Standardized ileal digestible lysine requirement for laying hens

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Preface

In 2017 a new Table has been introduced called; Table ‘Standardized ileal digestibility of amino acids in feedstuffs for poultry’ and has been described in the CVB Documentation report nr. 61. As a feed evaluation system has two pillars – the supply of nutrients by the diet on the one hand and the requirement for these nutrients by the animals on the other hand (both expressed in the same units) – it was also necessary to also update and express the amino acid requirements on a standardized ileal digestibility (SID) basis.
Therefore a large meta-analysis dataset was constructed from studies in which amino acid requirements in laying hens were estimated. The SID amino acid concentrations of the diets used in these studies were recalculated based on the new CVB SID amino acid Table presented in CVB documentation report nr. 61 and the requirements for SID lysine were subsequently estimated. The results of this meta-analysis for standardized ileal digestible lysine (SID-LYS) are presented in the present CVB Documentation report. Compared to the former CVB apparent faecal digestible LYS recommendation for laying hens described in CVB Documentation report nr. 18 and published in 1996 the present established SID-LYS amino acid recommendations for laying hens are:

1. Based on a substantial larger dataset of requirement studies
2. Based on studies with modern laying hen types in the period 1990 – 2017
3. Based on standardized ileal digestible amino acid values in feedstuffs instead of apparent faecal digestible amino acid values.

The in this report estimated requirement of SID-LYS will be incorporated in the Dutch CVB Tabellenboek Veevoeding Pluimvee 2018 and in the English version CVB Table Poultry Nutrition 2018.

This study was guided and assessed by the Technical Committee of CVB and the Ad hoc group ‘SID amino acid requirements for laying hens’

Wageningen, June 2018

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Contents

Abbreviations

AA	Amino acids
AFD	Apparent faecal digestible
ARG	Arginine
BW	body weight
BWG	Body weight gain
CP	Crude protein
EM	Egg mass
FCR	Feed conversion ratio
ILE	Isoleucine
LYS	Lysine
Max	Maximum value
ME	Metabolic energy
MElh	Metabolic energy for laying hens
MET	Methionine
Min	Minimum value
M+C	Methionine plus Cysteine
N	Number
R^2	Coefficient of determination
Req	Requirement
SID	Standardized ileal tract digestible
Std. Dev.	Standard deviation
Std. Err.	Standard error
THR	Threonine
TRP	Tryptophan
VAL	Valine
%CV	Coefficient of variation

1 Introduction

In 2012 a large meta-analysis was carried out by van Krimpen and others in order to determine the dietary requirements for standardized ileal tract digestible (SID) amino acids (AA) for laying hens. This study resulted in a report published by van Krimpen et al. (2015). Before the start of this meta-analysis carried out by van Krimpen et al. another large meta-analysis was carried out in order to determine the SID-AA levels for the various feed ingredients. This meta-analysis resulted in a CVB table with SID-AA concentrations for the various feed ingredients and this Table was used by van Krimpen et al. (2015) in order to recalculate the dietary SID-AA levels for the individual AA titration studies in order to estimate AA requirements. However, in 2017 this CVB Table was updated with new data published in the years between 2012 and 2017 as there were questions about the SID cysteine digestibility value for soybean meal. As a result, not only the SID-AA values for soybean meal have been updated but also for other feedstuffs. As a consequence it was necessary to recalculate all the diets used in the AA titration studies that van Krimpen et al. (2015) used to determine AA-requirements. In this study the results of estimated dietary SID lysine (SID-LYS) requirements based on the new Table values as presented in CVB documentation report nr. 61 are presented. Furthermore, the dataset used by van Krimpen et al. has been extended with new studies that were not included in the study of van Krimpen et al..

In the study of van Krimpen et al. only those titration studies were selected in which synthetic dietary LYS was added at increasing levels whereas the rest of the diet did not change. However, this resulted in only a few studies that could be included in the meta-analysis. Most studies that investigated the LYS requirement were set up in such a way that not only LYS was varied but also certain other amino acids as well in order to keep certain essential amino acid : LYS ratios at a desired constant level. Because of the few LYS studies remaining it was decided to also take into account the LYS requirement studies in which also other amino acids were increased alongside LYS. This resulted in a substantial larger dataset than used by van Krimpen et al..

Furthermore, two models for estimation of SID-LYS requirements were compared. The first model consisted of a quadratic broken-line model as described and used in the estimation of SID-LYS requirements in broilers (CVB documentation report nr. 62) and the second model consisted of a quadratic broken-line model as well but with this difference that the slope of the line following the estimated break-point value was allowed to vary whereas in the first broken quadratic broken line model the line was forced to be horizontal.

2 Materials and Methods

Lysine requirement studies were selected from literature (1990 – 2017) in which not only the dietary LYS content was varied by means of addition of graded levels of dietary synthetic LYS but also those studies where included in which also other amino acids were increased alongside LYS. In some studies next to increasing levels of synthetic LYS also levels of other synthetic amino acids such as MET, THR, TRP, VAL and ILE were increased in order to maintain certain essential amino acid : LYS ratios constant or to prevent these ratios to become sub-optimal and thereby limit the response to increases in dietary SID-LYS.
In one study a diet rich in protein and levels of amino acids was mixed with increasing levels of a nitrogen free diet ensuring constant dietary amino acid : LYS ratios for all amino acids (study of Silva et al., 2015), in some studies only the dietary LYS content was varied by means of addition of synthetic LYS. Furthermore, performance characteristics such as daily egg mass (EM: g/d/hen) and feed conversion ratio (FCR; g feed : g egg mass) had to be recorded and information with respect to dietary composition and age of the laying hens had to be provided in the studies.

Requirements were estimated using two models, a quadratic single-slope broken-line model with a horizontal plateau and a quadratic two-slope broken-line model in which the slope of the line beyond the estimated break point value was allowed to vary. The quadratic two-slope broken-line model was also taken into account as in some cases a decrease in EM or an increase in FCR was observed after the lowest FCR or highest EM was observed at further increases in SID-LYS concentration. These two models were adopted from a publication of Robbins et al. (2006).

The quadratic single-slope broken-line model (model 1) is as follows:

If (SID-LYS (%) < R) then EM or FCR = L + U × (R – SID-LYS)$^{\wedge 2}$;
Else EM or FCR = L + U × 0;
Where:
L = plateau value for EM or FCR
R = break-point value for SID-LYS (%)
U = slope value, representing the increase in EM or decrease in FCR per unit increase in dietary SID-LYS.

The quadratic two-slope broken-line model in which the slope of the line beyond the estimated break point values is allowed to vary is as follows:

If (SID-LYS (%) < R) then EM or FCR = L + U × (R – SID-LYS)$^{\wedge 2}$;
Else EM or FCR = L + V × (SID-LYS – R)
Where:
L = plateau value for EM or FCR
R = break-point value for SID-LYS (%)
U = slope value, representing the increase in EM or decrease in FCR per unit increase in dietary SID-LYS at SID-LYS levels before the break-point value for SID-LYS.
V = slope value, representing the decrease in EM or increase in FCR per unit increase in dietary SID-LYS at SID-LYS levels after the break-point value for SID-LYS has been reached. The possible slope values were restricted to zero and positive values for FCR and restricted to zero and negative values for EM.

Via the PROC MIXED procedure and the PROC NLMIXED procedures of SAS estimated SID-LYS requirements for EM and FCR were regressed against factors such as EM, FCR, egg production rate, age, and the dietary factors CP, ME and CP : ME ratio with study effect included as a random factor. Furthermore, the estimated SID-LYS requirement levels were also used to calculate ratios of other non-test SID-AA that coincided with the estimated SID-LYS requirement levels and it was checked whether some of the non-test SID-AA could negatively affect the estimated SID-LYS levels for FCR and EM.

3 Results and Discussion

In Table 1. Some characteristics of the studies included in the meta-analysis is given. The dataset consisted of 16 studies with in total 26 trials and 152 observations.

In Appendix A for each titration trial the relationship between dietary SID-LYS content and FCR and between dietary SID-LYS content and EM is presented graphically together with the estimated SID-LYS requirements for the quadratic single- and two-slope broken-line models. A visual analysis of these graphs show that in most cases the estimated SID-LYS requirements are similar for both models and also that in a number of cases the estimated SID-LYS requirements using the two-slope model are clearly higher than for the single-slope model and seems, from a visual interpretation, to overestimate optimal SID-LYS requirements (for example, see the SID-LYS requirement estimates for trials 1, 10, 11 and 17 in Appendix A). <u>Therefore it was decided to use the estimated SID-LYS requirements based on the quadratic single-slope broken-line model as the basis for deriving SID-LYS recommendations for EM and FCR.</u>
In Appendix B the estimated single-slope quadratic broken-line model parameters for each titration trial is given.

In Table 2 the average estimated optimal SID-LYS concentrations and SID-LYS intake statistics are presented. The average calculated optimal SID-LYS intake levels were 766 mg/d for EM and 732 mg/d for FCR. When correcting for study effect the average SID-LYS requirements were 777 mg/d for maximum EM and 766 mg/d for minimum FCR.

Table 2. Estimated optimal SID-LYS requirements (in % of the diet, as mg/d and as mg per g of egg mass) for maximum egg mass (EM) and minimum FCR.

	Parameter	N*	Mean	Std. Dev.	Min	Max	%CV
SID-LYS (%)	EM	14	0.712	0.0676	0.584	0.841	9.5
	FCR	17	0.698	0.1316	0.465	1.047	18.9
SID-LYS intake (mg/d)	EM	14	766	122	565	975	15.9
	FCR	17	732	160	472	1117	21.9
SID-LYS intake per g of EM (mg/g)	EM	14	14.1	1.75	11.7	16.9	12.4
	FCR	17	14.1	2.63	9.7	20.6	18.7

*Number of titration trials. A study might contain multiple titration trials.

Results in Table 2 show a wide range in optimal estimated SID-LYS concentrations or SID-LYS intake levels. This wide range can be the result of various process such as the quantity of EM (determined by egg production percentage and egg weight), the energy content of the feed, body weight changes of the animals, the weight of the birds, temperature, subclinical infections, genetics and the setup of the experiment. With respect to the setup of the experiment; it was observed that the effect of the model estimated steepness of the curve was related to the estimated requirement for SID-LYS for minimum FCR (Fig. 1) and also that the difference between minimum and maximum observed FCR in an experiment did affect the estimated SID-LYS requirement for FCR (Fig. 2). Furthermore, the steepness of the curve was related to the difference in minimum and maximum FCR within an experiment (R^2 = 0.642).

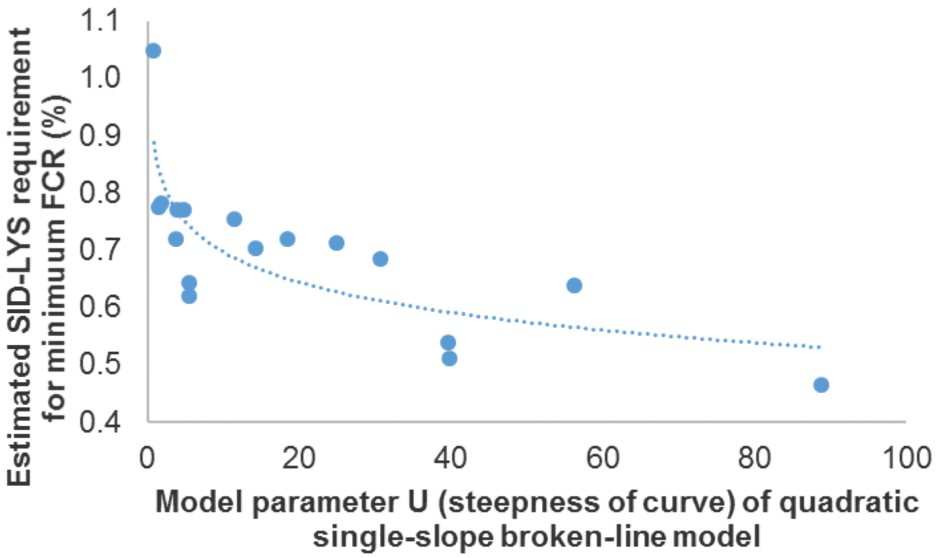

Figure 1. Relationship between the model estimated steepness of the decrease in estimated requirement of dietary SID-LYS for minimum FCR per unit increase in dietary SID-LYS and the estimated SID-LYS requirement for minimum FCR using the quadratic single-slope broken-line model. Model parameters: Estimated requirement for SID-LYS (%) = -0.0761±0.0150 × LN(U) + 0.8719±0.0399; R^2 = 0.631.

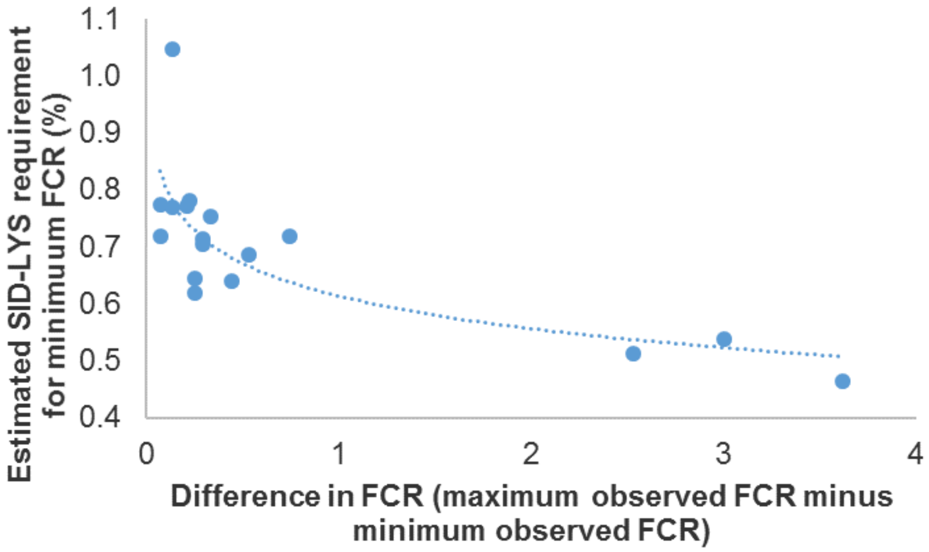

Figure 2. Relationship between the difference in maximum and minimum observed FCR and the estimated SID-LYS requirement for minimum FCR using the quadratic single-slope broken-line model. Model parameters: Estimated requirement for SID-LYS (%) = -0.0826±0.0190 × LN(difference in FCR) + 0.614±0.0292; R^2 = 0.557.

However, the effect of steepness of the curve on estimated SID-LYS requirements for maximum EM was less significant (R^2 = 0.270) compared the effect of steepness of the curve on estimated SID-LYS requirements for minimum FCR (Fig. 1). These relationships suggest that for experiments with lower basal SID-LYS concentrations also lower estimated SID-LYS requirements can be expected due to the fitting characteristics of the model compared to experiments with higher basal levels of SID-LYS.

Table 1. Summary of the total dataset

Study	Trial	Breed	Starting Age (weeks)	Duration of experiment (weeks)	Max obs. rate of lay (%)	Max obs. egg mass	Max obs. feed intake	Min SID-LYS (%)	Max SID-LYS (%)	Constant AA:LYS ratio for	Max. FCR minus Min. FCR	Max. egg mass minus Min. egg mass
Schmidt et al. (2009)	1	Lohman Brown	79	16	80	55	117	0.584	0.790	M+C and TRP	0.29	7.4
Schmidt et al. (2008)	2	Lohman LSL	79	16	85	56	116	0.584	0.790		0.33	11.7
Moraes Sa et al. (2015)	3	Lohman LSL	34	16	92	57	125	0.611	0.814	M+C, TRP	0.29	5.4
	4	Lohman Brown	34	16	91	58	115	0.611	0.814		0.13	4.7
Pastore et al. (2015)	5	Hy-Line W-36	60	16	83	55	98	0.600	0.904	M+C, THR, TRP, VAL, ILE	0.07	2.6
Nunes et al. (2015)	6	Shaver Brown	50	16	91	60	119	0.784	0.904	M+C, TRP, THR	0.18	5.1
Silva et al. (2015)	7	Dekalb White	37	3	97	61	106	0.267	0.892	All amino acids	2.53	45.0
	8	Dekalb White	41	3	96	63	103	0.267	0.892		3.00	49.0
	9	Dekalb White	45	3	99	64	111	0.267	0.892		3.62	50.0
Santos et al. (2014)	10	Isa Brown	28	16	93	58	117	0.632	1.011	M+C, TRP, THR, ILE, VAL	0.13	5.6
Bouyeh and Gevorgian (2011)	11	Hy-line W-36	52	12	73	44	107	0.544	0.762	M+C	0.74	5.7
	12	Hy-line W-36	52	12	76	47	121	0.586	0.797		0.87	5.9
Rocha et al. (2009)	13	Hy-line W-36	24	16	94	53	100	0.547	0.778	M+C, TRP, THR, ILE, VAL	0.21	7.7
Cupertino et al. (2009)	14	Lohmann LSL	54	16	83	52	113	0.555	0.761	M+C, TRP, ILE,	0.53	13.1
	15	Lohmann Br.	54	16	79	52	110	0.555	0.761		0.44	10.5
Faria et al. (2003)	16	Hy-line W-36	44	7	85	49	100	0.419	0.675		0.25	13.2
	17	Hy-line W-36	58	7	80	46	93	0.445	0.700		0.25	6.2
Liu et al. (2005)	18	Hy-line W-36	37		85	51	101	0.598	0.671	M+C, TRP	0.12	2.4
	19	Hy-line W-36	37		80	48	102	0.550	0.624		0.13	4.4
Fihlho et al. (2006)	20	Hisex Brown	30	16	91	59	109	0.736	0.976		0.09	2.2
Schutte and Smink	21	Lohmann LSL	24	12	97	57	112	0.578	0.858		0.07	1.3
Star and van krimpen (2016)	22	Dekalb White	61	7	93	59	124	0.527	0.765		0.07	1.2
	23	Bovans Brown	61	7	85	55	123	0.527	0.765		0.13	2.0
	24	Dekalb White	69	7	88	57	126	0.527	0.765		0.12	2.6
	25	Bovans Brown	69	7	81	53	124	0.527	0.765		0.16	2.9
Shahir et al. (2006)	26	Hy-Line W36	26	16	83	47	101	0.465	0.746		0.22	3.8

This is also clearly shown in Appendix A with trials 7 – 9 of the study of Silva et al. (2015). In the study of Silva et al. large contrasts in SID-LYS were made. When fitting the quadratic single-slope broken-line model on the full dataset of observations of Silva et al. and on a reduced dataset (in which the 3 lowest SID-LYS observations were omitted) substantially higher SID-LYS requirement values for FCR were estimated based on the reduced dataset. It was therefore decided to base the estimated SID-LYS requirement values for FCR and EM from the study of Silva et al. on the reduced dataset in which the 3 lowest SID-LYS levels in each trial were omitted. In Appendix A and Appendix B the titration results of the study of Silva et al. (2015) with the lowest 3 SID-LYS levels removed before estimation of the SID-LYS requirement are represented with the letter 'a'.
Furthermore, the observations of the study of Bouyeh and Gevorgian were also removed because of the observed low maximum laying rate percentage. In Table 3 the average estimated optimal SID-LYS concentrations and SID-LYS intake statistics are presented in which the estimated SID-LYS requirement values for FCR and EM from the study of Silva et al. (2015) are based on the reduced dataset in which the 3 lowest SID-LYS levels in each trial were omitted and in which the results of the study of Bouyeh and Gevorgian were excluded due to the observed low maximum laying rate percentage.

Table 3. Estimated optimal SID-LYS requirements (as % and as daily intake) for maximum egg mass (EM) and minimum FCR in which the estimated SID-LYS requirement values for FCR and EM from the study of Silva et al. were based on the reduced dataset in which the 3 lowest SID-LYS levels were omitted and in which the results of the study of Bouyeh and Gevorgian were excluded due to the observed low maximum laying rate percentage.

	Parameter	N*	Mean	Std. Dev.	Min.	Max	%CV
SID-LYS (%)	EM	13	0.724	0.0814	0.584	0.859	11.3
	FCR	16	0.732	0.0998	0.619	1.047	13.6
	FCR**	15	0.711	0.0557	0.619	0.781	7.8
SID-LYS intake (mg/d)	EM	13	784	132	565	975	16.9
	FCR	16	777	134	593	1157	17.2
	FCR**	15	751	91	593	882	12.0
SID-LYS intake per g of EM (mg/g)	EM	13	14.5	1.59	11.9	16.9	11.0
	FCR	16	14.4	2.15	11.6	20.6	15.0
	FCR**	15	13.9	1.40	11.6	15.5	10.0

*Number of titration trials. A study might contain multiple titration trials.
**The estimated SID-LYS requirements for FCR from the study of Santos et al. (2014) were outlier values that were situated around 3 standard deviations away from the average and therefore excluded from the other observations.

3.1 SID-LYS requirement for maximum EM based on model estimated requirements for SID-LYS

The estimated requirements for SID-LYS intake and dietary SID-LYS content for maximum EM was observed to be strongly related to EM itself:

[F1] SID-LYS intake for maximum EM (mg/d) = -387±294 + 21.6±5.43 × EM (g/d); n = 13, R^2=0.613

[F2] SID-LYS content for maximum EM (%) = 0.084±0.1685 + 0.0119±0.00311 × EM (g/d); n = 13, R^2=0.569

There was one outlier observation (observation of trial number 7a from the study of Silva et al. (2015)). Removal of this value resulted in the following relationship between SID-LYS intake for maximum EM and maximum EM itself:

[F3] SID-LYS intake for maximum EM (mg/d)= -528±190 + 24.6±3.53 × EM (g/d); n = 12 R^2=0.829

[F4] SID-LYS content for maximum EM (%) = -0.062±0.1298 + 0.0148±0.00242 × EM (g/d); n = 12, R^2=0.789.

The explained variation of SID-LYS intake for maximum EM by the model was further substantially increased when adding minimum FCR as a covariable next to maximum EM to the model, however, the factors FCR and EM were not significant (P=0.061 and P=0.171 for EM and FCR, respectively, probably due to the small dataset):

[F5] SID-LYS intake for maximum EM (mg/d)= -1591±319 + 31.0±2.96 × EM (g/d) + 354±97.5 × FCR; n = 12 R^2=0.931

When using the relationship [F5]. and the feed requirements for laying hens at various BW, egg production rates and egg weights as stated in Table 6.4a in the Tabellenboek Veevoeding 2012 it appeared that estimated SID-LYS requirements for maximum EM using formula [F5] increased substantially at increasing BW and that this increase in SID-LYS requirement per unit increase in BW was substantially higher than the estimated SID-LYS requirement for maintenance of 32 mg SID-LYS per kg BW. Therefore it was decided to not consider relationship [F5] with respect to establishing SID-LYS requirements.

Furthermore, the explained variation of SID-LYS intake requirements for maximum EM was also increased when BW was added as a covariable to the diet next to the factor EM (R^2 increased from 0.829 to 0.894), although to a lesser degree then was the case for the covariable FCR. In the case when BW was added as a covariable it was necessary to make an estimation of the BW for 5 out of the 12 observations as the BW of the birds in these 5 titration trials were not provided in the publication. Due to the uncertainty of the estimated BW of these 5 titration trials the model results with BW as a covariable in de model are not presented in this document.

According to the American Egg Board 1 gram of EM contains 9.3 mg LYS. However, in [F3] and [F5] the estimated SID-LYS requirements per g of EM are 24.6 and 31.0 mg respectively suggesting a low marginal conversion efficiency of less than 40%. This fact, together with a negative intercept value in formula [F1] and [F3] suggesting a negative SID-LYS requirement of more than 350 mg/d for maintenance which is physiologically impossible, shows that the regression formula cannot be used for extrapolation in order to estimate SID-LYS requirements at egg production rates lower than 80%.
It was also tried to regress the estimated requirement for SID-LYS to SID-LYS requirement for maintenance, EM and EM squared simultaneously assuming a LYS requirement for maintenance of 38 mg/kg BW. This value of 38±25.7 mg/kg BW was adopted as the average LYS requirement per kg BW value from the studies of McDonald and Morris (1985) (73 mg LYS per kg BW), Bowmaker and Gous (1991) (11 mg LYS per kg BW), Nonis et al. (2008) (37 mg LYS per kg BW) and Venturini et al. (2011) (32 mg LYS per kg BW). Assuming an average SID-LYS digestibility of 85% it means a SID-LYS maintenance requirement of 32 mg/kg per kg BW. However, this exercise was not successful as it resulted in slightly negative SID-LYS requirements for EM production at EM below 25 g/d.

Assuming a SID-LYS maintenance requirement of 32 mg per kg BW the average SID-LYS intake for maximum EM per g of EM was 13.7±1.46 mg and the average SID-LYS intake for minimum FCR per g of EM was 13.1±1.34 which means that SID-LYS would be converted into EM lysine with an efficiency of around 68-71%. However, when calculating the SID-LYS requirement in this way only a low percentage of the variation could be explained.

A factor that possibly contributed to the variation in SID-LYS requirement per g of EM was the change in BW over time. For example, when regressing the change in daily BW against the estimated SID-LYS requirement for maximum EM expressed per g of EM corrected for maintenance (Fig. 1), an almost significant (P=0.061) relationship was observed with decreasing SID-LYS requirement per g of EM at increases in daily BWG. A similar relationship, although less clear (P=0.158), was observed between changes in daily BW and SID-LYS requirement for minimum FCR expressed per g of EM and corrected for maintenance (Fig. 2).

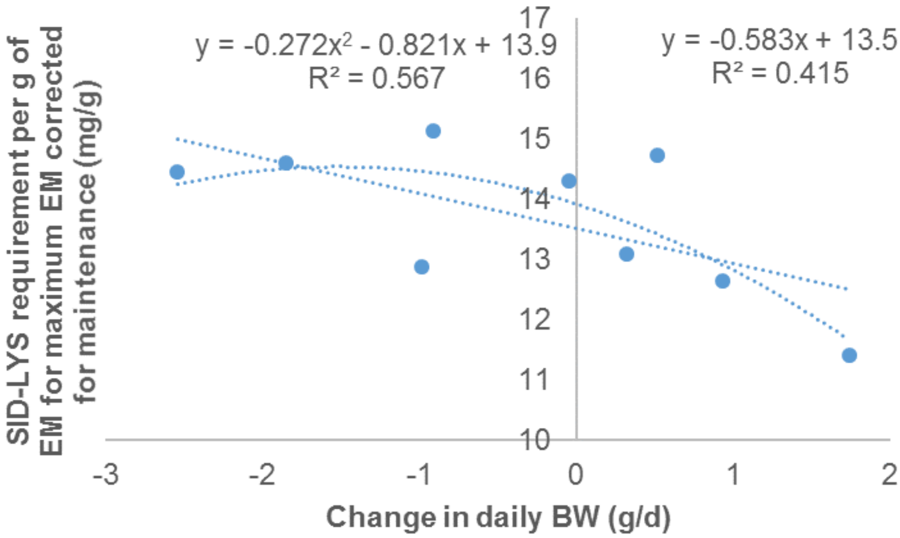

Figure 1. Relationship between change in daily BW (g/d) and SID-LYS requirement for maximum egg mass (EM) expressed per g of EM and corrected for maintenance requirement (estimated SID-LYS maintenance requirement of 32 mg/kg per kg BW).

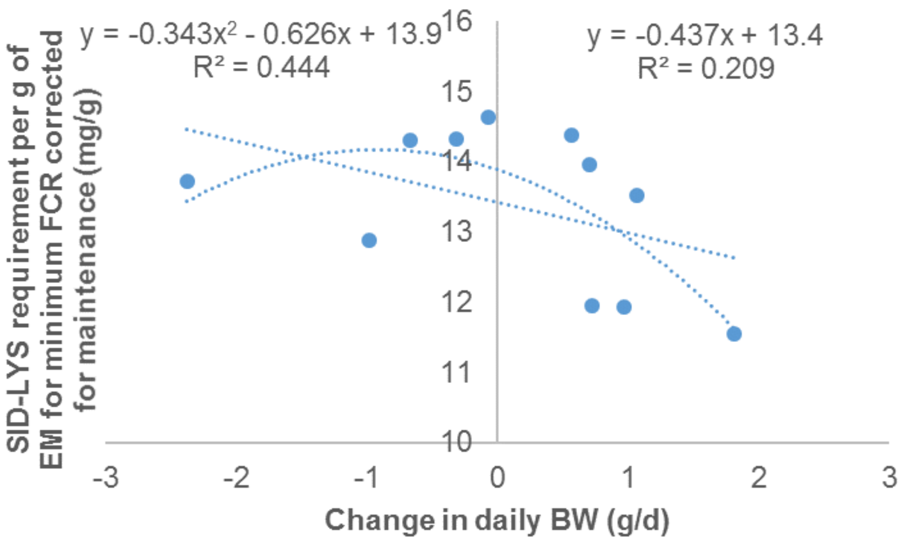

Figure 2. Relationship between change in daily BW (g/d) and SID-LYS requirement for minimum FCR expressed per g of egg mass (EM) and corrected for maintenance requirement.

When working out the assumption of an SID-LYS requirement of 32 mg/kg per kg BW, an average SID-LYS requirement per g of EM of 13.7 mg, an egg weight of 60 g and the feed requirements for laying hens at various BW, egg production rates and egg weights as stated in Table 6.4a in the Tabellenboek Veevoeding 2012 the calculated SID-LYS requirements expressed in mg/d and in % of the diet are then as shown in Table 4.

Table 4. Estimated optimal SID-LYS requirements expressed in mg/d, as a percentage of the diet for maximum egg mass (EM), and as a SID-LYS:EM ratio at various egg production rates based on an SID-LYS requirement of 32 mg/kg per kg BW, an average SID-LYS requirement per g of EM of 13.7 mg, an egg weight of 60 g and the feed requirements for laying hens at various BW, egg production rates and egg weights as stated in Table 6.4a in the Tabellenboek Veevoeding 2012*.

	SID-LYS (mg/d)				Dietary SID-LYS (%)				SID-LYS:EM ratio			
	Egg production rate (%)											
BW (kg)	60	75	85	95	60	75	85	95	60	75	85	95
1.4	538	661	744	826	0.595	0.663	0.702	0.737	14.9	14.7	14.6	14.5
1.5	541	665	747	829	0.580	0.649	0.687	0.722	15.0	14.8	14.6	14.5
1.6	544	668	750	832	0.567	0.635	0.674	0.708	15.1	14.8	14.7	14.6
1.7	548	671	753	835	0.555	0.622	0.661	0.695	15.2	14.9	14.8	14.7
1.8	551	674	756	839	0.544	0.610	0.648	0.683	15.3	15.0	14.8	14.7
1.9	554	677	760	842	0.533	0.599	0.637	0.671	15.4	15.1	14.9	14.8
2.0	557	681	763	845	0.523	0.588	0.626	0.660	15.5	15.1	15.0	14.8

*Feed intake is calculated based on: a feed with a MElh content of 11.8 MJ/kg, a requirement of 12.1 kJ per g egg mass, a maintenance requirement of 435 kJ ME per kg MBW (BW^0.75), a requirement of 21.5 kJ ME per gram BWG, a daily BWG of 1.5 g, and 9.5 kJ ME per kg BW per unit decrease in ºC below 25 ºC and a daily temperature of 22 ºC.

However, this approach of which the results are shown in Table 4 does not account for the effect of a decreasing dietary SID-LYS conversion efficiency into EM at increasing EM as will be shown later on in this document in chapter 3.3.

3.2 SID-LYS requirement for minimum FCR based on model estimated requirements for SID-LYS

With respect to estimation of the optimal SID-LYS content for minimum FCR the estimated SID-LYS requirement value from the study of Santos et al. (2014) of 1.05% was an outlier value (more than 3 standard deviations removed from the average estimated SID-LYS requirement value) and this value therefore was not included in the analysis. The estimated SID-LYS intake requirement and estimated dietary SID-LYS content requirement for minimum FCR was not related to the estimated minimum FCR itself (P=0.543 for SID-LYS intake and P=0.340 for SID-LYS content). Also, the estimated SID-LYS requirements expressed as intake (mg/d), as percentage, and as mg SID-LYS per g of EM were not significantly related to the dietary energy content, protein level, or protein: energy ratio.

There was almost a trend (P=0.101) for a positive effect of the estimated minimum FCR on SID-LYS intake req. for minimum FCR per g of EM corrected for BW (mg/g):

[F6] SID-LYS intake req. for minimum FCR (per g of EM corrected for BW; mg/g) = 6.4±3.31 + 3.48±1.644 × FCR (g feed: g EM); n = 16, R^2=0.464.

Because of the low percentage of variation in SID-LYS requirement for minimum FCR that can be explained compared to the percentage of variation in SID-LYS requirement for maximum EM it is concluded that it is desirable to provide recommendations for SID-LYS for laying hens based on EM.

3.3 *Conversion efficiency of SID-LYS into EM based on all titration observations*

When considering the efficiency in which dietary SID-LYS is converted into EM it becomes clear from Fig. 4 that, as dietary SID-LYS intake and dietary SID-LYS concentration increase, the efficiency from around 100 percent at a dietary SID-LYS concentration of around 0.4% and a SID-LYS intake level of around 300 mg/d decreases. This observation together with the finding in this study that the average dietary SID-LYS conversion efficacy at which dietary SID-LYS is converted into EM for maximum EM is 68% and for minimum FCR is 71% suggests that, in order to obtain maximum performance, one should reckon with an unavoidable inefficiency in SID-LYS utilization that increases at increased production levels and at increased dietary SID-LYS intake levels.

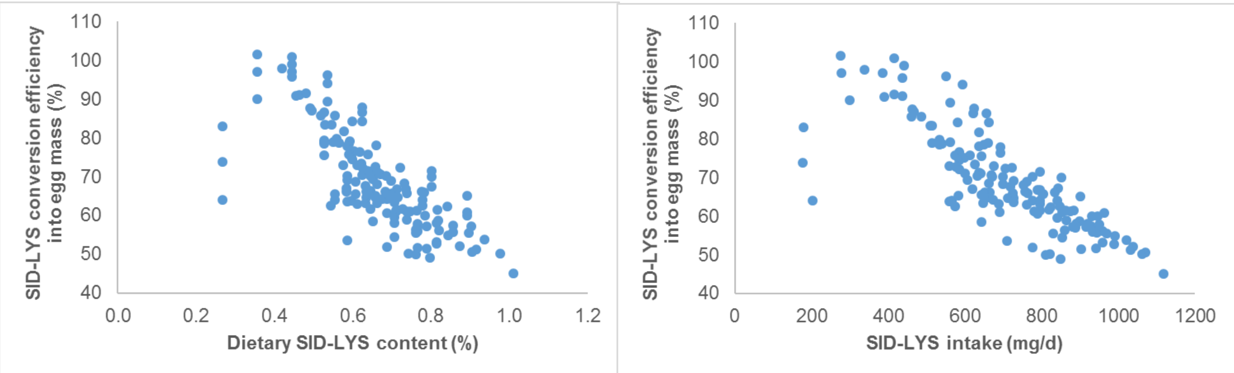

Figure 4. Relationship between dietary SID-LYS content (%) and dietary SID-LYS conversion efficiency into egg mass (%) (left panel) and the relationship between dietary SID-LYS intake (mg/d) and dietary SID-LYS conversion efficiency into egg mass (%) (right panel) based on all the titration data in the meta-analysis dataset (n = 152 observations from 26 titration trials). The SID-LYS conversion efficiency was calculated as: (egg mass (g/d) × 9.3) / SID-LYS intake (mg/d) × 100.

In Fig. 5 the relationship between SID-LYS intake and dietary SID-LYS conversion efficiency into EM is again presented but then the values with FCR values higher than 2.4 and lower than 1.9 were excluded from the analysis in order to reduce the effect of body protein deposition or mobilization on the relationship between SID-LYS intake and SID-LYS conversion efficiency into EM.

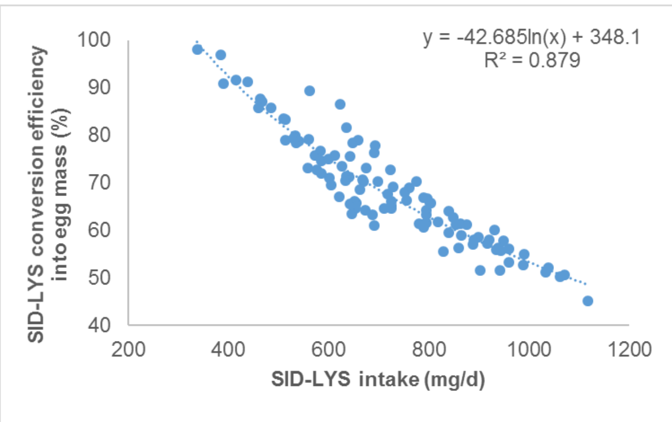

Figure 5. Relationship between dietary SID-LYS intake (mg/d) and dietary SID-LYS conversion efficiency into egg mass (%) based on all the titration data in the meta-analysis dataset in which the feed conversion ratio was in between the range 1.9 – 2.4 (n = 104 observations from 26 titration trials). The SID-LYS conversion efficiency was calculated as: (egg mass (g/d) × 9.3) / SID-LYS intake (mg/d) × 100.

In Fig. 6 the relationship between SID-LYS intake and dietary SID-LYS conversion efficiency into EM is again presented with extreme FCR values higher than 2.4 and lower than 1.9 being excluded from the analysis as to reduce the effect of body protein deposition or mobilization on the relationship between SID-LYS intake and SID-LYS conversion efficiency into EM and, furthermore, those observations were removed that had titration levels beyond the titration level at which maximum observed EM was achieved within each titration trial.

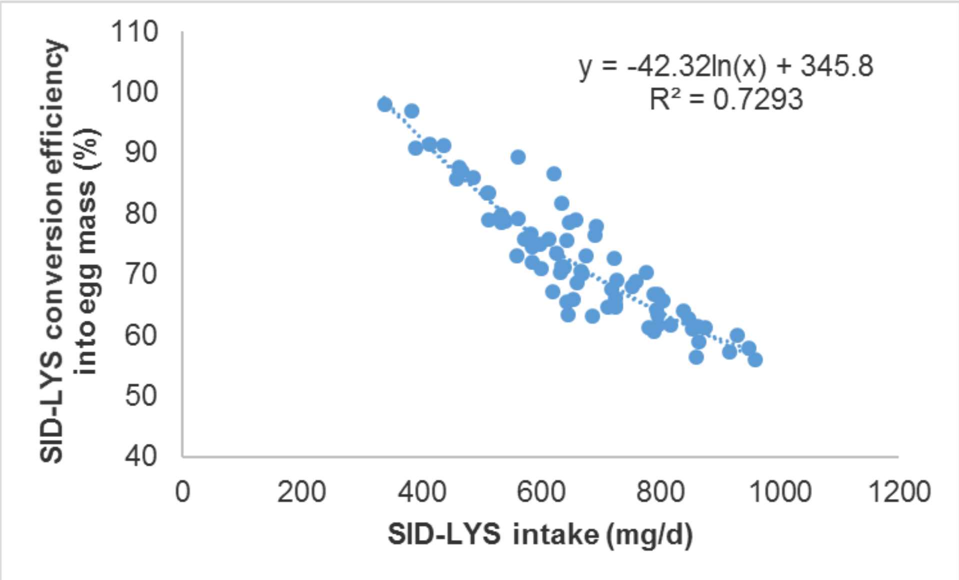

Figure 6. Relationship between dietary SID-LYS intake (mg/d) and dietary SID-LYS conversion efficiency into egg mass (%) based on all the titration data in the meta-analysis dataset in which the feed conversion ratio was in between the range 1.9 – 2.4 and without those titration levels that were beyond the titration level at which maximum EM was achieved within each titration trial (n = 78 observations). The SID-LYS conversion efficiency was calculated as: (egg mass (g/d) × 9.3) / SID-LYS intake (mg/d) × 100.

In Figures 4 – 6 a clear decrease in SID-LYS conversion efficiency into EM is observed that should be accounted for in the determination of SID-LYS requirement levels for laying hens.

3.4 SID-LYS requirement for EM production based on all titration observations with FCR values in the range 1.9 - 2.4 and without those titration observations that were beyond the titration level at which maximum EM was achieved.

In Fig. 7 the relationship between EM production and dietary SID-LYS intake is presented. This relationship together with the various models fitted to the data clearly shows that it is very difficult to estimate a maintenance requirement of SID-LYS based on data from egg producing laying hens due to the fact that extrapolation over a wide SID-LYS intake distance is required. Another complicating factor is the fact that a laying hen can only produce an egg of a certain weight (lowest egg weight in the meta-analysis dataset was 52 gram at a SID-LYS intake of 179 mg). A laying hen cannot ‘decide’ to produce an egg of let’s say 20 gram because it has an SID-LYS intake above maintenance that is enough to provide for an egg of 20 gram. Instead, a laying hen may ‘decide’ to produce an egg of around 52 gram every second or third day and using its LYS reserves to compensate for shortage of dietary LYS intake.

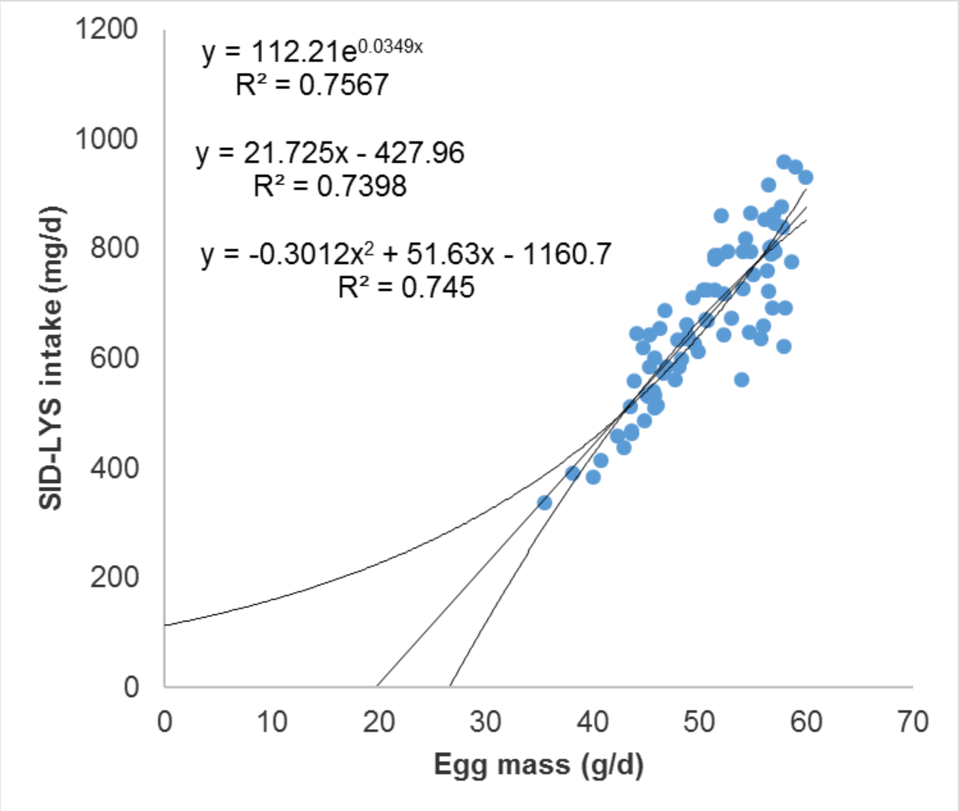

Figure 7. Relationship between egg mass (g/d/hen) and dietary SID-LYS intake (mg/d) based on all the titration data in the meta-analysis dataset in which the feed conversion ratio was in between the range 1.9 – 2.4 and without those titration levels that were beyond the titration level at which maximum EM was achieved within each titration trial (n = 78 observations).

In Fig. 8 the relationship between dietary SID-LYS intake (mg/d) and SID-LYS intake per g of egg mass produced (mg SID-LYS intake/g of egg mass) is presented based on all the titration data in the meta-analysis dataset in which the feed conversion ratio was in between the range 1.9 – 2.4 and without those titration levels that were beyond the titration level at which maximum EM was achieved within each titration trial (n=78). The open squares are the model outcomes of SID-LYS requirements for maximum egg mass and the open triangles are the model outcomes of SID-LYS requirements for minimum FCR. The regression line is based on all observations. Results in Fig. 8 show that the relationship between SID-LYS requirements per g of EM for maximum EM and minimum FCR as estimated by the model and SID-LYS intake are in line with the relationship between SID-LYS intake and SID-LYS intake per g of EM for all the observations. This means that for the estimation of SID-LYS requirements for laying hens the data from all studies can be used which results in a more robust dataset with a larger range in dietary SID-LYS intake levels.

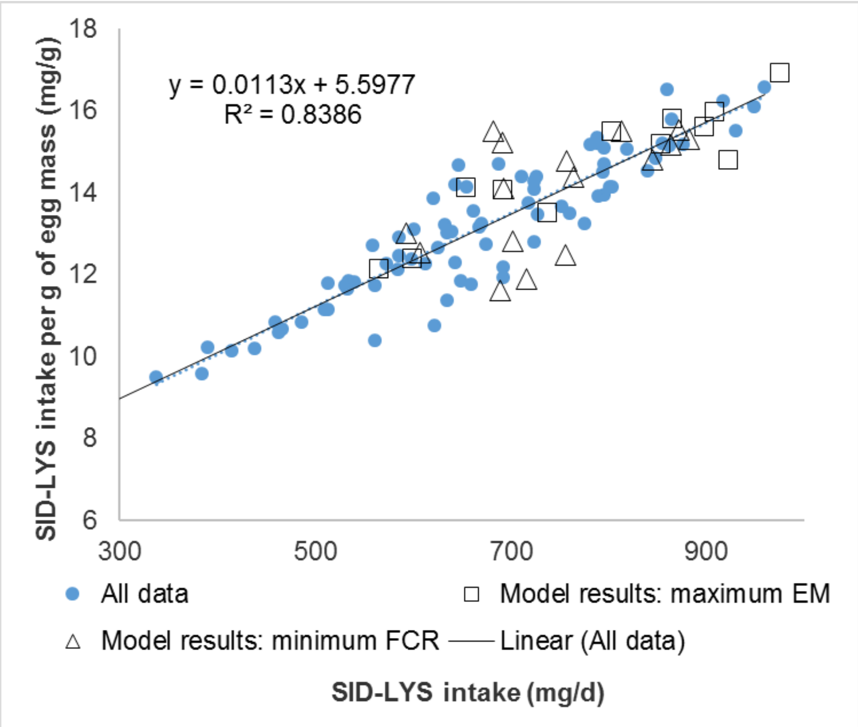

Figure 8. Relationship between dietary SID-LYS intake (mg/d) and SID-LYS intake per g of egg mass produced (mg SID-LYS intake/g of egg mass) based on all the titration data in the meta-analysis dataset in which the feed conversion ratio was in between the range 1.9 – 2.4 and without those titration levels that were beyond the titration level at which maximum EM was achieved within each titration trial (n = 78 observations). The open squares are the individual titration trial model outcomes of SID-LYS requirements for maximum egg mass and the open triangles are the individual titration trial model outcomes of SID-LYS requirements for minimum FCR. The regression line is based on all titration data.

The SID-LYS intake was significantly related to egg mass and egg production rate (no significant interaction between egg mass and egg production rate):

[F7] SID-LYS intake (mg/d) = -324±112.5 + 35.6±4.31 × EM (g/d) – 9.61±3.076 × egg production rate (%): n = 78, R^2 = 0.786.

Using the same model as in [F7] but then excluding the data from the study of Bouyeh and Gevorgian that had low maximum observed egg production rates lower than 80% resulted in the following model:

[F8] SID-LYS intake (mg/d) = -363±115.0 + 35.3±4.23 × EM (g/d) – 9.04±3.034 × egg production rate (%): n = 75, R^2 = 0.795.

Although there are differences in estimated regression factors between models [F7] and [F8] the predicted SID-LYS requirement for a normal production situation of an egg production rate of 90% and an egg weight of 62 g results in almost similar predicted SID-LYS requirement of 798 mg/d for formula [F7] and 793 mg/d for formula [F8].

The consequence of using relationship [F8]. for predicted SID-LYS requirements at two different production rates and using an average egg weight of 60 g are presented in Table 5.

Table 5. Estimated optimal SID-LYS requirements expressed in mg/d and as a percentage of the diet for maximum egg mass (EM) at various egg production rates based on formula [F8], an average egg weight of 60 g and the feed requirements for laying hens at various BW, egg production rates and egg weights as stated in Table 6.4a in the Tabellenboek Veevoeding 2012*.

	Feed intake (g/d)		Egg mass (g/d)		SID-LYS (mg/d)		Dietary SID-LYS (%)		SID-LYS:EM ratio (%)	
	Egg production rate (%)									
BW (kg)	90	95	90	95	90	95	90	95	90	95
1.5	112	115	54	57	731	792	0.655	0.690	13.5	13.9
1.6	114	117	54	57	731	792	0.639	0.674	13.5	13.9
1.7	117	120	54	57	731	792	0.624	0.659	13.5	13.9
1.8	120	123	54	57	731	792	0.611	0.645	13.5	13.9
1.9	122	125	54	57	731	792	0.598	0.631	13.5	13.9
2.0	125	128	54	57	731	792	0.585	0.619	13.5	13.9

*Feed intake is calculated based on: a feed with a MElh content of 11.8 MJ/kg, a requirement of 12.1 kJ per g egg mass, a maintenance requirement of 435 kJ ME per kg MBW (BW^0.75), a requirement of 21.5 kJ ME per gram BWG, a daily BWG of 1.5 g, and 9.5 kJ ME per kg BW per unit decrease in ºC below 25 ºC and a daily temperature of 22 ºC.

4 Conclusions

Based on the results of this study it is concluded that SID-LYS requirements for EM production can be estimated with more precision than SID-LYS requirements for FCR. The conclusion of the Ad hoc group ‘SID amino acid requirements for laying hens’ was that formula [F8] was preferable above the other SID-LYS requirement prediction formulas for EM production. This conclusion of the Ad hoc group was accepted and taken over by the Technical Committee of CVB.

List of studies included in the meta-analysis

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Schmidt, M., P. C. Gomes, H. S. Rostagno, L. F. T. Albino, R. V. Nunes, and A. A. Calderano. 2008. Nutritional requirement of digestible lysine to white-egg laying hens on the second cycle of production. Revista Brasileira de Zootecnia 37(6):1029-1035.

Schmidt, M., P. C. Gomes, H. S. Rostagno, L. F. T. Albino, R. V. Nunes, and E. S. Cupertino. 2009. Nutritional requirement of digestible lysine for brown-egg laying hens in the 2nd production cycle. Revista Brasileira de Zootecnia 38(10):1956-1961.

Schutte, J. B. and W. Smink. 1998. Requirement of the Laying Hen for Apparent Fecal Digestible Lysine. Poultry Science 77(5):697-701.

Shahir, M. H., F. Shariatmadari, S. A. Mirhadi, and A. Chwalibog. 2006. Determination of lysine requirement of laying hen using serum biochemical indicators. Archiv fur Geflugelkunde 70(2):74-79.

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Nonis, M. K. and R. M. Gous. 2008. Threonine and lysine requirements for maintenance in chickens. South African Journal of Animal Sciences 38(2):75-82.

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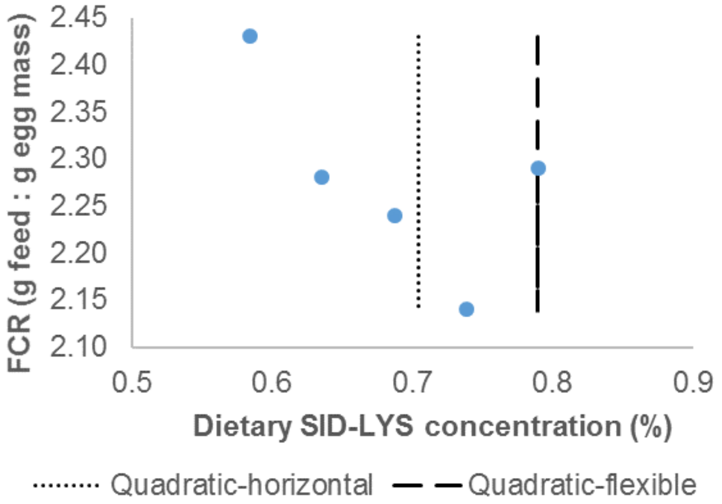
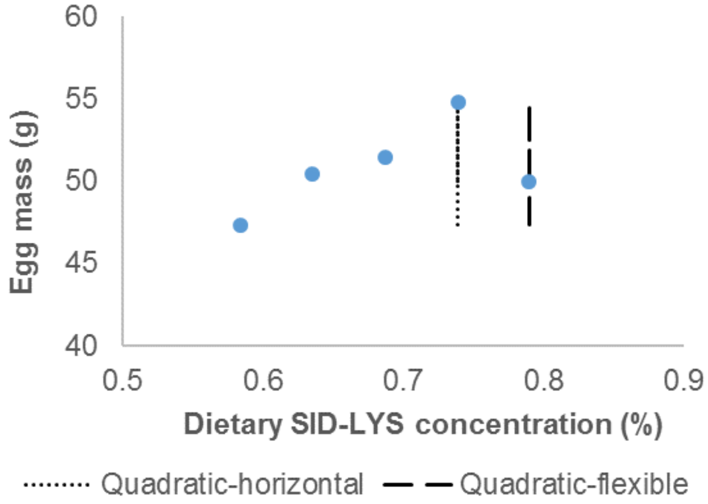
Venturini, K. S., E. B. Malheiros, N. K. Sakomura, M. F. Sarcinelli, E. P. Silva, and L. Hauschild. 2011. A model to estimate lysine requirements of laying hens. https://en.engormix.com/poultry-industry/articles/model-estimate-lysine-requirements-t34981.htm.

Appendix A. Relationship between dietary SID-LYS supply and performance parameters FCR and EM for the various titration trials including the estimated SID-LYS requirements based on the quadratic single-slope broken-line model and the quadratic two-slope broken-line model.

The letter 'a' behind the trial number (shown in the first column) means the model is fitted on all observations except the 3 observations with the lowest dietary SID-LYS levels. If no letter is shown behind the trial number it means that the model is fitted based on all observations of the trial.

Trial	FCR (g feed : g EM)	EM (g/day/hen)
1. Schmidt et al. (2009) Trial 1 Optim. SID-LYS FCR (%) Quadratic single-slope: 0.704 Quadratic two-slope: 0.789 Optimal SID-LYS EM (%) Quadratic single-slope: 0.739 Quadratic two-slope: 0.790		

<table>
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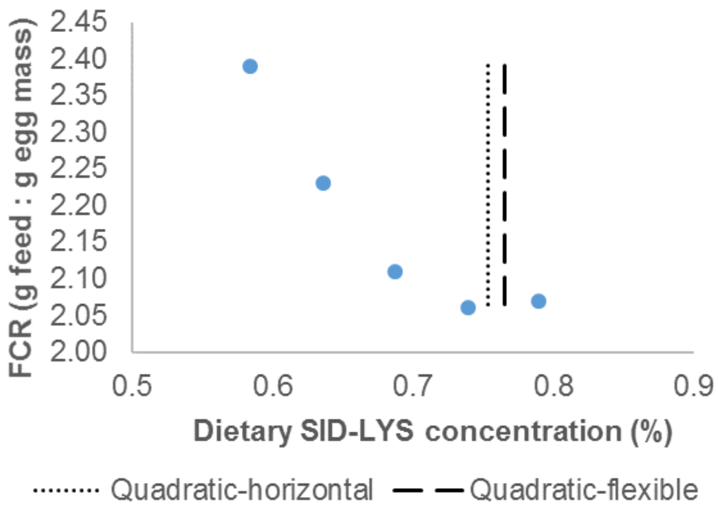
Schmidt et al. (2008)
Trial 1

Optim. SID-LYS FCR (%)
Quadratic single-slope:
0.754
Quadratic two-slope:
0.766

Optimal SID-LYS EM (%)
Quadratic single-slope:
0.738
Quadratic two-slope:
0.738</td>
<td>
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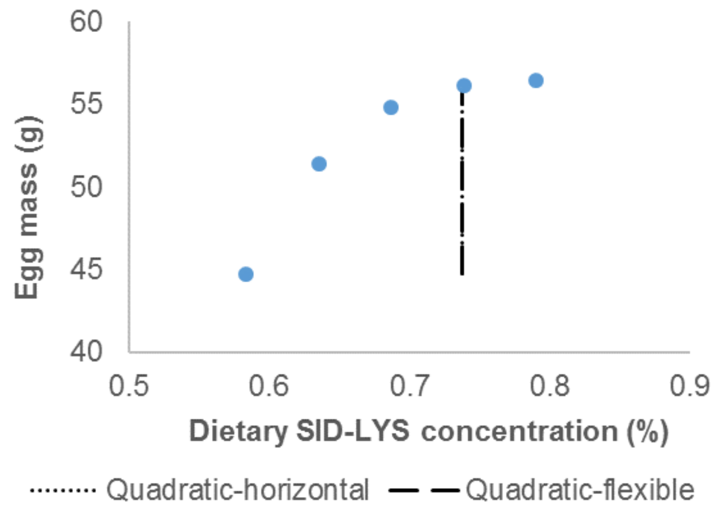
<td>
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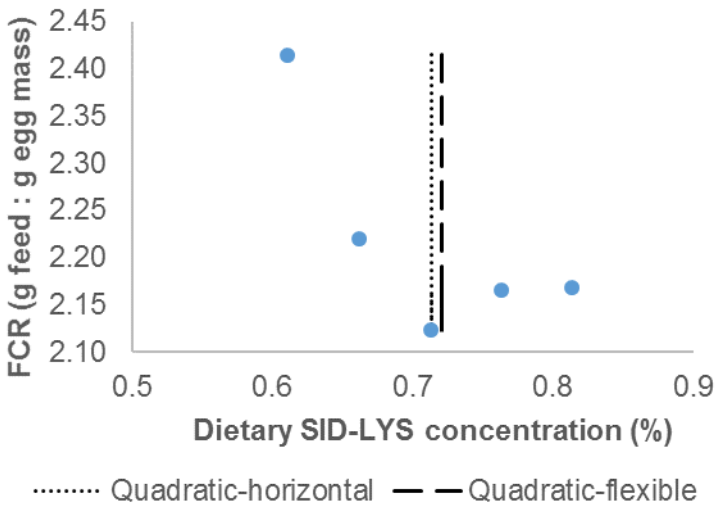
<td>3.
Moraes Sa et al. (2007)
Trial 1

Optim. SID-LYS FCR (%)
Quadratic single-slope:
0.714
Quadratic two-slope:
0.721

Optimal SID-LYS EM (%)
Quadratic single-slope:
0.743
Quadratic two-slope:
0.756</td>
<td>
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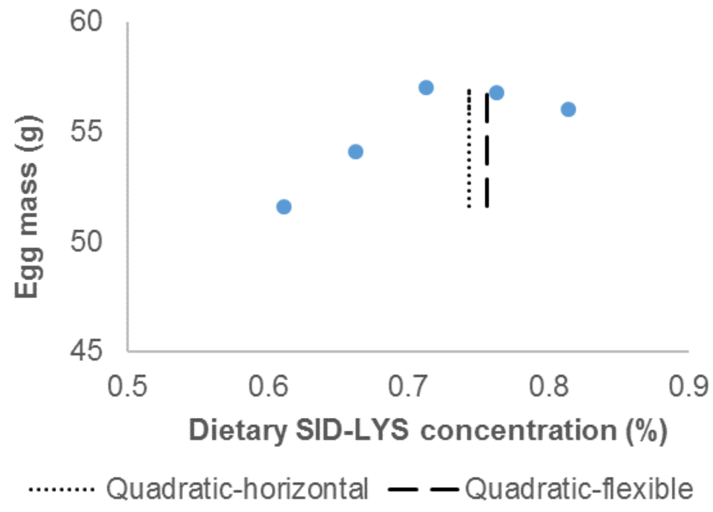
<td>
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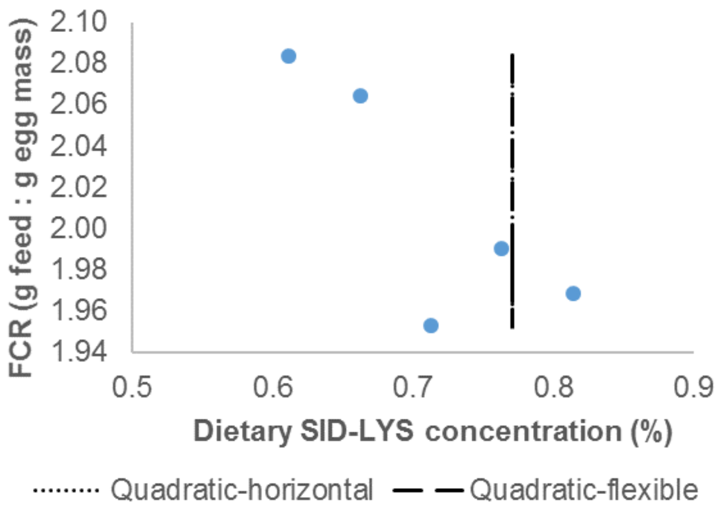
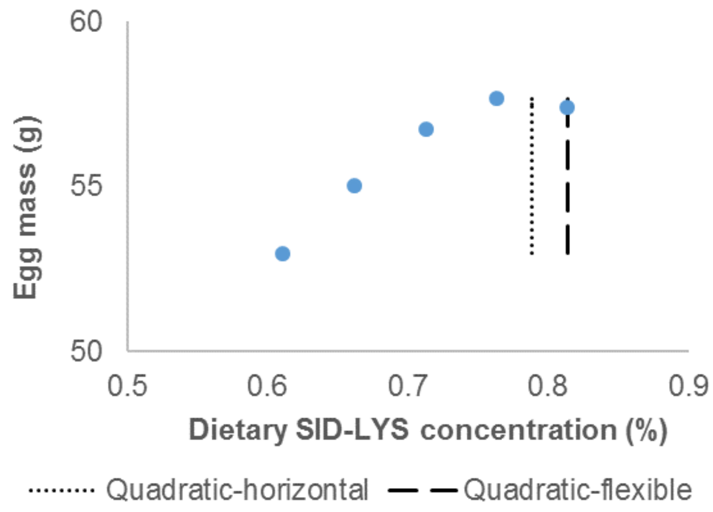
4.
Moraes Sa et al. (2007)
Trial 2

Optim. SID-LYS FCR (%)
Quadratic single-slope:
0.771
Quadratic two-slope:
0.771

Optimal SID-LYS EM (%)
Quadratic single-slope:
0.788
Quadratic two-slope:
0.814

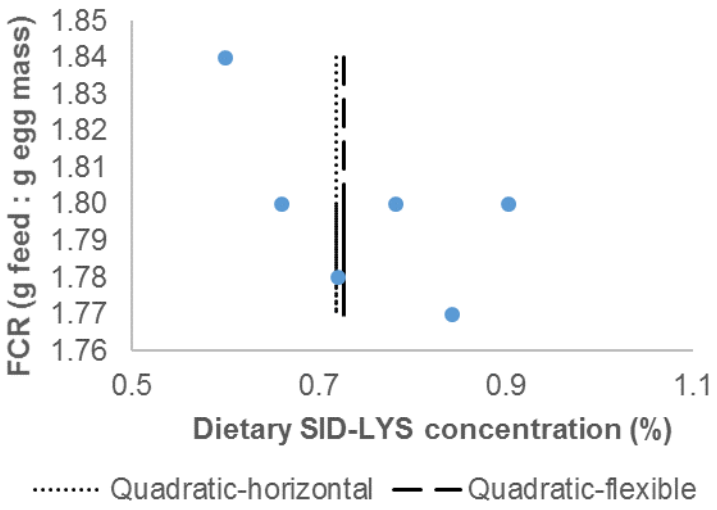


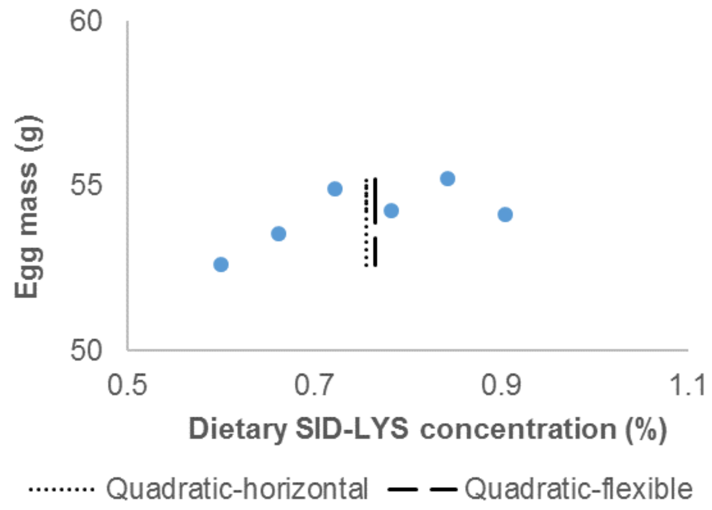


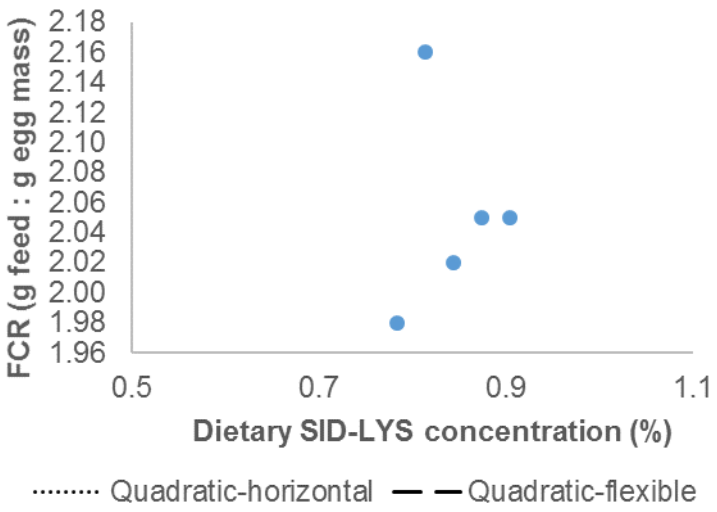
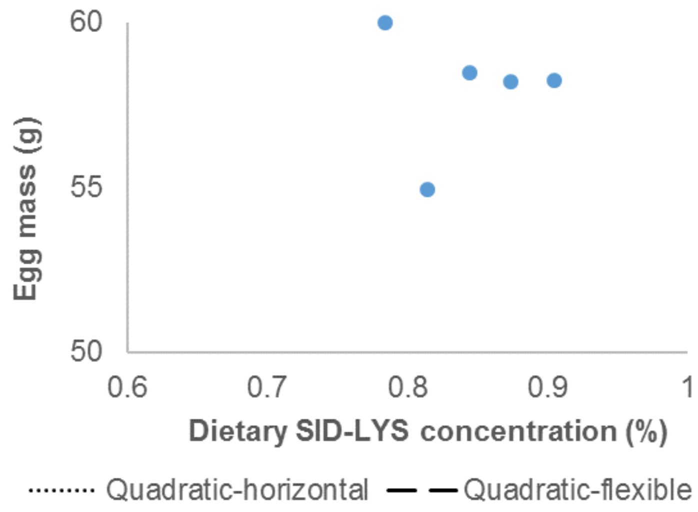
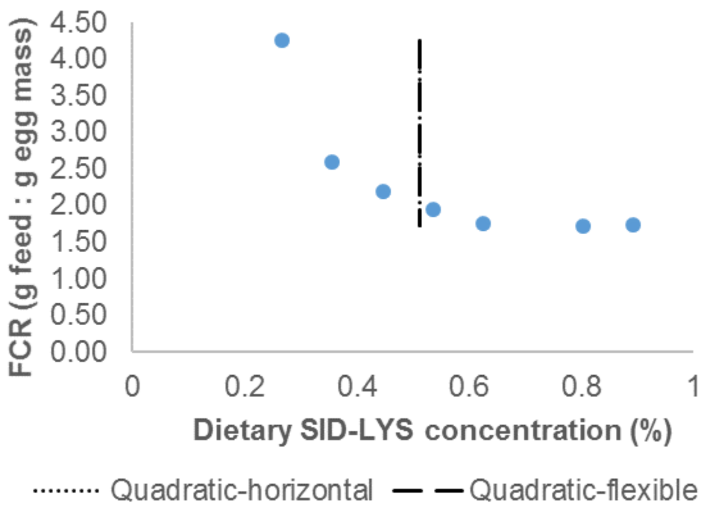
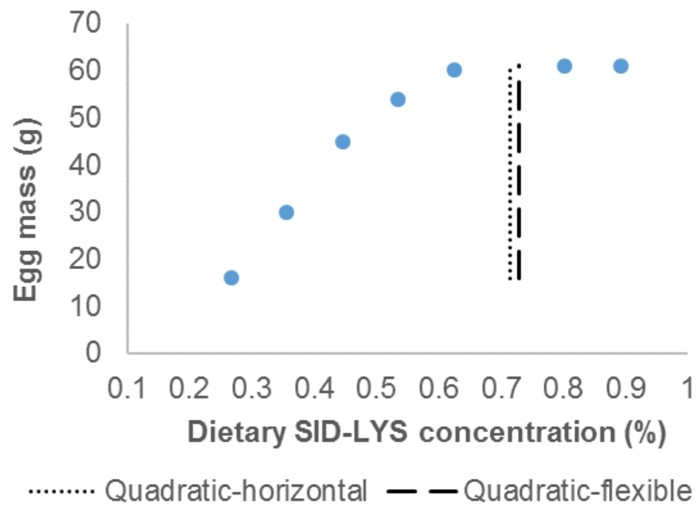
5.
Pastore et al. (2015)

Optim. SID-LYS FCR (%)
Quadratic single-slope:
0.771
Quadratic two-slope:
0.771

Optimal SID-LYS EM (%)
Quadratic single-slope:
0.788
Quadratic two-slope:
0.814



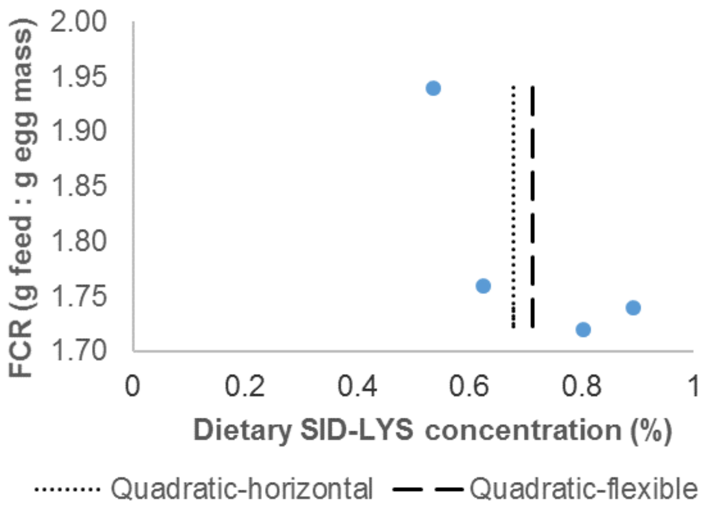


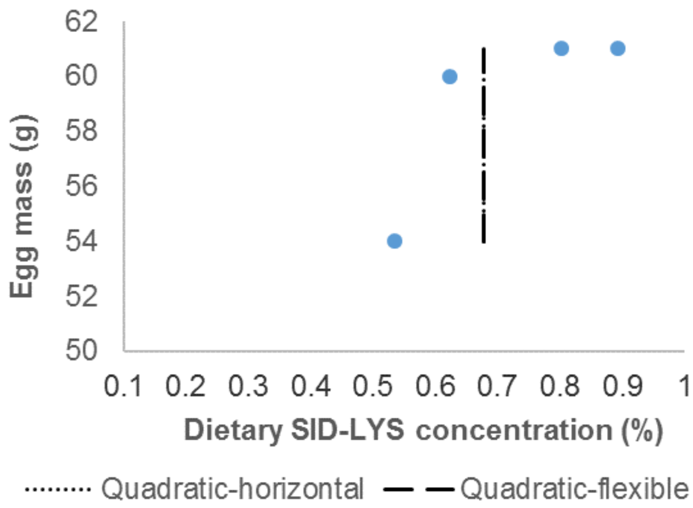
6. Nunes et al. (2015) Optim. SID-LYS FCR (%) Could not be estimated Optimal SID-LYS EM (%) Could not be estimated		
7. Silva et al. (2015) Trial 1 Optim. SID-LYS FCR (%) Quadratic single-slope: 0.512 Quadratic two-slope: 0.512 Optimal SID-LYS EM (%) Quadratic single-slope: 0.715 Quadratic two-slope: 0.729		

7a.
Silva et al. (2015)
Trial 1

Optim. SID-LYS FCR (%)
Quadratic single-slope:
0.678
Quadratic two-slope:
0.713

Optimal SID-LYS EM (%)
Quadratic single-slope:
0.678
Quadratic two-slope:
0.678

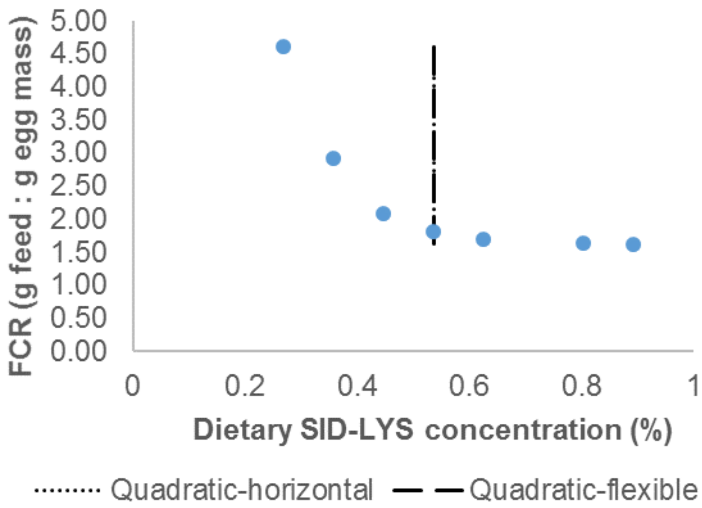


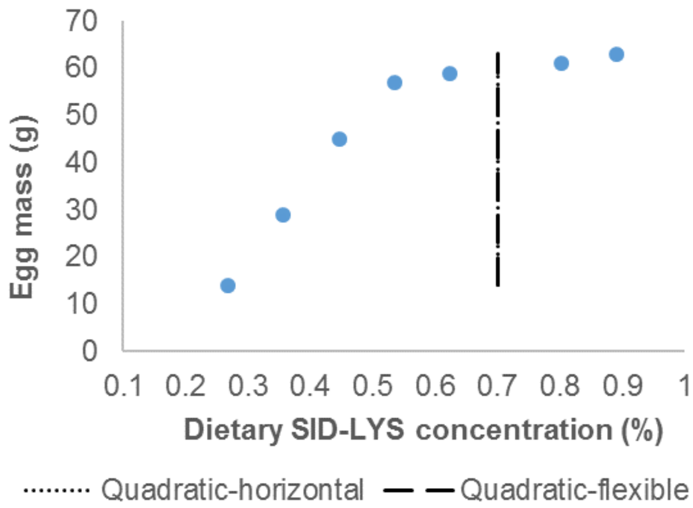


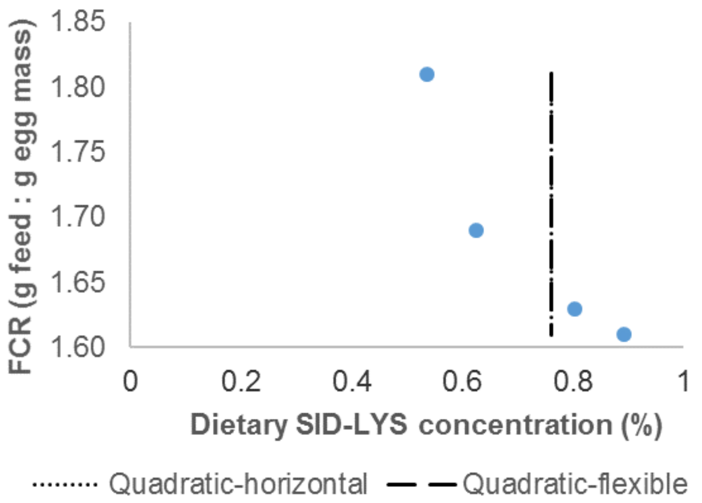
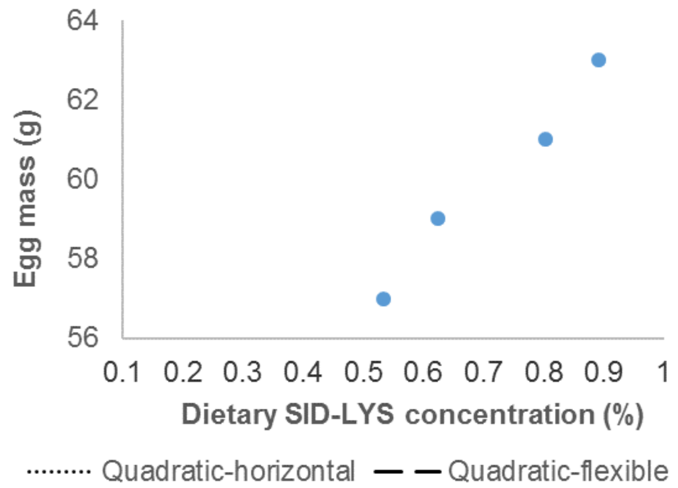
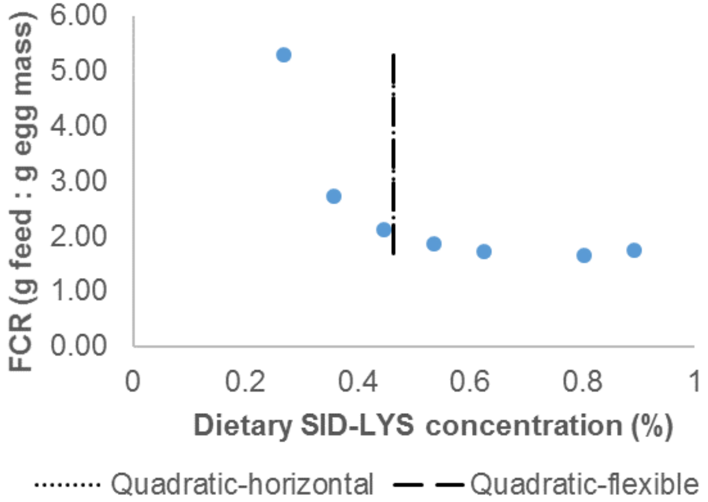
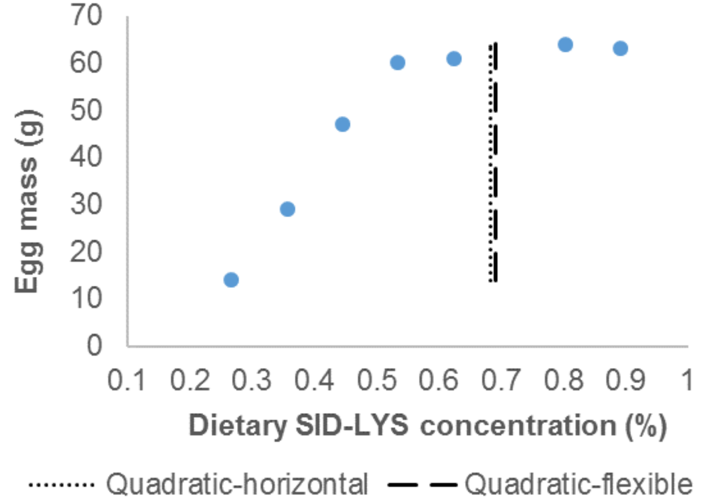
8.
Silva et al. (2015)
Trial 2

Optim. SID-LYS FCR (%)
Quadratic single-slope:
0.538
Quadratic two-slope:
0.538

Optimal SID-LYS EM (%)
Quadratic single-slope:
0.701
Quadratic two-slope:
0.701



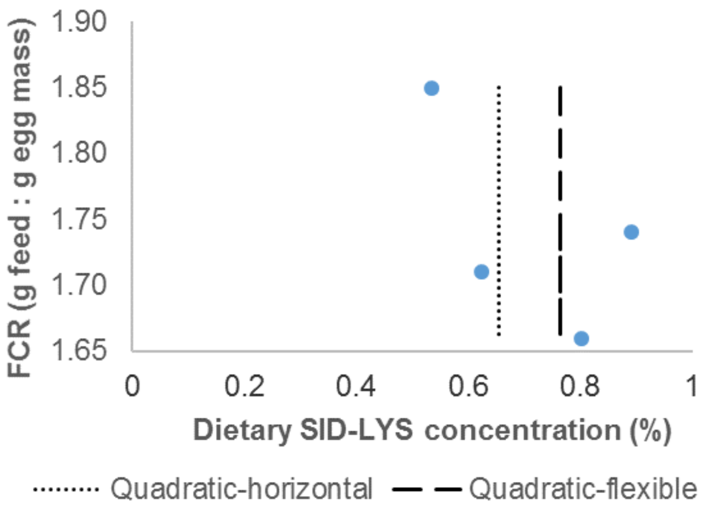


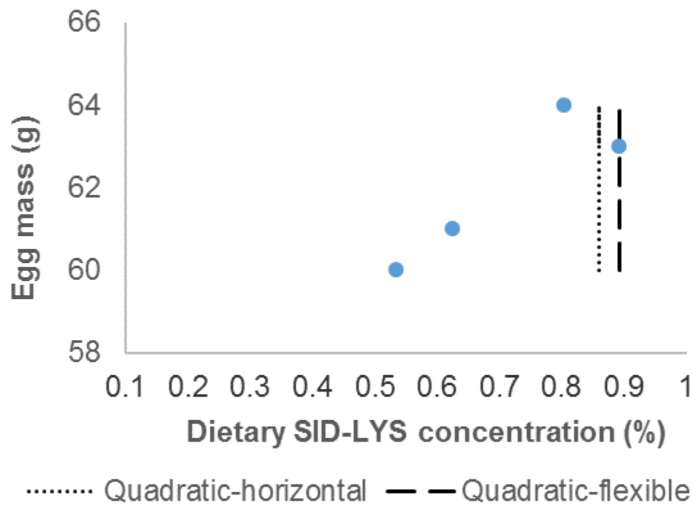
8a. Silva et al. (2015) Trial 2 Optim. SID-LYS FCR (%) Quadratic single-slope: 0.762 Quadratic two-slope: 0.762 Optimal SID-LYS EM (%) Estimate far outside measurement range		
9. Silva et al. (2015) Trial 3 Optim. SID-LYS FCR (%) Quadratic single-slope: 0.465 Quadratic two-slope: 0.465 Optimal SID-LYS EM (%) Quadratic single-slope: 0.682 Quadratic two-slope: 0.691		

9a.
Silva et al. (2015)
Trial 3

Optim. SID-LYS FCR (%)
Quadratic single-slope:
0.655
Quadratic two-slope:
0.765

Optimal SID-LYS EM (%)
Quadratic single-slope:
0.859
Quadratic two-slope:
0.892

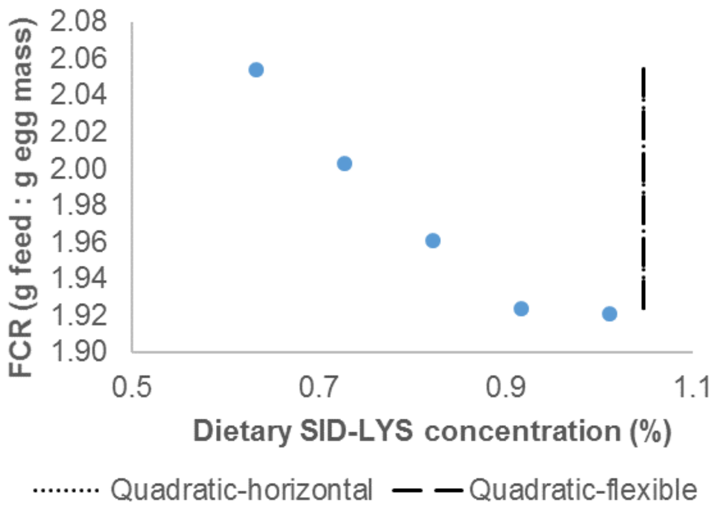


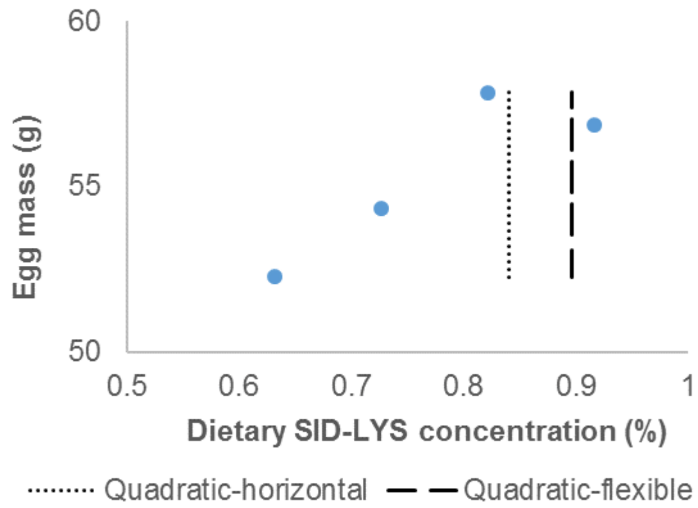


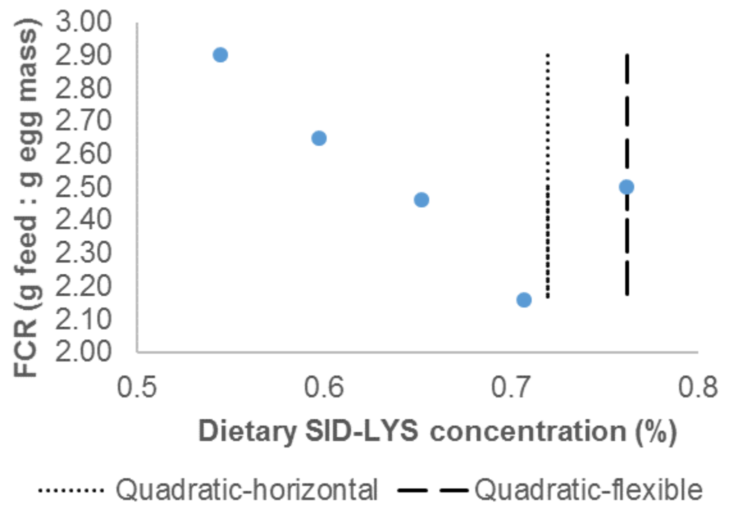
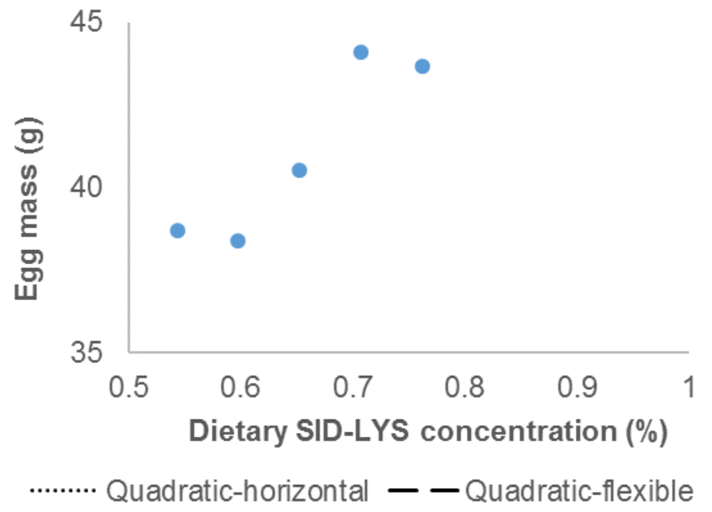
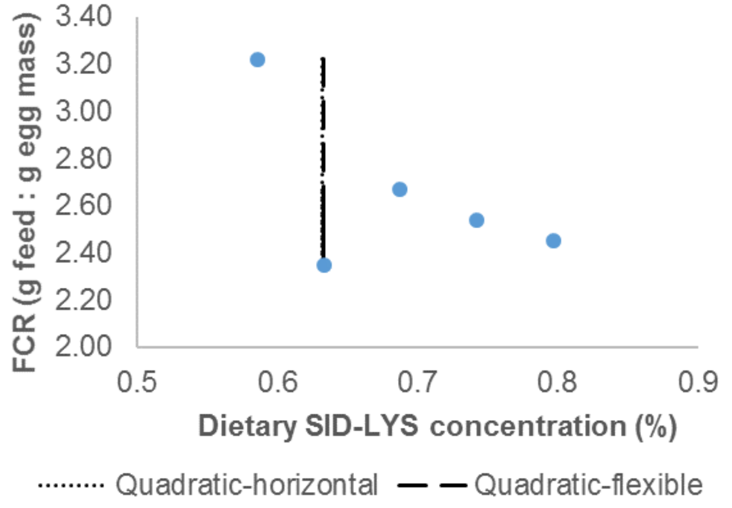
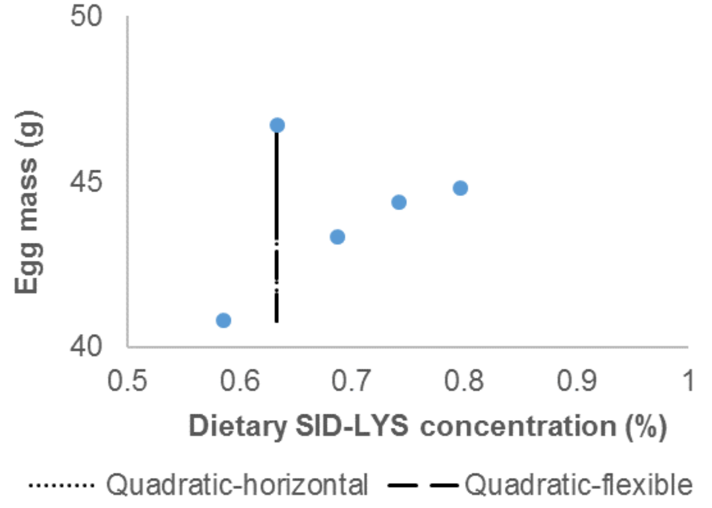
10.
Santos et al. 2014

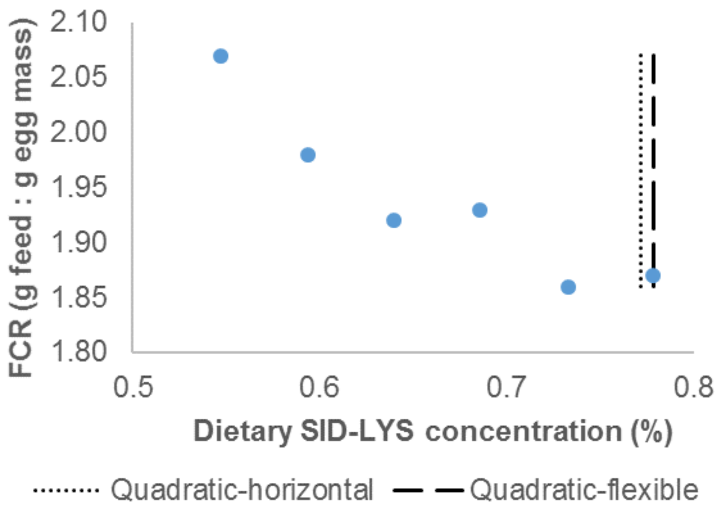
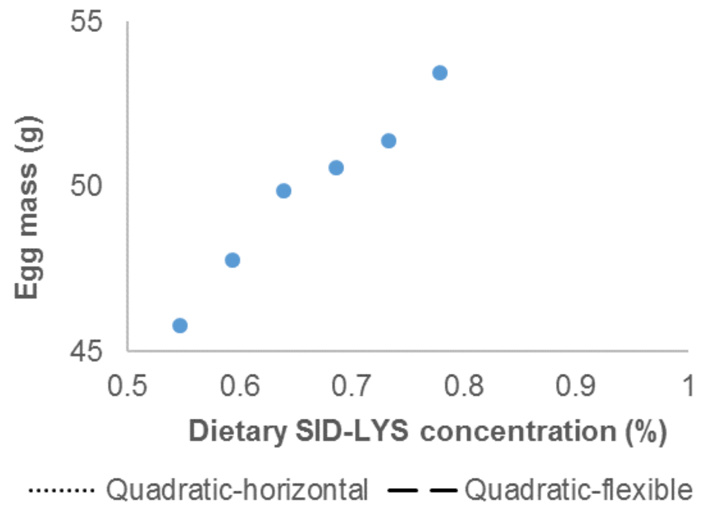
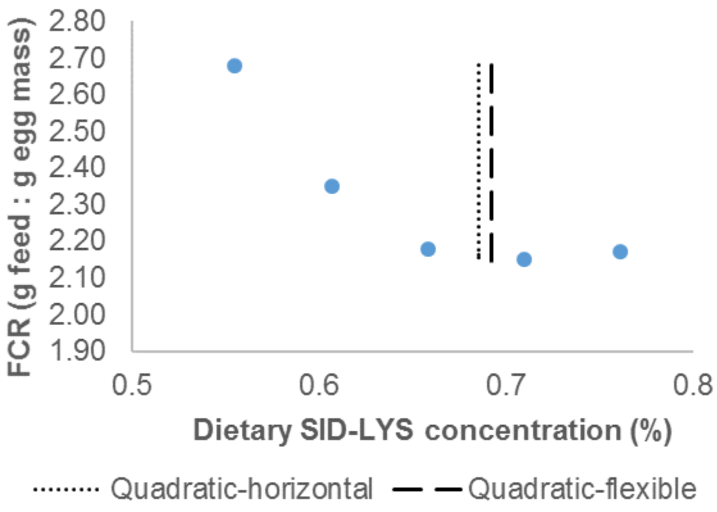
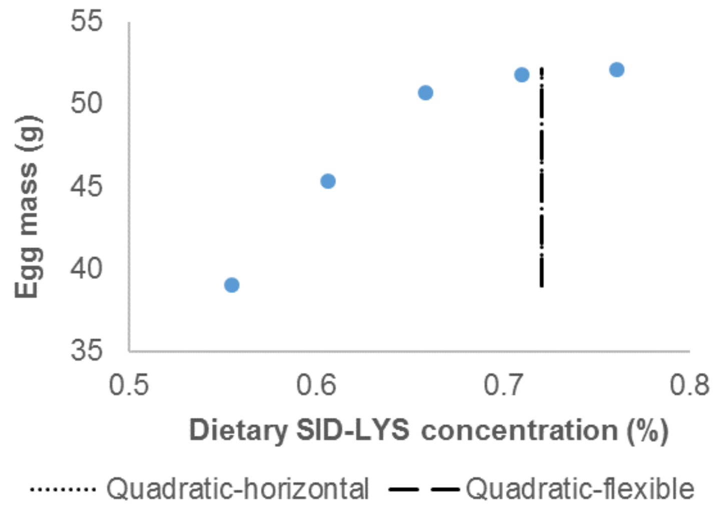
Optim. SID-LYS FCR (%)
Quadratic single-slope:
1.047
Quadratic two-slope:
1.047

Optimal SID-LYS EM (%)
Quadratic single-slope:
0.841
Quadratic two-slope:
0.896





11. Bouyeh and Gevorgian 2011 Trial 1 Optim. SID-LYS FCR (%) Quadratic single-slope: 0.719 Quadratic two-slope: 0.762 Optimal SID-LYS EM (%) Estimated optimal SID-LYS content far outside measurement range		
12. Bouyeh and Gevorgian 2011 Trial 2 Optim. SID-LYS FCR (%) Quadratic single-slope: 0.632 (not uniquely estimateble) Quadratic two-slope: 0.633 Optimal SID-LYS EM (%) Quadratic single-slope: 0.633 Quadratic two-slope: 0.633		

13. Rocha et al. 2009 Optim. SID-LYS FCR (%) Quadratic single-slope: 0.771 Quadratic two-slope: 0.778 Optimal SID-LYS EM (%) Estimated optimal SID-LYS content far outside measurement range		
14. Cupertino et al. 2009 Trial 1 Optim. SID-LYS FCR (%) Quadratic single-slope: 0.685 Quadratic two-slope: 0.692 Optimal SID-LYS EM (%) Quadratic single-slope: 0.721 Quadratic two-slope: 0.721		

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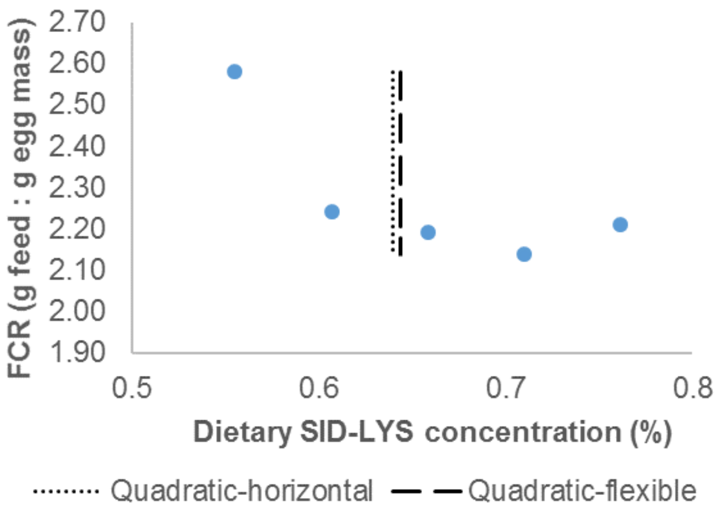
<td>15.
Cupertino et al. 2009
Trial 2

Optim. SID-LYS FCR (%)
Quadratic single-slope:
0.640
Quadratic two-slope:
0.644

Optimal SID-LYS EM (%)
Quadratic single-slope:
0.639
Quadratic two-slope:
0.639</td>
<td>
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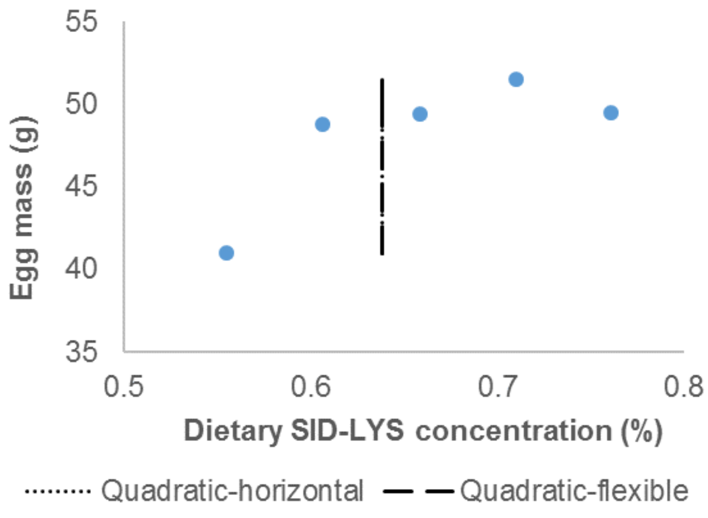
<td>
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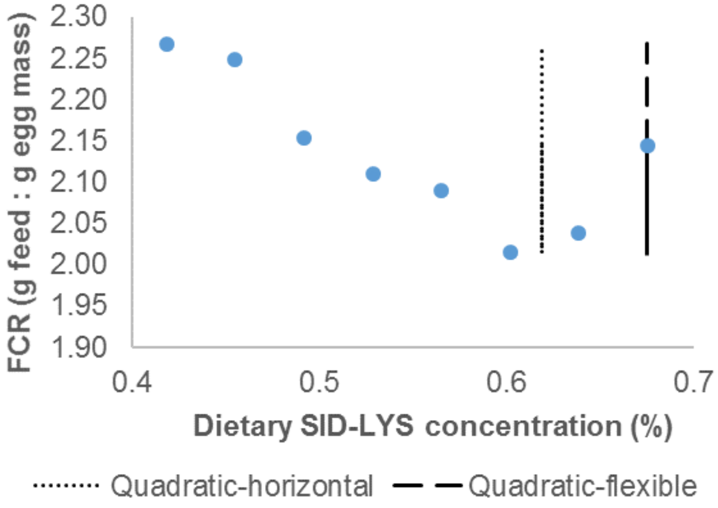
<td>16.
Faria et al. 2003
Trial 1

Optim. SID-LYS FCR (%)
Quadratic single-slope:
0.619
Quadratic two-slope:
0.675

Optimal SID-LYS EM (%)
Quadratic single-slope:
0.613
Quadratic two-slope:
0.639</td>
<td>
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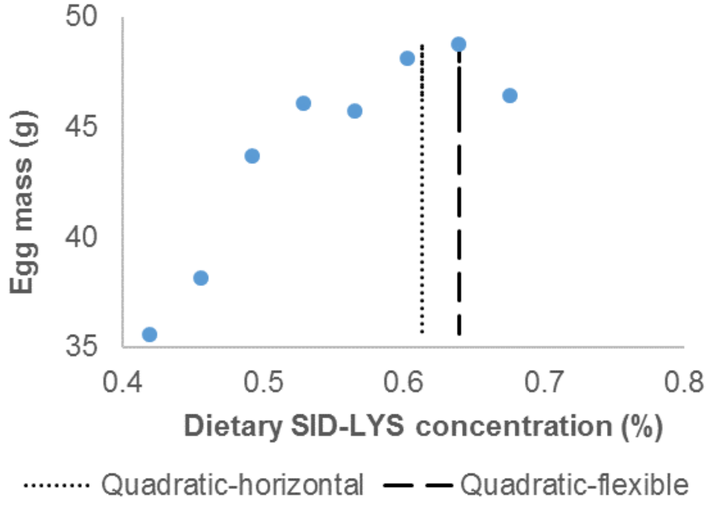
<td>
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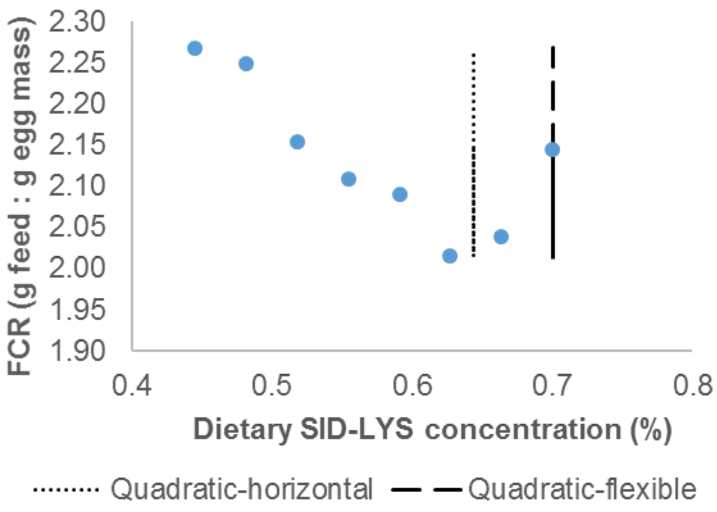
<td>17.
Faria et al. 2003
Trial 2

Optim. SID-LYS FCR (%)
Quadratic single-slope:
0.619
Quadratic two-slope:
0.675

Optimal SID-LYS EM (%)
Quadratic single-slope:
0.613
Quadratic two-slope:
0.639</td>
<td>
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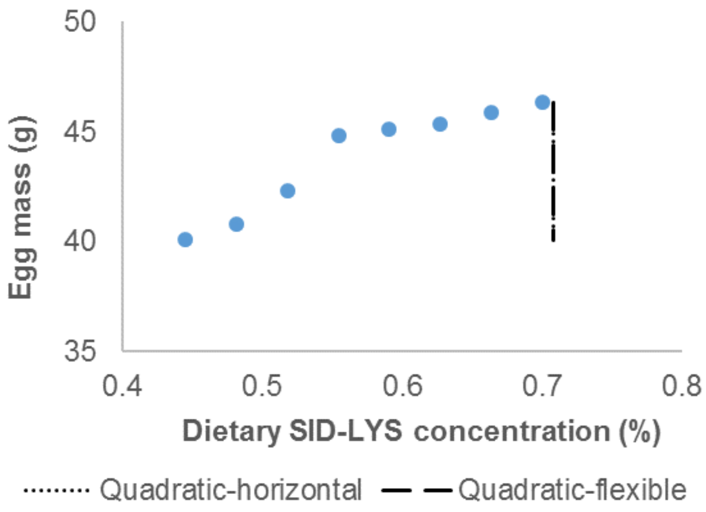
<td>
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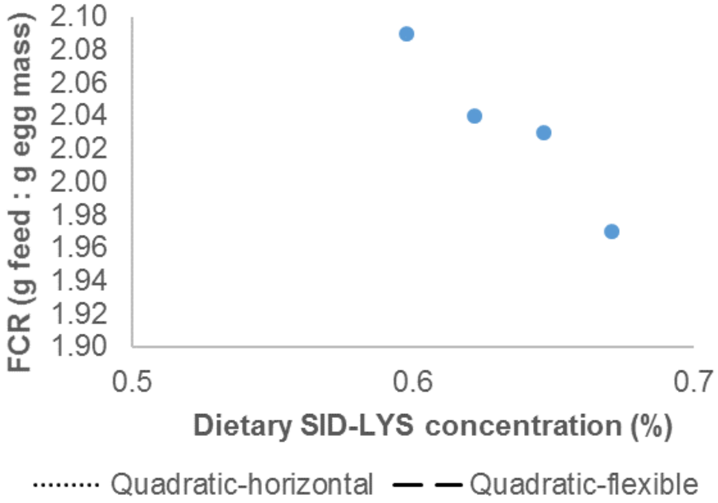
<td>18.
Liu et al. 2005
Trial 1

Optim. SID-LYS FCR (%)
Could not be estimated

Optimal SID-LYS EM (%)
Could not be estimated</td>
<td>
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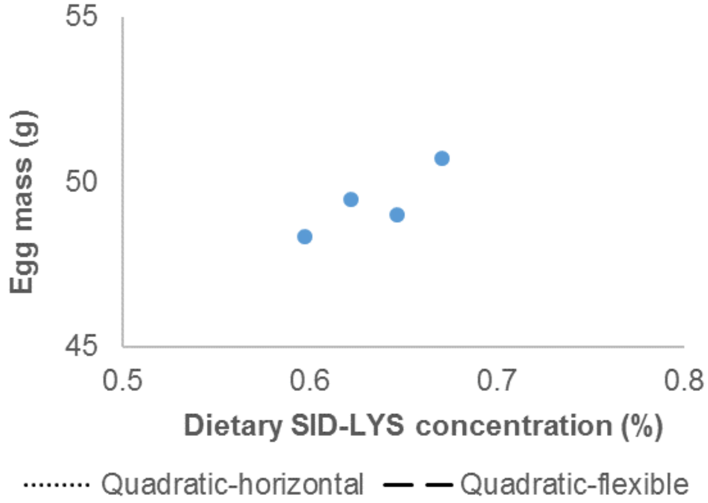
<td>
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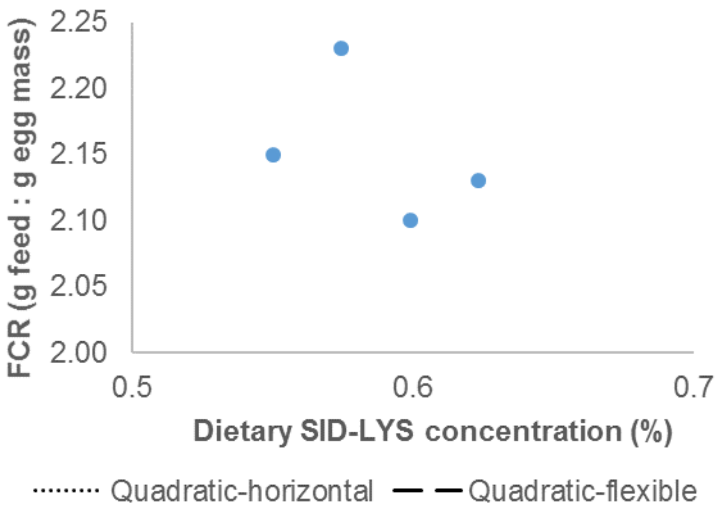
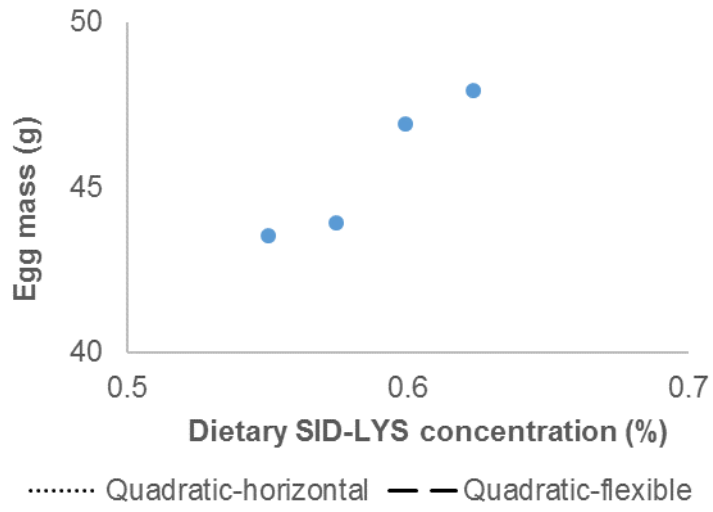
19.
Liu et al. 2005
Trial 2

Optim. SID-LYS FCR (%)
Could not be estimated

Optimal SID-LYS EM (%)
Could not be estimated

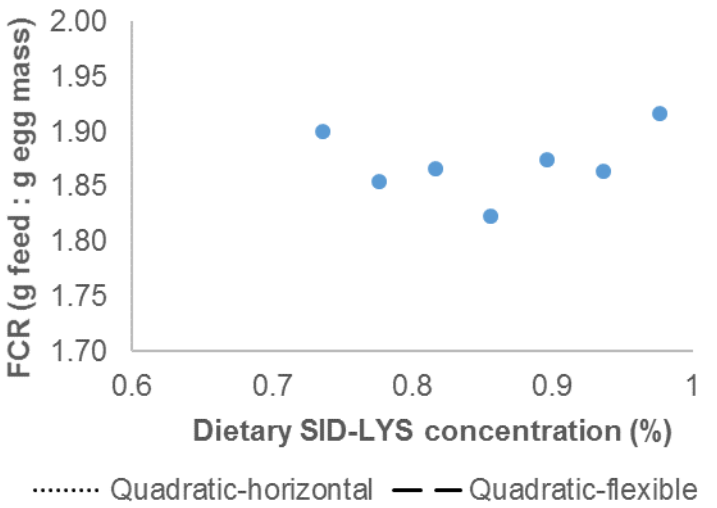


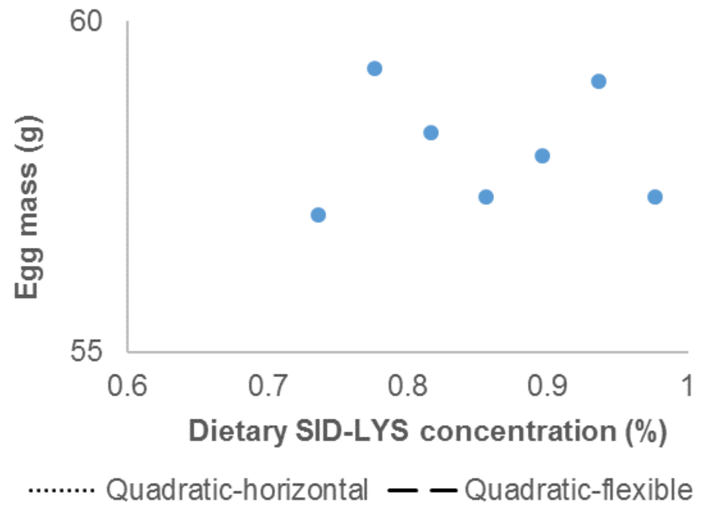


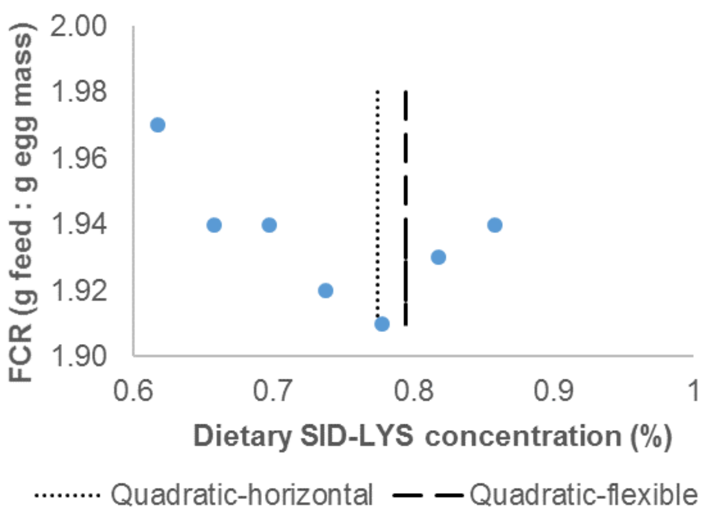
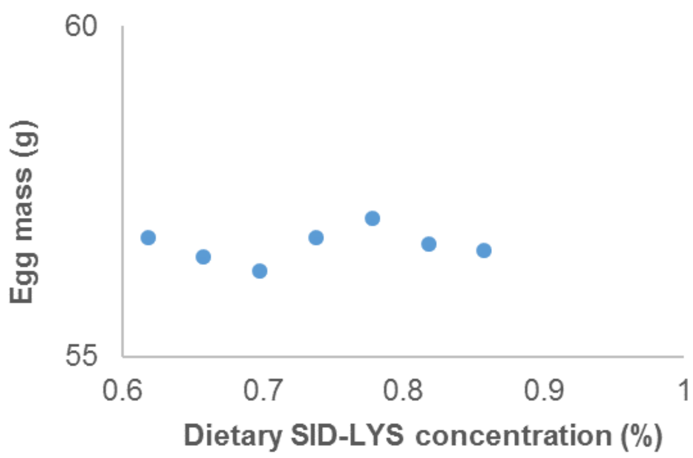
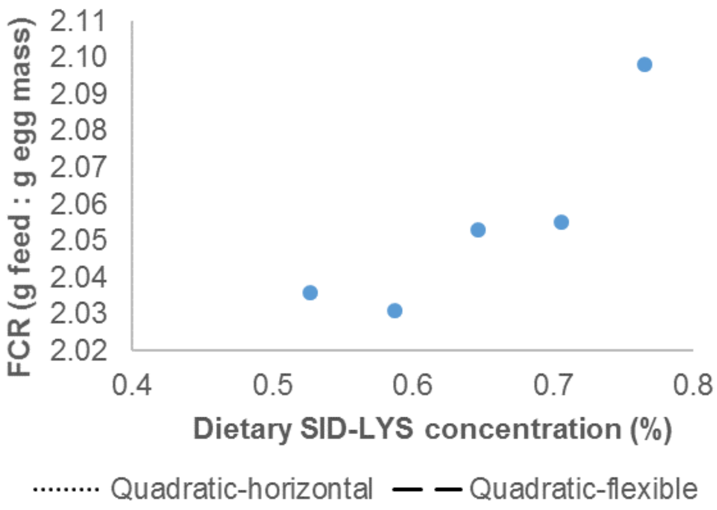
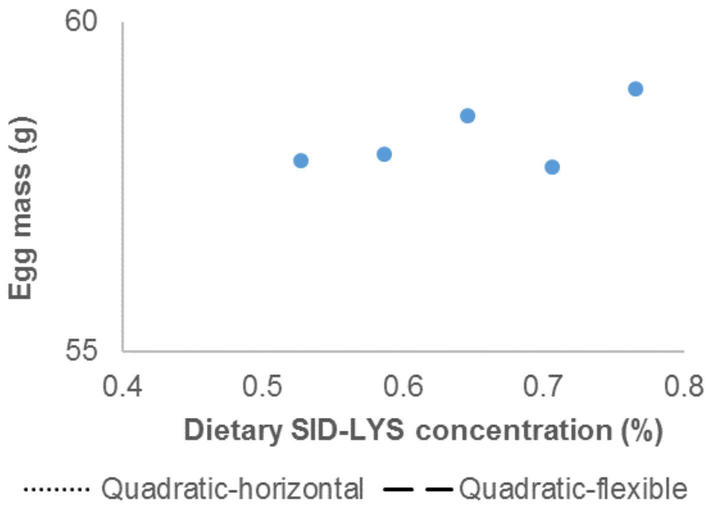
20.
Fihlho al. 2006

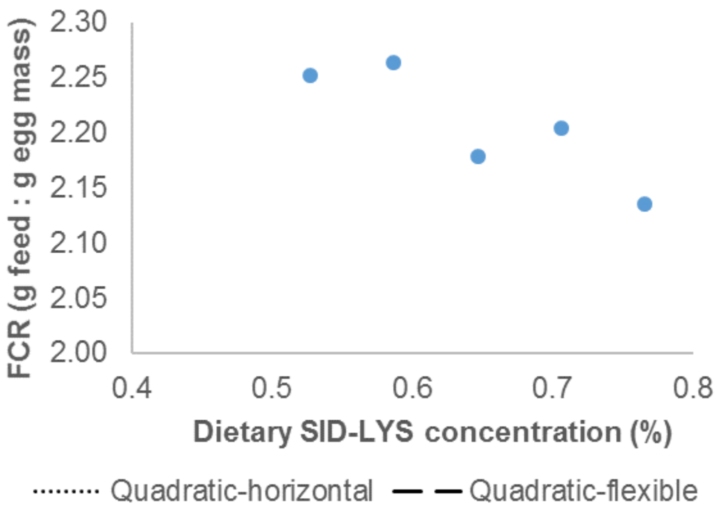
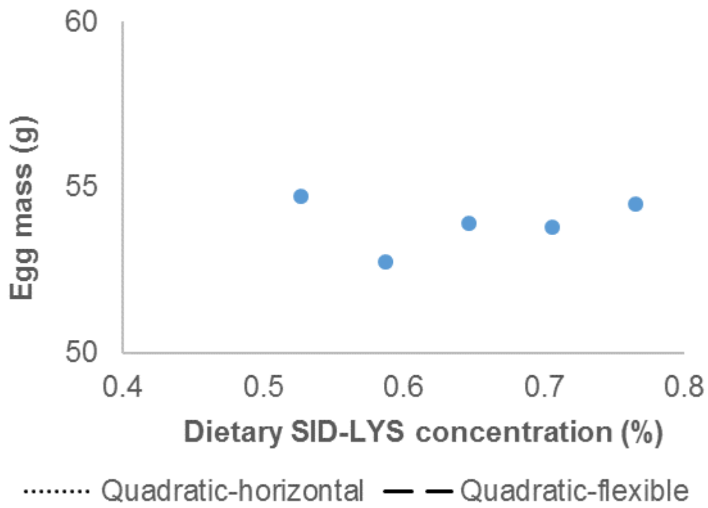
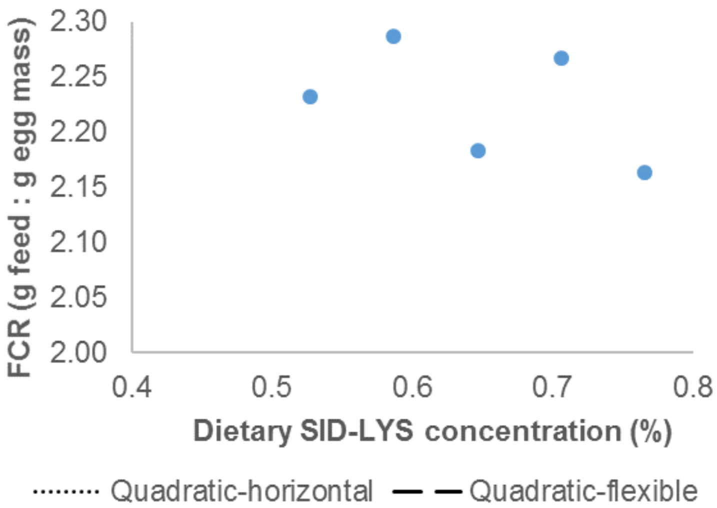
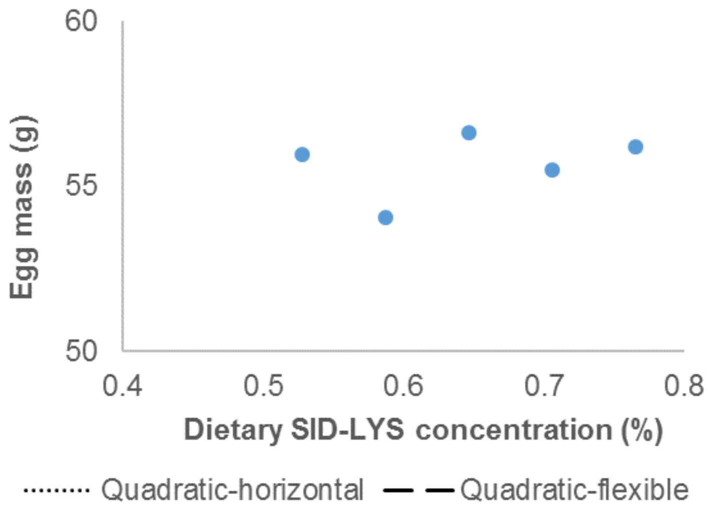
Optim. SID-LYS FCR (%)
Could not be estimated

Optimal SID-LYS EM (%)
Could not be estimated





21. Schutte and Smink 1998 Optim. SID-LYS FCR (%) Quadratic single-slope: 0.775 Quadratic two-slope: 0.794 Optimal SID-LYS EM (%) Could not be estimated		
22. Star and van Krimpen 2016 Trial 1 Optim. SID-LYS FCR (%) Could not be estimated Optimal SID-LYS EM (%) Could not be estimated		

23. Star and van Krimpen 2016 Trial 2 Optim. SID-LYS FCR (%) Could not be estimated Optimal SID-LYS EM (%) Could not be estimated		
24. Star and van Krimpen 2016 Trial 3 Optim. SID-LYS FCR (%) Could not be estimated Optimal SID-LYS EM (%) Could not be estimated		

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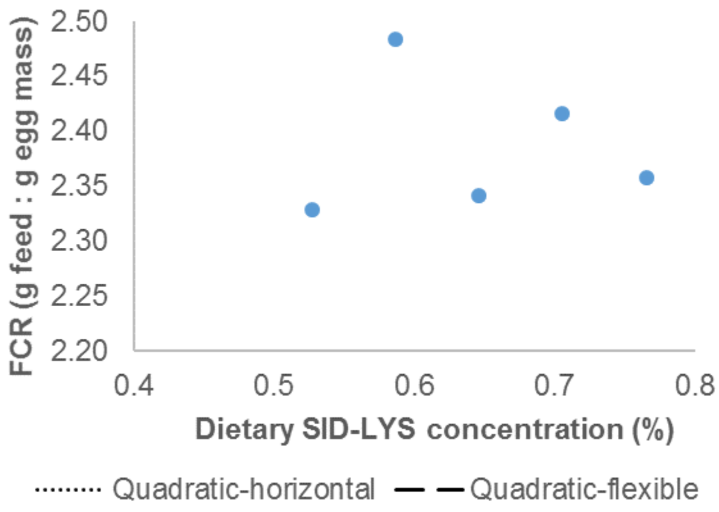
Star and van Krimpen
2016
Trial 4

Optim. SID-LYS FCR (%)
Could not be estimated

Optimal SID-LYS EM (%)
Could not be estimated</td>
<td>
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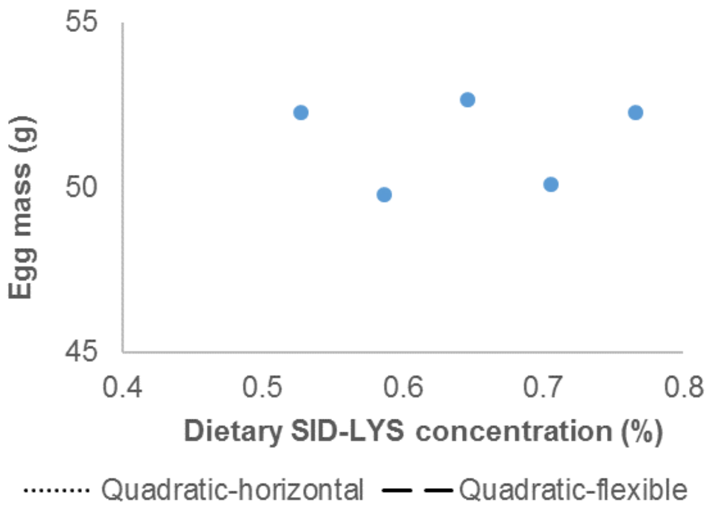
<td>
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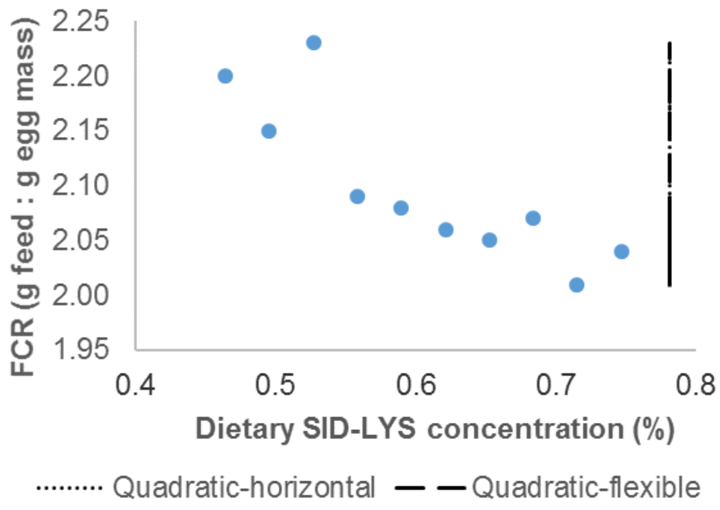
<td>26.
Shahir et al. 2005

Optim. SID-LYS FCR (%)
Quadratic single-slope:
0.781
Quadratic two-slope:
0.781

Optimal SID-LYS EM (%)
Quadratic single-slope:
0.584
Quadratic two-slope:
0.584</td>
<td>
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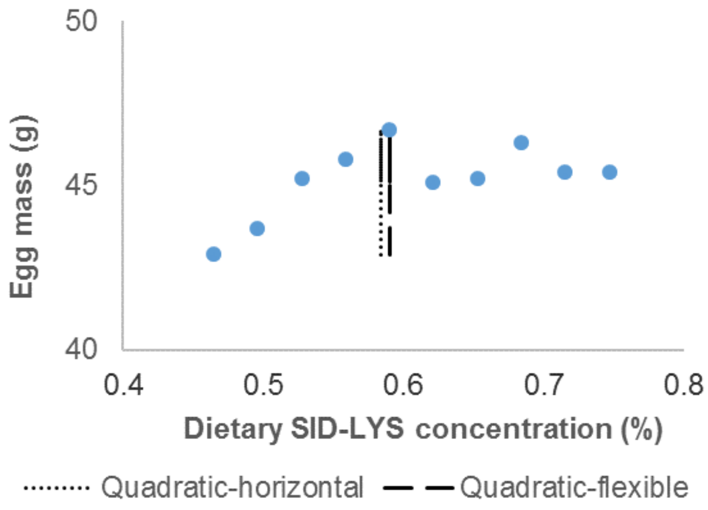
<td>
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Appendix B. SID-LYS model estimates using the quadratic single-slope broken-line model for minimum FCR and maximum EM

SID-LYS model estimates using the single-slope quadratic broken-line model for minimum FCR. The letter ‘a’ behind the trial number (shown in the first column) means the model is fitted on all observations except the 3 observations with the lowest dietary SID-LYS levels. If no letter is shown behind the trial number it means that the model is fitted based on all observations of the trial.

Trial nr.	Estimate L	Std. Err. L	Estimate R	Std. Err. R	Estimate U	Std. Err. U	R^2
1	2.22	0.050	0.704	0.1014	14.3	24.28	0.734
2	2.06	0.007	0.754	0.0100	11.4	1.33	0.998
3	2.15	0.014	0.714	0.0218	24.9	10.92	0.977
4	1.97	0.029	0.771	0.1085	4.8	6.57	0.786
5	1.79	0.008	0.719	0.0748	3.7	4.82	0.766
6							
7	1.81	0.081	0.512	0.0327	39.9	11.28	0.978
7a	1.73	0.010	0.678	0.0242	10.2	3.39	0.994
8	1.69	0.045	0.538	0.0158	39.7	4.92	0.996
8a	1.62	0.010	0.762	0.0409	3.7	1.39	0.992
9	1.79	0.085	0.465	0.0223	88.9	21.01	0.987
9a	1.70	0.040	0.655	0.1420	10.4	23.69	0.835
10	1.92	0.008	1.047	0.0546	0.8	0.19	0.994
11	2.35	0.137	0.719	0.1195	18.5	25.15	0.791
12	2.50	0.068	0.632	.	341.3	72.33	0.881
13	1.87	0.021	0.771	0.0594	3.8	1.96	0.943
14	2.16	0.007	0.685	0.0056	30.7	2.66	0.999
15	2.18	0.021	0.640	0.0180	56.2	24.12	0.979
16	2.07	0.029	0.619	0.0767	5.4	4.29	0.804
17	2.07	0.029	0.644	0.0761	5.5	4.36	0.804
18							
19							
20							
21	1.93	0.006	0.775	0.0672	1.5	1.07	0.829
22							
23	1.14	51.886	4.979	210.4000	0.1	2.77	0.763
24							
25							
26	2.03	0.048	0.781	0.1675	1.9	1.73	0.772

SID-LYS model estimates using the quadratic broken-line model for maximum EM. The letter 'a' behind the trial number (shown in the first column) means the model is fitted on all observations except the 3 observations with the lowest dietary SID-LYS levels. If no letter is shown behind the trial number it means that the model is fitted based on all observations of the trial.

Trial nr.	Estimate L	Std. Err. L	Estimate R	Std. Err. R	Estimate U	Std. Err. U	R^2
1	52	1.7	0.739	0.1611	-200	423	0.590
2	56	0.2	0.738	0.0075	-484	48	0.999
3	57	0.5	0.743	0.0421	-293	189	0.945
4	58	0.2	0.788	0.0202	-148	33	0.993
5	55	0.3	0.755	0.0883	-87	102	0.781
6							
7	61	0.7	0.715	0.0185	-230	19	0.998
7a	61	0.0	0.678	0.0000	-341	0	1.000
8	62	1.3	0.701	0.0308	-261	38	0.993
8a							
9	63	1.6	0.682	0.0363	-296	53	0.990
9a	63	0.9	0.859	0.2493	-35	53	0.904
10	56	1.3	0.841	0.2397	-93	217	0.604
11							
12	45	0.7	0.633	.	-1802	717	0.678
13	56	5.9	1.039	0.3941	-41	43	0.975
14	52	0.4	0.721	0.0149	-481	86	0.995
15	50	0.7	0.639	0.0258	-1306	810	0.958
16	48	0.7	0.613	0.0335	-331	119	0.952
17	46	0.5	0.708	0.0478	-95	32	0.962
18							
19							
20							
21	57	0.1	0.618	.	-554	170	0.639
22							
23							
24							
25							
26	46	0.2	0.584	0.0398	-210	154	0.789