Standardized ileal digestible methionine and cysteine 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 methionine and cysteine was subsequently estimated. The results of this meta-analysis for standardized ileal digestible methionine and cysteine (SID-M+C) requirement and separately for the SID-methionine (SID-MET) requirement are presented in the present CVB Documentation report.
Compared to the former CVB apparent faecal digestible M+C recommendation for laying hens described in CVB Documentation report nr. 18 and published in 1996 the present established SID-M+C 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 requirements of SID-M+C and SID-MET 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
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 methionine and cysteine (SID-M+C) requirement 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-M+C 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

Methionine +cysteine requirement studies were selected from literature (1990 – 2017) in which only the dietary M+C content was varied by means of addition of graded levels of dietary synthetic MET. 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-M+C : AFD-LYS ratio needed to be at least 20% below the CVB (2012) AFD-M+C : AFD-LYS requirement level.

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

Via the PROC MIXED procedure of SAS estimated SID-M+C requirements for EM and FCR were regressed against factors such as EM, FCR, age, and the dietary factors CP, ME and CP : ME ratio with study effect included as a random factor.

3 Results and Discussion

3.1 SID-M+C requirements

In Table 1. Some characteristics of the studies included in the meta-analysis is given. The dataset consisted of 16 studies with in total 27 trials and 133 observations. In all titration experiments included in this study, DL-methionine was the MET source.

In Appendix A for each titration trial the relationship between dietary SID-M+C (%) and FCR and between dietary SID-M+C (%) and EM is presented graphically together with the estimated SID-M+C 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 2, 15 and 21) also model estimates are provided in case the basal treatment (or the treatment with the lowest SID-M+C content) was removed as it was expected that for these trials this would significant affect model estimates of R (or requirement estimates for SID-M+C).

In Table 2 the average estimated optimal SID-M+C concentrations and SID-M+C intake statistics are presented.

Table 2. Estimated optimal SID-M+C requirements (% and daily intake) for maximum egg mass (EM) and minimum FCR excluding these values in which estimated SID-M+C requirements values were outside the measurement range. (also including observations 2a, 15a and 21 a).

	Parameter	N*	Mean	Std. Dev.	Min.	Max	%CV
SID-M+C (%)	EM	19	0.597	0.1063	0.465	0.783	17.8
	FCR	24	0.652	0.1029	0.497	0.842	15.8
SID-M+C intake (mg/d)	EM	19	661	94.8	521	789	14.3
	FCR	24	692	64.3	576	807	9.3
SID-M+C intake per g of EM (mg/g)	EM	19	11.9	1.63	9.2	14.9	13.7
	FCR	24	12.5	1.22	10.3	14.0	9.8
SID-M+C:SID-LYS ratio	EM	19	84	15.7	61	115	18.6
	FCR	24	90	13.4	65	112	14.9
SID-M+C:SID-LYS ratio**	EM	19	87	12.4	66	116	14.2
	FCR	24	93	11.3	71	112	12.1

*number of titration trials (total number of titration trials is 30 (27 trials + 3 titration trials for which R values were estimated again after excluding the diet containing the lowest dietary SID-M+C level). Titration trials excluded for EM were 2, 2a, 3, 6, 10, 13, 14, 15a, 16, 19 and 20. Titration trials excluded for FCR were 1, 6, 10, 13, 15a and 19.
**This ratio was 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-M+C : SID-LYS ratio, otherwise the observed SID-LYS intake at which maximum EM was estimated was used for calculation of the SID-M+C : SID-LYS ratio.

Results in Table 2 show a wide range in optimal estimated SID-M+C concentrations and optimal SID-M+C intake levels. This wide range can be the result of various processes such as the quantity of EM (determined by egg production percentage and egg weight), the energy and protein and amino acid content of the feed, body weight changes of the animals during the measurement period, 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-M+C for maximum EM (Fig. 1) and also that the difference between minimum and maximum EM in an experiment did affect the estimated SID-M+C requirement for maximum EM (Fig. 2).
Similar relationships were observed between estimated SID-M+C requirements for minimum FCR and model estimated steepness of the curve (Fig.3) and between estimated SID-M+C requirements for minimum FCR and the differences between minimum and maximum observed FCR values in the titration trials (Fig. 4).

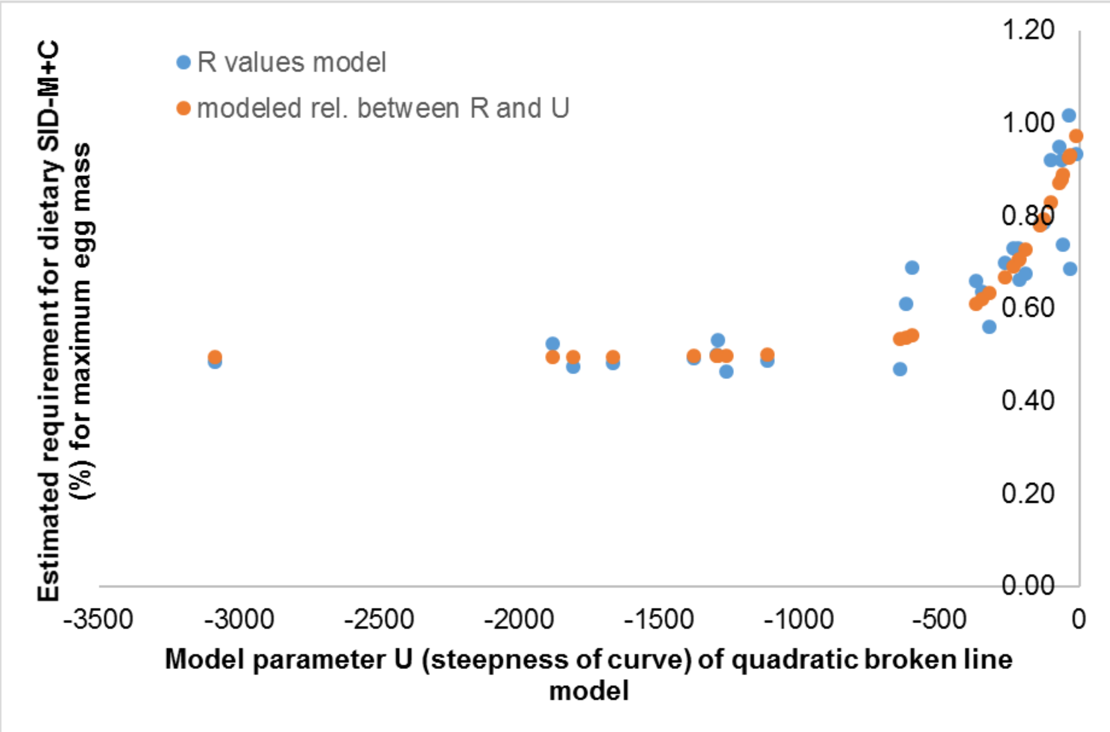

Figure 1. Relationship between the steepness of the increase in egg mass (g/d) per unit increase in dietary SID-M+C (%) and the estimated SID-M+C requirement (%) for maximum egg mass using the quadratic broken-line model. Model parameters: Estimated requirement for SID-M+C (%) = 0.495 + 0.498×EXP(0.00401 × U); R^2 = 0.800.

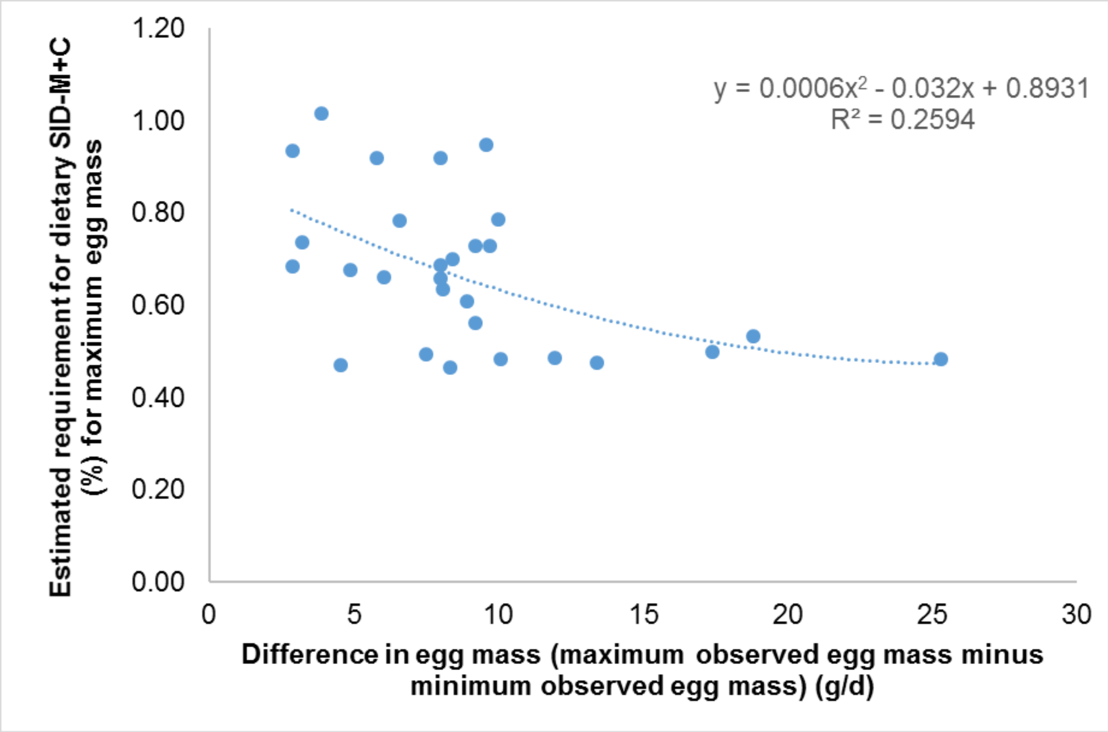

Figure 2. Relationship between the difference in maximum and minimum observed egg mass and the estimated SID-M+C requirement for maximum egg mass using the quadratic broken-line model.

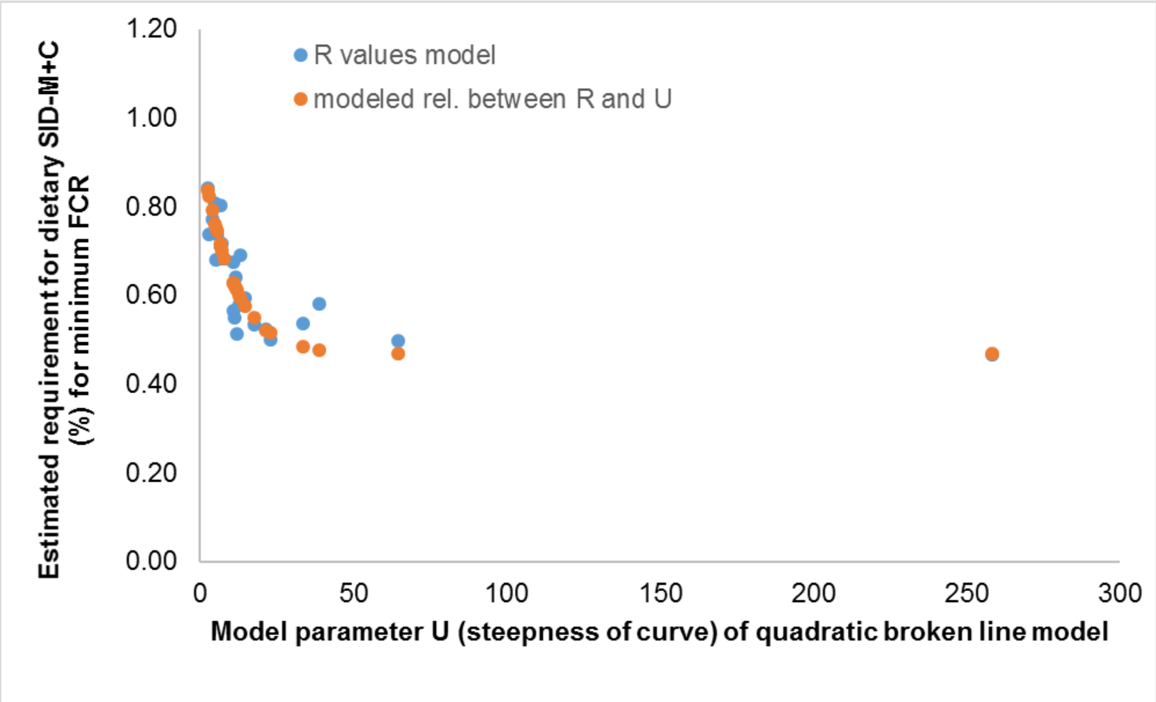

Figure 3. Relationship between the model estimated steepness of the decrease in FCR (g feed/g EM) per unit increase in dietary SID-M+C (%) and the estimated SID-M+C requirement (%) for minimum FCR using the quadratic broken-line model. Model parameters: Estimated requirement for SID-M+C (%) = 0.468 + 0.487×EXP(-0.1019 × U); R^2 = 0.785.

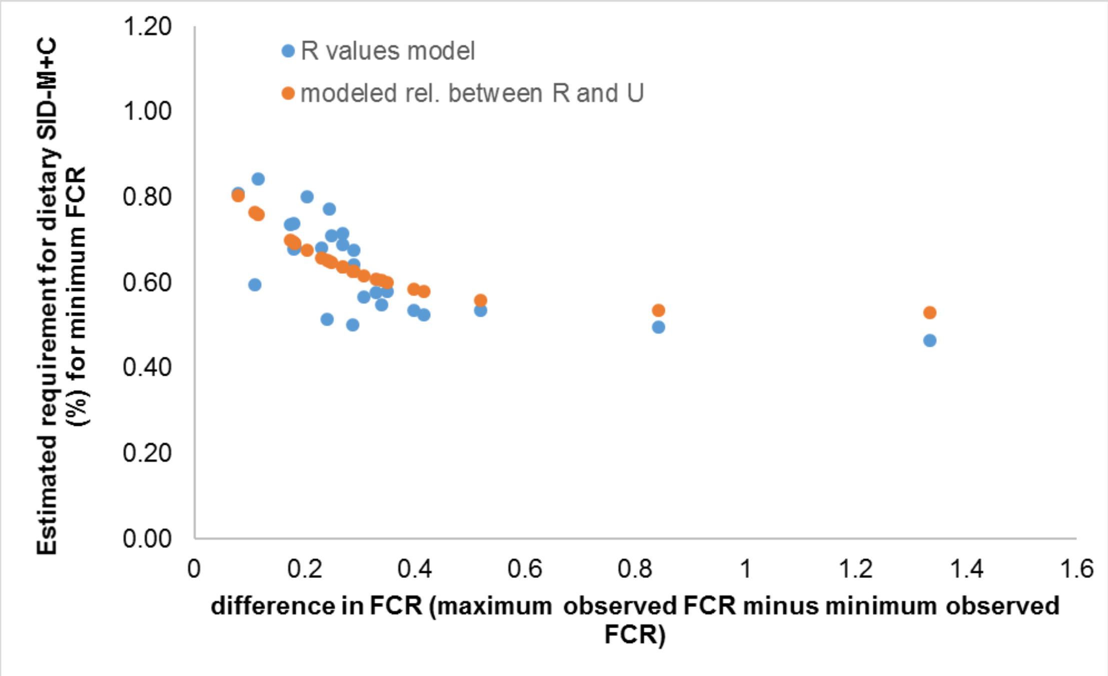

Figure 4. Relationship between the difference in maximum and minimum observed FCR and the estimated SID-M+C requirement for minimum FCR using the quadratic broken-line model.

These relationships indicate that at experiments with lower dietary basal SID-M+C concentrations also lower estimated SID-M+C requirements may be expected due to the fitting characteristics of the model compared to experiments with higher basal levels of SID-M+C. This becomes very clear from the data from Schutte (trial 15 and 15a). Trial 15 based on all observations result in an estimated dietary SID-M+C requirement for maximum EM of 0.476% whereas the same trial but then excluding the lowest dietary SID-M+C treatment (trial 15a) result in an estimated dietary SID-M+C requirement of 0.934% (Figure 5).

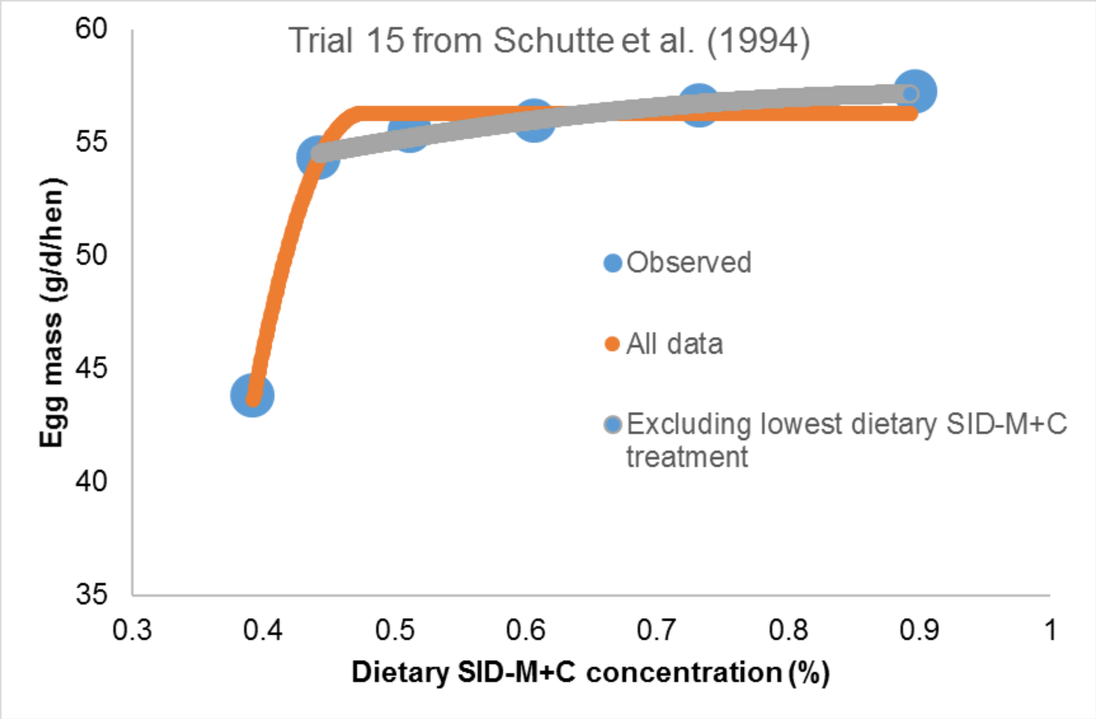

Figure 5. Effect of excluding the lowest dietary SID-M+C treatment on estimated relationship between dietary SID (%) and egg mass production (g/hen/day for trial 15 from the study of Schutte et al. (1994). Estimated SID-M+C requirement value including lowest dietary SID-M+C treatment is 0.476% and the estimated SID-M+C requirement value excluding the lowest dietary SID-M+C treatment is 0.934%.

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-M+C (%)	Max SID-M+C (%)	Max. FCR minus Min. FCR	Max. egg mass minus Min. egg mass
Gomez et al. (2009)	1	Hy-Line W36	100	6	14.5	80	52	98	0.393	0.783	0.29	9.2
Brumano et al. (2010)	2	Hy-Line W36	24	16	16.9	95	55	90	0.637	0.887	0.12	5.8
Brumano et al. (2010)	3	Hy-Line W36	42	16	16.9	87	55	94	0.637	0.887	0.21	8.0
M. Schmidt et al. (2009)	4	Lohmann Brown	79	16	15.4	79	54	113	0.492	0.700	0.29	8.1
Cupertino et al. (2009)	5	Lohmann LSL	54	16	15.4	86	57	112	0.492	0.700	0.35	8.9
Cupertino et al. (2009)	6	Lohmann Brown	54	16	15.4	82	55	113	0.492	0.700	0.34	10.0
M. Schmidt et al. (2011)	7	Lohmann LSL	79	16	15.6	87	58	114	0.497	0.709	0.18	6.0
Geraldo et al. (2010)	8	Hy-Line W36	25	16	16.5	93	53	101	0.565	0.799	0.18	6.6
Narvaez-Solarte et al. (2005)	9	Lohmann White	22	16	14.5	94	57	108	0.417	0.667	0.52	18.8
Filho et al. (2006)	10	Hisex Brown	20	24	17.2	91	55	110	0.526	0.806	0.15	4.4
Sa et al. (2007)	11	Lohmann White	34	16	15.7	95	60	120	0.513	0.713	0.29	8.0
Sa et al. (2007)	12	Lohmann Brown	34	16	15.7	93	58	115	0.513	0.713	0.18	4.9
Novak et al. (2004)	13	Dekalb Delta	20	23	17.2	85	50	97	0.523	0.765	0.13	2.9
Novak et al. (2004)	14	Dekalb Delta	44	19	17.2	87	52	98	0.522	0.765	0.17	3.2
Schutte et al. (1994)	15	Lohmann LSL	25	12	14.0	97	57	119	0.392	0.897	0.34	13.4
Schutte et al. (1994)	16	Lohmann LSL	25	12	14.9	98	56	113	0.508	0.657	0.11	0.6
Dänner and Bessei (2002)	17	Lohmann LSL	24	24	14.9	95	58	113	0.423	0.573	0.33	7.5
Bertram et al. (1995)	18	Lohmann LSL	24	12	15.7	97	58	109	0.407	0.607	0.31	10.1
Lemme et al. (2004)	19	Lohmann Brown	22	24	14.7		59	123	0.394	0.514	1.34	25.3
Kakhi et al. (2016)	20	Hy-line layers	32	4	16.3	92	58	98	0.528	0.728	0.25	9.6
Kakhi et al. (2016)	21	Hy-line layers	36	4	16.3	92	58	104	0.528	0.728	0.27	9.7
Kakhi et al. (2016)	22	Hy-line layers	40	4	16.3	90	57	105	0.528	0.728	0.23	8.4
Kakhi et al. (2016)	23	Hy-line layers	32	12	16.3	91	57	102	0.528	0.728	0.25	9.2
Star and van Krimpen (2016)	24	Dekalb White	61	7	13.9	93	59	124	0.386	0.636	0.24	4.6
Star and van Krimpen (2016)	25	Bovans Brown	61	7	13.90	86	56	120	0.386	0.636	0.40	12.0
Star and van Krimpen (2016)	26	Dekalb White	69	7	13.9	89	57	129	0.386	0.636	0.42	8.3
Star and van Krimpen (2016)	27	Bovans Brown	69	7	13.9	83	54	124	0.386	0.636	0.84	17.4

For some titration trials the estimated plateau values of FCR were lower than 1.8 (trials 2, 3, 6 and 20) and for one trial (trial 27) an estimated plateau value of FCR higher than 2.3 was observed. Removing these extreme observations resulted in average estimated optimal SID-M+C concentrations and SID-M+C intake statistics as presented in Table 3. A comparison between results in Table 2 (including observations with extreme FCR values) and Table 3 (results excluding very low and very high FCR values) show that excluding these extreme values resulted in lower differences in average estimated SID-M+C requirements between EM and FCR due to lower estimated SID-M+C requirements for FCR.

Table 3. Estimated optimal SID-M+C requirements (% and daily intake) for maximum egg mass (EM) and minimum FCR excluding these values in which estimated SID-M+C requirements values were outside the measurement range and where FCR values were lower than 1.8 or higher than 2.3. Observations 2a, 15a and 21a were included in the analysis.

	Parameter	N*	Mean	Std. Dev.	Min.	Max	%CV
SID-M+C (%)	EM	18	0.602	0.1067	0.465	0.783	17.7
	FCR	19	0.628	0.0783	0.514	0.738	12.5
SID-M+C intake (mg/d)	EM	18	663	97.0	521	789	14.6
	FCR	19	687	66.9	576	807	9.7
SID-M+C intake per g of EM (mg/g)	EM	18	11.9	1.68	9.2	14.9	14.1
	FCR	19	12.3	1.24	10.3	14.0	10.1
SID-M+C:SID-LYS ratio	EM	18	85	15.4	61	115	18.1
	FCR	19	88	11.2	67	109	12.7
SID-M+C:SID-LYS ratio**	EM	18	88	12.7	66	116	14.5
	FCR	19	90	10.1	71	111	11.2

*number of titration trials (total number of titration trials is 30 (27 trials + 3 titration trials for which R values were estimated again after excluding the diet containing the lowest dietary SID-M+C level). Titration trials excluded for EM were 2, 2a, 3, 6, 10, 13, 14, 15a, 16, 19, 20 and 27. Titration trials excluded for FCR were 1, 2, 2a, 3, 6, 10, 13, 15a and 19, 20 and 27.

**This ratios was 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-M+C : SID-LYS ratio, otherwise the observed SID-LYS intake at which maximum EM was estimated was used for calculation of the SID-M+C : SID-LYS ratio.

From a visual analysis of Fig. 6 and 7 it seems that dietary SID-M+C intake is only weakly related to EM production (Fig. 6) and that dietary SID-M+C concentration is only weakly related to FCR (Fig. 7).

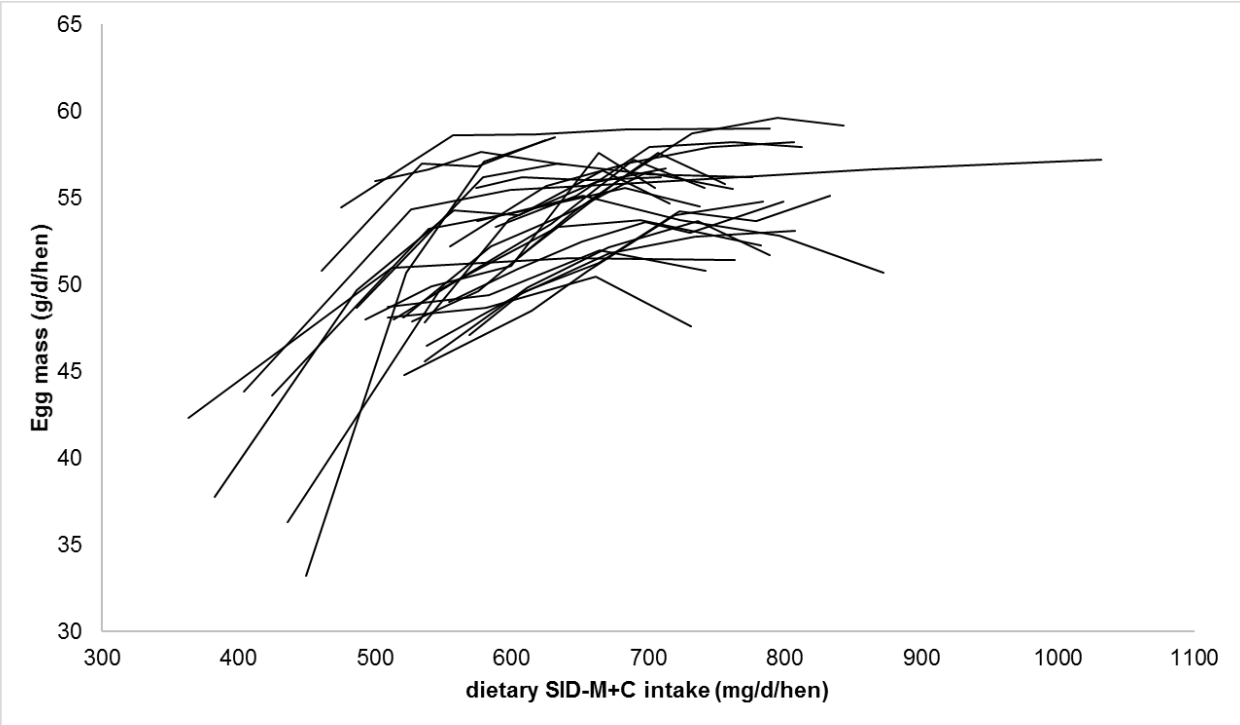

Figure 6. Relationship between dietary SID-M+C intake (mg/d/hen) and egg mass (g/d/hen) for the 27 individual titration experiments.

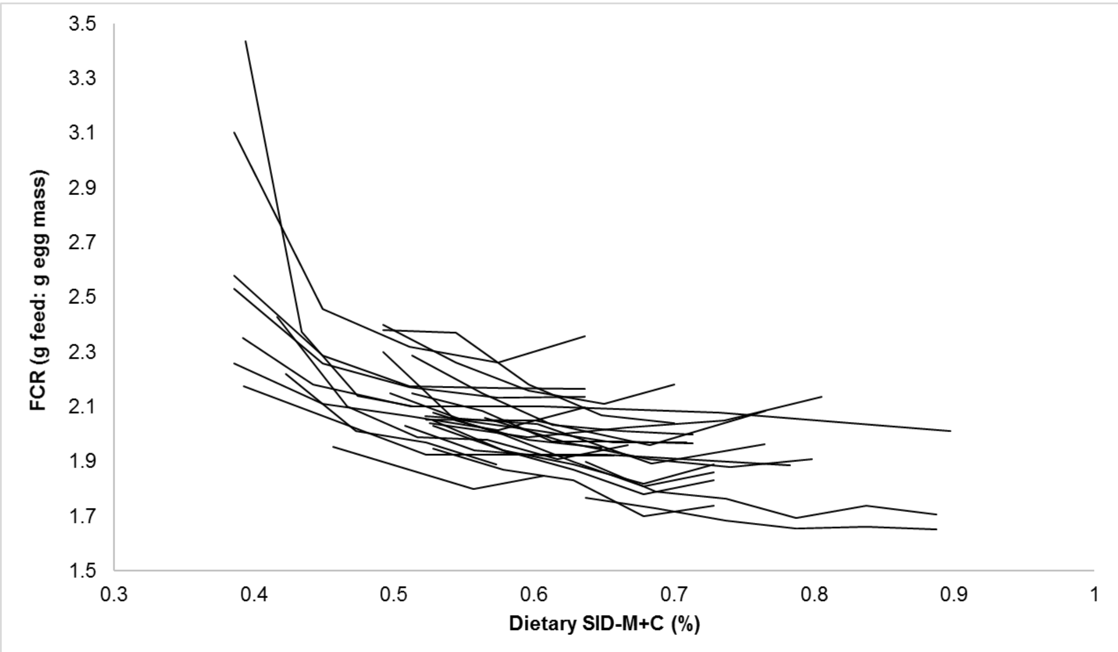

Figure 7. Relationship between dietary SID-M+C concentration (% in feed) and feed conversion ratio (FCR; g feed : g egg mass) for the 27 individual titration experiments.

This also becomes clear when the estimated SID-M+C requirements for maximum EM (expressed as mg/d) were regressed against EM which resulted in a very weak relationship (R^2 = 0.004). However, when regressing the estimated SID-M+C requirements for minimum FCR (expressed as %) against FCR a moderate relationship was observed as is shown in Figure 8.

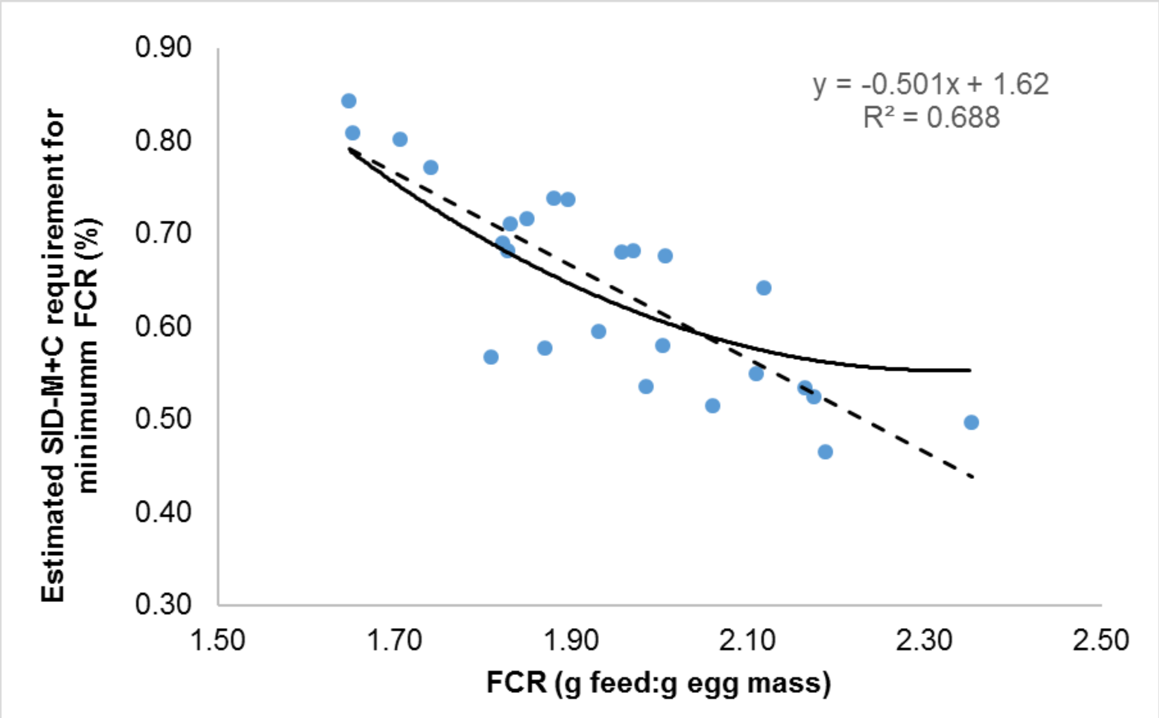

Figure 8. Relationship between FCR observed at the estimated SID-M+C requirement for minimum FCR and the estimated SID-M+C requirement for minimum FCR. When correcting for study (including study as a random factor in the model) the relationship would be: SID-M+C req. for minimum FCR (%) = 1.16±0.149 – 0.2705±0.0754 × FCR (g feed : g egg mass). In case study was included as a random factor a quadratic relationship also became significant (having a lower AIC value): SID-M+C req. for minimum FCR (%) = 3.39±0.860 – 2.447±0.8285 × FCR (g feed : g egg mass) + 0.5276±0.1988 × FCR (g feed : g egg mass). This quadratic relationship is shown in the Figure as the solid line. The linear relationship between FCR and estimated SID-M+C requirement for minimum FCR without correcting for study is shown in the figure by the regression formula and the dashed line.

In Figure 9 the relationship between FCR observed and estimated SID-M+C requirement for minimum FCR is shown again but then the observations with minimum FCR values lower than 1.8 and higher than 2.3 are excluded from the analysis including the observation from Trial 19 because of the low maximum dietary SID-M+C concentration used.

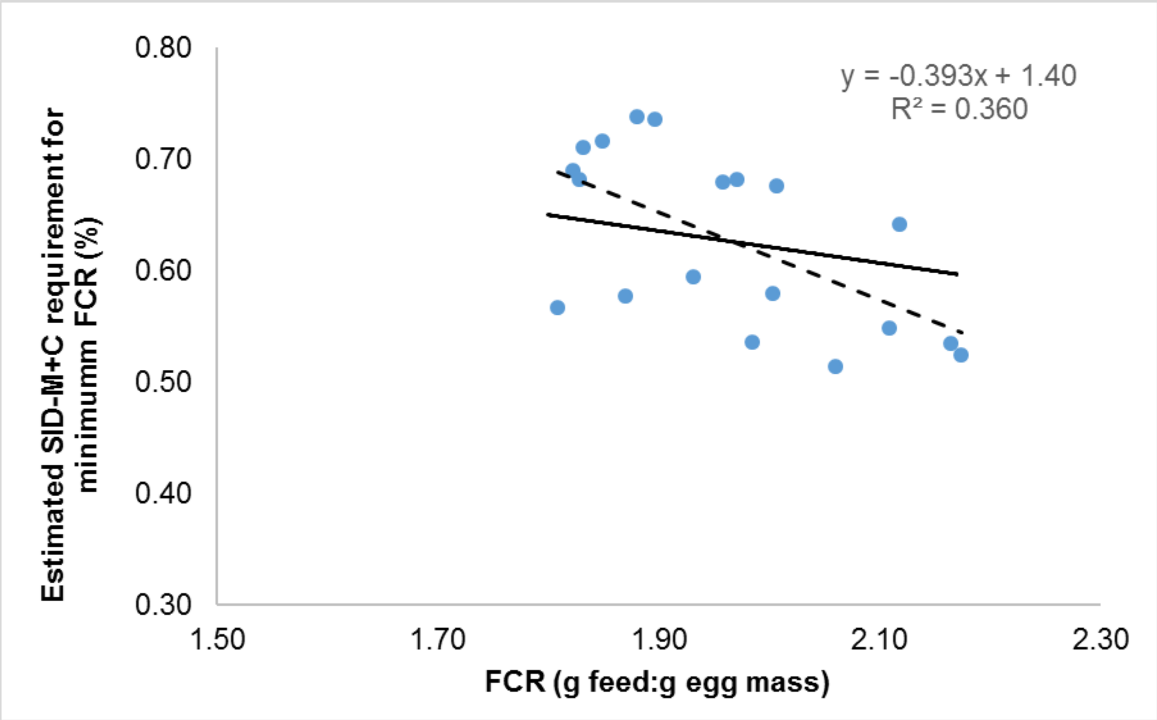

Figure 9. Relationship between FCR observed at the estimated SID-M+C requirement for minimum FCR and the estimated SID-M+C requirement for minimum FCR. Observations with FCR lower than 1.8 and higher than 2.3 were excluded from the analysis including the observation from Trial 19 because of the low maximum dietary SID-M+C concentration used. When correcting for study (including study as a random factor in the model) the relationship is as follows: SID-M+C req. for minimum FCR (%) = 0.909±0.1899 – 0.1437±0.09640 × FCR (g feed : g egg mass). This relationship is shown in Figure 9 as the solid line. The effect of FCR on SID-M+C req. for minimum FCR was not significant (P=0.187) when study was included as a random factor whereas in case study was not included as a random factor the effect of FCR was significant (P=0.007). The relationship without including study as a random factor in the model is shown in Figure 9 as the dashed line and the regression formula shown in the Figure.

Furthermore, it was observed that the estimated SID-M+C requirements were strongly related to the calculated concentration of dietary protein. This is shown in Figure 10 for the association between estimated SID-M+C requirements for FCR and dietary CP. As well the estimated SID-M+C requirements (%) were also strongly related to various individual amino acids (but not to dietary SID-LYS concentration; R^2 = 0.056 for FCR and 0.000 for EM). For example, dietary SID-HIS concentration was strongly correlated to the estimated SID-M+C requirement for minimum FCR (r = 0.950) and dietary SID-SER was strongly correlated to the estimated SID-M+C requirement for minimum FCR (r = 0.943).

Strathe et al. (2011) carried out a meta-analysis on the requirement of digestible MET in laying hens and observed a significant effect of BW on digestible MET requirement for both maximum EM and minimum FCR. However, in our meta-analysis BW data was not presented in most of the studies and was therefore not taken into account. Even when, based on the type of bird, body weights were estimated, the factor body weight was not helpful in explaining variation in estimated SID-M+C requirements. Furthermore, it is likely that the effect of BW is also incorporated in the estimated SID-M+C requirement for FCR as BW is positively correlated with FCR.

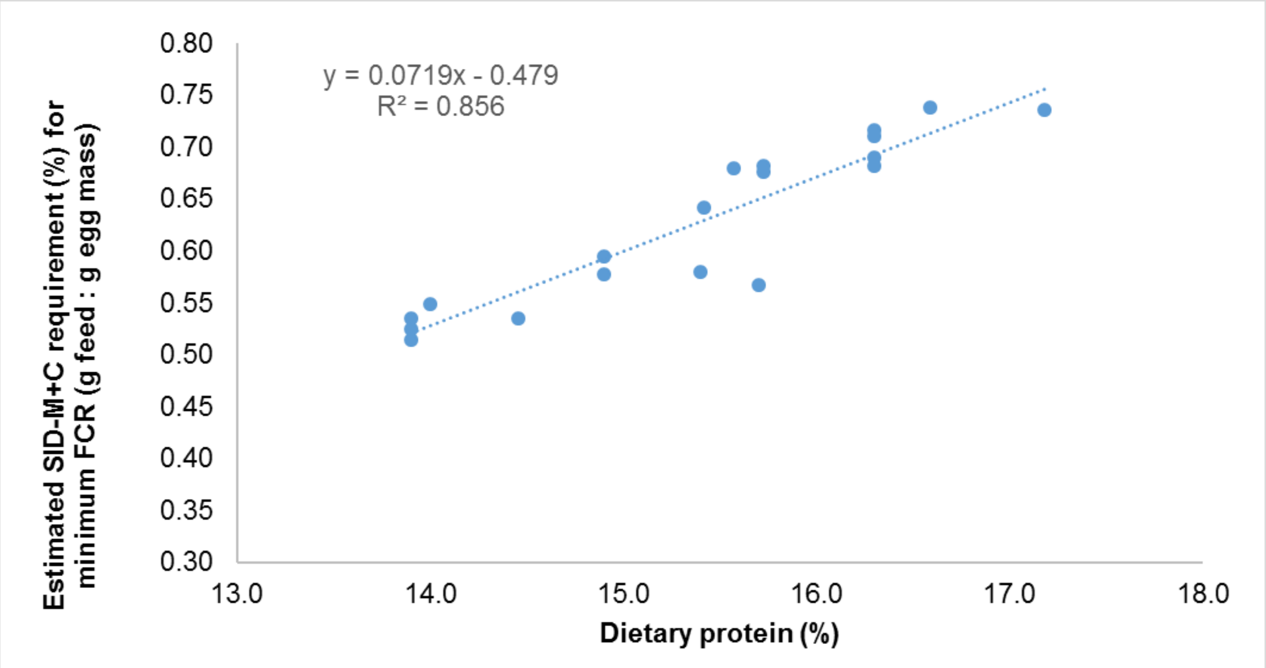

Figure 10. Relationship between dietary protein (%) and estimated SID-M+C requirements for minimum FCR (% in feed). Observations with minimum FCR lower than 1.8 and higher than 2.3 were excluded from the analysis including the observation from Trial 19 because of the low maximum dietary SID-M+C concentration used in this trial.

The estimated SID-M+C requirements for maximum EM : SID-LYS ratios and estimated SID-M+C requirements for minimum FCR : SID-LYS ratios were also expressed against egg mass (Fig. 11) and FCR (Fig. 12).

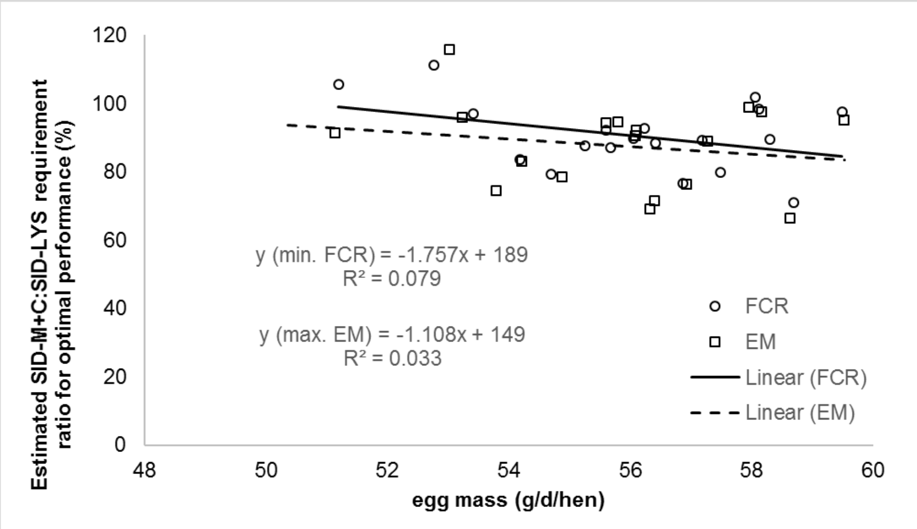

Figure 11. Relationship between egg mass (g/d/hen) and the estimated SID-M+C requirements for minimum FCR and maximum egg mass expressed as a ratio to SID-LYS. Observations with FCR lower than 1.8 and higher than 2.3 were excluded from the analysis including the observation from Trial 19 because of the low maximum dietary SID-M+C concentration used. This ratio was 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 or minimum FCR was estimated, then this formula was used to calculate the M+C:LYS ratio, otherwise the observed SID-LYS intake at which maximum EM or minimum FCR was estimated was used.

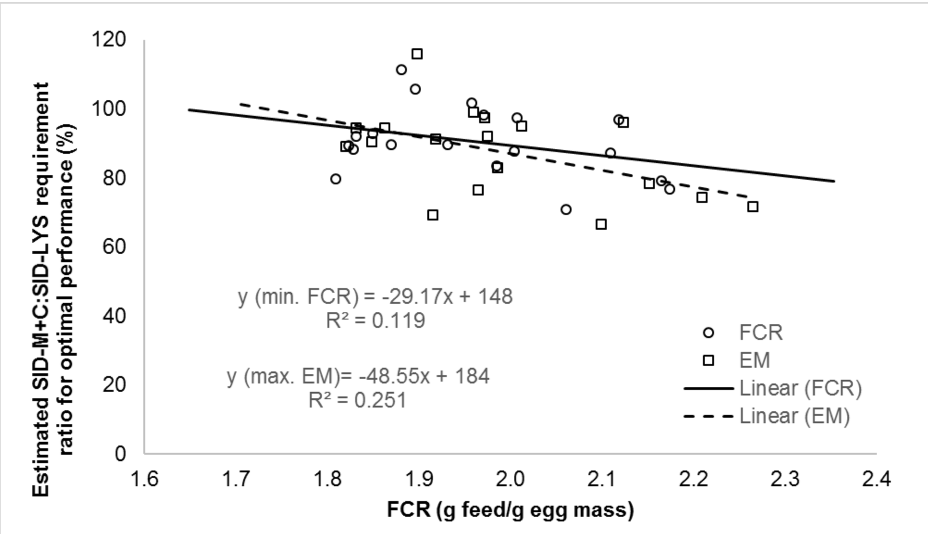

Figure 12. Relationship between FCR (g feed:g egg mass) and the estimated SID-M+C requirements for minimum FCR and maximum egg mass expressed as a ratio to SID-LYS. Observations with FCR lower than 1.8 and higher than 2.3 were excluded from the analysis including the observation from Trial 19 because of the low maximum dietary SID-M+C concentration used. This ratio was 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 or minimum FCR was estimated, then this formula was used to calculate the M+C:LYS ratio, otherwise the observed SID-LYS intake at which maximum EM or minimum FCR was estimated was used.

Apparently, the variation in estimated SID-M+C requirements for maximum EM and minimum FCR is more related to the dietary protein concentration or concentrations of other SID-amino acid concentrations (except for SID-LYS) than to EM or FCR. It is, however, undesirable to provide SID-M+C recommendations based on the dietary protein content or amino acid content of the diet as these may change in time. Therefore, it seems more rational to base SID-M+C recommendations on the expected FCR as is shown in Figure 9. When doing so this resulted in the recommendations as shown in Table 4 below.

Table 4. Estimated optimal SID-M+C requirements for minimum FCR expressed in mg/d and as a percentage of the diet for minimum FCR at various egg production rates based on the formula presented in Figure 9 based on a linear relationship and accounting for a study effect. 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-M+C (mg/d)		Dietary SID-M+C (%)		SID-M+C:SID-LYS ratio**	
	Egg production rate (%)									
BW (kg)	90	95	90	95	90	95	90	95	90	95
1.5	112	115	54	57	683	711	0.612	0.620	94	90
1.6	114	117	54	57	692	720	0.605	0.613	95	91
1.7	117	120	54	57	700	728	0.597	0.606	96	92
1.8	120	123	54	57	707	736	0.590	0.599	97	93
1.9	122	125	54	57	714	744	0.583	0.593	98	94
2.0	125	128	54	57	720	750	0.577	0.586	99	95

*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-M+C:SID-LYS ratio for minimum FCR is calculated based on the ratio between SID-M+C intake and SID-LYS intake. The calculated SID-LYS intake is based on formula [F8] described in CVB documentation report nr. 69.

In Table 5 the same exercise is carried out as in Table 4 with this difference that the dietary SID-M+C requirement for minimum FCR and expressed in percentage was estimated using the linear relationship without accounting for a study effect. Results in Table 5 show that at increasing BW (and thereby also increasing FCR values) the calculated SID-M+C:SID-LYS ratio decrease. This is in agreement with the relationship portrayed in Figure 12 (without correcting for study effect). Furthermore, Figure 13 shows that at increasing dietary protein concentrations also estimated SID-M+C:SID-LYS ratios for minimum FCR and maximum EM increase. It is likely that heavier birds that require more energy for maintenance also require lower dietary concentrations of protein compared to lighter birds. However, results in Table 5 also show that SID-M+C requirements expressed in mg/d decline at increasing body weight (or increasing FCR). From a physiological point of view this doesn’t make sense.

Table 5. Estimated optimal SID-M+C requirements for minimum FCR expressed in mg/d and as a percentage of the diet for minimum FCR at various egg production rates based on the formula presented in Figure 9 based on a linear relationship without accounting for a study effect. 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-M+C (mg/d)		Dietary SID-M+C (%)		SID-M+C:SID-LYS ratio**	
	Egg production rate (%)									
BW (kg)	90	95	90	95	90	95	90	95	90	95
1.5	112	115	54	57	654	697	0.586	0.607	90	88
1.6	114	117	54	57	647	691	0.566	0.589	89	87
1.7	117	120	54	57	640	685	0.546	0.570	88	87
1.8	120	123	54	57	631	678	0.527	0.552	87	86
1.9	122	125	54	57	622	669	0.508	0.534	85	85
2.0	125	128	54	57	611	660	0.489	0.516	84	84

*Feed intake is calculated based on: a feed with a MEIh 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-M+C:SID-LYS ratio for minimum FCR is calculated based on the ratio between SID-M+C intake and SID-LYS intake. The calculated SID-LYS intake is based on formula [F8] described in CVB documentation report nr. 69.

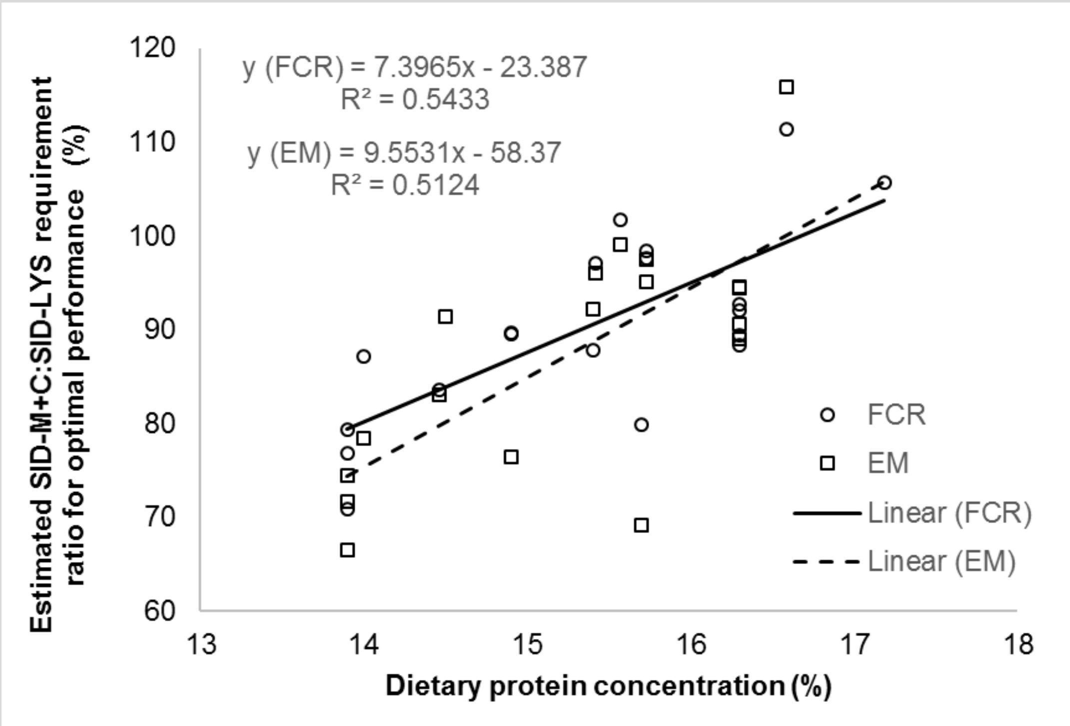

Figure 13. Relationship between calculated dietary protein and the estimated SID-M+C:SID-LYS requirement ratios for minimum FCR and maximum egg mass. Observations with minimum FCR lower than 1.8 and higher than 2.3 were excluded in this relationship including the observation from Trial 19 because of the low maximum dietary SID-M+C concentration used. The SID-LYS levels were calculated as follows. In case the calculated SID-LYS requirements using formula [F8] in CVB documentation report nr. 69 resulted in a lower SID-LYS requirement than the observed SID-LYS intake at which maximum EM or minimum FCR was estimated, then this formula was used to calculate the M+C:LYS ratio, otherwise the observed SID-LYS intake at which maximum EM or minimum FCR was estimated was used.

In Table 6 the requirements for dietary SID-M+C are provided when based on an average SID-M+C requirement of 12.3 mg per g of EM for minimum FCR as shown in Table 3. Results in Table 6 does not result in increasing SID-M+C:SID-LYS ratios at increasing body weight (or FCR) as shown in Table 4 which are opposite to the relationship between FCR and SID-M+C:SID-LYS ratio as shown in Figures 11 and 12 or the physiological unexplainable decreasing SID-M+C requirements at increasing body weight (FCR) as shown in Table 5.

Table 6. Estimated optimal SID-M+C requirements for minimum FCR expressed in mg/d and as a percentage of the diet for minimum FCR at various egg production rates based on a average estimated SID-M+C requirement for minimum FCR of 12.3 mg per g of egg mass as presented in Table 3. 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-M+C (mg/d)		Dietary SID-M+C (%)		SID-M+C:SID-LYS ratio**	
	Egg production rate (%)									
BW (kg)	90	95	90	95	90	95	90	95	90	95
1.5	112	115	54	57	664	701	0.595	0.611	91	89
1.6	114	117	54	57	664	701	0.581	0.597	91	89
1.7	117	120	54	57	664	701	0.567	0.583	91	89
1.8	120	123	54	57	664	701	0.555	0.571	91	89
1.9	122	125	54	57	664	701	0.543	0.559	91	89
2.0	125	128	54	57	664	701	0.532	0.548	91	89

*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-M+C:SID-LYS ratio for minimum FCR is calculated based on the ratio between SID-M+C intake and SID-LYS intake. The calculated SID-LYS intake is based on formula [F8] described in CVB documentation report nr. 69.

In conclusion, contrary to estimation of SID-LYS requirements for laying hens where a clear relationship between dietary SID-LYS intake and EM was observed (see CVB documentation report nr. 69) this was not the case for SID-M+C. Furthermore, a weak negative relationship was observed between optimal dietary concentration of SID-M+C and FCR. Using this weak relationship between FCR and SID-M+C requirement as shown in Fig. 9 resulted in estimated SID-M+C requirements and SID-M+C:SID-LYS requirement ratios as shown in Table 4 and 5. These requirements as shown in Table 4 and 5 resulted in increased (Table 4) or decreased (Table 5) SID-M+C:SID-LYS requirement ratios at increasing BW that are difficult to explain physiologically. Therefore it is concluded that it is most safe to base SID-M+C requirements on the average SID-M+C requirement for minimum FCR of 12.3 mg per g of EM as shown in Table 3. As in general the estimated SID-M+C requirements for minimum FCR are higher than for maximum EM this also guarantees a sufficient supply of SID-M+C for maximum EM.

It was observed that high estimated SID-M+C requirement estimates (> 0.5% SID-M+C) for FCR were accompanied by low estimated steepness decreases in FCR per unit increase of SID-M+C (Fig. 3). This suggests that dietary levels of SID-M+C higher than 0.5% are likely to result in only small benefits with respect to a reduction in FCR. Figures 1 and 3 indicate that a dietary SID-M+C concentration of around 0.5% can be seen as an absolute minimum value and that a strong decline in EM and increase in FCR may be expected at dietary SID-M+C levels lower than 0.5%. The calculated dietary SID-M+C percentage requirements in Table 6 based on SID-M+C requirements of 12.3 mg SID-M+C per g of EM are well above this minimum level of 0.5%.

3.2 SID-MET requirement

Another question is related to the individual SID-MET requirement level for laying hens. In the study of Gomez and Angeles (2016) the effect of the ratio of digestible MET : digestible CYS at a constant digestible M+C concentration (0.52%) was investigated. The digestible MET and CYS levels both varied from 0.20 – 0.32%. It was observed that the ratio with the highest dig MET:CYS ratio (ratio of 160%, with a digestible MET concentration of 0.32% and a digestible CYS concentration of 0.20%) resulted in the highest EM and lowest FCR. The EM at the highest dig. MET concentration ratio of 0.20% was 49.5 g. Using a quadratic broken-line model as described in this document resulted in a estimated digestible MET : digestible CYS ratio of 150% for minimum FCR and 150% for maximum EM as well (Fig. 14).

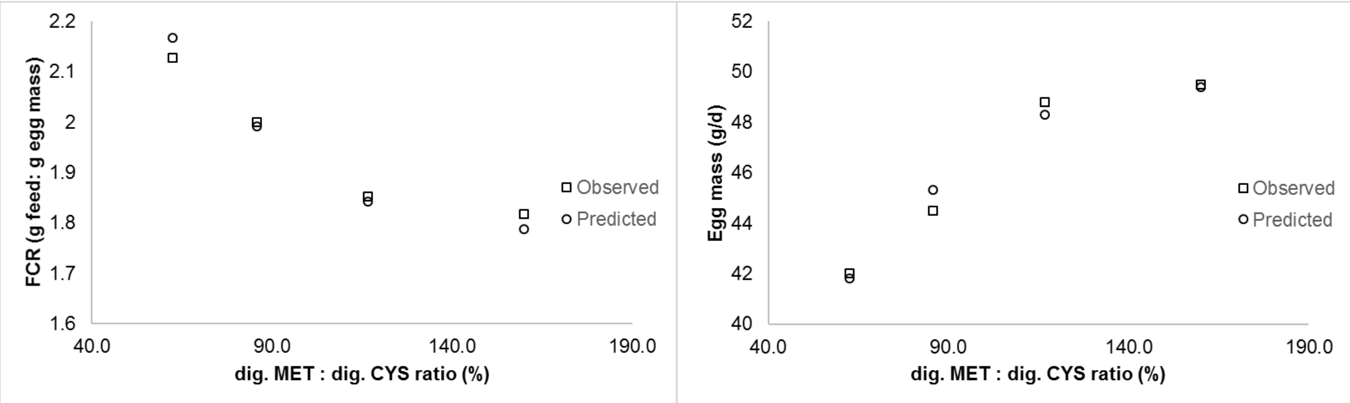

Figure 14. Effect of digestible MET : digestible CYS ratio (%) on FCR (g feed: g egg mass) and egg mass (g/d/hen) based on the data of Gomez and Angeles (2016). Using the quadratic broken-line model as described in the M&M section of this document optimal dig. MET : dig. CYS ratios (%) of 150% were estimated for both minimum FCR and maximum egg mass.

Based on the data of Gomez and Angeles (2016) it seems that that increasing the digestible CYS percentage (while keeping the total SID-M+C constant) might reduce optimal performance at a constant dietary M+C supply or might increase the total digestible M+C requirement. It therefore seems logical to also set a requirement for SID-MET next to a SID-M+C requirement. It should be noted that the variation in SID-CYS between the various diets used in the titration studies in this study was very low (0.21±0.014%). This allows for estimation of a SID-MET requirements based on the estimated SID-M+C requirements and by subtracting a SID-CYS percentage of 0.21% of the estimated SID-M+C requirements.
In case SID-MET is expressed as a ratio relative to SID-LYS this result in an optimal SID-MET:SID-LYS ratios varying from 59% (BW of 1.5 kg) to 55% (BW of 2.0 kg) for a laying hen producing 57 g of EM in case a SID-M+C requirement of 12.3 mg per g of EM is used. The current CVB (2012) SID-MET:SID-LYS requirement is 50%. Because of the limited data on SID-MET requirements, it seems wise to set the optimal dietary SID-MET:SID-LYS ratio at 55%.

4 Conclusions

In conclusion, contrary to estimation of SID-LYS requirements for laying hens where a clear relationship between dietary SID-LYS intake and EM was observed (see CVB documentation report nr. 69) this was not the case for SID-M+C. Furthermore, a weak negative relationship was observed between optimal dietary concentration of SID-M+C and FCR. Using this weak relationship between FCR and SID-M+C requirement as shown in Fig. 9 resulted in estimated SID-M+C requirements and SID-M+C:SID-LYS requirement ratios as shown in Table 4 and 5. These requirements as shown in Table 4 and 5 resulted in increased (Table 4) or decreased (Table 5) SID-M+C:SID-LYS requirement ratios at increasing BW making it difficult to determine which relationship is to be preferred. Therefore it was concluded by the Ad hoc group that it is most safe to base SID-M+C requirements on the average SID-M+C requirement for minimum FCR of 12.3 mg per g of EM as shown in Table 3. As in general the estimated SID-M+C requirements for minimum FCR are higher than for maximum EM this also guarantees a sufficient supply of SID-M+C for maximum EM.

With respect to dietary SID-MET requirements it is concluded that only limited information is available with respect to dietary SID-MET requirements that also includes dietary levels of SID-CYS. Based on the limited data that is available it is concluded that dietary SID-MET:SID-LYS ratio of 55% is sufficient to guarantee a sufficient supply of dietary SID-MET for maximum performance.

List of studies included in the meta-analysis

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Schmidt, M., Gomes, P. C., Rostagno, H. S., Albino, L. F. T., Nunes, R. V. & Brumano, G. 2009. Nutrition levels of digestible methionine + cystine for brown-egg laying hens in the 2nd production cycle. *Revista Brasileira de Zootecnia,* 38**,** 1962-1968.

Schmidt, M., Gomes, P. C., Rostagno, H. S., Albino, L. F. T., Nunes, R. V. & Mello, H. H. C. 2011. Nutrition levels of digestible methionine+cystine for white-egg laying hens in the second production cycle. *Revista Brasileira de Zootecnia,* 40**,** 142-147.

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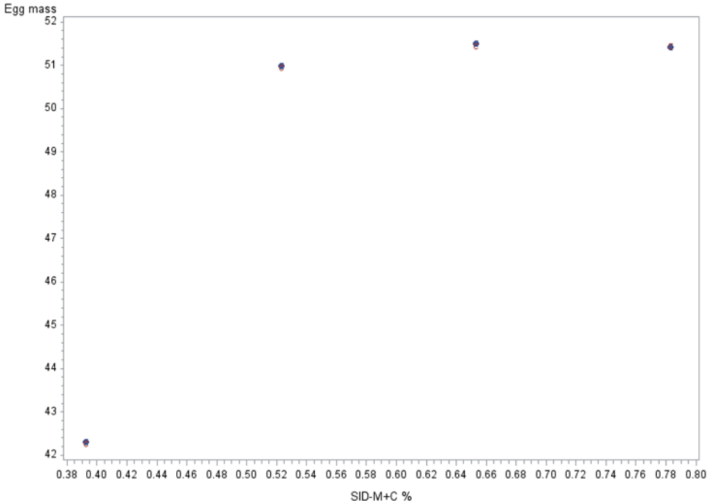
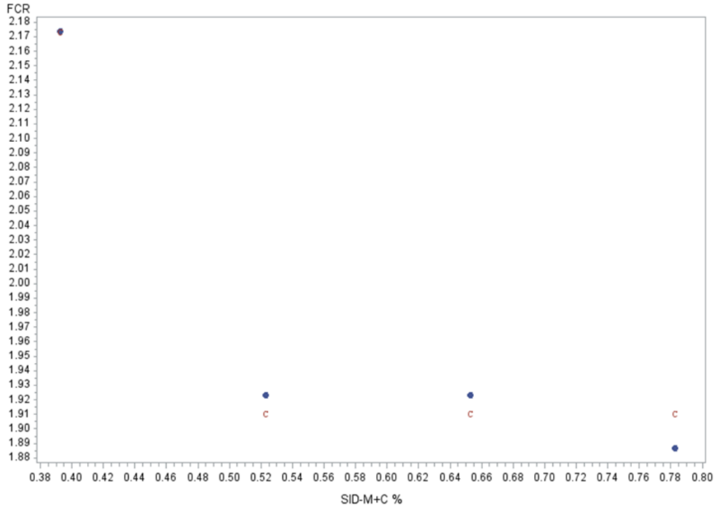
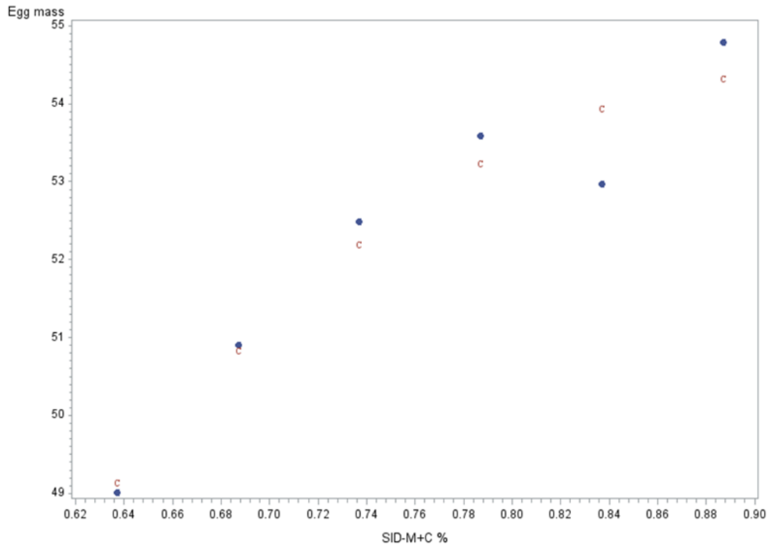
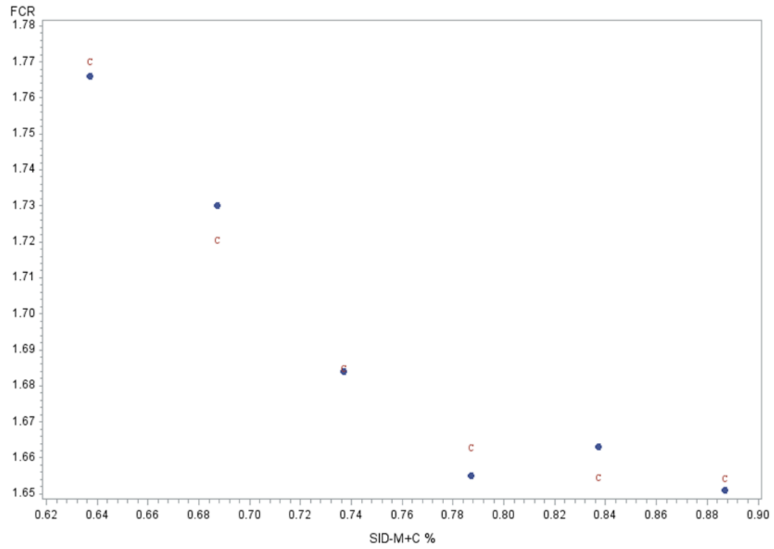
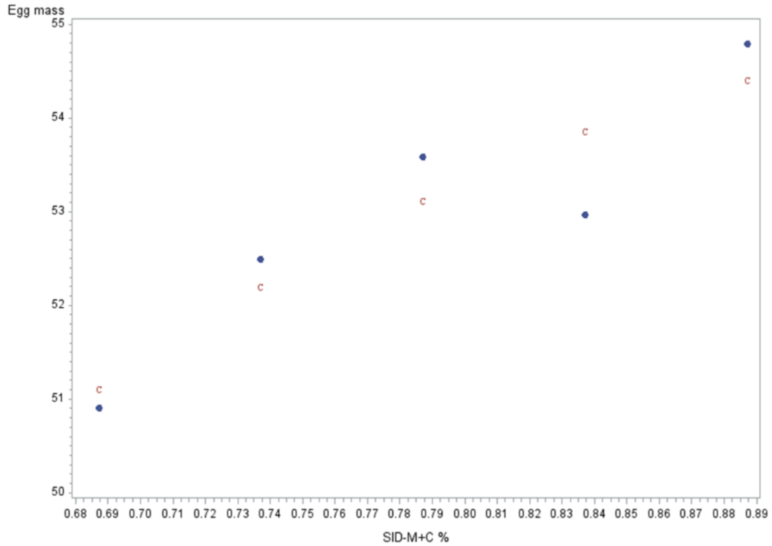
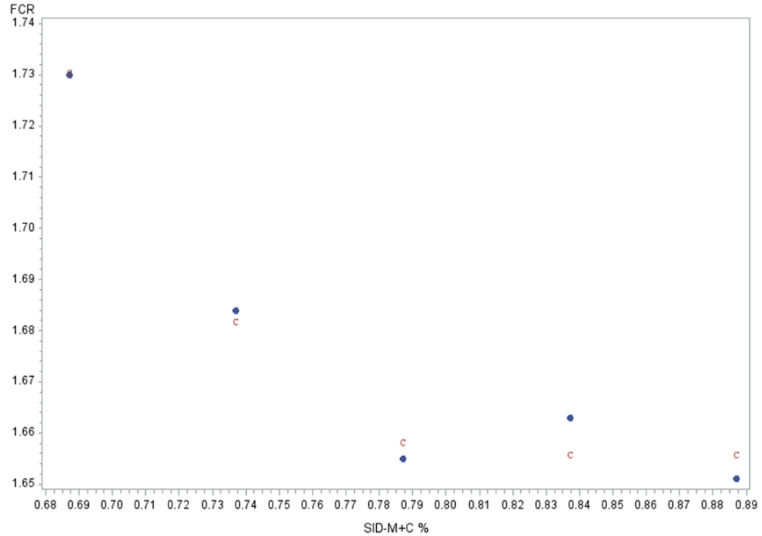
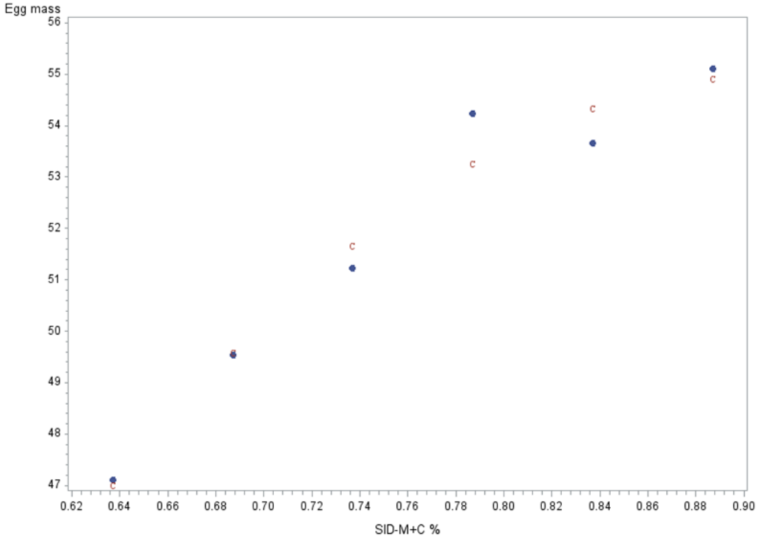
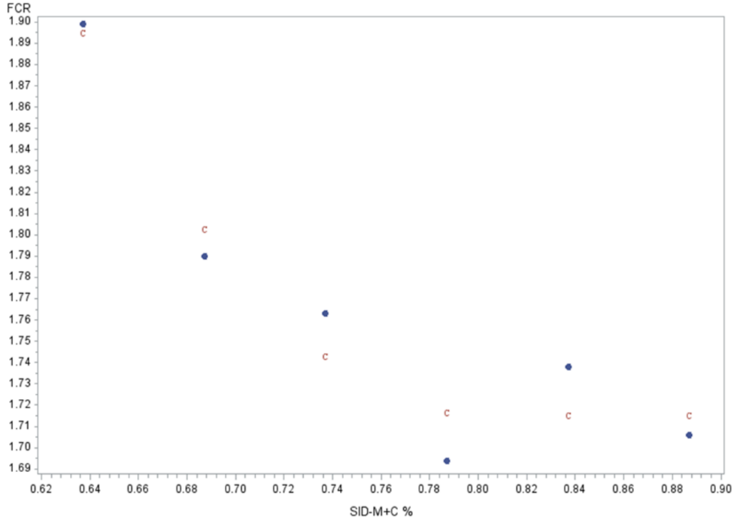
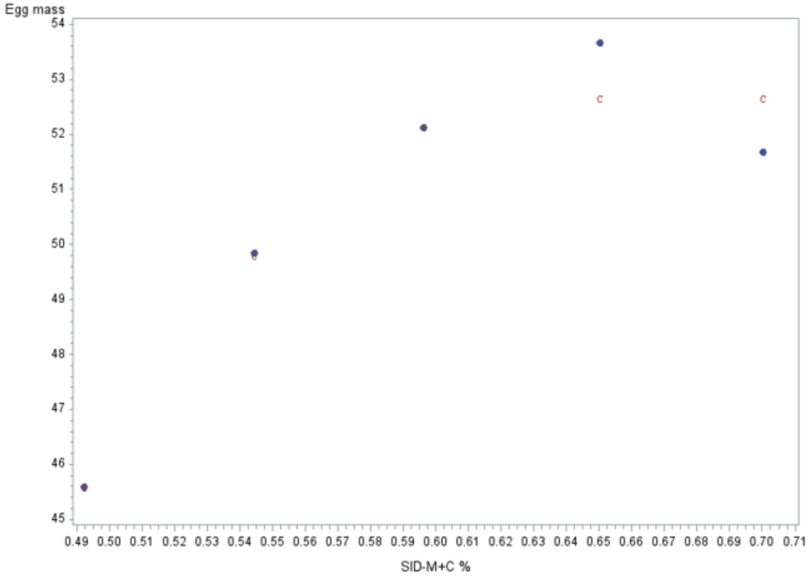
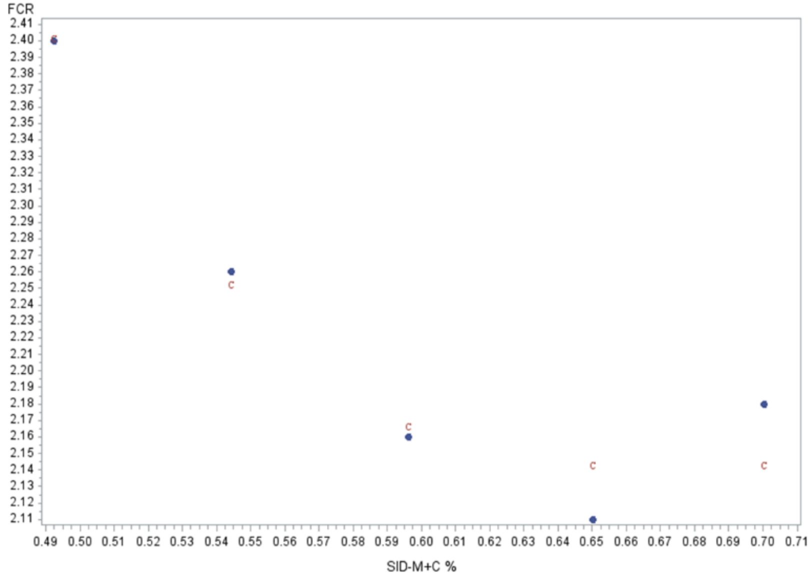
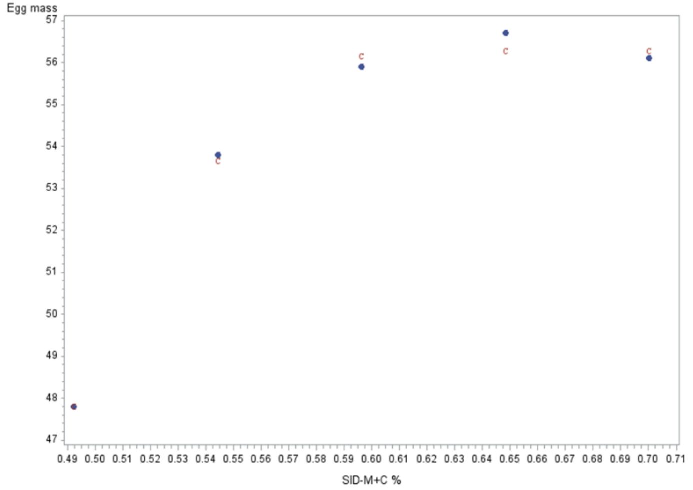
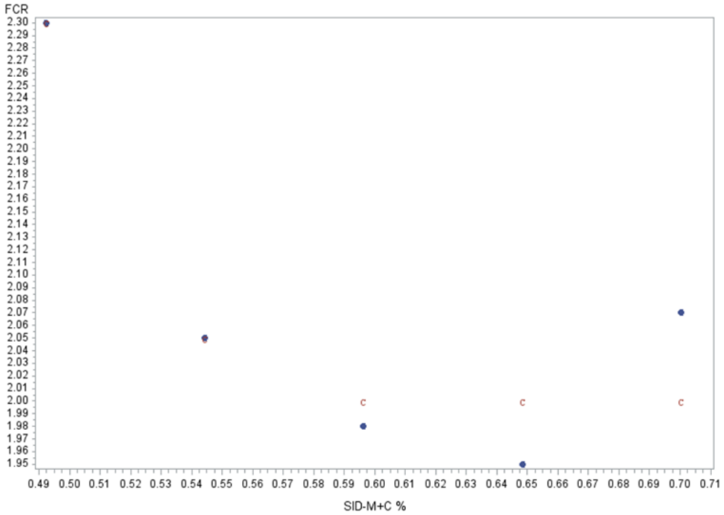
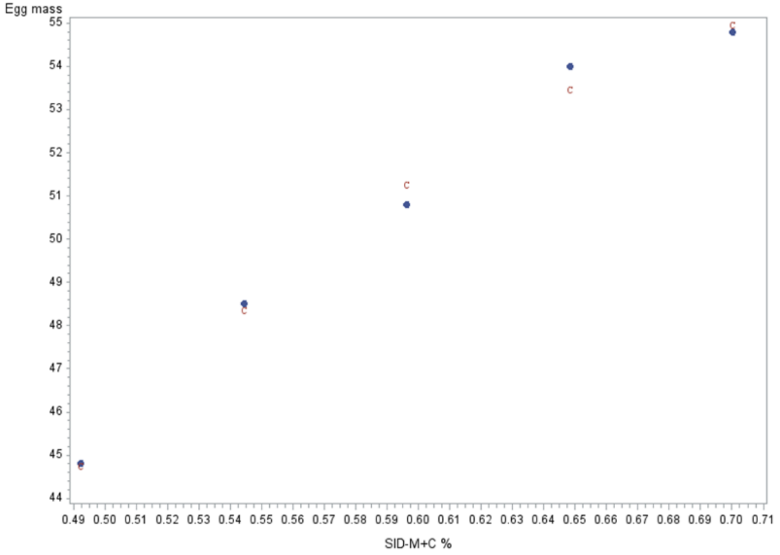
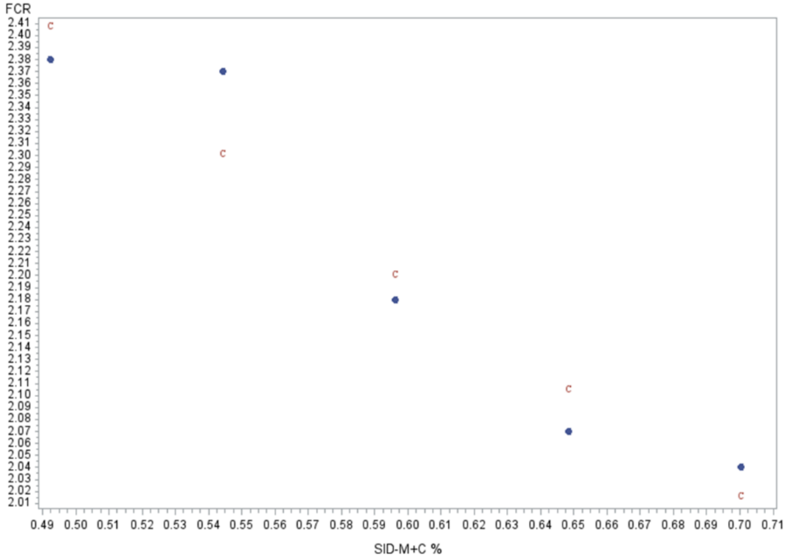
Appendix A. Relationship between dietary SID-M+C supply and performance parameters FCR and EM for the various titration trials including the estimated SID-M+C requirements based on the quadratic broken-line model

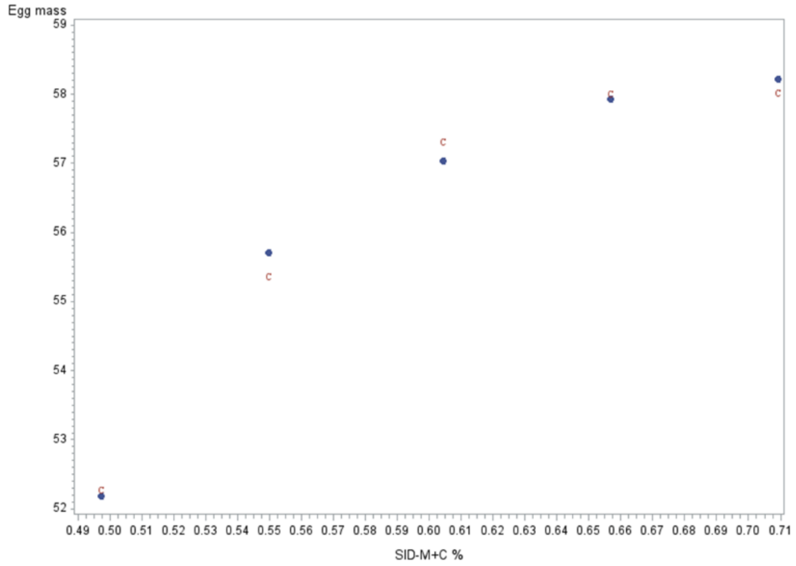
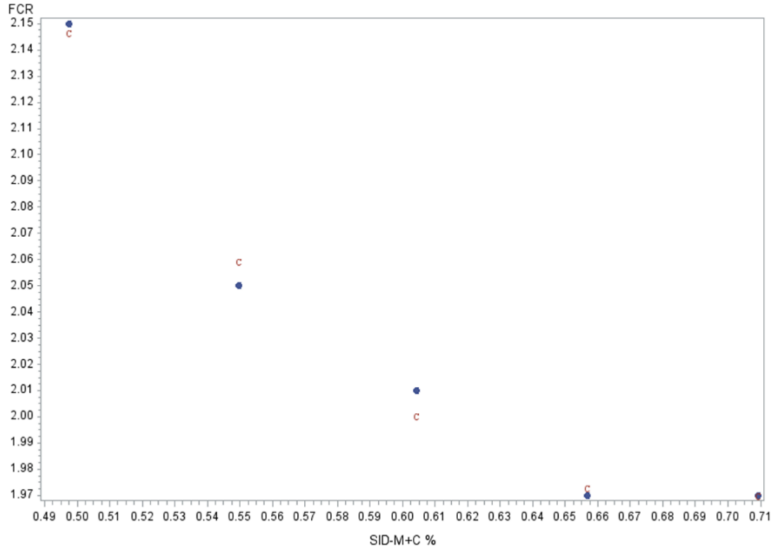
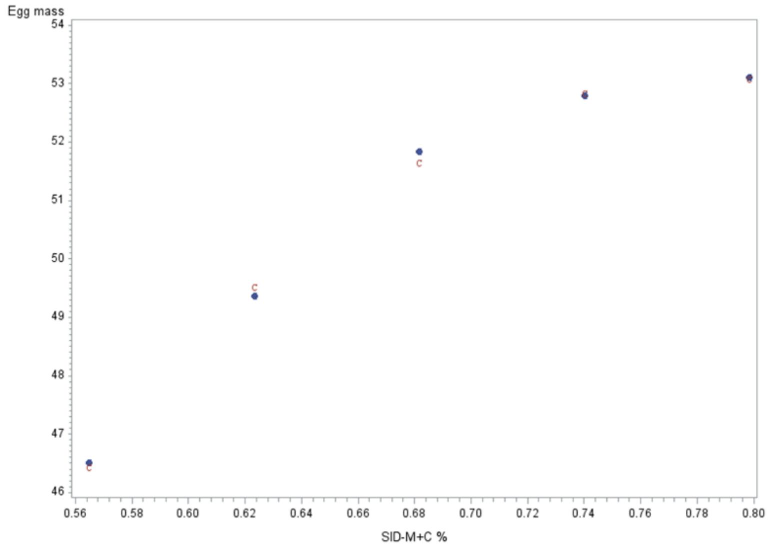
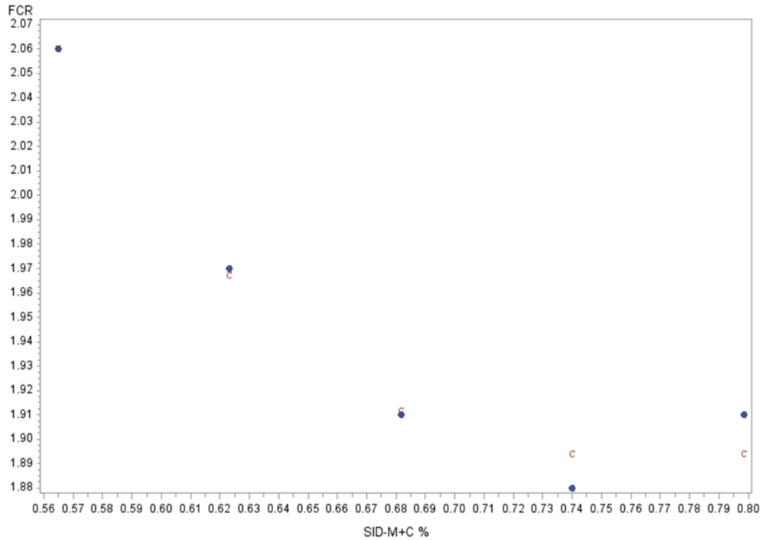
The letter ‘a’ behind the trial number (shown in the first column) means the model is fitted on all observations except the observation with the lowest dietary SID-M+C level. If no letter is shown behind the trial number it means that the model is fitted based on all observations of the trial.

Trial	Relationship between SID-M+C (%) and EM (g/hen/day)	Relationship between SID-M+C (%) and FCR (g feed/g EM)
1. Gomez and Angeles (2009) SID-M+C EM (%) 0.561 SID-M+C FCR (%) 0.500 (no unique estimate possible)		

2. Brumano et al. (2010) Trial 1 SID-M+C EM (%) 0.920 (extrapolation) SID-M+C FCR (%) 0.842		
2a. Brumano et al. (2010) Trial 1 SID-M+C EM (%) 1.017 (extrapolation) SID-M+C FCR (%) 0.809		

3. Brumano et al. (2010) Trial 2 SID-M+C EM (%) 0.920 (extrapolation) SID-M+C FCR (%) 0.802		
4. Schmidt et al. (2009) SID-M+C EM (%) 0.635 SID-M+C FCR (%) 0.641		

5. Cupertino et al. (2009) Trial 1 SID-M+C EM (%) 0.609 SID-M+C FCR (%) 0.580		
6. Cupertino et al. (2009) Trial 2 SID-M+C EM (%) 0.786 (extrapolated value) SID-M+C FCR (%) 1.488 (extrapolated value)		

7. Schmidt et al. (2011) SID-M+C EM (%) 0.661 SID-M+C FCR (%) 0.680		
8. Geraldo et al. (2010) SID-M+C EM (%) 0.783 SID-M+C FCR (%) 0.738		

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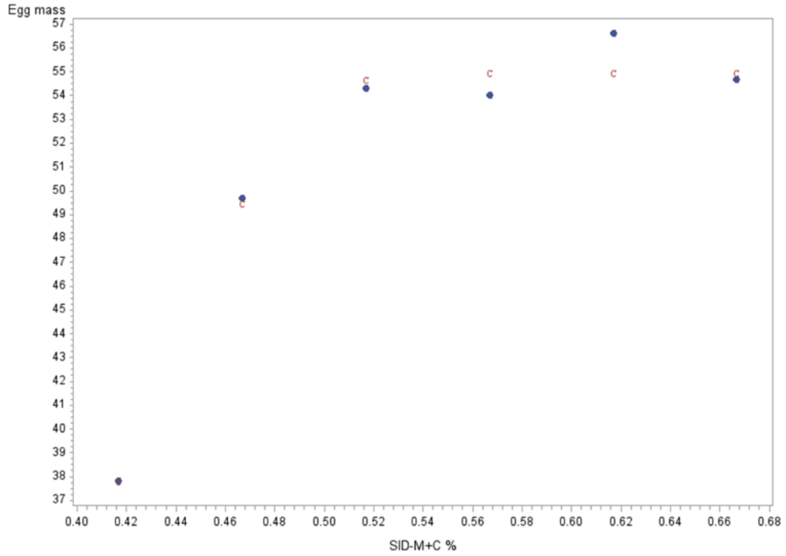
Narvaez-Solarte et al. (2005)

SID-M+C EM (%) 0.532

SID-M+C FCR (%) 0.536</td>
<td>
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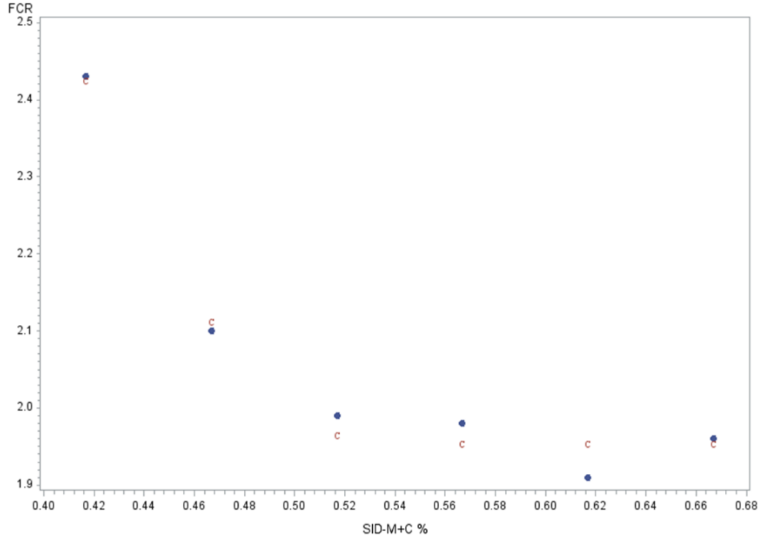
<td>
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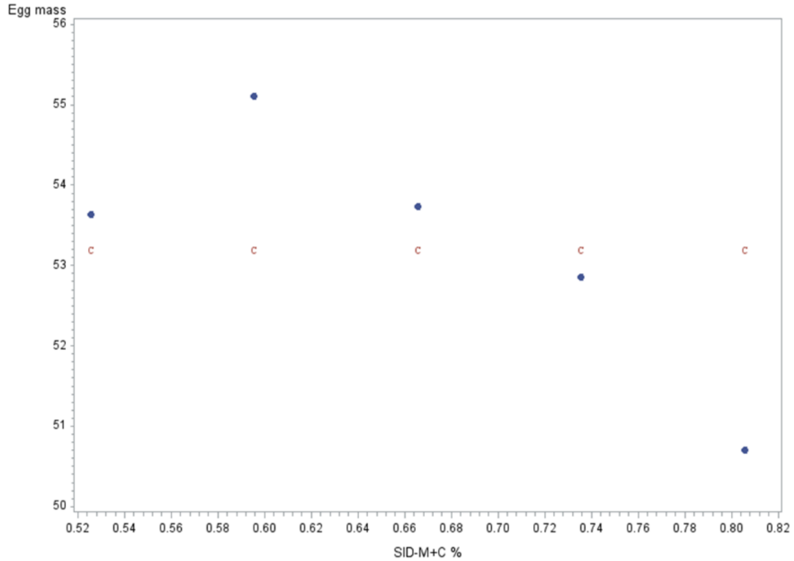
Filho et al. (2006)

SID-M+C EM (%) Not possible to estimate

SID-M+C FCR (%) Not possible to estimate</td>
<td>
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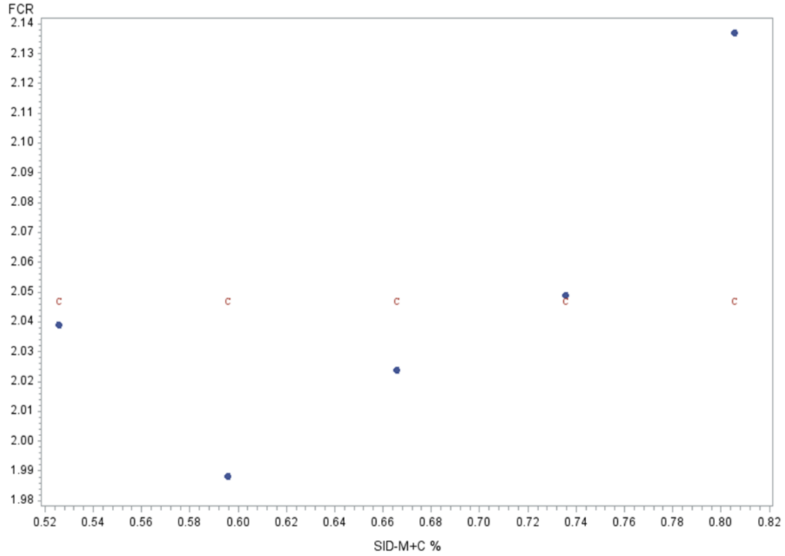
<td>
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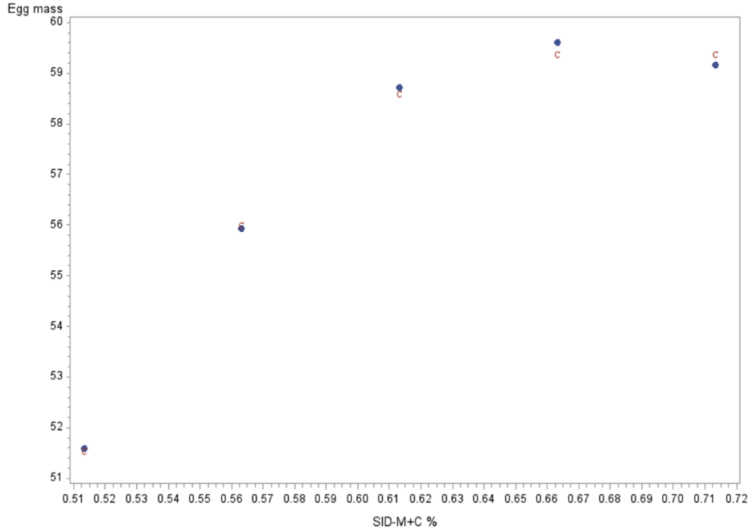
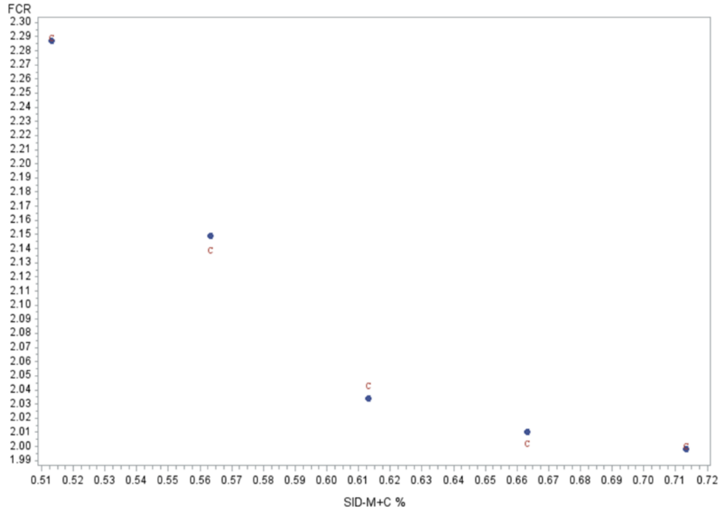
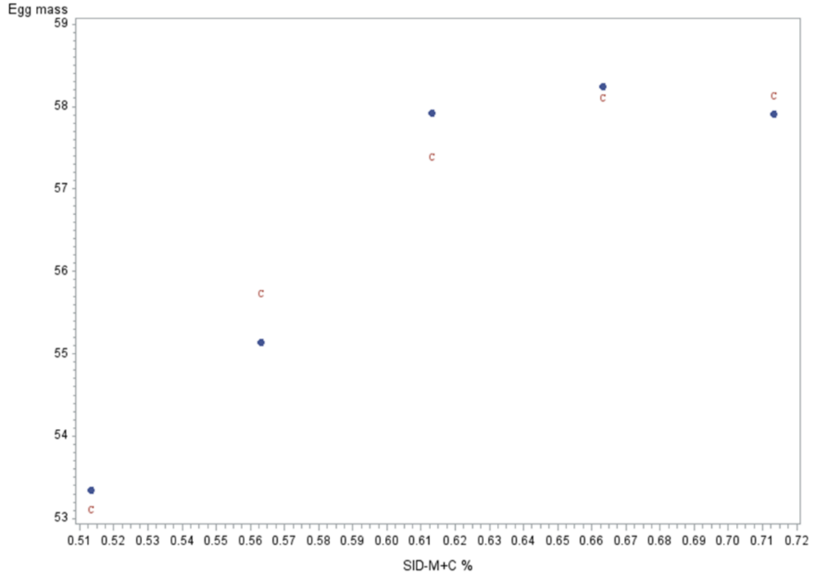
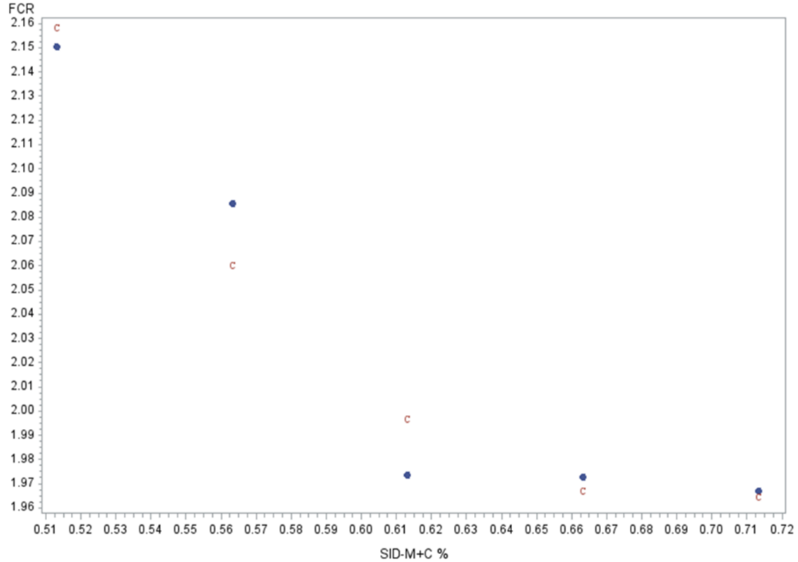
11. Sa et al. (2007) Trial 1 SID-M+C EM (%) 0.659 SID-M+C FCR (%) 0.676		
12. Sa et al. (2007) Trial 2 SID-M+C EM (%) 0.675 SID-M+C FCR (%) 0.682		

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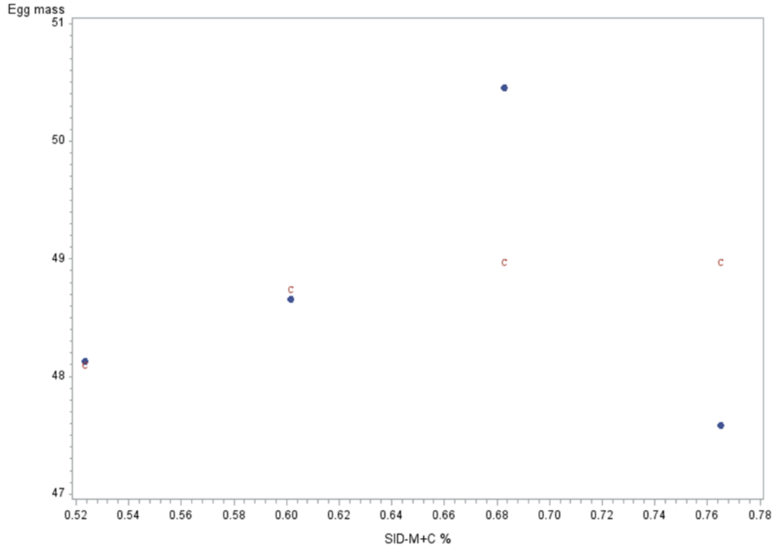
<td>13.
Novak et al. (2004)
Trial 1

SID-M+C EM (%) 0.685

SID-M+C FCR (%) not possible to estimate</td>
<td>
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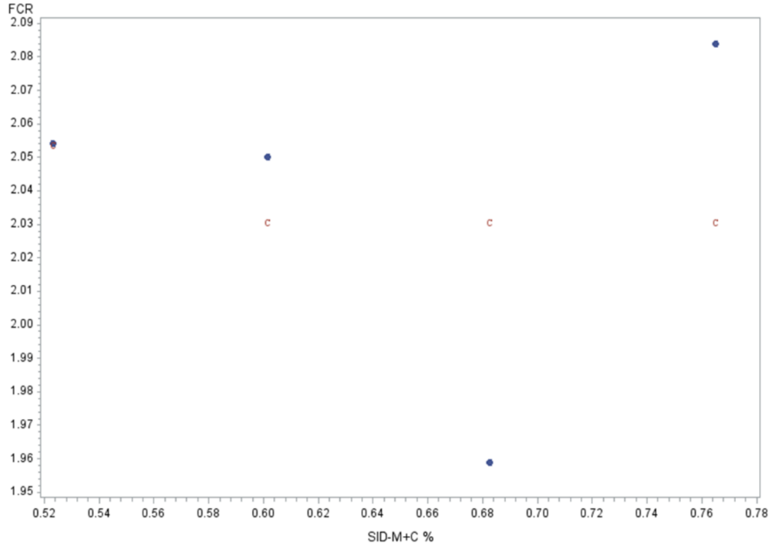
<td>
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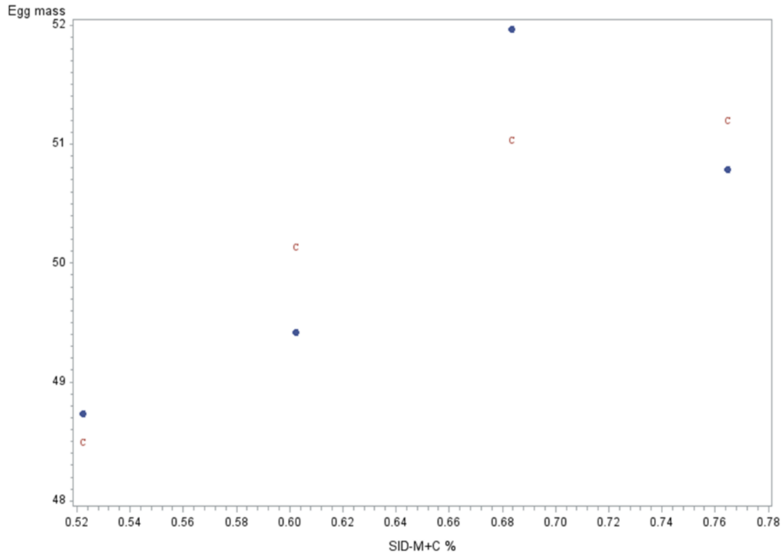
Novak et al. (2004)
Trial 2

SID-M+C EM (%) 0.737

SID-M+C FCR (%) 0.737</td>
<td>
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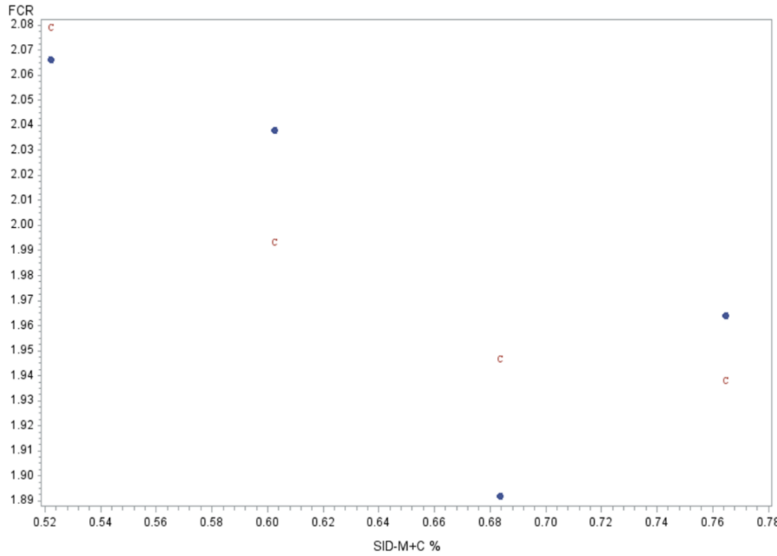
<td>
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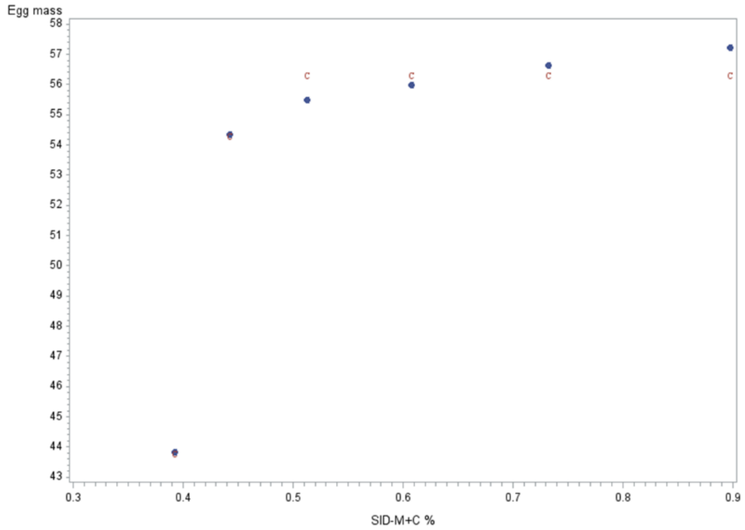
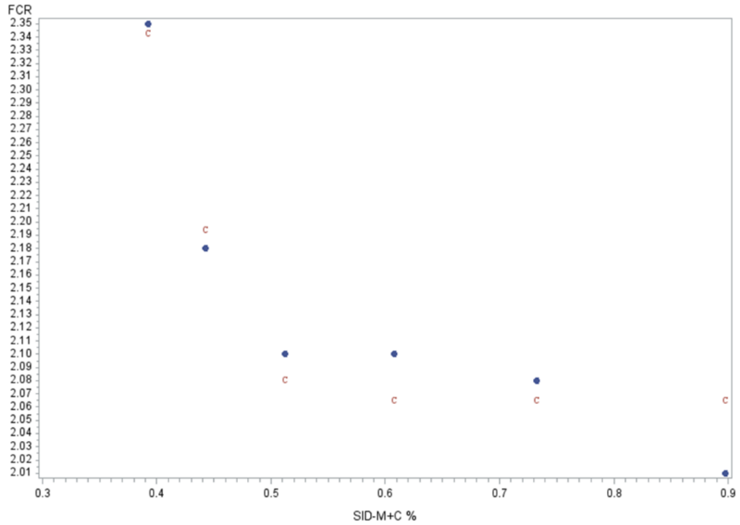
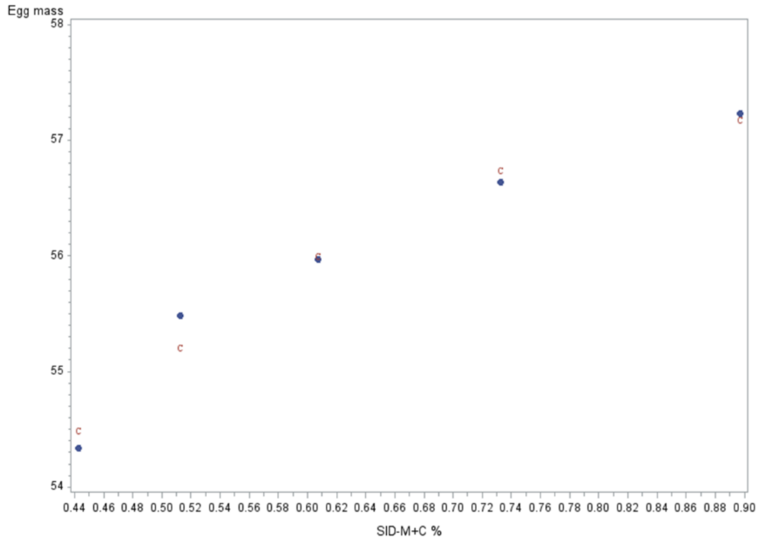
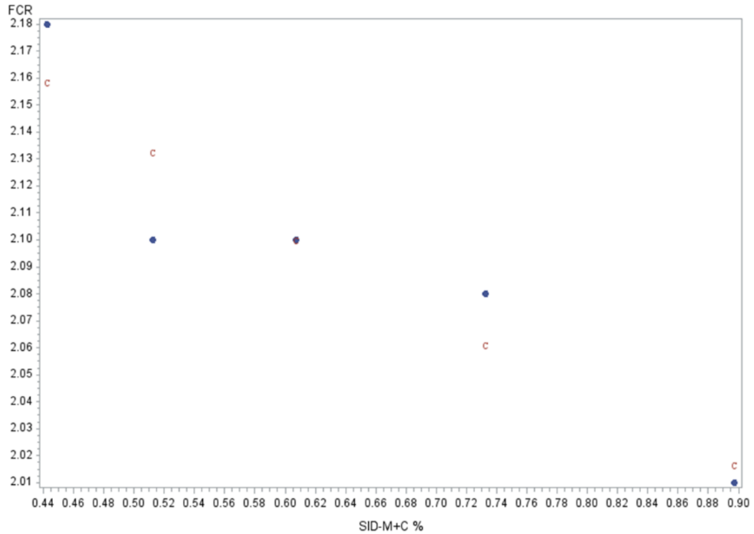
15. Schutte et al. (1994) Trial 1 SID-M+C EM (%) 0.476 SID-M+C FCR (%) 0.549		
15a. Schutte et al. (1994) Trial 1 SID-M+C EM (%) 0.934 SID-M+C FCR (%) 1.724 (extrapolated value)		

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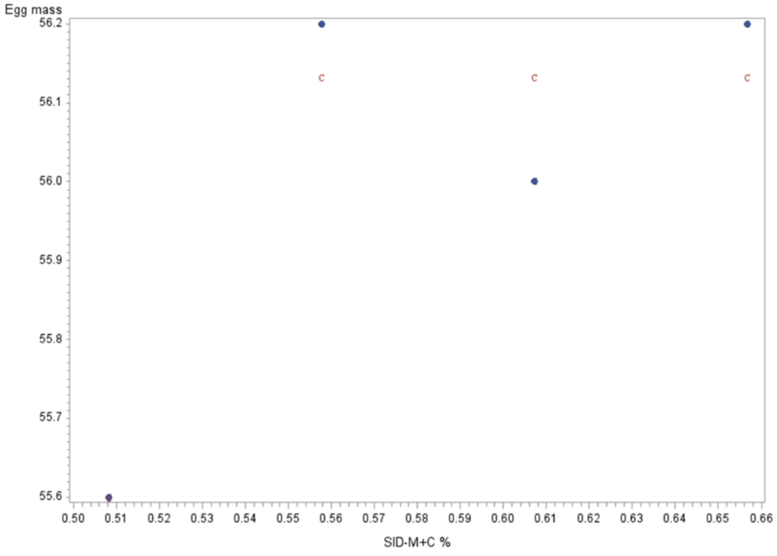
Schutte et al. (1994)
Trial 2

SID-M+C EM (%) 0.525 (no unique estimation possible)

SID-M+C FCR (%) 0.595</td>
<td>
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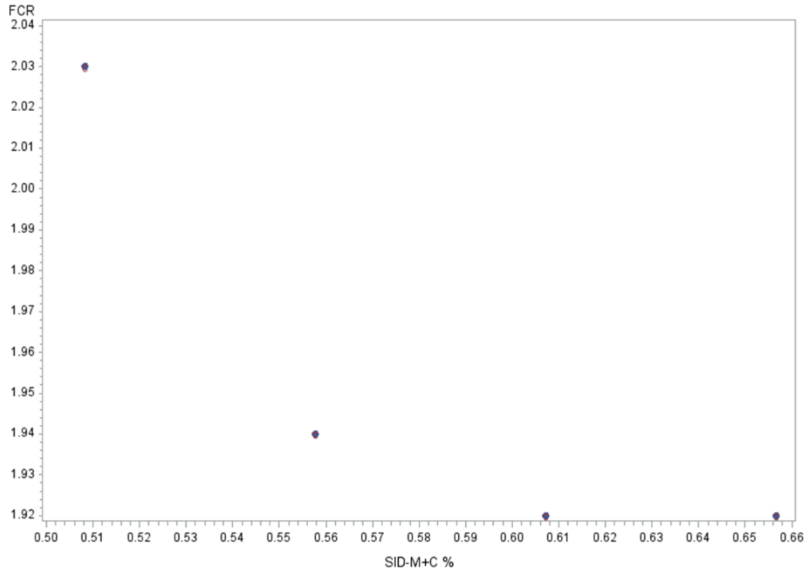
<td>
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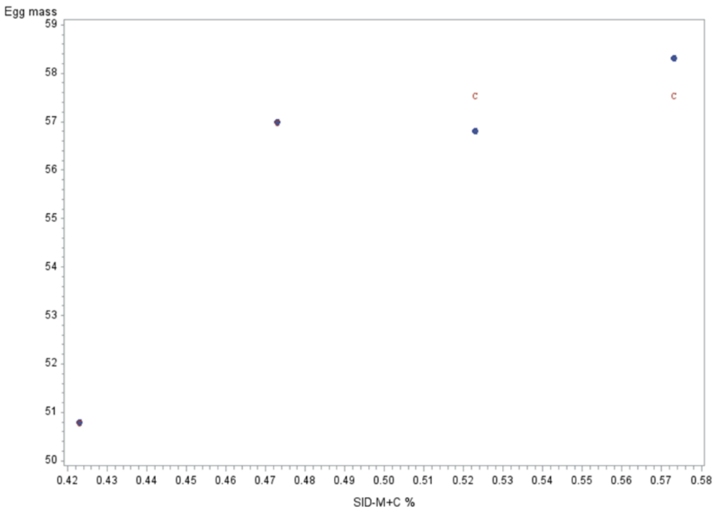
<td>17.
Danner and Bessei (2002)

SID-M+C EM (%) 0.493

SID-M+C FCR (%) 0.577</td>
<td>
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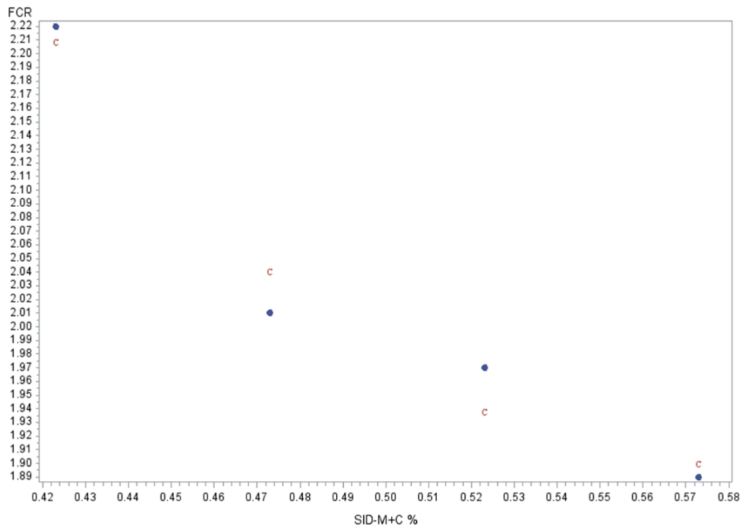
<td>
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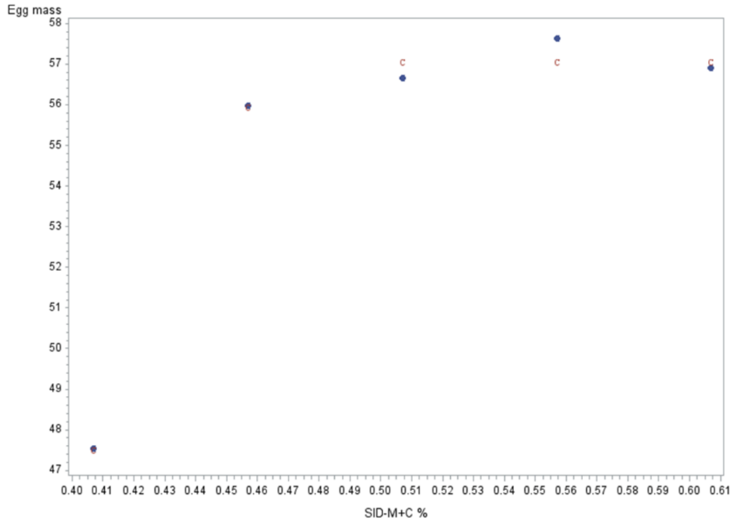
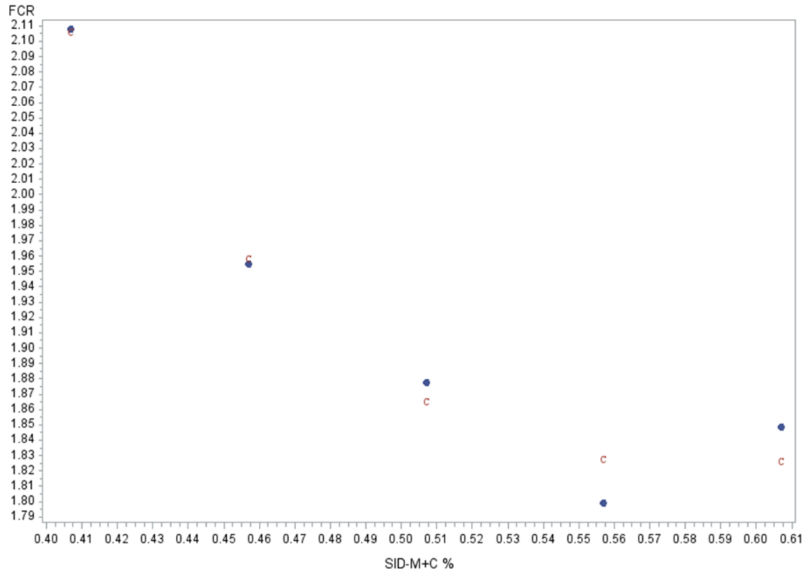
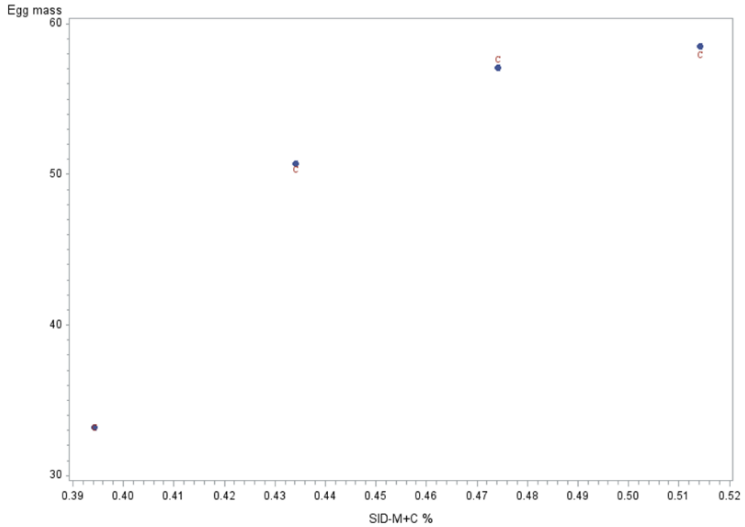
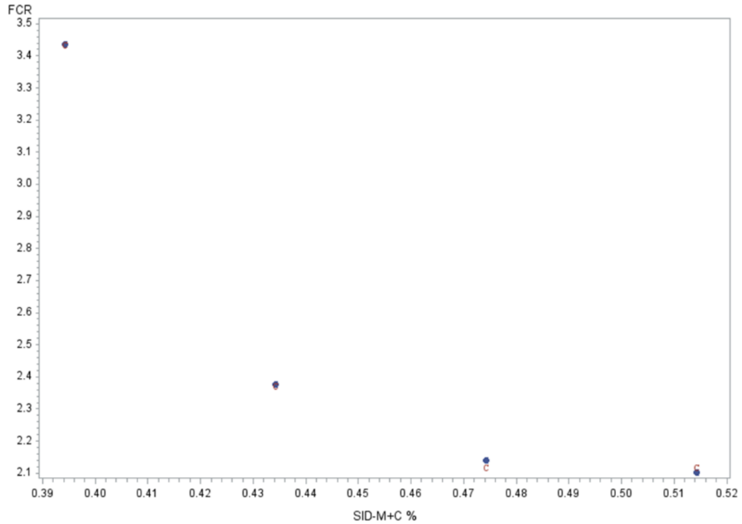
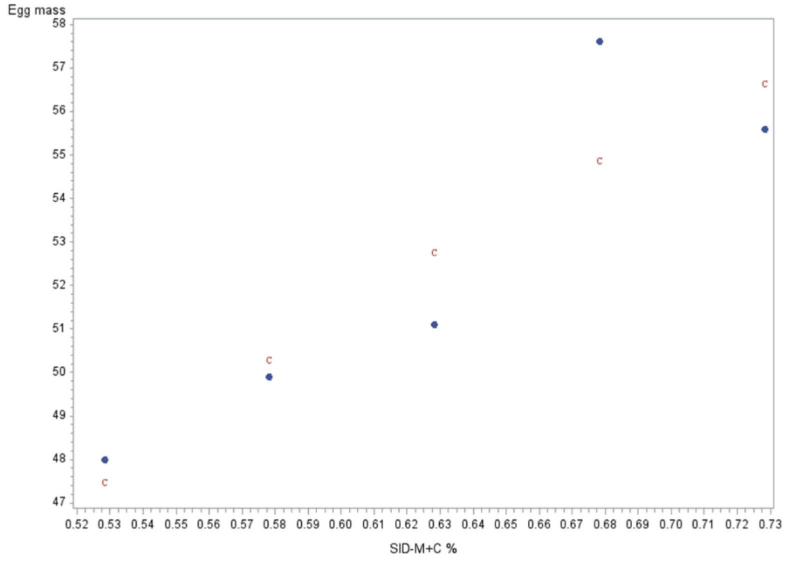
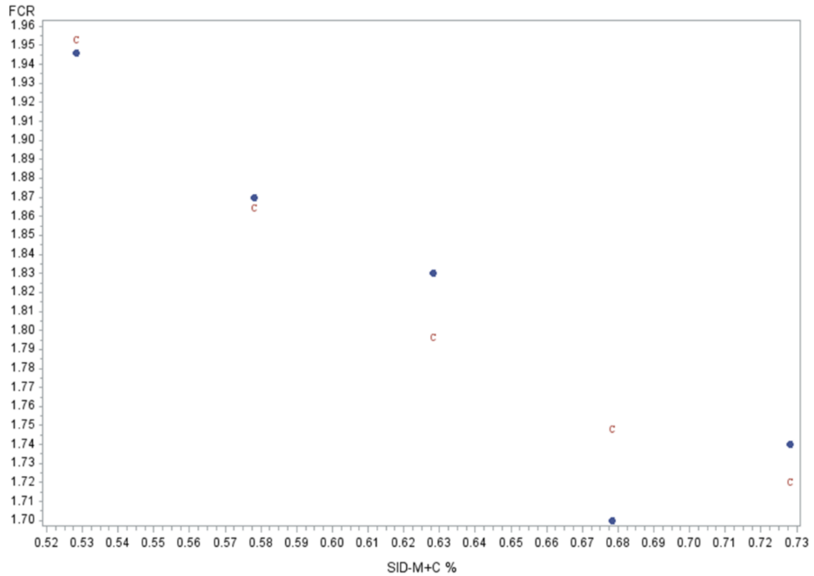
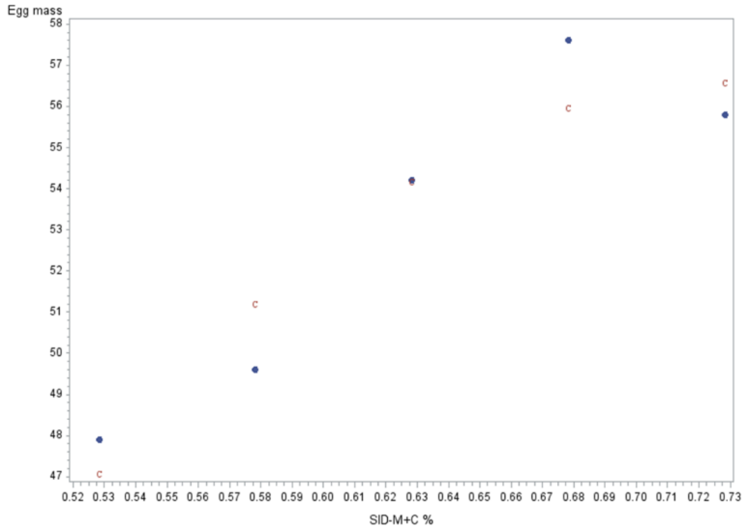
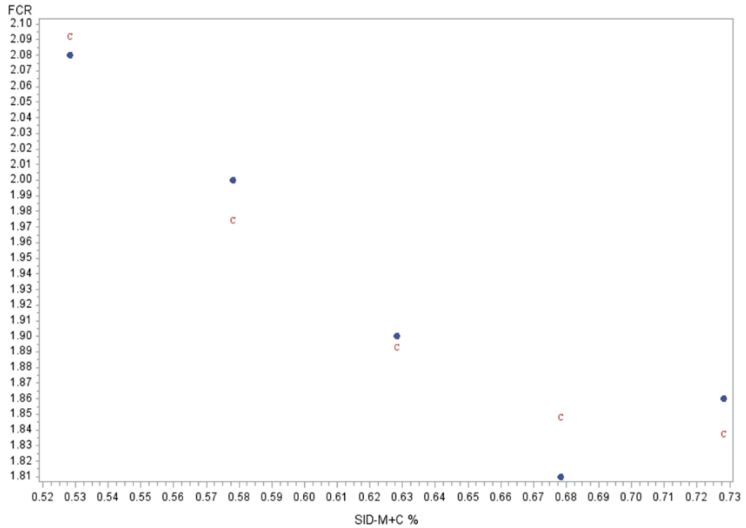
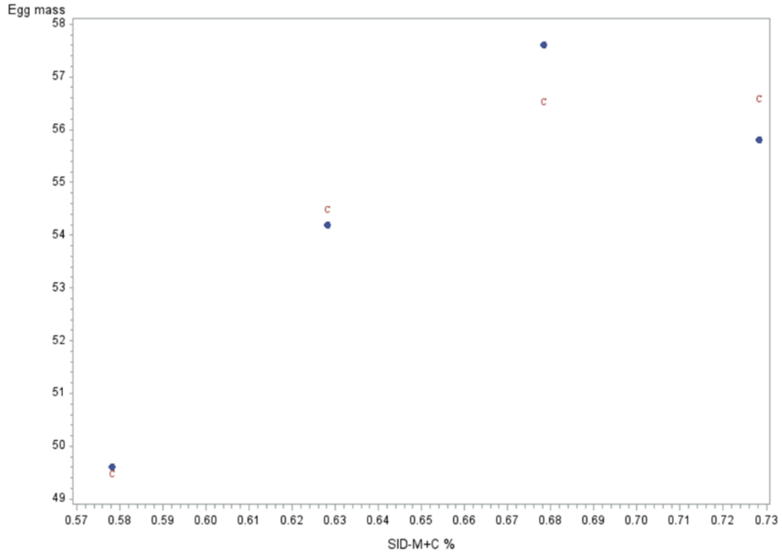
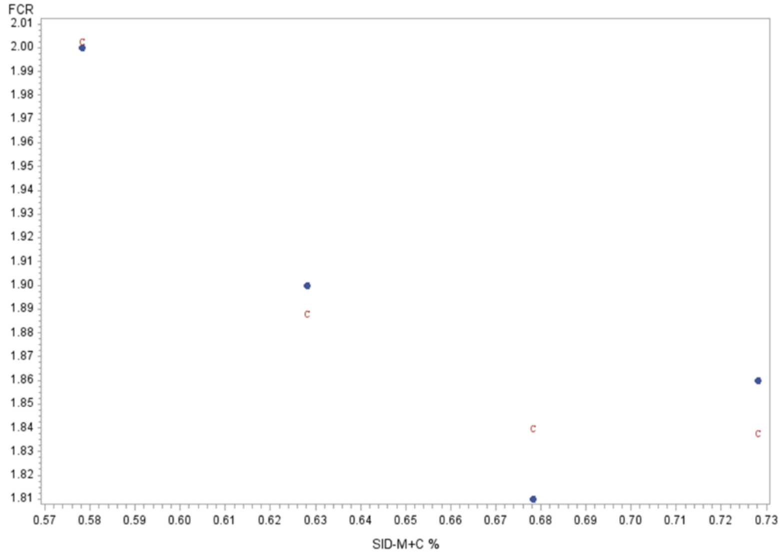
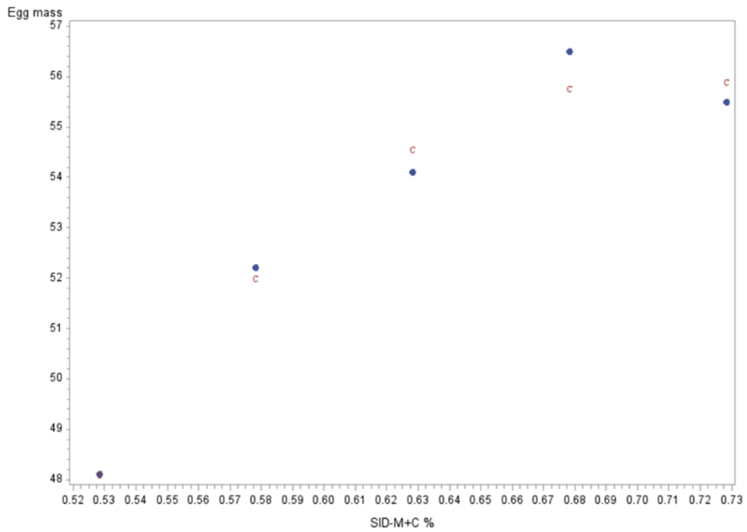
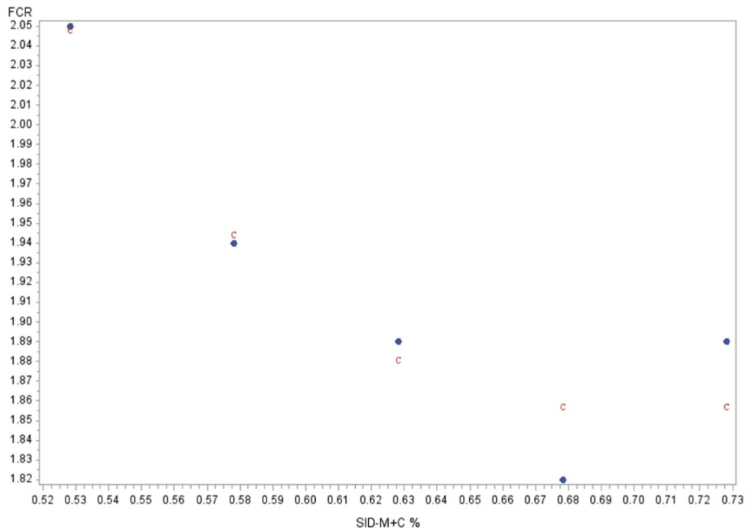
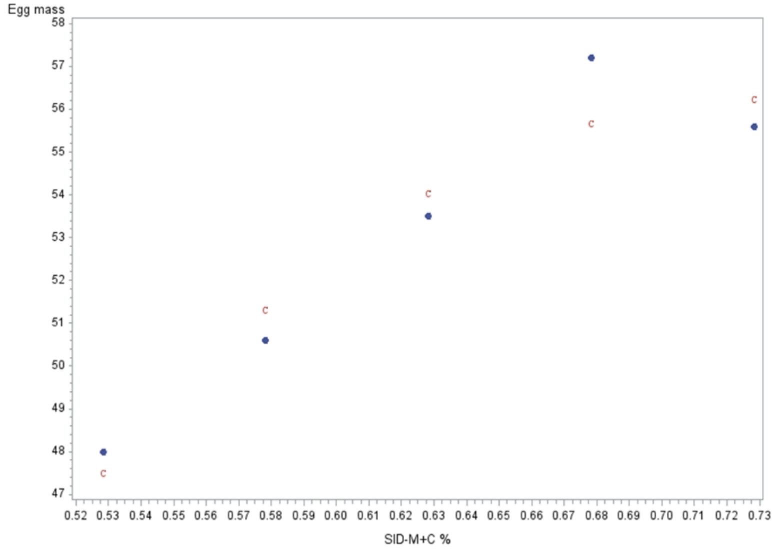
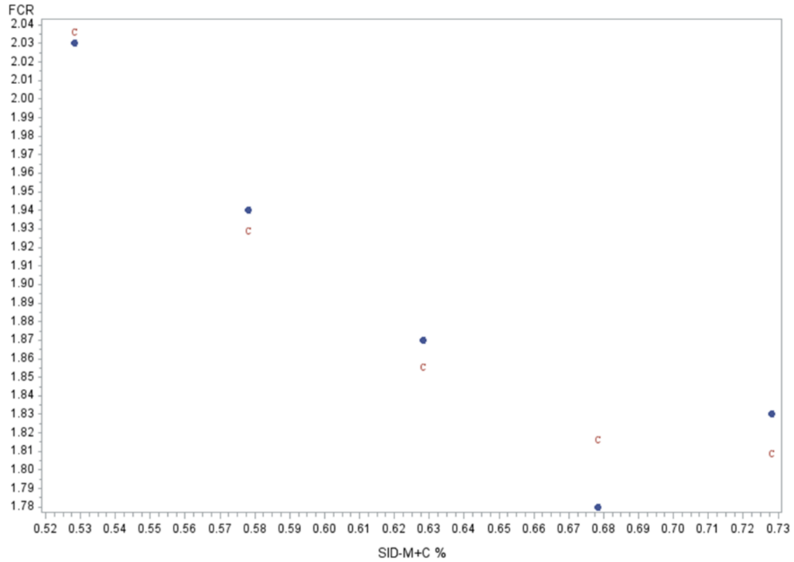
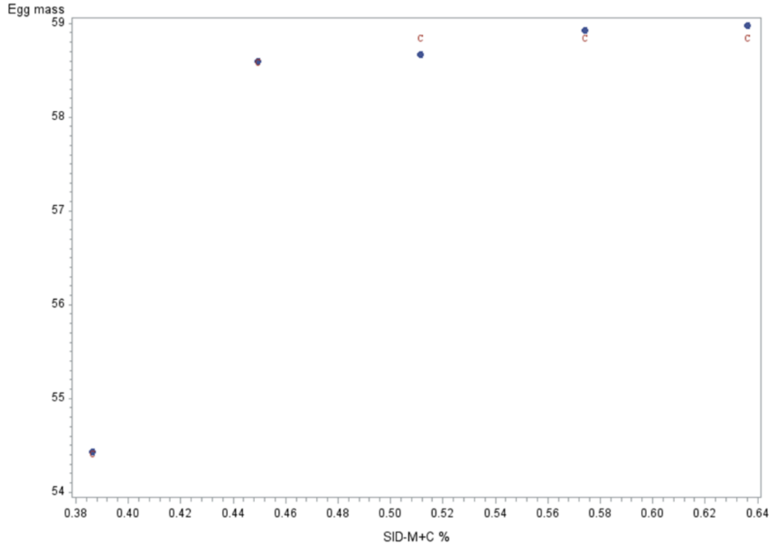
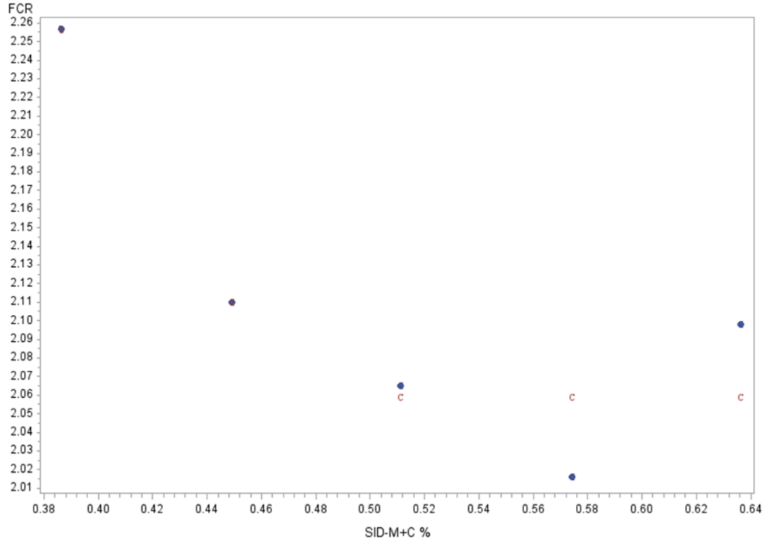
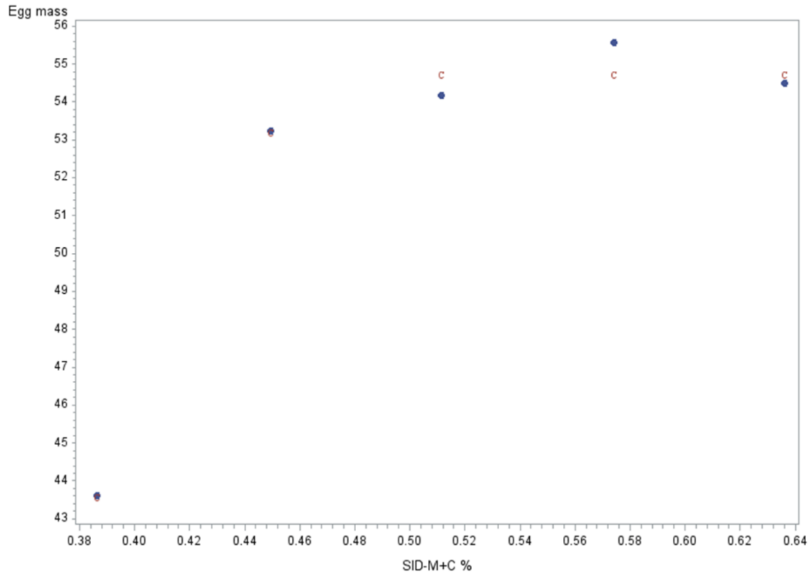
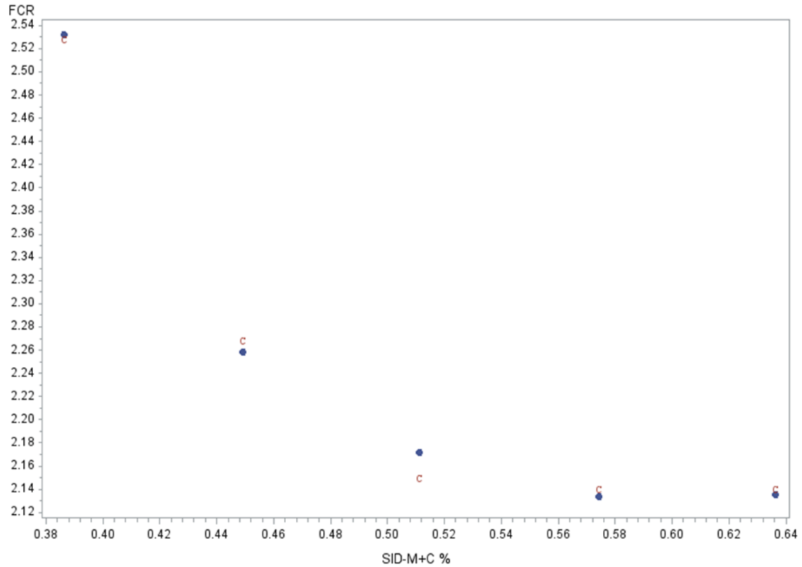
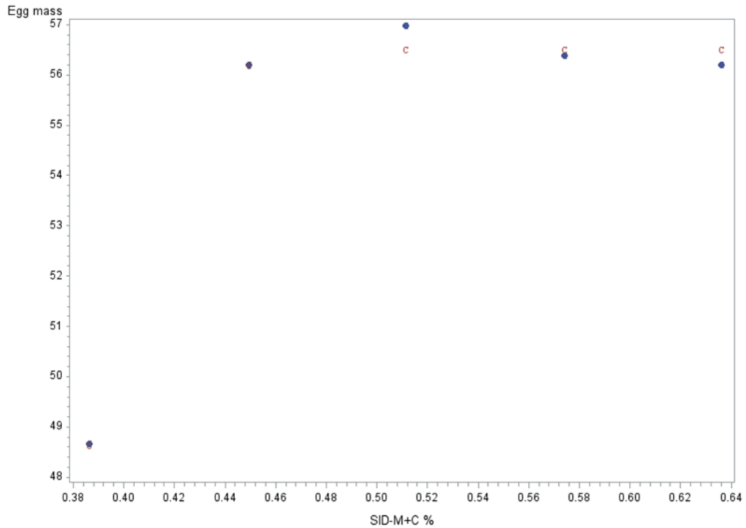
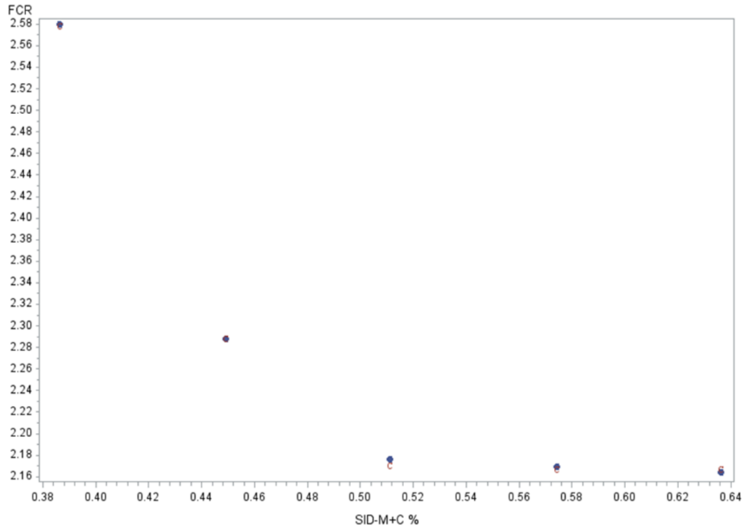
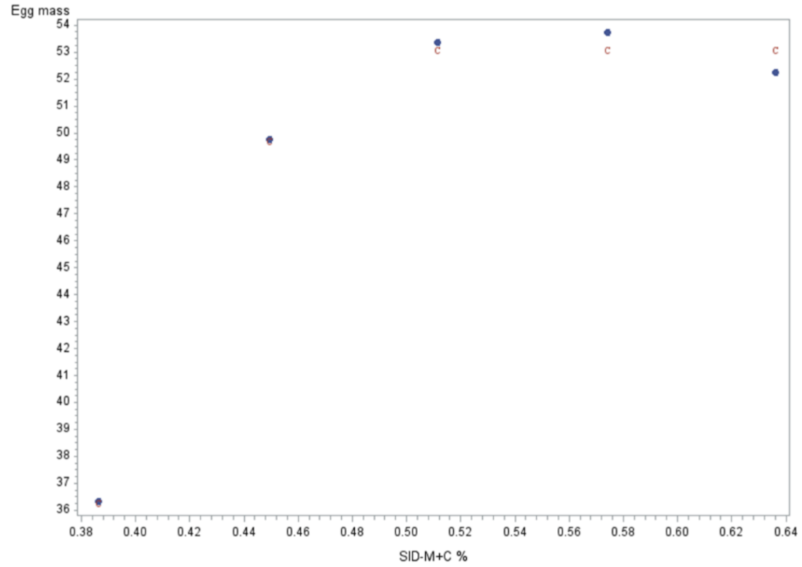
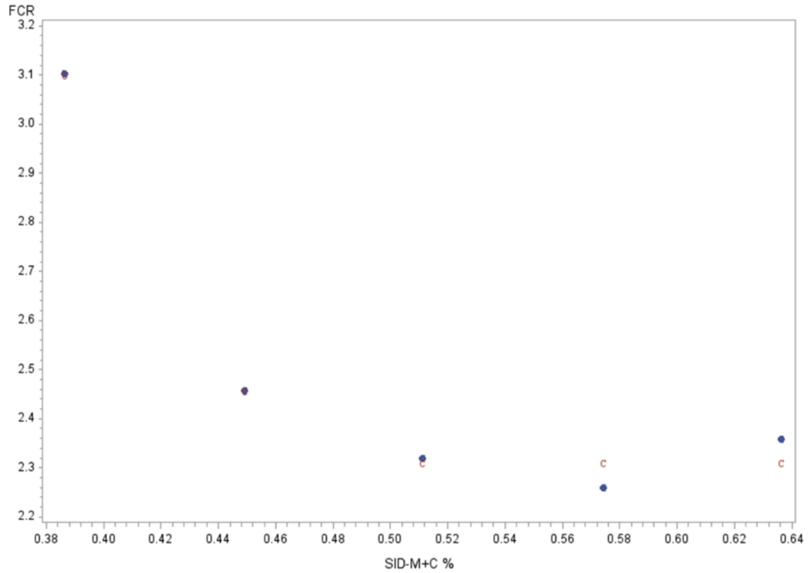
18. Bertram et al. (1995) SID-M+C EM (%) 0.483 SID-M+C FCR (%) 0.567		
19. Lemme et al. (2004) SID-M+C EM (%) 0.484 SID-M+C FCR (%) 0.466		

20. Kakhi et al. (2016) Trial 1 SID-M+C EM (%) 0.949 (extrapolation) SID-M+C FCR (%) 0.772		
21. Kakhi et al. (2016) Trial 2 SID-M+C EM (%) 0.730 SID-M+C FCR (%) 0.716		

21a. Kakhi et al. (2016) Trial 2 SID-M+C EM (%) 0.687 SID-M+C FCR (%) 0.690		
22. Kakhi et al. (2016) Trial 3 SID-M+C EM (%) 0.699 SID-M+C FCR (%) 0.682		

23. Kakhi et al. (2016) Trial 4 SID-M+C EM (%) 0.729 SID-M+C FCR (%) 0.711		
24. Star and van Krimpen (2016) Trial 1 SID-M+C EM (%) 0.469 SID-M+C FCR (%) 0.514		

25. Star and van Krimpen (2016) Trial 2 SID-M+C EM (%) 0.486 SID-M+C FCR (%) 0.535		
26. Star and van Krimpen (2016) Trial 3 SID-M+C EM (%) 0.465 SID-M+C FCR (%) 0.524		

27. Star and van Krimpen (2016) Trial 4 SID-M+C EM (%) 0.500 SID-M+C FCR (%) 0.497		

Appendix B. SID-M+C model estimates for minimum FCR and maximum EM

SID-M+C model estimates 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 observation with the lowest dietary SID-M+C level. If no letter is shown behind the trial number it means that the model is fitted based on all observations of the trial. Values of R that are bold are estimated requirement values for SID-M+C that are situated beyond the measurement range.

Trial nr.	Estimate L	Std. Err. L	Estimate R	Std. Err. R	Estimate U	Std. Err. U	R^2
1	1.911	0.0121	0.500	.	22.9	2.11	0.983
2	1.655	0.0066	0.842	0.0322	2.7	0.87	0.977
2a	1.656	0.0045	0.809	0.0247	5.0	2.05	0.979
3	1.716	0.0149	0.802	0.0440	6.6	3.62	0.939
4	2.144	0.0248	0.641	0.0431	11.6	6.83	0.950
5	2.000	0.0361	0.580	0.0425	38.9	37.92	0.897
6	1.362	4.8642	**1.488**	5.1878	1.1	6.14	0.926
7	1.970	0.0086	0.680	0.0239	5.3	1.36	0.991
8	1.895	0.0106	0.738	0.0328	5.5	2.12	0.978
9	1.955	0.0186	0.536	0.0183	33.4	10.66	0.981
10							
11	2.001	0.0091	0.676	0.0144	10.9	1.90	0.996
12	1.965	0.0210	0.682	0.0495	6.8	3.94	0.955
13							
14	1.939	0.0720	0.737	0.2849	3.1	7.82	0.683
15	2.066	0.0233	0.549	0.0505	11.3	7.60	0.927
15a	1.916	0.7076	**1.724**	4.6247	0.1	0.65	0.869
16	1.920	0.0000	0.595	0.0000	14.8	0.00	1.000
17	1.900	0.0504	*0.577*	0.0595	13.0	9.39	0.963
18	1.827	0.0208	0.567	0.0337	11.0	4.61	0.975
19	2.121	0.0200	0.466	0.0036	258.2	26.20	0.999
20	1.713	0.0867	0.772	0.1717	4.0	4.74	0.900
21	1.839	0.0339	0.716	0.0621	7.2	4.60	0.942
21a	1.838	0.0323	0.690	0.0669	13.2	15.44	0.922
22	1.858	0.0262	0.682	0.0615	8.1	6.49	0.911
23	1.810	0.0292	0.711	0.0594	6.8	4.29	0.944
24	2.060	0.0244	0.514	0.0611	12.0	11.76	0.896
25	2.141	0.0122	0.535	0.0161	17.6	3.83	0.994
26	2.168	0.0028	0.524	0.0034	21.6	1.09	1.000
27	2.313	0.0285	0.497	0.0158	64.8	18.83	0.990

SID-M+C model estimates 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 observation with the lowest dietary SID-M+C level. If no letter is shown behind the trial number it means that the model is fitted based on all observations of the trial. Values of R that are bold are estimated requirement values for SID-M+C that are situated beyond the measurement range.

Trial nr.	Estimate L	Std. Err. L	Estimate R	Std. Err. R	Estimate U	Std. Err. U	R^2
1	51.5	0.05	0.561	0.0044	-322	16	1.000
2	54.4	0.98	**0.920**	0.1101	-66	45	0.934
2a	55.0	4.24	**1.017**	0.5482	-36	85	0.845
3	55.0	1.07	**0.920**	0.0789	-100	49	0.965
4	52.7	0.69	0.635	0.0426	-348	210	0.949
5	56.3	0.25	0.609	0.0124	-618	133	0.994
6	55.9	1.70	**0.786**	0.0767	-129	52	0.992
7	58.0	0.27	0.661	0.0222	-213	58	0.989
8	53.1	0.16	0.783	0.0131	-140	16	0.998
9	55.0	0.62	0.532	0.0167	-1291	386	0.984
10							
11	59.4	0.17	0.659	0.0092	-368	47	0.998
12	58.1	0.48	0.675	0.0437	-191	102	0.961
13	49.0	1.48	0.685	0.8917	-33	371	0.111
14	51.2	1.20	0.737	0.2481	-59	131	0.739
15	56.3	0.38	0.476	0.0116	-1808	516	0.986
15a	57.2	0.30	**0.934**	0.1189	-11	5	0.977
16	56.1	0.07	0.525	.	-1882	470	0.889
17	57.6	0.75	0.493	0.0322	-1379	1228	0.967
18	57.1	0.29	0.483	0.0101	-1663	439	0.993
19	58.0	0.70	0.484	0.0077	-3088	520	0.998
20	60.1	25.76	**0.949**	1.1647	-71	258	0.819
21	56.6	1.75	*0.730*	0.0874	-234	194	0.904
21a	56.6	1.10	0.687	0.0526	-595	564	0.948
22	55.9	0.57	0.699	0.0337	-267	103	0.979
23	56.3	1.30	*0.729*	0.0706	-217	147	0.932
24	58.9	0.10	0.469	0.0097	-641	147	0.996
25	54.7	0.42	0.486	0.0159	-1114	355	0.989
26	56.5	0.24	0.465	0.0146	-1262	458	0.993
27	53.1	0.44	0.500	0.0117	-1294	272	0.995