Standardized ileal digestible isoleucine 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 requirement for SID isoleucine was subsequently estimated. The results of this meta-analysis for standardized ileal digestible isoleucine (SID-ILE) requirement are presented in the present CVB Documentation report.
Compared to the former CVB apparent faecal digestible ILE recommendation for laying hens described in CVB Documentation report nr. 18 and published in 1996 the present established SID-ILE amino acid recommendations for laying hens are:

1. Based on a 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 requirements of SID-ILE 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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Abbreviations

AA	Amino acids
AFD	Apparent faecal digestible
ARG	Arginine
BW	Body weight
BWG	Body weight gain
CP	Crude protein
CYS	Cysteine
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 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 has been 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 isoleucine (SID-ILE) 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..
Furthermore, compared to the study of van Krimpen another model for estimation of SID-ILE requirements has been used. This model consisted of a quadratic broken-line model as described and used in the estimation of SID-LYS requirements for laying hens as well (CVB documentation report nr. 69).

2 Materials and Methods

Isoleucine requirement studies were selected from literature (1990 – 2017) in which the dietary ILE content was varied by means of addition of graded levels of dietary synthetic ILE. Furthermore, performance characteristics such as 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. The apparent faecal digestible (AFD) non-test-AA : AFD-LYS ratios needed to be at least 90% of the CVB (2012) requirement level and the basal AFD-ILE : AFD-LYS ratio needed to be at least 20% below the CVB (2012) AFD-ILE : AFD-LYS requirement level.
Ass well, also studies were considered in which the ILE requirement was investigated based on summit and dilution diets that were mixed in various ratios in order to obtain the desired differences in ILE.

Requirements were estimated using a quadratic broken-line model as described below. This model was adopted from a publication of Robbins et al. (2006).

The quadratic broken-line model is as follows:

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

Estimated SID-ILE requirements for EM and FCR were regressed against factors such as EM, FCR, age, and the dietary factors CP, ME and CP : ME ratio. Unfortunately, it was not possible to also include body weight as a variable due to lack of information.

3 Results and Discussion

In Table 1. Some characteristics of the studies included in the meta-analysis is given. The dataset consisted of 7 studies with in total 16 trials and 100 observations. Of these studies, 4 studies (including 9 trials) were titration trials whereas the other studies consisted of summit and dilution diets.

In Appendix A for each trial the relationship between dietary SID-ILE (%) and FCR and between dietary SID-ILE (%) and EM is presented graphically together with the estimated SID-ILE requirements for the quadratic broken-line model.
In Appendix B the estimated quadratic broken-line model parameters for each titration trial is given. In some cases (for trials 1, 3, 10, 11, 12, 15 and 16) also model estimates are provided after removing the basal treatment (or the treatment with the lowest SID-ILE content) as it was expected that for these trials this would significant affect model estimates of R (or requirement estimates for SID-ILE). The model predictions for these trials were the basal treatment was removed prior to fitting the model are shown with the letter “a” (for example; trial 1 becomes trial 1a).

For a number of titration trials it was not possible to estimate (unique) SID-ILE requirements for maximum EM (for trials 3, 4, 5, 6, 7 and 9) and for minimum FCR (for trials 1, 2, 3, 4, 5, 6, 7, 9, and 14). Especially with respect to estimation of SID-ILE requirement for minimum FCR few titration trials allowed for estimation of a requirement. With respect to estimation of SID-ILE requirements for FCR it also seemed that in a number of cases birds had a negative energy balance complicating the possibility to estimate reliable SID-ILE requirements for FCR. Therefore, in this study the focus is on establishing SID-ILE requirements for maximum EM. Furthermore, for the dilution studies optimal SID-ILE concentrations for maximum EM were estimated at high dietary SID-LYS intake levels (1.01%;1156 mg/d) and dietary concentrations of dietary protein (on average19.8%). As these high dietary SID-LYS and high dietary protein concentrations are not used in practice it might be undesirable to translate these results to practice.

The titration results from trials 1, 2, 3, 4, 5, 6 and 7 (studies from Peganova and Eder; 2002 and 2003) indicate that an oversupply of dietary SID-ILE reduces EM and should be prevented. For trials 4, 5, 6 and 7 the lowest dietary SID-ILE treatment resulted in the highest EM yield. It is, therefore, unknown what the optimal SID-ILE concentration would have been in trials 4, 5, 6 and 7. Probably these optimal SID-ILE concentrations would be lower than the basal SID-ILE level.

In Table 2 the average estimated optimal SID-ILE concentrations and SID-ILE intake statistics for maximum EM are presented in which is distinguished between titration trials and dilution diets.

Table 2. Estimated optimal SID-ILE requirements (as % and as daily intake) for maximum egg mass (EM).

	Parameter	N*	Mean	Std. Dev.	Min.	Max	%CV
SID-ILE (%)	Titration*	9	0.531	0.0970	0.417	0.685	18.3
	Dilution**	12	0.554	0.0635	0.494	0.708	11.5
	Dilution***	9	0.541	0.0431	0.495	0.630	8.0
SID-ILE intake (mg/d)	Titration*	9	608	92.0	461	758	15.1
	Dilution**	12	634	94.2	474	772	14.9
	Dilution***	9	636	89.0	474	759	14.0
SID-ILE intake per g of EM (mg/g)	Titration*	9	11.1	1.56	8.6	13.0	14.1
	Dilution**	12	12.9	2.01	9.4	17.0	15.6
	Dilution***	9	12.3	1.60	9.4	14.9	13.0
SID-ILE:SID-LYS ratio	Titration*	9	91.3	13.93	69.3	117.8	15.3
	Dilution**	12	55.5	6.50	49.4	69.5	11.7
	Dilution***	9	56.8	7.16	49.4	69.5	12.6
SID-ILE:SID-LYS ratio****	Titration*	9	91.4	13.90	69.3	117.8	15.2
	Dilution**	12	99.7	20.46	75.4	133.7	20.5
	Dilution***	9	91.5	14.50	75.4	113.8	15.9

* number of titration trials (total number of titration trials is 9 (8 trials + 1 titration trials for which R values were estimated again after excluding the diet containing the lowest dietary SID-ILE level). For trials 4, 5, 6 and 7 the lowest observed dietary SID-ILE level was used.

**number of dilution trials (total number of dilution trials is 12 (7 dilution trials + 5 dilution trials for which R values were estimated again after excluding the diet containing the lowest dietary SID-ILE level).

*** number of dilution trials (total number of dilution trials is 9 (5 dilution trials + 4 dilution trials for which R values were estimated again after excluding the diet containing the lowest dietary SID-ILE level). Dilution trials 12 and 13 were excluded because of the low maximum observed rate of lay of 75 and 71 percent, respectively.

****This ratio is calculated using formula [F8] in CVB Documentation report nr. 69 to predict SID-LYS requirement. In case the formula [F8] resulted in a lower SID-LYS requirement than the observed SID-LYS intake at which maximum EM was estimated, then this formula was used to calculate the SID-ILE:SID-LYS ratio, otherwise the observed SID-LYS intake at which maximum EM was estimated was used.

Results in Table 2 show a wide range in optimal estimated SID-ILE concentrations and optimal SID-ILE intake levels. Because of the high dietary concentrations of protein and lysine in the dilution trials that corresponded with the estimated SID-ILE requirements that are substantially higher than used in practise it was decided to focus on the results of the titration trials. In Table 2 the average estimated SID-ILE requirements are shown. As the results of the individual titration trials in Appendix A and B shows, in a number of cases it is difficult or even impossible to estimate reliable SID-ILE requirements. Therefore, it was also chosen to consider the observed SID-ILE intake levels at which maximum EM production was observed. In case the SID-ILE requirements of the titration studies were based on the observations with the highest EM the average SID-ILE:SID-LYS requirement was 90±19.4% and SID-ILE : EM ratio 10.3±1.98 mg/g.

An average observed dietary SID-ILE requirement of 10.3 mg/g EM corresponds better with optimal dietary ILE requirements per g of EM as estimated by Huyghebaert et al. (1991) and Mannion et al. (1993) than the average model estimated SID-ILE requirement of 11.1 mg SID-ILE per g of EM. Huyghebaert et al. (1991) estimated an ILE requirement of 44.47 mg/kg body weight and 9.48 mg per g of EM. Mannion et al. (1991) estimated an ILE requirement of

33.56 mg/kg body weight and 9.91 mg per g of EM. The average calculated SID digestibility of ILE in both the study of Huyghebaert et al. (1991) and Mannion et al. (1991) was 84%. Converting from total to SID level his means that the relationships observed by Huyghebaert et al. and Mannion et al. are as follows: for Huyghebaert et al.; 37.4 mg/kg body weight and 7.96 mg per g of EM and for Mannion et al.; 28.2 mg/kg body weight and 8.32 mg per g of EM. In 8 of the 9 titration trials in the present study Lohman Brown laying hens were used that produced an average maximum EM of 55.6 g/d. Assuming a body weight of 1.9 kg this would mean SID-ILE requirements of 9.2 and 9.3 mg per g EM according to the studies of Huyghebaert and Mannion, respectively.

In Table 3 the estimated optimal SID-ILE requirements for maximum EM is given expressed in mg/d and as a percentage of the diet for maximum EM at various egg production rates based on SID-ILE requirements of 10.3 mg per g of EM produced.

Table 3. Estimated optimal SID-ILE requirements for maximum EM expressed in mg/d and as a percentage of the diet for maximum EM at various egg production rates based on SID-ILE requirements of 10.3 mg per gram of EM produced. The calculated feed intake required for an average egg weight of 60 g and at egg production rates of 90 and 95% are based on the assumptions presented as a footnote (*) underneath this Table.

	Feed intake (g/d)		Egg mass (g/d)		SID-ILE (mg/d)		Dietary SID-ILE (%)		SID-ILE:SID-LYS ratio**	
	Egg production rate (%)									
BW (kg)	90	95	90	95	90	95	90	95	90	95
1.5	112	115	54	57	556	587	0.498	0.512	76	74
1.6	114	117	54	57	556	587	0.486	0.500	76	74
1.7	117	120	54	57	556	587	0.475	0.489	76	74
1.8	120	123	54	57	556	587	0.465	0.478	76	74
1.9	122	125	54	57	556	587	0.455	0.468	76	74
2.0	125	128	54	57	556	587	0.445	0.459	76	74

*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.

**The optimal SID-ILE:SID-LYS ratio for maximum EM is calculated based on the ratio between SID-ILE intake (SID-ILE requirements calculated as 10.3 mg per gram of EM produced) and SID-LYS intake which is based on formula [F8] described in CVB Documentation report nr. 69.

Table 1. Summary of the total dataset

Study	Trial	Breed	Starting Age (weeks)	Duration of experiment (weeks)	Dietary CP (%)	Max obs. rate of lay (%)	Max obs. egg mass	Max obs. feed intake	Min SID-ILE (%)	Max SID-ILE (%)	Max. FCR minus Min. FCR	Max. egg mass minus Min. egg mass
Peganova and Eder (2002)	1	Lohman Brown	25	8	11.8	95	54	112	0.328	0.748	0.140	9.4
	2	Lohman Brown	24	9	13.2	98	58	117	0.333	1.013	0.190	9.6
	3	Lohman Brown	46	9	13.2	91	57	115	0.333	1.013	0.170	9.6
Peganova and Eder (2003)	4	Lohman Brown	25	3	11.4	94	52	115	0.486	1.066	0.230	11.9
	5	Lohman Brown	25	3	11.4	94	54	119	0.486	1.066	0.130	3.9
	6	Lohman Brown	25	3	11.4	98	56	130	0.486	1.066	0.230	16.6
	7	Lohman Brown	25	3	11.4	97	57	130	0.486	1.066	0.370	5.3
Shivazad et al. (2002)*	8	Hy-line W36	35	8	10-14	89	50	91	0.373	0.540	0.300	16.2
Carvalho Mello et al. (2012)	9	Hy-Line W36	42	16	15.3	83	53	94	0.556	0.753	0.052	1.5
Mannion et al. (1993)*	10	Hy-Line	42	4	11-28	85	52	121	0.253	0.745	2.078	30.9
	11	Tegel	42	4	11-28	86	50	116	0.253	0.745	2.427	32.5
	12	Hy-Line	66	4	11-28	75	45	111	0.238	0.728	2.236	29.4
	13	Tegel	66	4	11-28	71	42	105	0.238	0.728	2.759	28.4
Dong et al. (2016)	14	Lohman Brown	28	12	14.5	94	58	113	0.515	0.915	0.102	1.4
Huyghebaert et al. (1991)*	15	ISA Brown	32	4	11-20	90	57	125	0.290	0.594	1.081	20.1
	16	ISA Brown	52	4	11-20	82	55	130	0.290	0.594	1.087	18.2

*Studies with an asterix are dilution studies meaning that AA other than ILE changed as well alongside ILE.

Results from Table 3 indicate a SID-ILE:SID-LYS requirement ratio of 74%.

The SID-ILE requirement for dietary SID-ILE for EM production was also estimated directly on the pooled data from Mannion et al. and from Huyghebaert et al.. In this case we excluded the highest SID-ILE observations that did not result in meaningful increases in EM (i.e. less than 0.025 g EM per mg of SID-ILE supply) (Fig. 2). Including also body weight as a variable besides EM (not allowing for an intercept value) resulted in an estimate for body weight that was not significant and therefore was not included in the model.

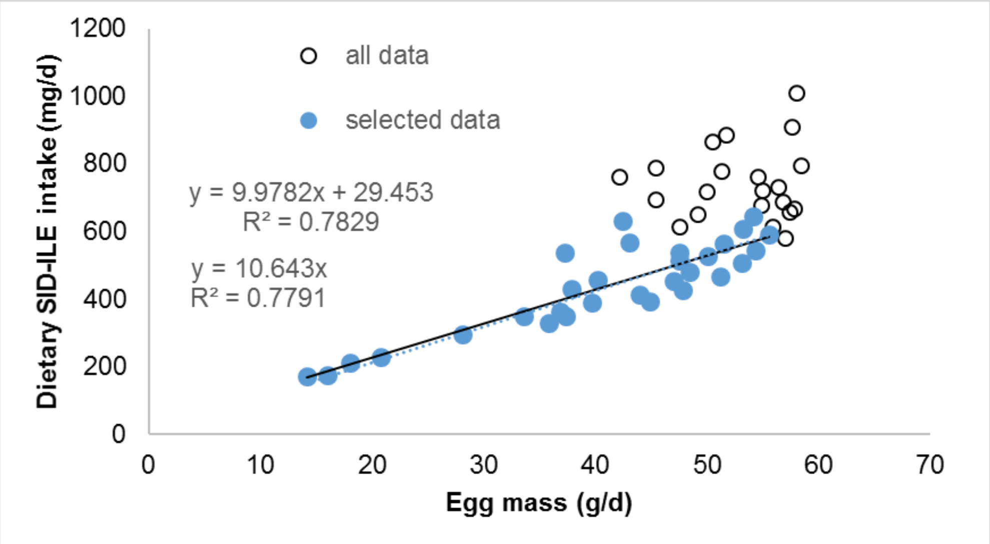

Figure 1. Relationship between egg mass produced (mg/d) and dietary SID-ILE for the pooled dataset of Huyghebaert et al. (1991) and Mannion et al. (1993). Regression equation is based on the selected data of the pooled (excluding the highest SID-ILE observations that did not result in meaningful increases in EM (i.e. less than 0.025 g increase in egg mass produced per mg of SID-ILE supply)).

Just as in Figure 1, the requirement for dietary SID-ILE for EM production was directly estimated on the pooled data from Mannion et al. and from Huyghebaert et al. but then results from trials 12 and 13 from the study of Mannion et al. were excluded due to the low maximum egg production rates for these two trials. Furthermore, just as was done in Figure 1, the highest SID-ILE observations were excluded that did not result in meaningful increases in EM (i.e. less than 0.025 g EM per mg of SID-ILE supply) (Fig. 2).

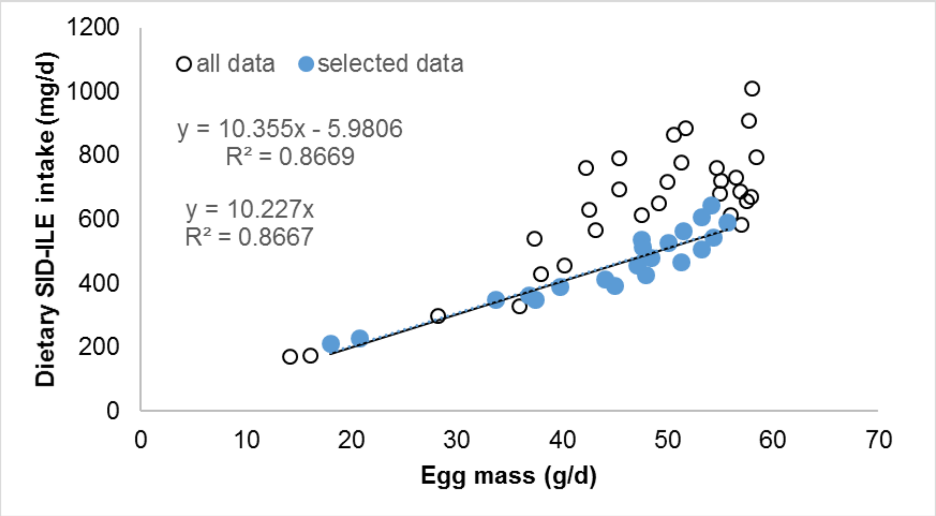

Figure 2. Relationship between egg mass produced (mg/d) and dietary SID-ILE for the pooled dataset of Huyghebaert et al. (1991) and Mannion et al. (1993) but excluding results from trials 12 and 13 from the study of Mannion et al. due to the low maximum egg production rates for these two trials. Regression equation is based on the selected data of the pooled (excluding the highest SID-ILE observations that did not result in meaningful increases in EM (i.e. less than 0.025 g increase in egg mass produced per mg of SID-ILE supply)).

The regression formula shown in Figure 2 shows a dietary SID-ILE requirement of 10.2 mg per g of EM produced. This estimate is very similar to the observed average optimal SID-ILE level of 10.3 mg per g of EM produced.

In Table 4 the estimated optimal SID-ILE requirements for maximum EM expressed in mg/d and as a percentage of the diet for maximum EM at various egg production rates are given based on the average model estimated SID-ILE requirement of 11.1 mg per gram of EM produced as shown in Table 2.

Table 4. Estimated optimal SID-ILE requirements for maximum EM expressed in mg/d and as a percentage of the diet for maximum EM at various egg production rates based on the average model estimated SID-ILE requirement of 11.1 mg per gram of EM produced as shown in Table 2. The calculated feed intake required for an average egg weight of 60 g and at egg production rates of 90 and 95% are based on the assumptions presented as a footnote (*) underneath this Table.

	Feed intake (g/d)		Egg mass (g/d)		SID-ILE (mg/d)		Dietary SID-ILE (%)		SID-ILE:SID-LYS ratio**	
	Egg production rate (%)									
BW (kg)	90	95	90	95	90	95	90	95	90	95
1.5	112	115	54	57	599	633	0.537	0.551	82	**80**
1.6	114	117	54	57	599	633	0.524	0.539	82	**80**
1.7	117	120	54	57	599	633	0.512	0.526	82	**80**
1.8	120	123	54	57	599	633	0.501	0.515	82	**80**
1.9	122	125	54	57	599	633	0.490	0.504	82	**80**
2.0	125	128	54	57	599	633	0.480	0.494	82	**80**

*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.

**The optimal SID-ILE:SID-LYS ratio for maximum EM is calculated based on the ratio between SID-ILE intake (SID-ILE requirements calculated as 11.1 mg per gram of EM produced) and SID-LYS intake which is based on formula [F8] described in CVB documentation report nr. 69.

It is not easy to select a SID-ILE:SID-LYS requirement ratio. Based on average data from titration and dilution studies optimal SID-ILE:SID-LYS ratios may be 91% (Table 2), 11.1 – 12.3 mg SID-ILE per gram of egg mass (Table 2) resulting in SID-ILE:SID-LYS ratios of 80 – to 89% or around 10.3 mg SID-ILE per gram of EM when choosing the approach shown in Figure 2 or when using the observed average optimal SID-ILE requirement per g of EM resulting in a SID-ILE:SID-LYS requirement ratio of 74%. The high ratio of 91% reported in Table 2 can be explained by the fact that low SID-LYS levels were used in the titration trials (on average 0.578 % resulting in a high efficiency in which dietary lysine was converted into EM of 12.2 mg SID-LYS per g of EM). For example, when using the relation F8 described in CVB Documentation report nr. 69 an average SID-LYS of 13.4 mg per g of egg mass would be expected. Using the SID-LYS requirement as predicted by formula F8 in CVB Documentation report nr. 69 the average SID-ILE:SID-LYS ratio of the titration experiments would become 84%,

In Figure 3 the relationship between dietary SID-ILE intake (mg/d) and egg mass production (g/d/hen) for the various trials are presented. The vertical lines represent the SID-ILE intake required for an EM production of 57 g and assuming SID-ILE:SID-LYS requirement ratios of 91%, 80% and 74%.

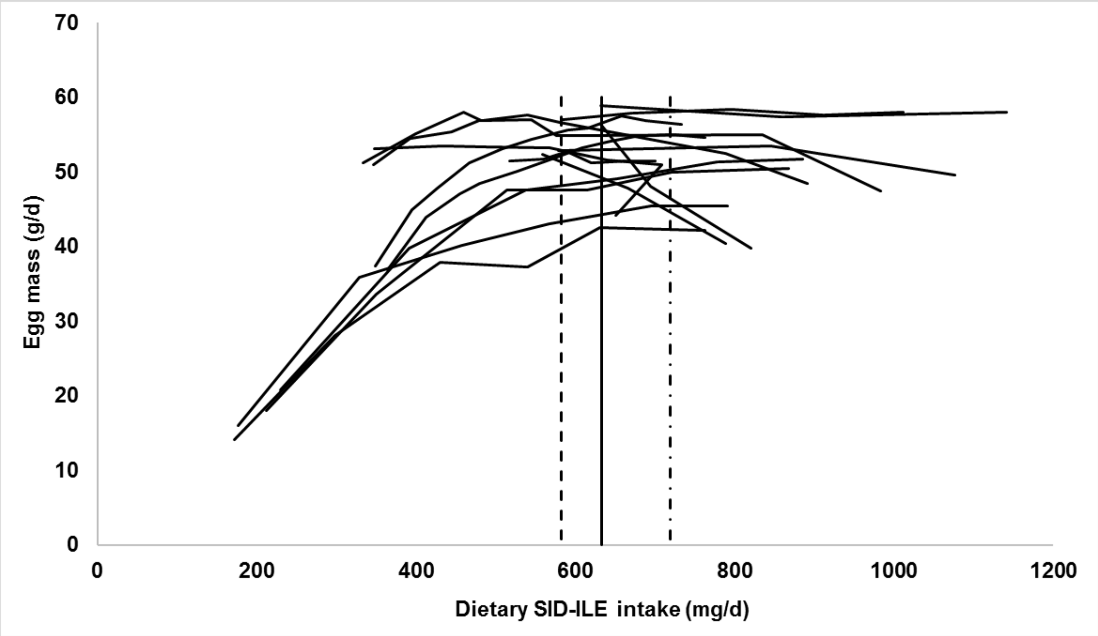

Figure 3. Relationship between dietary SID-ILE intake (mg/d) and egg mass production (g/d/hen). Based on average data from titration and dilution studies optimal SID-ILE:SID-LYS ratios may be 91% (vertical dash dotted line), 11.1 mg SID-ILE per gram of egg mass resulting in a SID-ILE:SID-LYS ratio of 80% (vertical solid line) or around 10.3 mg SID-ILE per gram of egg mass when choosing the approach shown in Figure 2 resulting in a SID-ILE:SID-LYS requirement ratio of 74% (dashed line).

4 Conclusions

It is concluded that it is most prudent to estimate requirement estimates of dietary SID-ILE on the titration studies and not on the dilution studies as the dietary protein levels in the dilution studies were much higher than observed in practice. These high dietary protein levels used in the dilution studies might affect the requirement estimates for SID-ILE. It is furthermore concluded that it seems not wise to use the estimated SID-ILE : SID-LYS requirement ratios that are based on the titration studies due to the low dietary SID-LYS concentrations used in the test diets resulting in high efficiencies in which dietary lysine was converted into EM. Therefore it is concluded that it is best to base the dietary SID-ILE requirement for laying hens on a SID-ILE requirement ratio of 11.1 mg per g of EM. This requirement is the average estimated SID-ILE requirement ratio for maximum EM per g of egg mass based on the titration studies as presented in Table 2. This requirement results in a SID-ILE:SID-LYS requirement ratio of 80% for a hen producing 57 g of EM.

List of studies included in the meta-analysis

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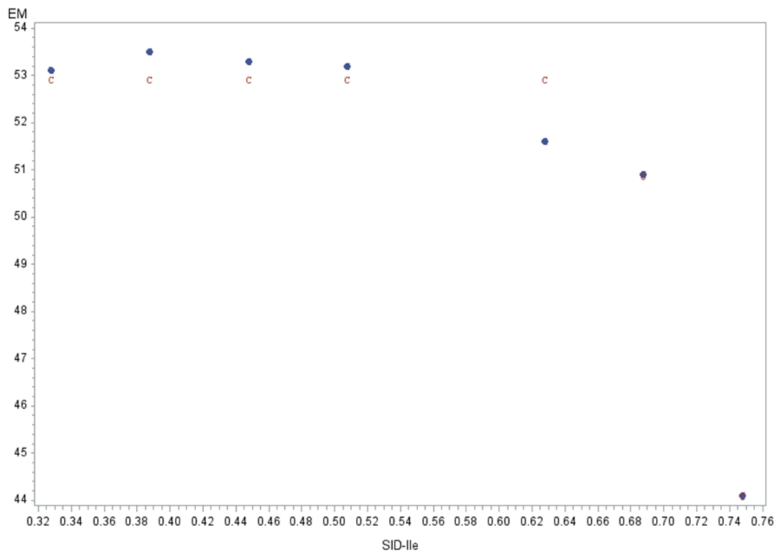
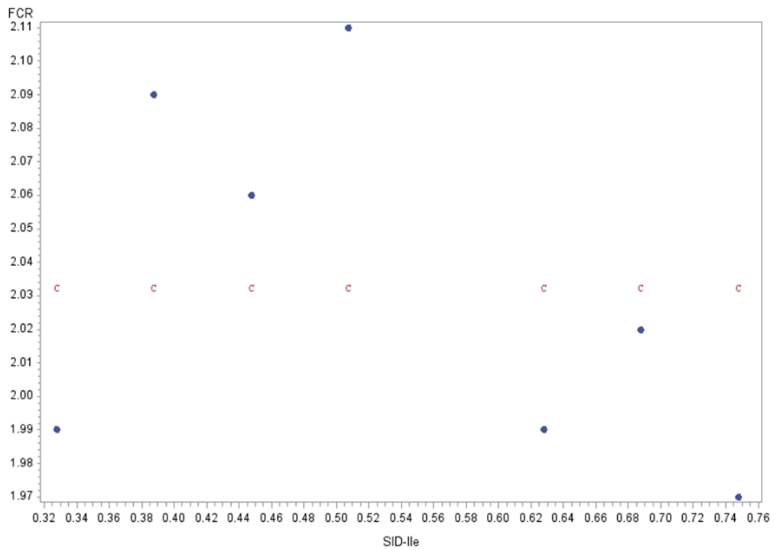
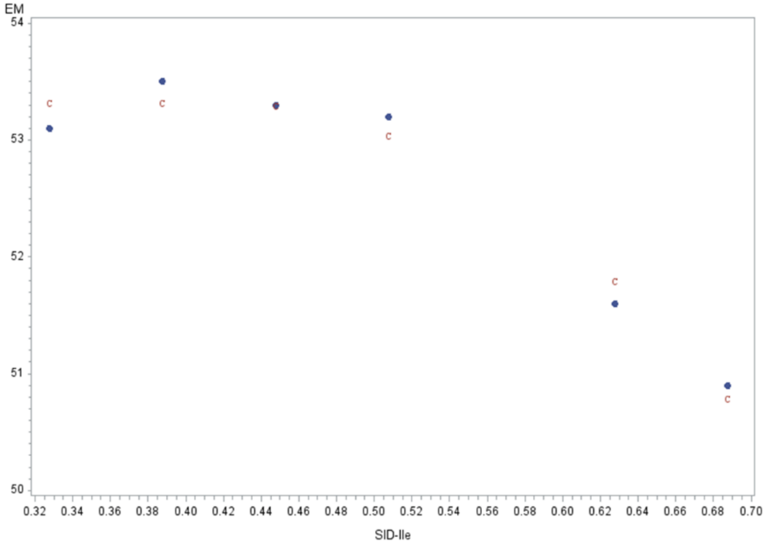
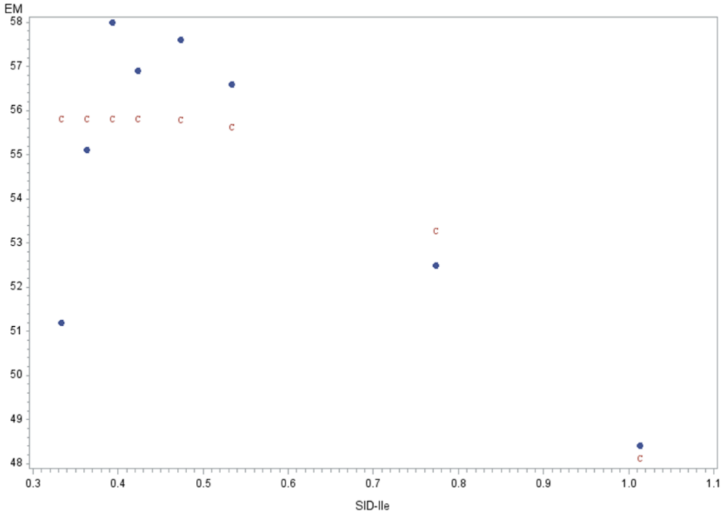
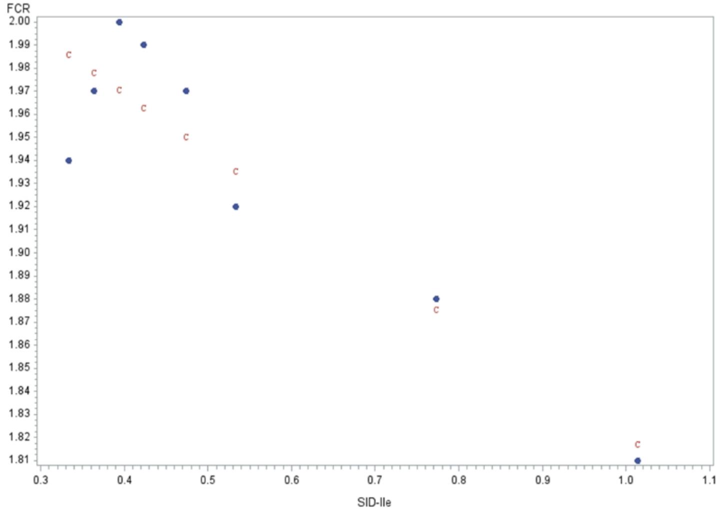
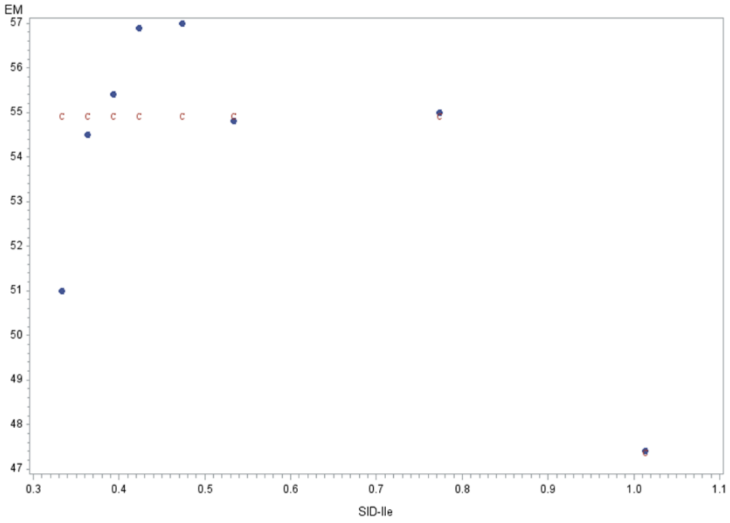
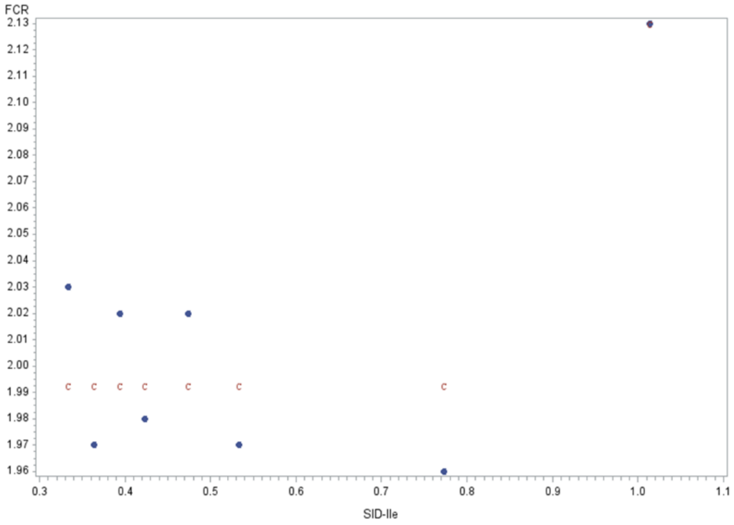
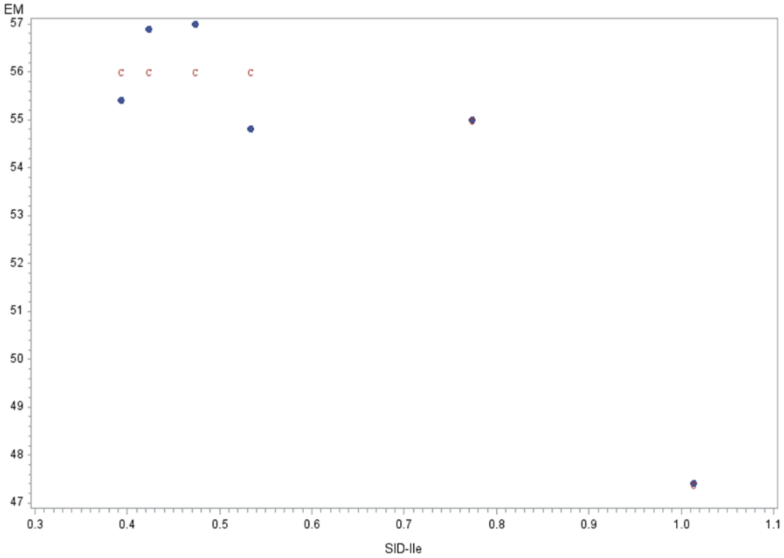
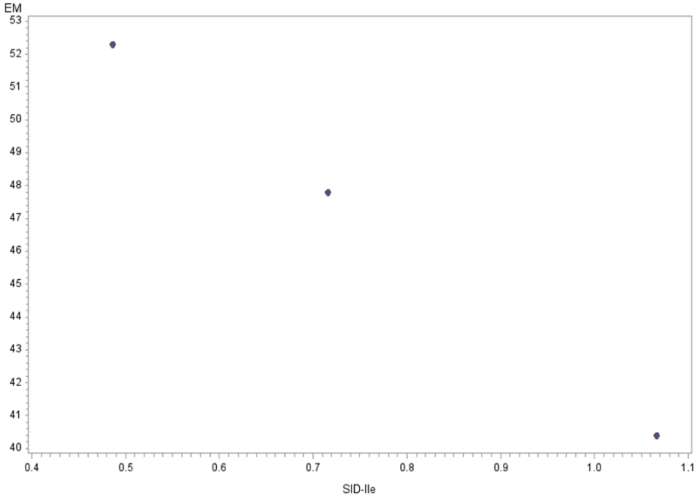
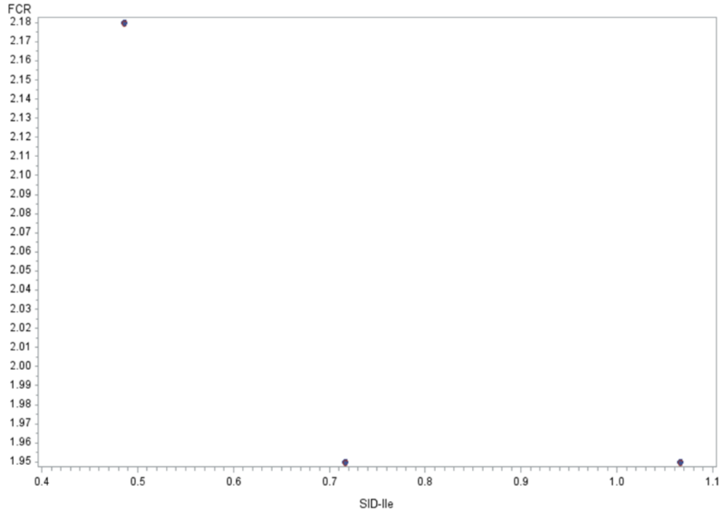
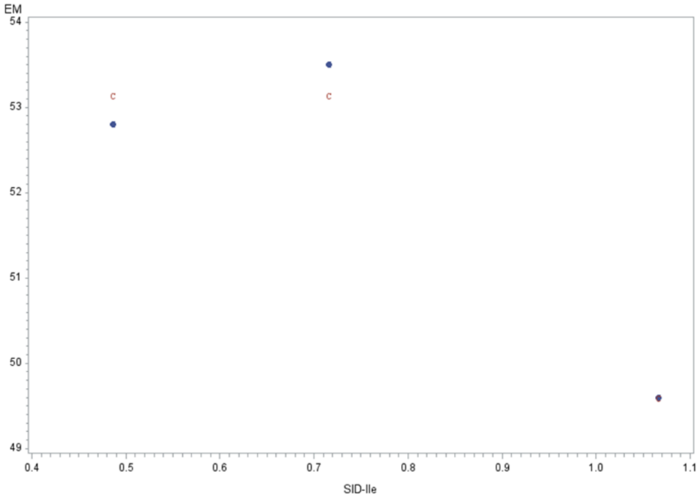
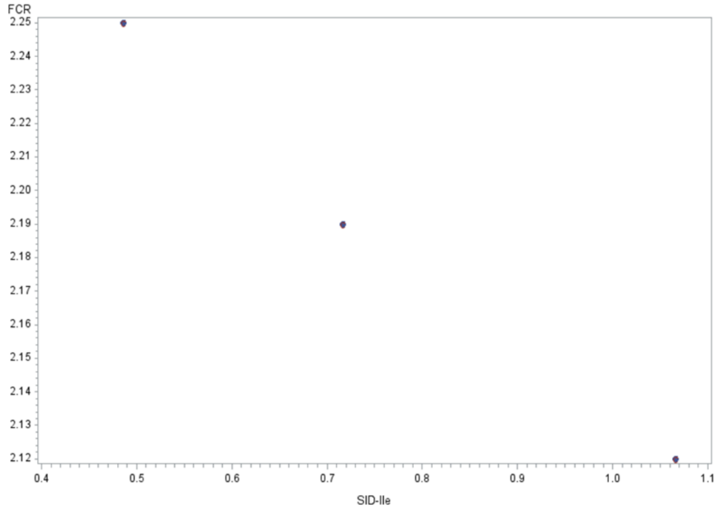
Appendix A. Relationship between dietary SID-ILE supply and performance parameters FCR and EM for the various titration trials including the estimated SID-ILE requirements based on the quadratic broken-line model

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

Study	Relationship between SID-ILE (%) and EM (g/hen/day)	Relationship between SID-ILE (%) and FCR (g feed/g EM)
1. Peganova and Eder (2002) Trial 1 SID-ILE req. EM. (%) 0.632 SID-ILE req. FCR. (%) Could not be estimated		

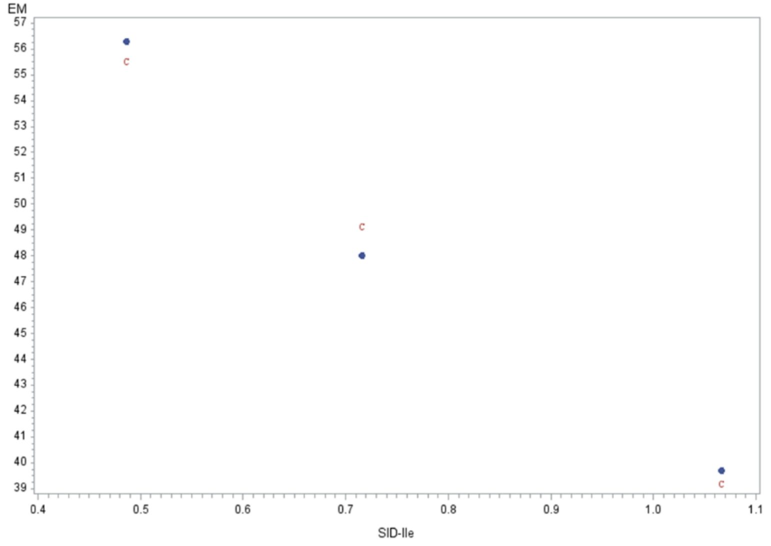
1a. Peganova and Eder (2002) Trial 1 SID-ILE req. EM. (%) 0.417 SID-ILE req. FCR. (%)		
2. Peganova and Eder (2002) Trial 2 SID-ILE req. EM. (%) 0.448 SID-ILE req. FCR. (%) Extrapolated value		

3. Peganova and Eder (2002) Trial 3 SID-ILE req. EM. (%) 0.774 SID-ILE req. FCR. (%) Could not be estimated		
3a. Peganova and Eder (2002) Trial 3 SID-ILE req. EM. (%) 0.647 SID-ILE req. FCR. (%)		

4. Peganova and Eder (2003) Trial 1 SID-ILE req. EM. (%) Extrapolated value SID-ILE req. FCR. (%) 0.650		
5. Peganova and Eder (2003) Trial 2 SID-ILE req. EM. (%) 0.722 SID-ILE req. FCR (%) Extrapolated value		

<table>
<tr>
<td>6.
Peganova and Eder (2003)
Trial 3

SID-ILE req. EM. (%)
Extrapolated value

SID-ILE req. FCR (%)
0.600</td>
<td>
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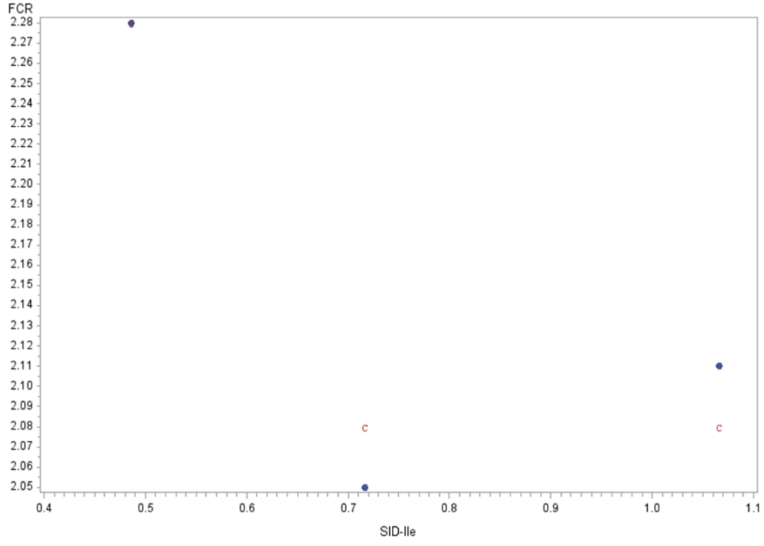
<td>
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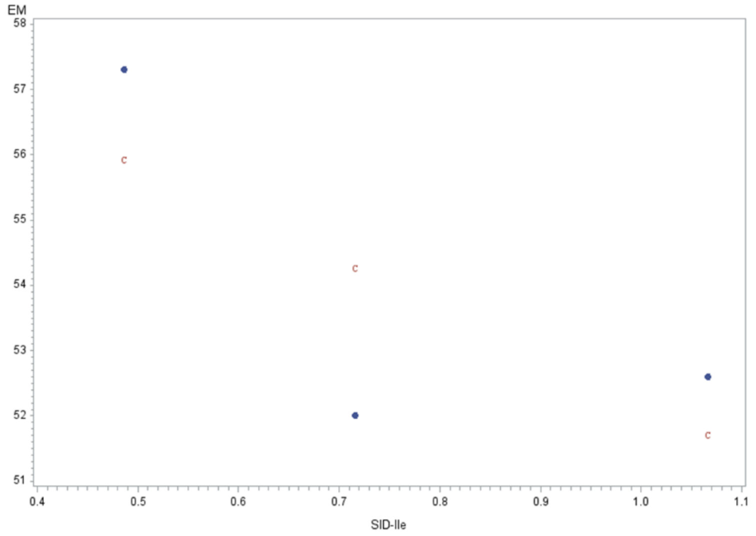
Peganova and Eder (2003)
Trial 4

SID-ILE req. EM. (%)
Extrapolated value

SID-ILE req. FCR (%)
0.600</td>
<td>
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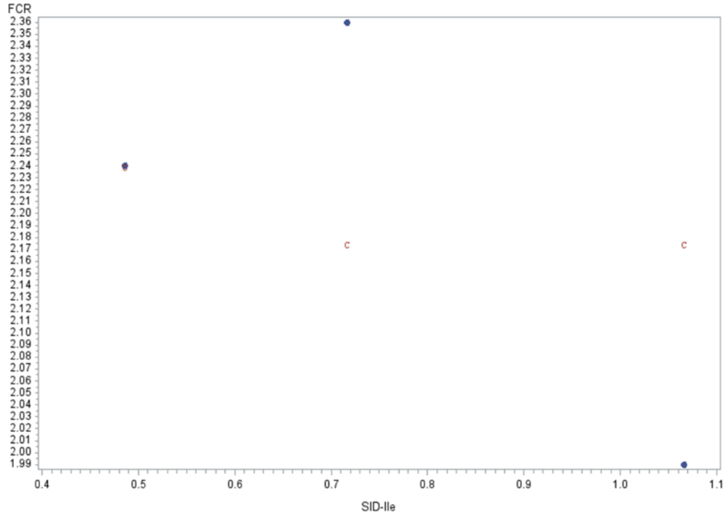
<td>
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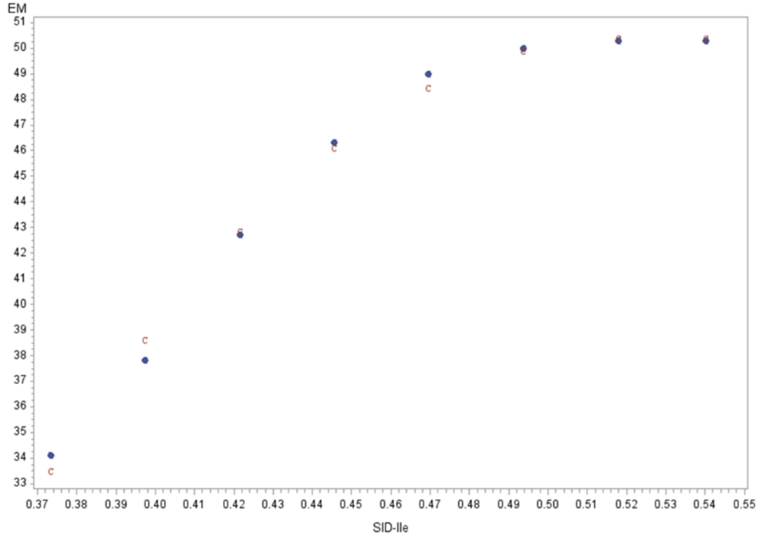
<td>8.
Shivazad et al. (2002)

SID-ILE req. EM. (%) 0.519

SID-ILE req. FCR (%) 0.561</td>
<td>
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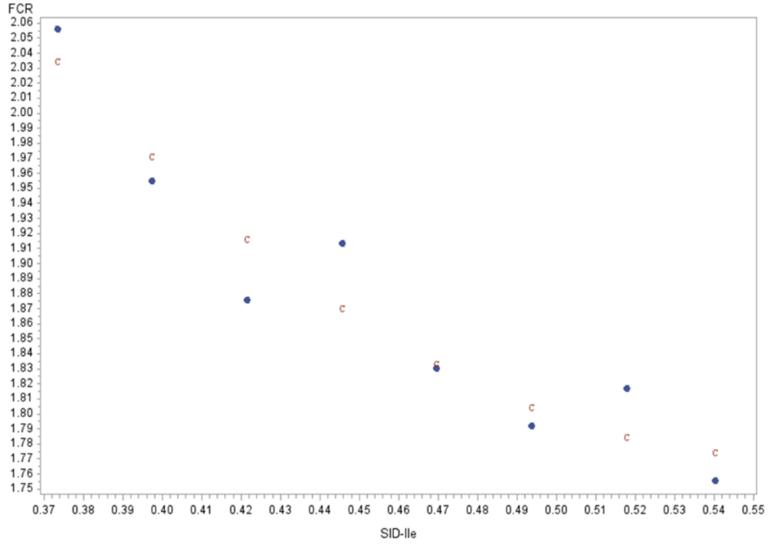
<td>
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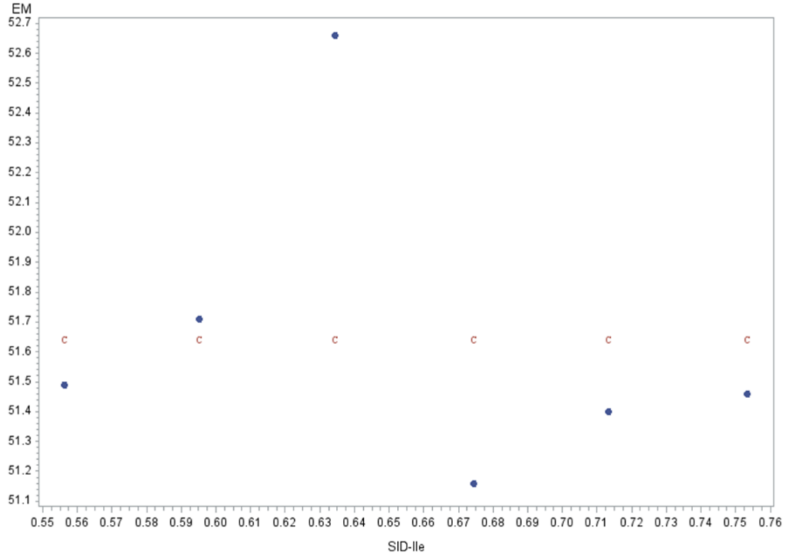
Carvalho Mello et al. (2012)

SID-ILE req. EM. (%) Could not be estimated

SID-ILE req. FCR (%) Could not be estimated</td>
<td>
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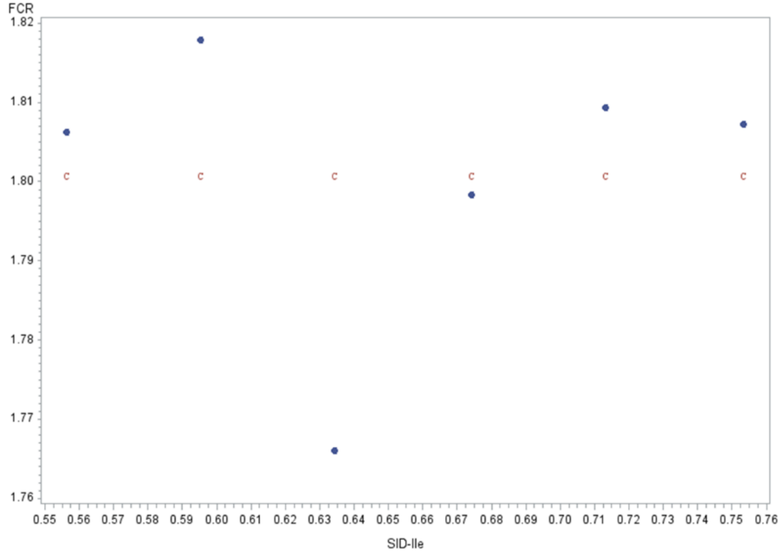
<td>
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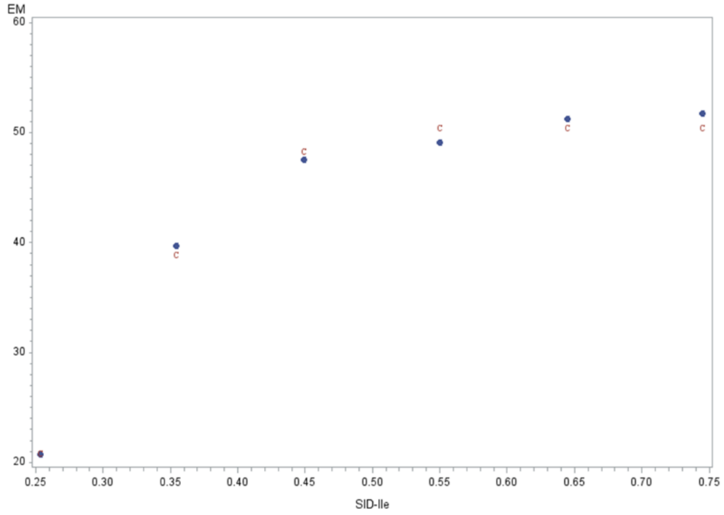
Mannion et al. (1993)
Trial 1

SID-ILE req. EM. (%) 0.522

SID-ILE req. FCR (%) 0.434</td>
<td>
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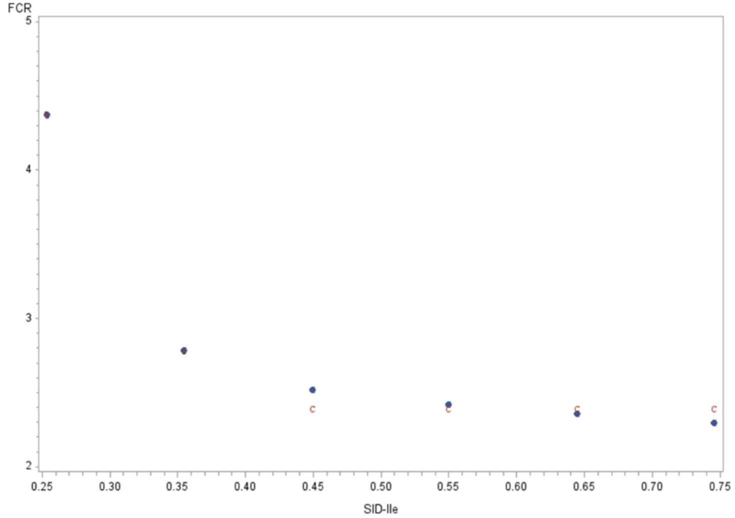
<td>
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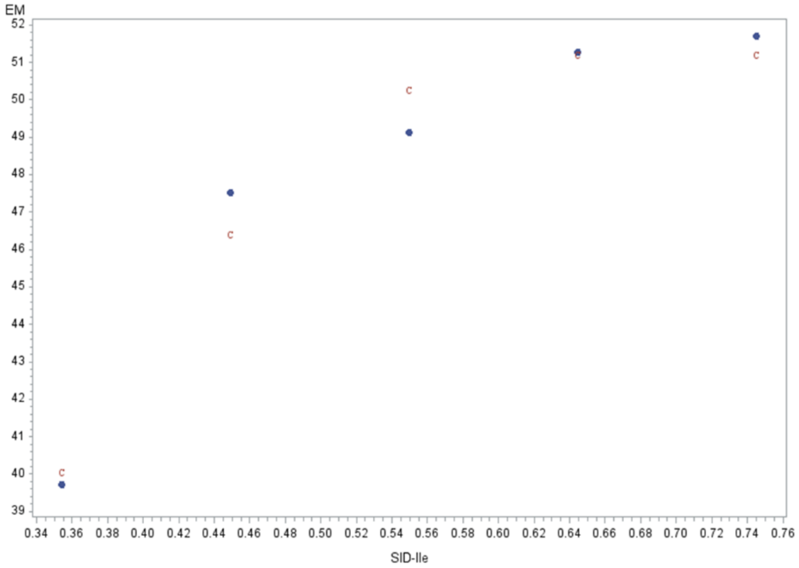
<td>10a.
Mannion et al. (1993)
Trial 1

SID-ILE req. EM. (%) 0.630

SID-ILE req. FCR (%) 0.698</td>
<td>
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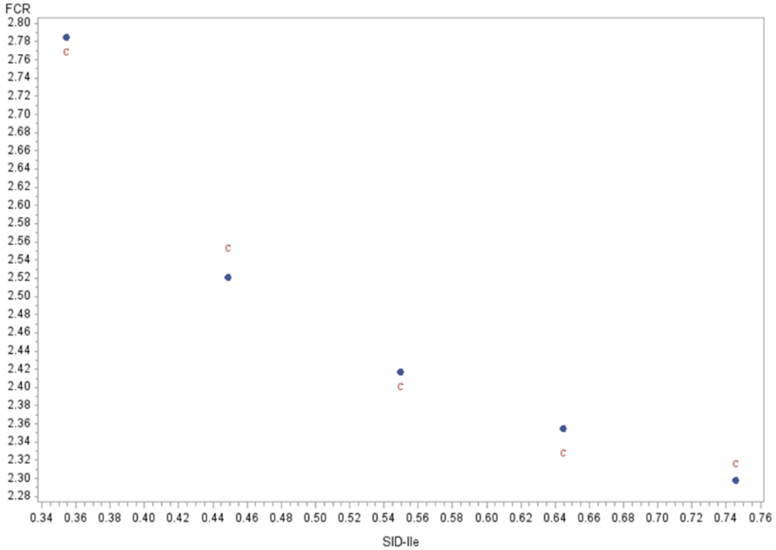
<td>
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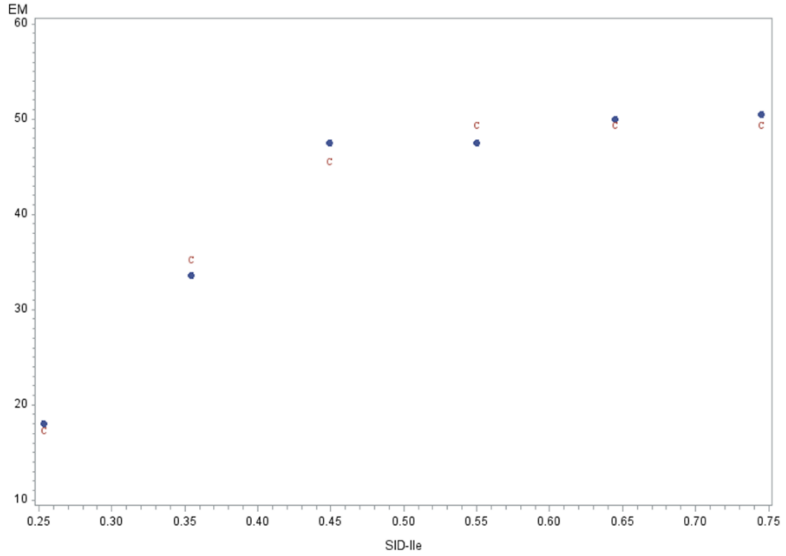
<td>11.
Mannion et al.
(1993)
Trial 2

SID-ILE req.
EM. (%) 0.522

SID-ILE req.
FCR (%)
0.465</td>
<td>
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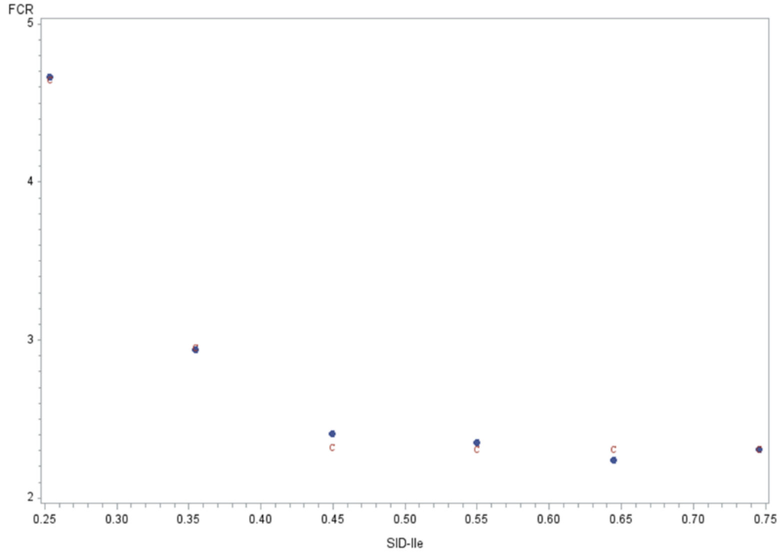
<td>
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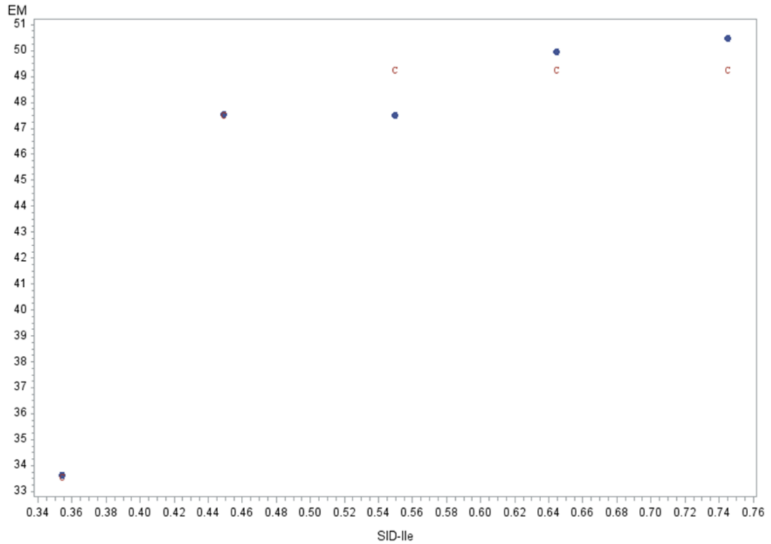
<td>11a.
Mannion et al.
(1993)
Trial 2

SID-ILE req.
EM. (%) 0.497

SID-ILE req.
FCR (%)
0.516</td>
<td>
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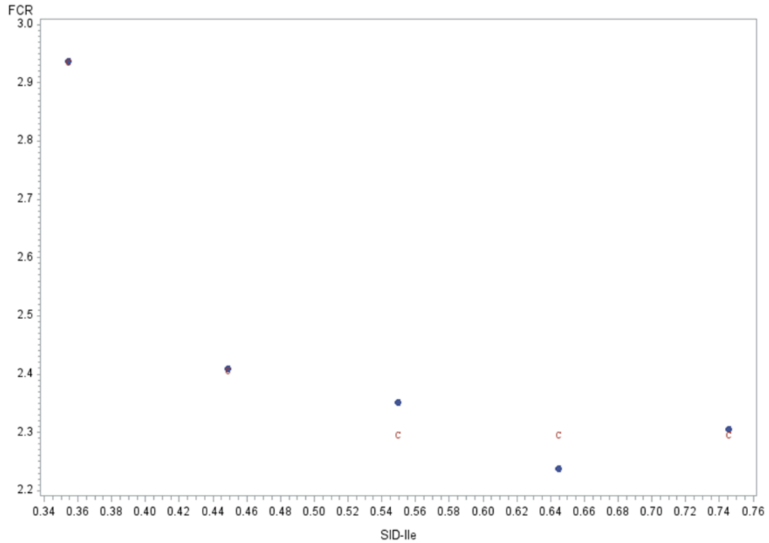
<td>
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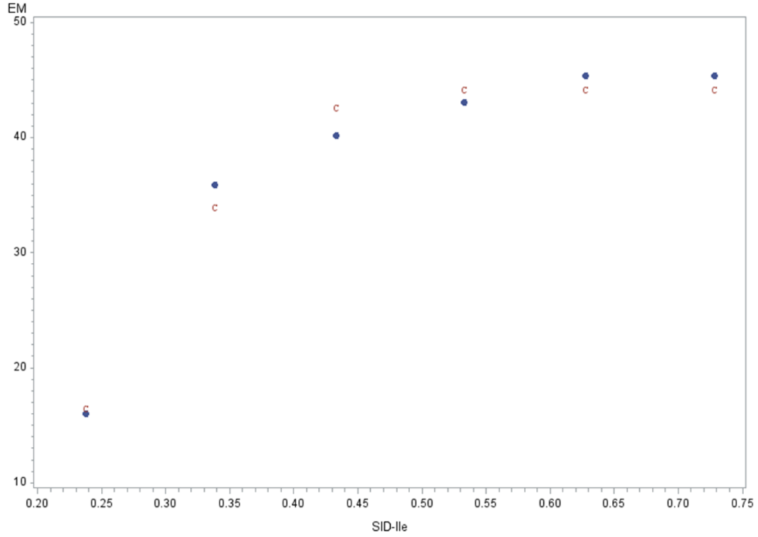
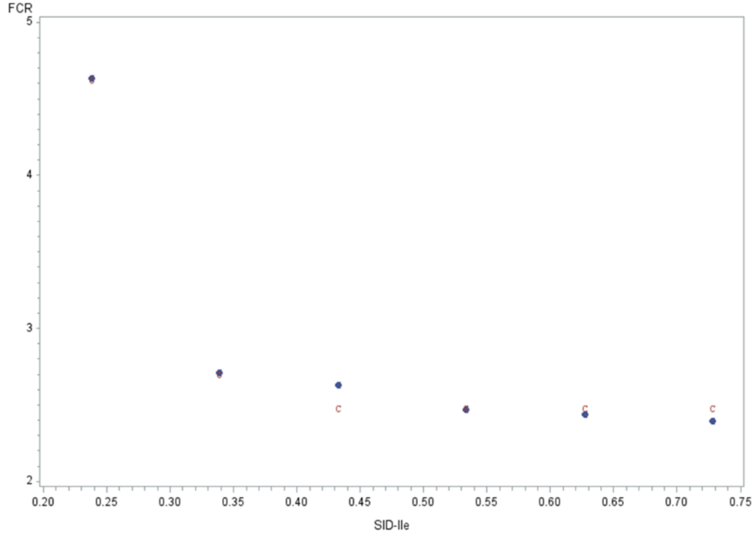
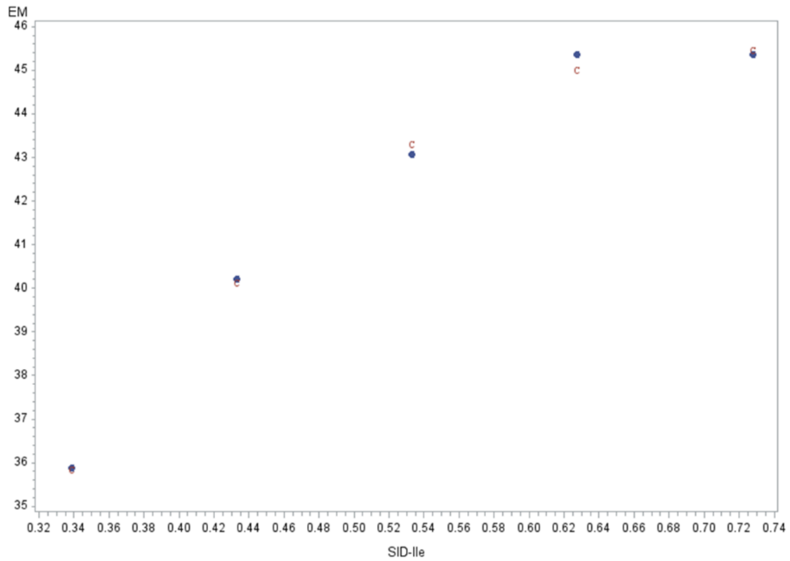
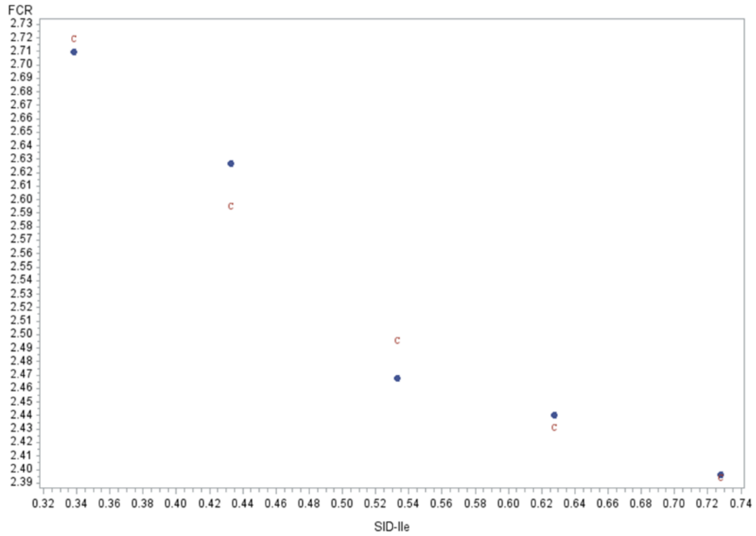
12. Mannion et al. (1993) Trial 3 SID-ILE req. EM. (%) 0.494 SID-ILE req. FCR (%) 0.387		
12a. Mannion et al. (1993) Trial 3 SID-ILE req. EM. (%) 0.708 SID-ILE req. FCR (%) 0.791		

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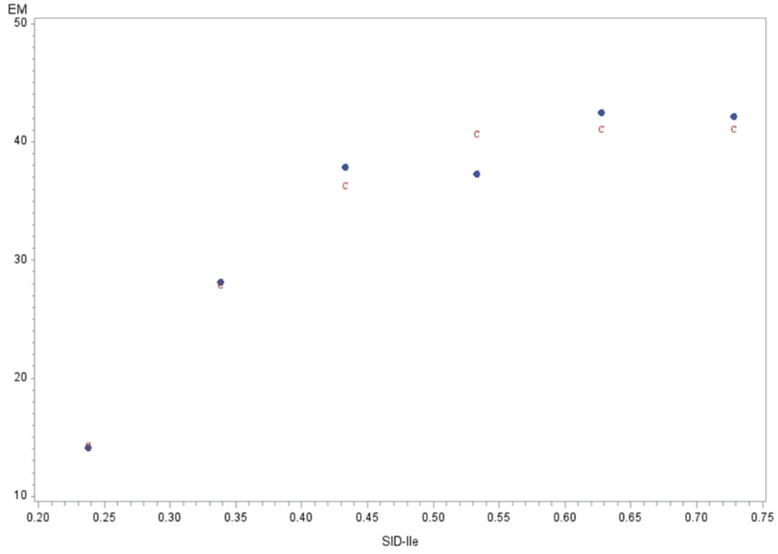
<td>13.
Mannion et al. (1993)
Trial 3

SID-ILE req. EM. (%) 0.576

SID-ILE req. FCR (%) 0.430</td>
<td>
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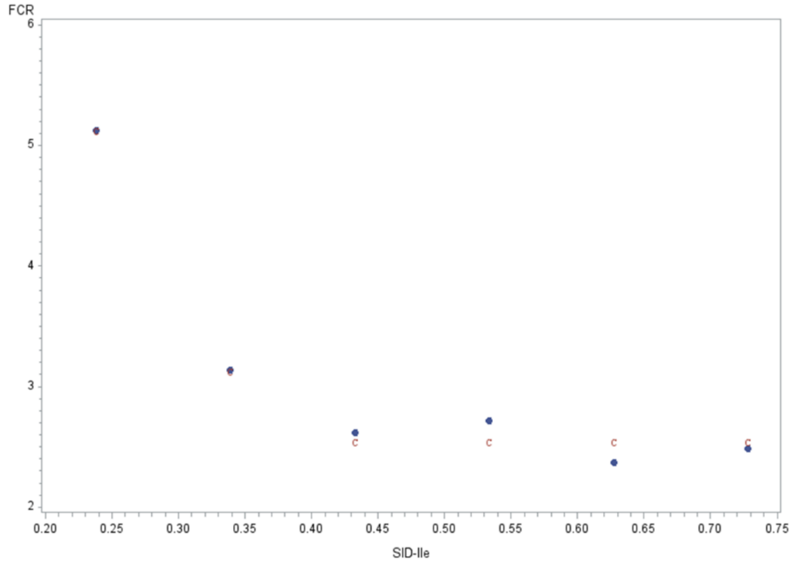
<td>
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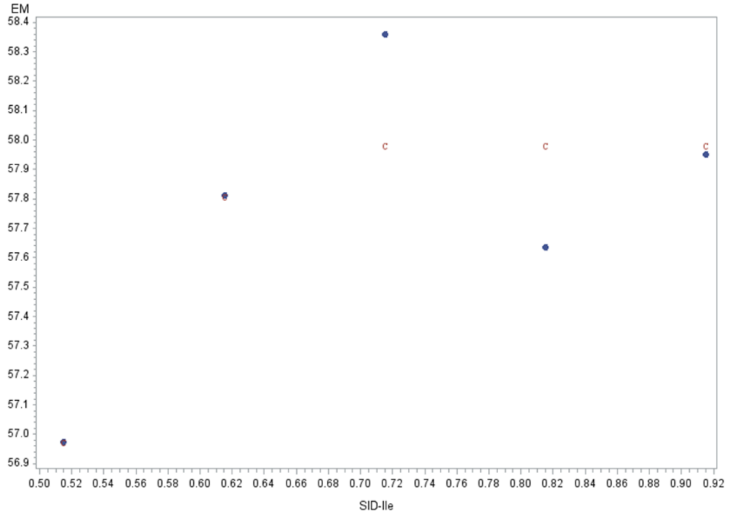
<td>14.
Dong et al. (2016)

SID-ILE req. EM. (%) 0.685

SID-ILE req. FCR (%) 0.610</td>
<td>
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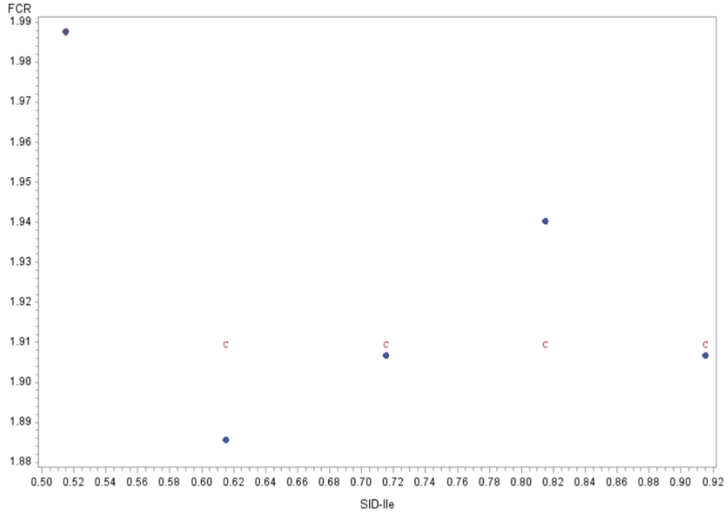
<td>
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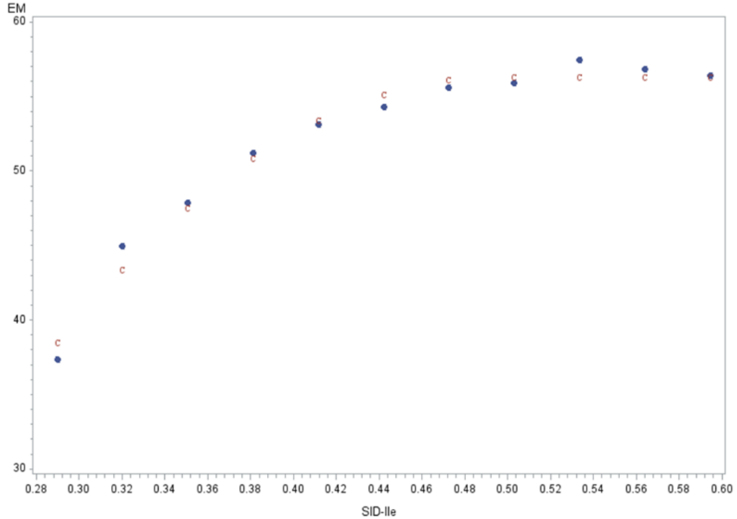
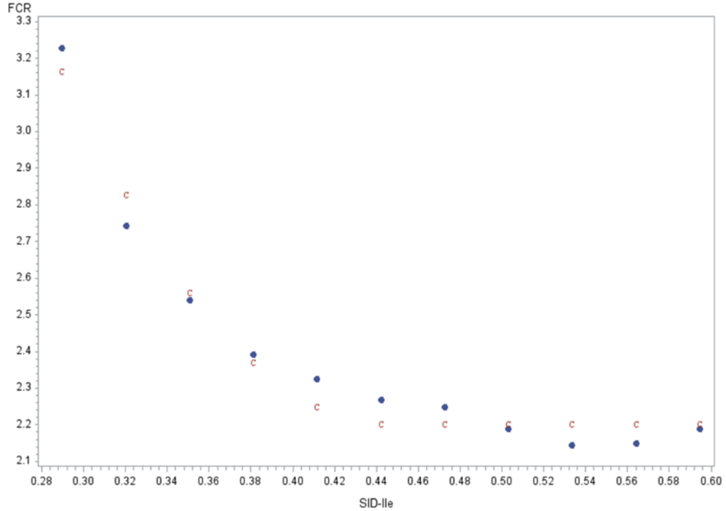
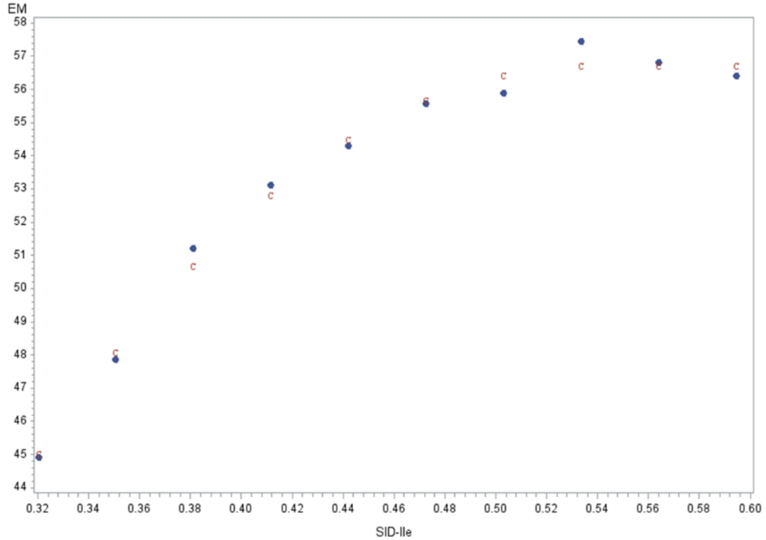
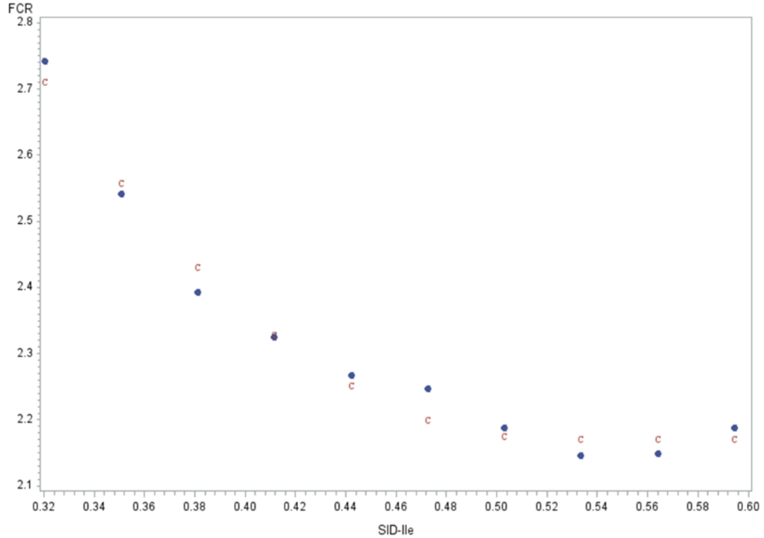
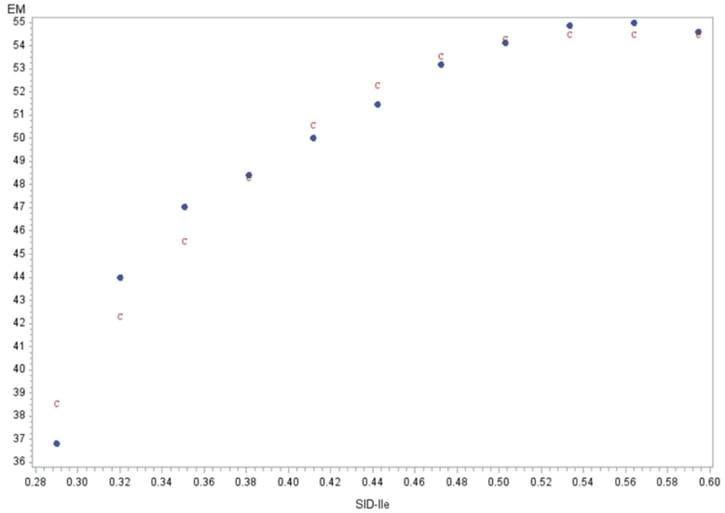
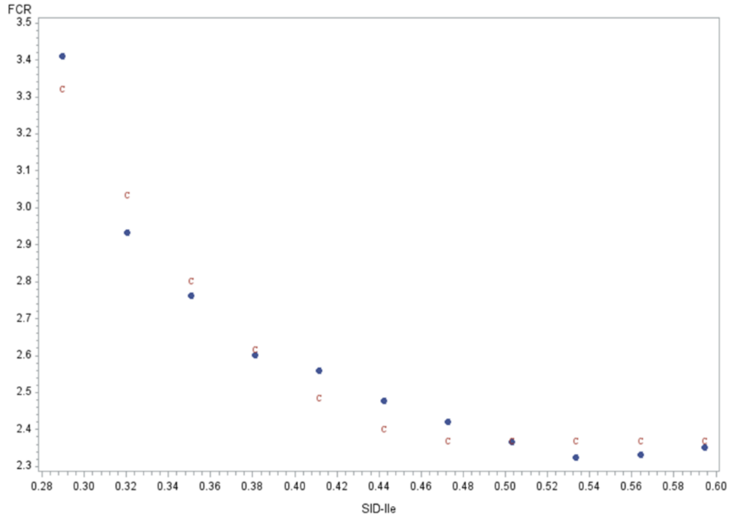
15. Huyghebaert et al. (1991) Trial 1 SID-ILE req. EM. (%) 0.495 SID-ILE req. FCR (%) 0.447		
15a. Huyghebaert et al. (1991) Trial 1 SID-ILE req. EM. (%) 0.536 SID-ILE req. FCR (%) 0.519		

16. Huyghebaert et al. (1991) Trial 2

SID-ILE req. EM. (%) 0.532

SID-ILE req. FCR (%) 0.477

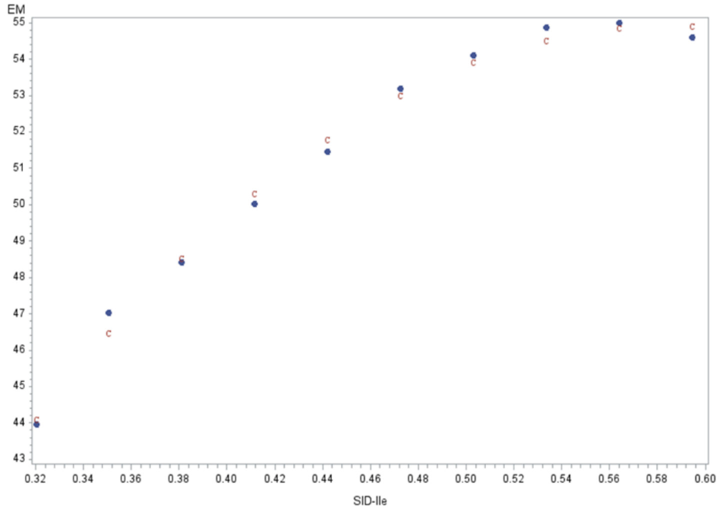


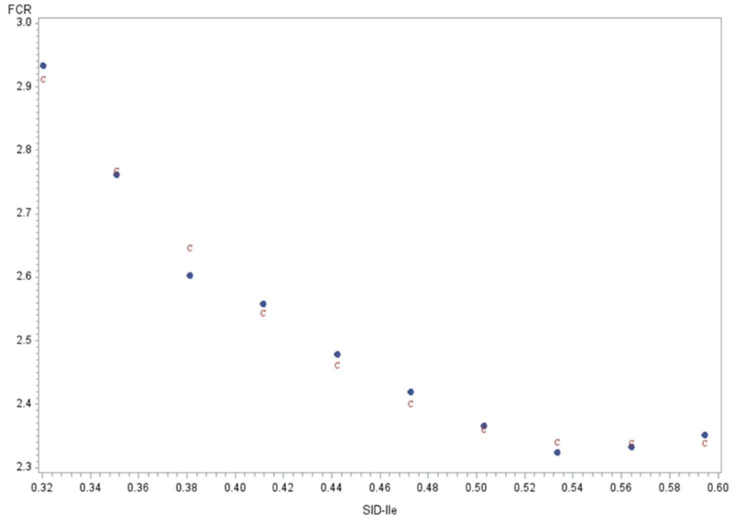


16a. Huyghebaert et al. (1991) Trial 2

SID-ILE req. EM. (%) 0.583

SID-ILE req. FCR (%) 0.548





Appendix B. SID-ILE model estimates for minimum FCR and maximum EM

SID-ILE model estimates for minimum FCR. Values of R that are bold are extrapolated estimated values. The letter ‘a’ behind the trial number (shown in the first column) means the model is fitted on all observations except the first observation with the lowest dietary SID-ILE level. 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							
1a							
2	0.57	31.565	**11.406**	254	0.0	0.3	0.844
3							
3a							
4	1.95	0.000	0.650	.	-8.6	0.0	1.000
5	2.06	.	**1.844**	.	-0.1	.	1.000
6	2.08	0.021	0.600	.	-15.4	2.8	
7	2.18	0.185	0.600	.	-5.0	24.8	
8	1.77	0.044	0.561	0.0653	-7.5	4.6	0.914
9							
10	2.40	0.048	0.434	0.0193	-60.6	13.4	0.991
10a	2.32	0.032	0.698	0.0634	-3.8	1.4	0.982
11	2.32	0.037	0.465	0.0139	-52.5	7.1	0.997
11a	2.30	0.033	0.516	0.0338	-24.3	10.2	0.979
12	2.48	0.050	0.387	0.0174	-97.4	22.9	0.992
12a	2.39	0.052	0.791	0.1440	-1.6	0.9	0.973
13	2.55	0.076	0.430	0.0249	-70.3	18.9	0.987
14	1.91	0.011	0.610	.	-8.6	2.8	0.760
15	2.20	0.025	0.447	0.0156	-39.2	8.6	0.972
15a	2.17	0.016	0.519	0.0181	-13.7	2.7	0.980
16	2.37	0.030	0.477	0.0204	-27.2	6.5	0.966
16a	2.34	0.014	0.548	0.0149	-11.1	1.5	0.990

SID-ILE model estimates for maximum EM. Values of R that are bold are extrapolated estimated values. The letter 'a' behind the trial number (shown in the first column) means the model is fitted on all observations except the first observation with the lowest dietary SID-ILE level. 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	53	0.3	0.632	0.0216	663	260	0.966
1a	53	0.1	0.417	0.0551	35	14	0.973
2	56	1.1	0.448	0.3964	24	35	0.614
3	55	0.8	0.774	.	132	37	0.674
3a	56	0.5	0.647	0.1133	64	40	0.943
4	85	.	**-2.995**	.	3	.	1.000
5	53	0.4	0.722	.	30	5	0.972
6	208	.	**-10.598**	.	1	.	0.984
7	119	.	**-17.100**	.	0	.	0.535
8	50	0.3	0.519	0.0079	801	89	0.995
9							
10	51	0.7	0.522	0.0237	410	75	0.993
10a	51	0.8	0.630	0.0632	147	68	
11	50	1.2	0.552	0.0365	359	92	0.985
11a	49	0.9	0.497	0.0365	772	393	0.974
12	44	1.2	0.494	0.0402	424	138	0.978
12a	45	0.3	0.708	0.0267	70	10	0.997
13	41	1.5	0.576	0.0603	253	86	0.971
14	58	0.2	0.685	0.1420	35	59	0.744
15	56	0.4	0.495	0.0156	425	70	0.983
15a	57	0.2	0.536	0.0130	250	32	0.991
16	55	0.6	0.532	0.0248	272	59	0.971
16a	55	0.3	0.583	0.0152	157	18	0.993