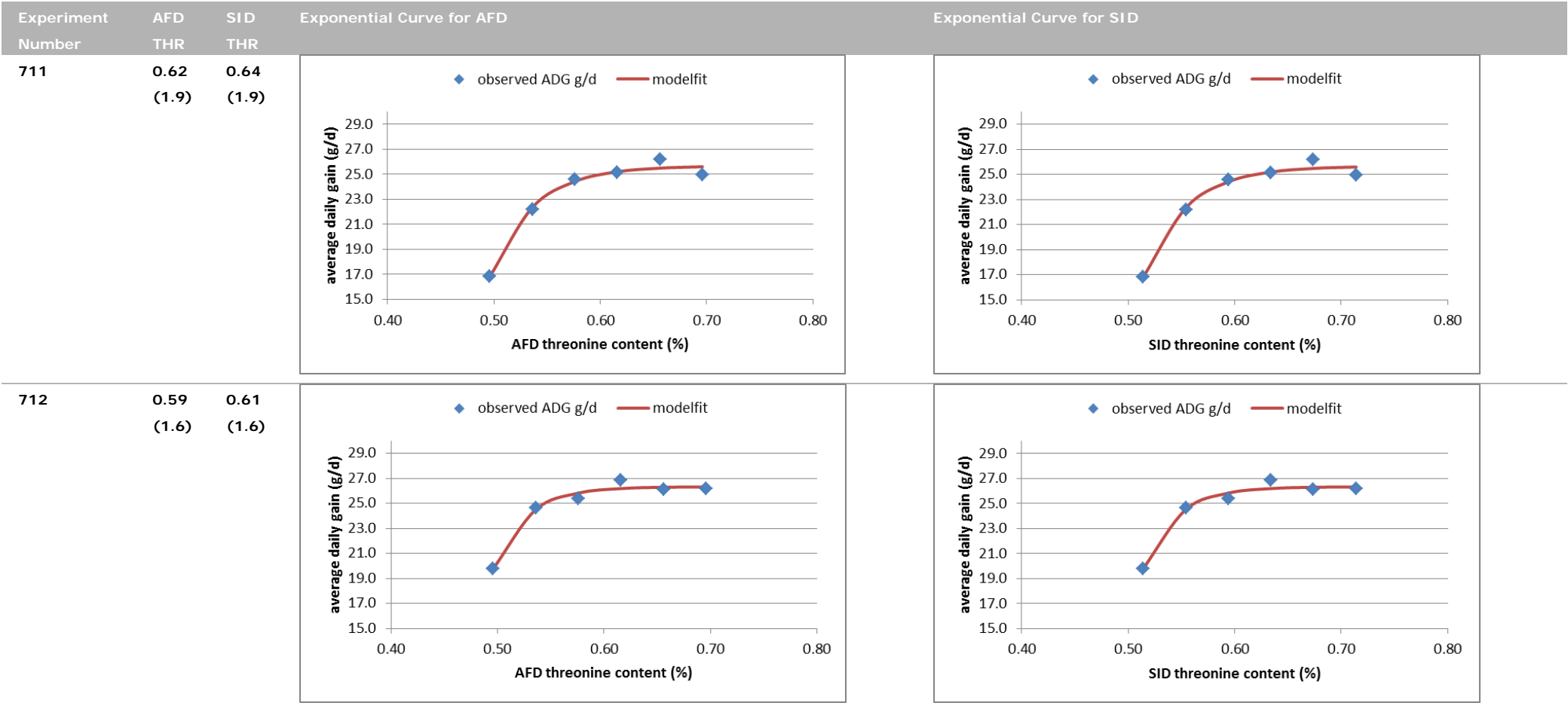
Appendix 1 List of references which have not been included in the database with reason

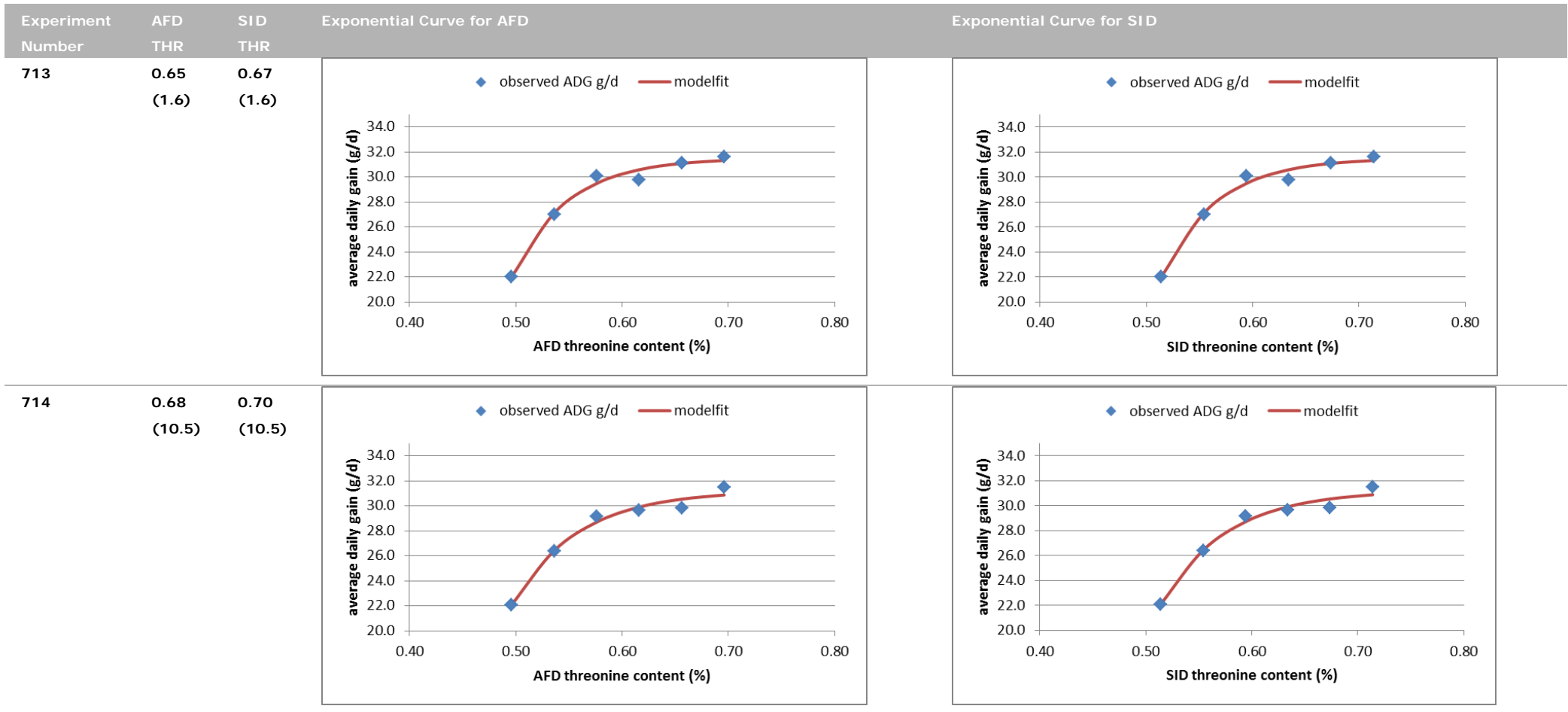
Reference	Journal	Reason
Kidd et al. 1996	Appl. Poultry Res.	Study, 2, 3 and 4: LYS and M+C <-10% of limiting level
Kidd et al. 1997	Poultry Science	Interaction between lysine and threonine
Penz et al. 1997	J. Appl. Poult. Res.	No significant BWG and FCR response
Kidd et al. 1999	J.Appl. Poultry Res.	LYS and M+C <-10% of limiting level
Alleman et al. 1999	British Poultry Science	Requirement not determined as concentration in the diet
Dozier et al. 2000a	J.Appl. Poultry Res.	LYS and M+C <-10% of limiting level
Dozier et al., 2000b	J. Appl. Poultry Res.	LYS, M+C and VAL <-10% of limiting level
Dozier III et al. 2000c	J. Appl. Poultry Res.	Only two dietary concentrations of threonine
Dozier III et al. 2001a	J. Appl. Poultry Res.	Only three dietary concentrations of threonine
Dozier III et al. 2001b	Poultry Science	Only two dietary concentrations of threonine
Kidd and Kerr 2009	J. Appl. Poultry Res.	>10% difference between published and calculated ME _{po}
Kidd et al. 2001	Animal Feed Science and Technology	Only three dietary concentrations of threonine
Kidd et al. 2002	J. Appl. Poult. Res.	Basal diet was not deficient in threonine concentration
Kidd et al. 2003	J.Appl. Poultry Res.	LYS and M+C <-10% of limiting level
Atencio et al. 2004	R. Bras. Zootec.	THR >-10% of limiting level
Kidd et al. 2004	Poultry Science	Strain is not mentioned
Ojano-Dirain and Waldroup	International Journal of Poultry Science	Only two dietary concentrations of threonine
Chee et al. 2010	British Poultry Science	Interactions of manno-oligosaccharides with dietary threonine and only three levels of threonine
Everett et al. 2010	J.Appl. Poultry Res.	THR >-10% of limiting level
Rao et al. 2011	Animal Feed Science and Technology	No significant BWG and FCR response
Ayasan et al. 2011	Süleyman Demirel Üniversitesi Ziraat Fakültesi Dergisi	THR >-10% of limiting level

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

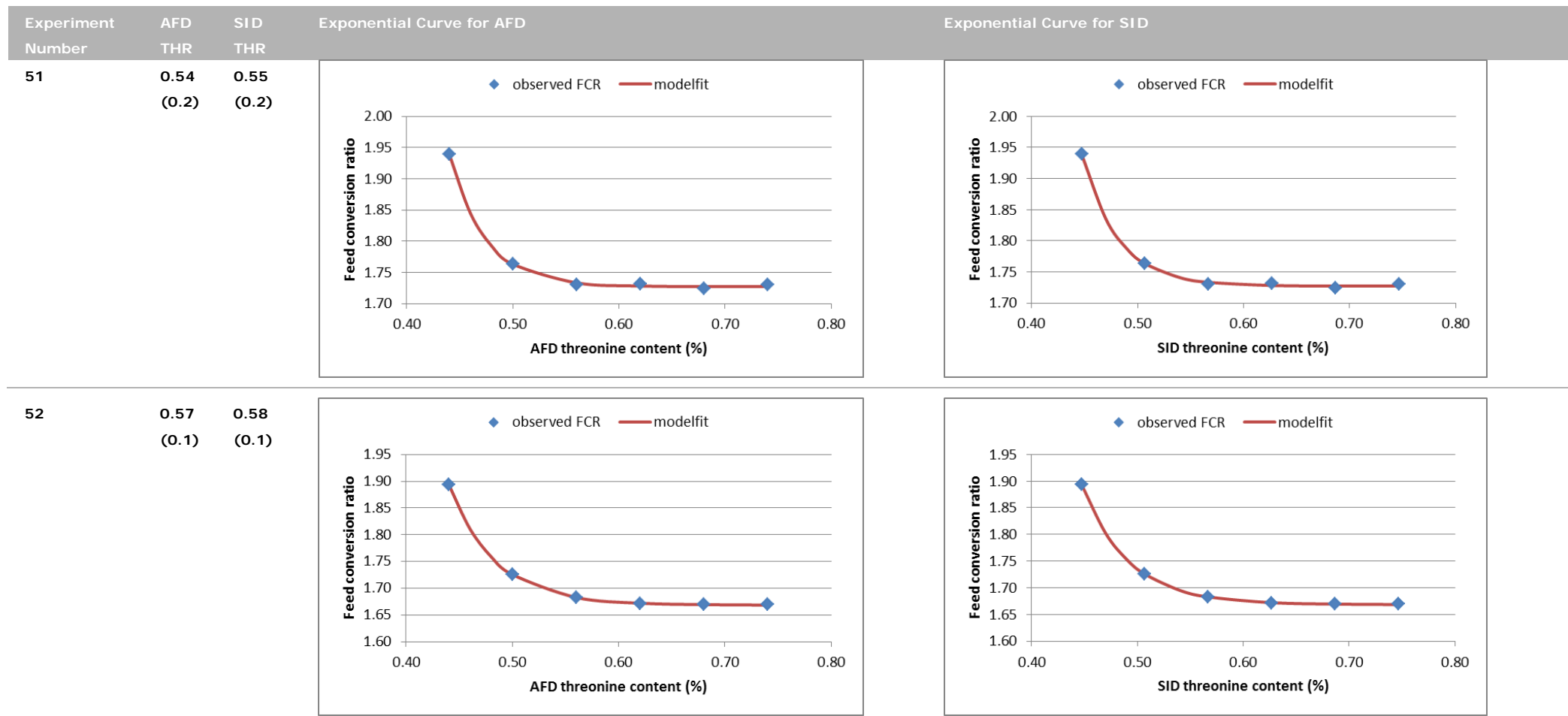




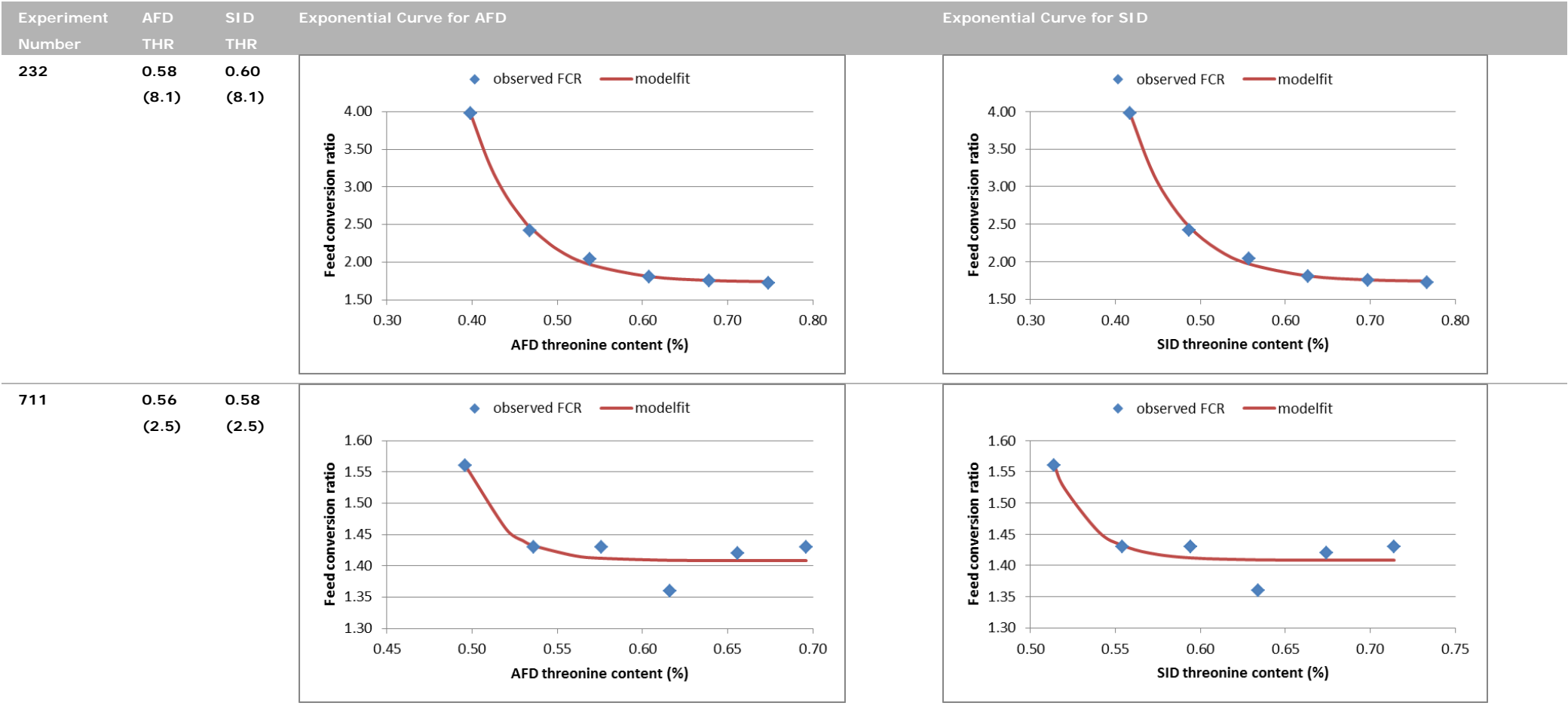


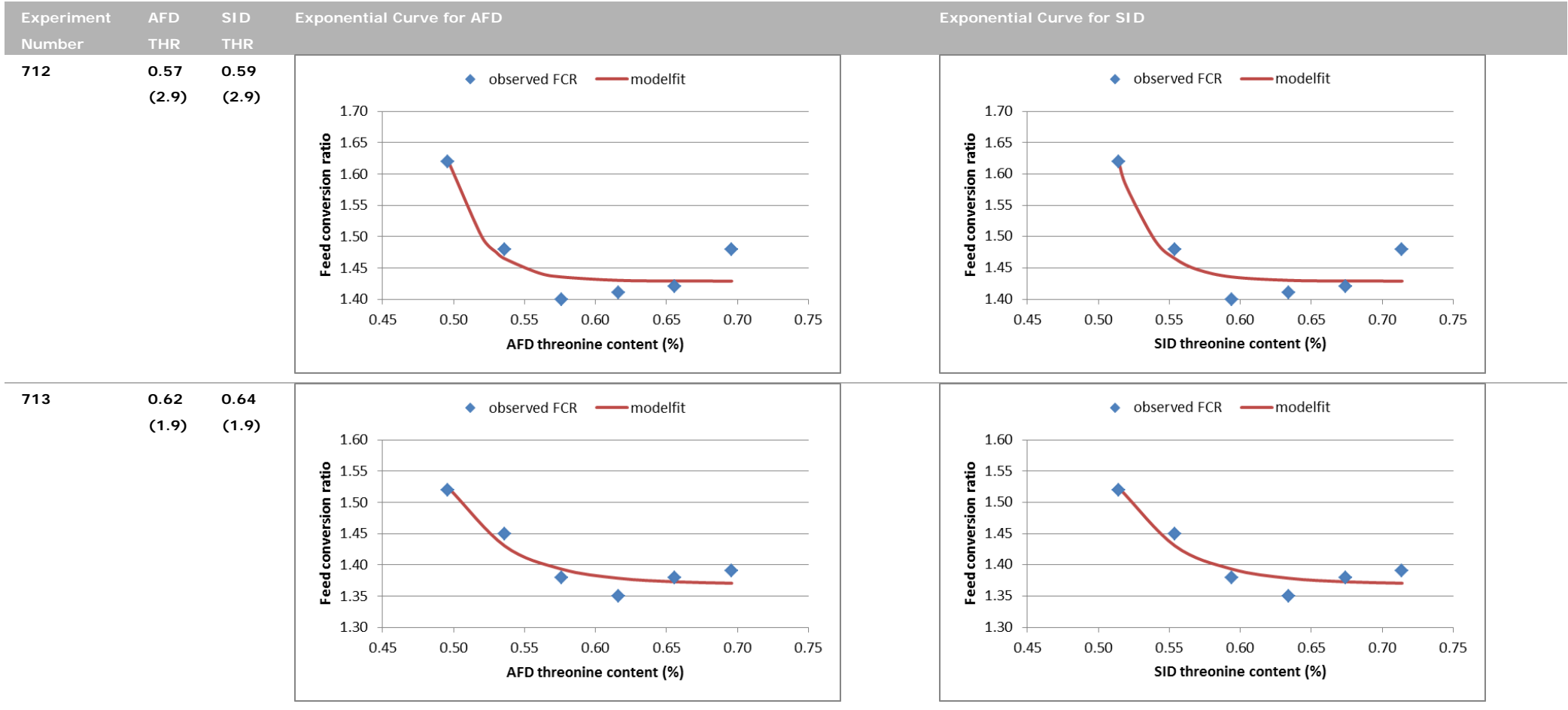


Appendix 3 The determined responses of feed conversion ratio as a function of the AFD and SID threonine content for each individual experiment (% CV=coefficient of variation; in brackets)



Experiment Number	AFD THR	SID THR	Exponential Curve for AFD	Exponential Curve for SID
181	0.54 (3.0)	0.55 (3.0)		
231	0.55 (6.4)	0.57 (6.4)		





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

Exp No.	Reference	AFD threonine concentration in the diet (%)	Gender	Strain	Age of birds	Published Requirement (%)	Models used by the reference	Re-calculated requirement using exponential models (%)	
								AFD threonine content for BWG	SID threonine content for BWG
231	Corzo et al. 2007	0.40, 0.47, 0.54, 0.61, 0.68, 0.75	Male	Ross 708	21-42	0.74	Quadratic	0.63	0.65
241	Ciftci and Ceylan 2004	0.49, 0.54, 0.60, 0.66	Male+Female	Ross PM3	1-22	0.72	Quadratic	0.60	0.60
243	Ciftci and Ceylan 2004	0.47, 0.53, 0.59, 0.65	Male+Female	Ross PM3	21-42	0.64	Quadratic	0.57	0.58
711	Rosa et al. 2001	0.50, 0.54, 0.58, 0.62, 0.66, 0.70	Male	Classic Arbor Acres	1-18	0.69	Quadratic	0.62	0.64
712	Rosa et al. 2001	0.50, 0.54, 0.58, 0.62, 0.66, 0.70	Male	High yield broiler chicken	1-18	0.68	Quadratic	0.59	0.61
713	Rosa et al. 2001	0.50, 0.54, 0.58, 0.62, 0.66, 0.70	Male	High yield broiler chicken	1-18	0.71	Quadratic	0.65	0.67
714	Rosa et al. 2001	0.50, 0.54, 0.58, 0.62, 0.66, 0.70	Female	High yield broiler chicken	1-18	0.70	Quadratic	0.68	0.70

Appendix 5 Experiments for which the requirement on the AFD and SID threonine 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 threonine requirement for FCR with age

Exp No.	Reference	SID threonine concentration in the diet (%)	Gender	Strain	Age of birds	Published Requirement (%)	Models used by the reference	Re-calculated requirement using exponential models (%)	
								AFD threonine content for FCR	SID threonine content for FCR
51	Mack et al. 1999	0.45, 0.51, 0.57, 0.63, 0.69, 0.75	Male	ISA	20-40	0.59	Exponential model	0.54	0.55
52	Mack et al. 1999	0.45, 0.51, 0.57, 0.63, 0.69, 0.75	Male	Ross 208	20-40	0.60	Exponential model	0.57	0.58
181	Corzo et al. 2003	0.45, 0.50, 0.55, 0.60, 0.65, 0.70	Female	Ross	30-42	0.69	Quadratic	0.54	0.55
231	Corzo et al. 2007	0.42, 0.49, 0.56, 0.63, 0.70, 0.77	Male	Ross 708	21-42	0.74	Quadratic	0.55	0.57
232	Corzo et al. 2007	0.42, 0.49, 0.56, 0.63, 0.70, 0.77	Male	Ross 708	21-42	0.73	Quadratic	0.58	0.60
711	Rosa et al. 2001	0.51, 0.55, 0.59, 0.63, 0.67, 0.71	Male	Classic Arbor Acres	1-18	0.69	Quadratic	0.56	0.58
712	Rosa et al. 2001	0.51, 0.55, 0.59, 0.63, 0.67, 0.71	Male	High yield broiler chicken	1-18	0.68	Quadratic	0.57	0.59
713	Rosa et al. 2001	0.51, 0.55, 0.59, 0.63, 0.67, 0.71	Male	High yield broiler chicken	1-18	0.71	Quadratic	0.62	0.64

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

Exp No.	Reference	Threonine concentration in the diet (%)		Gender	Strain	Age of birds	Published Requirement (%)	Models used by the reference	Re-calculated requirement for BWG using exponential models (threonine content in %)		Reason for exclusion*
		On AFD basis	On SID basis						On AFD basis	On SID basis	
51	Mack et al. 1999	0.44, 0.50, 0.56, 0.62, 0.68, 0.74	0.45, 0.51, 0.57, 0.63, 0.69, 0.75	Male	ISA	20-40	0.59	Exponential model	0.48	0.49	2
52	Mack et al. 1999	0.44, 0.50, 0.56, 0.62, 0.68, 0.74	0.45, 0.51, 0.57, 0.63, 0.69, 0.75	Male	Ross 208	20-40	0.60	Exponential model	0.53	0.54	2
71	Ayasan et al. 2009	0.58, 0.63, 0.68, 0.73, 0.78	0.56, 0.61, 0.66, 0.71, 0.76	Male	Ross 308	21-42	0.74	Linear	0.59	0.57	2
161	Kidd et al. 1996	0.55, 0.58, 0.61, 0.64, 0.68, 0.71	0.59, 0.62, 0.65, 0.68, 0.72, 0.75	Male+Female	Cobb	1-21	0.80	Linear	0.78	0.82	2
181	Corzo et el. 2003	0.44, 0.49, 0.54, 0.59, 0.64, 0.69	0.45, 0.50, 0.55, 0.60, 0.65, 0.70	Female	Ross	30-42	0.69	Quadratic	0.45	0.46	2
232	Corzo et al. 2007	0.40, 0.47, 0.54, 0.61, 0.68, 0.75	0.42, 0.49, 0.56, 0.63, 0.70, 0.77	Male	Ross 708	21-42	0.73	Quadratic	0.75	0.77	3
242	Ciftci and Ceylan 2004	0.49, 0.55, 0.61, 0.67	0.50, 0.56, 0.62, 0.68	Male+Female	Ross PM3	1-22	0.75	Quadratic	0.57	0.60	2
244	Ciftci and Ceylan 2004	0.46, 0.52, 0.58, 0.64	0.48, 0.54, 0.60, 0.66	Male+Female	Ross PM3	21-42	0.66	Quadratic	0.55	0.56	2

Exp No.	Reference	Threonine concentration in the diet (%)		Gender	Strain	Age of birds	Published Requirement (%)	Models used by the reference	Re-calculated requirement for BWG using exponential models (threonine content in %)		Reason for exclusion*
		On AFD basis	On SID basis						On AFD basis	On SID basis	
721	Mehri et al. 2010	0.46, 0.53, 0.60, 0.67, 0.74, 0.81	0.44, 0.51, 0.58, 0.65, 0.72, 0.79	Male+Female	Ross 308	15-28	0.76	Broken-line	0.90	0.89	3
731	Duarte et al. 2012	0.59, 0.64, 0.70, 0.75, 0.80, 0.86	0.58, 0.64, 0.69, 0.75, 0.80, 0.85	Male	Cobb	22_42	0.76	Broken-line	-0.49	-0.50	2
741	Corza et al. 2009	0.46, 0.54, 0.62, 0.70, 0.78, 0.86	0.45, 0.53, 0.61, 0.69, 0.77, 0.85	Female	Ross TP16	14_28	0.73	Broken-line	1.09	1.10	1

*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 SID threonine content

Exp No.	Reference	Threonine concentration in the diet (%)		Gender	Strain	Age of birds	Published Requirement (%)	Models used by the reference	Re-calculated requirement for FCR using exponential models (threonine content in %)		Reason for exclusion *
		On AFD basis	On SID basis						On AFD basis	On SID basis	
71	Ayasan et al. 2009	0.58, 0.63, 0.68, 0.73, 0.78	0.56, 0.61, 0.66, 0.71, 0.76	Male	Ross 308	21-42	0.74	Linear	0.59	0.57	2
161	Kidd et al. 1996	0.55, 0.58, 0.61, 0.64, 0.68, 0.71	0.59, 0.62, 0.65, 0.68, 0.72, 0.75	Male+Female	Cobb	1-21	0.80	Linear	0.66	0.70	2
241	Ciftci and Ceylan 2004	0.48, 0.54, 0.60, 0.66	0.48, 0.54, 0.60, 0.66	Male+Female	Ross PM3	1-22	0.72	Quadratic	0.60	0.61	2
242	Ciftci and Ceylan 2004	0.49, 0.55, 0.61, 0.67	0.50, 0.56, 0.62, 0.68	Male+Female	Ross PM3	1-22	0.75	Quadratic	0.51	0.55	2
243	Ciftci and Ceylan 2004	0.47, 0.53, 0.59, 0.65	0.48, 0.54, 0.60, 0.66	Male+Female	Ross PM3	21-42	0.64	Quadratic	-0.09	-0.09	2
244	Ciftci and Ceylan 2004	0.46, 0.52, 0.58, 0.64	0.48, 0.54, 0.60, 0.66	Male+Female	Ross PM3	21-42	0.66	Quadratic	0.72	0.73	1
714	Rosa et al. 2001	0.50, 0.54, 0.58, 0.62, 0.66, 0.70	0.51, 0.55, 0.59, 0.63, 0.67, 0.71	Female	High yield broiler chicken	1-18	0.70	Quadratic	0.63	0.65	3
721	Mehri et al. 2010	0.46, 0.53, 0.60, 0.67, 0.74, 0.81	0.44, 0.51, 0.58, 0.65, 0.72, 0.79	Male+Female	Ross 308	15-28	0.76	Broken-line	0.77	0.76	3

Exp No.	Reference	Threonine concentration in the diet (%)		Gender	Strain	Age of birds	Published Requirement (%)	Models used by the reference	Re-calculated requirement for FCR using exponential models (threonine content in %)		Reason for exclusion *
		On AFD basis	On SID basis						On AFD basis	On SID basis	
731	Duarte et al. 2012	0.59, 0.64, 0.70, 0.75, 0.80, 0.86	0.58, 0.64, 0.69, 0.75, 0.80, 0.85	Male	Cobb	22_42	0.76	Broken-line	3.50	3.49	2
741	Corza et al. 2009	0.46, 0.54, 0.62, 0.70, 0.78, 0.86	0.45, 0.53, 0.61, 0.69, 0.77, 0.85	Female	Ross TP16	14_28	0.73	Broken-line	1.11	1.13	1

*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).

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