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

Tryptophan requirement studies were selected from literature (1990 – 2017) in which the dietary TRP content was varied by means of addition of graded levels of dietary synthetic TRP. 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-TRP : AFD-LYS ratio needed to be at least 20% below the CVB (2012) AFD-TRP : 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-TRP (%) < R) then EM or FCR = L + U × (R – SID-TRP)^{2};
Else EM or FCR = L + U × 0;
Where:
L = plateau value for EM or FCR
R = break-point value for SID-TRP (%)
U = slope value, representing the increase in EM or decrease in FCR per unit increase in dietary SID-TRP.

Estimated SID-TRP requirements for EM and FCR were regressed against factors such as EM, FCR, age, and the dietary factors CP, ME and CP : ME ratio.

3   Results and Discussion

In Table 1 some characteristics of the studies included in the meta-analysis is given. The dataset consisted of 8 studies with in total 9 trials and 49 observations.

In Appendix A for each titration trial the relationship between dietary SID-TRP (%) and FCR and between dietary SID-TRP (%) and EM is presented graphically together with the estimated SID-TRP requirements for the quadratic broken-line model.
In Appendix B the estimated quadratic broken-line model parameters for each titration trial is given.

For one titration trial it was not possible to estimate a unique SID-TRP requirement for maximum EM (for trial 4) and for one titration trial it was not possible to estimate a unique SID-TRP requirement for minimum FCR (for trial 1). Furthermore, results from trial 9 were also excluded as this was a trial in which the amino acids ILE, VAL, LEU, PHE and TYR were fed in large excess of requirements.

In Table 2 the average estimated optimal SID-TRP concentrations and SID-TRP intake statistics for maximum EM minimum FCR are presented.

Table 2. Estimated optimal SID-TRP requirements (% and daily intake) for maximum egg mass (EM) and minimum FCR.

	Parameter	N*	Mean	Std. Dev.	Min.	Max	%CV
SID-TRP (%)	EM	7	0.160	0.0149	0.146	0.188	9.3
	FCR	7	0.157	0.0231	0.122	0.188	14.7
SID-TRP intake (mg/d)	EM	7	153	22.5	121	180	14.7
	FCR	7	151	26.0	101	175	17.3
SID-TRP intake per g of EM (mg/g)	EM	7	**3.0**	0.31	2.6	3.5	10.3
	FCR	7	**3.0**	0.43	2.3	3.6	14.3
SID-TRP:SID-LYS ratio	EM	7	23.0	3.26	18.2	27.7	14.2
	FCR	7	22.3	4.25	15.3	28.5	19.1
SID-TRP:SID-LYS ratio**	EM	7	25.3	4.33	21.3	33.8	17.2
	FCR	7	24.6	5.34	18.9	33.8	21.7
SID-TRP:SID-LYS ratio**	EM	6***	23.8	2.35	21.3	27.7	9.8
	FCR	7	24.6	5.34	18.9	33.8	21.7

* Total number of titration trials is 9. For two titration trials it was not possible to estimate unique SID-TRP requirements for FCR (trials 1 and 9), for one titration trial it was not possible to estimate a unique TRP requirement for EM and also it was decided not to include results from trial 9 in the analysis due to large excesses of a number of amino acids resulting in a total of 7 titration trials that could be used for estimation of SID-TRP requirements for maximum EM.
**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-TRP:SID-LYS ratio, otherwise the observed SID-LYS intake at which maximum EM was estimated was used.
***There was one estimated SID-TRP:SID-LYS requirement value for maximum EM that was an outlier value (from trial 2) according to the modified Z-score (a score used for small sample sizes). Therefore it was chosen to exclude this value.

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-TRP (%)	Max SID-TRP (%)	Max. FCR minus Min. FCR	Max. egg mass minus Min. egg mass
Esteve-Garcia et al. (2001)	1	Hy-line Brown	44	12	15.9	85	57	115	0.100	0.160	0.082	3.4
Calderano et al. (2012)	2	Hy-line W36	24	16	14.5	80	43	78	0.145	0.189	0.065	3.2
Calderano et al. (2011)	3	Hy-line W36	42	16	14.5	82	50	90	0.129	0.173	0.090	2.6
Deponti et al. (2007)	4	Hisex white	51	7	16.0	92	59	113	0.109	0.187	0.117	5.7
Russel and Harms (1999)	5	Hy-line W36	55	4	13.2	83	50	91	0.090	0.210	0.151	16.5
de Lima et al. (2012)	6	White laying hens	29	20	15.7	97	62	106	0.134	0.202	0.131	5.4
Mousavi et al. (2017)	7	Hy-line W36	30	6	14.0	86	49	92	0.083	0.233	0.344	15.0
Peganova et al. (2003)	8	Lohmann Brown	31	6	12.5	84	50	121	0.080	0.205	0.252	16.3
	9	Lohmann Brown	31	6	12.5	86	52	122	0.080	0.205	0.153	15.0

In Figure 1 the relationship between egg mass and dietary SID-TRP intake is presented for the various titration trials (titration trial 9 excluded). Furthermore, in Figure 1 the observations of the individual titration trials after the maximum egg mass has been reached have been excluded from the analysis. Various relationships were fitted through the data. The power relationship fitted the data best.
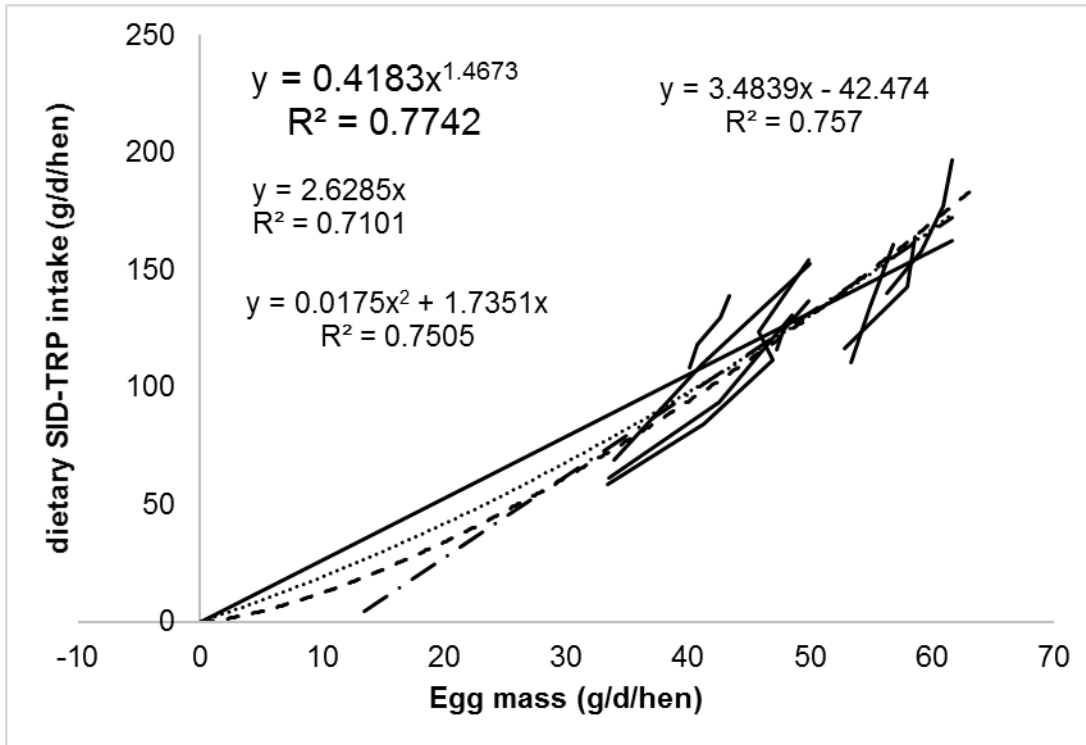

Figure 1. relationship between egg mass and dietary SID-TRP intake for the various titration trials (titration trial 9 excluded). In these relationships, observations of the individual titration trials after the maximum egg mass has been reached have been excluded. Various relationships were fitted through the data such as a linear relationship including an intercept value (long dash, dot), a linear relationship with intercept value zero (solid line), a polynomial relationship with intercept value zero (dotted line) and a power relationship (dashed line).

In Table 3 the estimated optimal SID-TRP requirements for maximum EM are shown based on SID-TRP requirements as estimated in Figure 1 using the power relationship. Furthermore, also the estimated SID-TRP:SID-LYS ratios are shown in Table 3 based on the SID-TRP requirements for EM as estimated in Fig. 1 using the power relationship and based on the SID-LYS requirements using formula [F8] in CVB Documentation report nr. 69.

Table 3. Estimated optimal SID-TRP 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 power relationship for estimation of SID-TRP requirement as shown in Figure 1. The calculated feed intake required for an average egg weight of 60 g and at egg production rates of 85, 90 and 95% are based on the assumptions presented as a footnote (*) underneath this Table.

BW (kg)	Feed intake (g/d)		Egg mass (g/d)		SID-TRP (mg/d)		Dietary SID-TRP (%)		SID-TRP:SID-LYS ratio**	
	Egg production rate (%)									
	90	95	90	95	90	95	90	95	90	95
1.5	112	115	54	57	146	158	0.130	0.137	20	20
1.6	114	117	54	57	146	158	0.127	0.134	20	20
1.7	117	120	54	57	146	158	0.124	0.131	20	20
1.8	120	123	54	57	146	158	0.122	0.128	20	20
1.9	122	125	54	57	146	158	0.119	0.126	20	20
2.0	125	128	54	57	146	158	0.117	0.123	20	20

*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-TRP:SID-LYS ratio for maximum EM is calculated based on the ratio between SID-TRP intake (SID-TRP requirements calculated using the power relationship in Figure 1) and SID-LYS intake which is based on formula [F8] described in CVB Documentation report nr. 69.

In Table 4 the estimated optimal SID-TRP requirements for maximum EM and minimum FCR are presented based on a SID-TRP requirement of 3.0 mg per g of EM (based on the average estimated SID-TRP requirement estimates for maximum EM and minimum FCR per gram of EM as presented in Table 2). Results in Table 4 show a SID-TRP:SID-LYS requirement ratio of 22%.

Summarised, in Table 2 the average estimated optimal SID-TRP:SID-LYS ratios vary between 22 and 23 for the estimated optimal SID-TRP:SID-LYS ratios and between 24 and 25 for the estimated optimal SID-TRP:SID-LYS ratios in which SID-LYS is estimated based on CVB Documentation report nr. 69. When relating SID-TRP requirement to EM as is done in Figure 1 and Table 3 using the power relationship an optimal SID-TRP:SID-LYS ratio of 20 is found. Furthermore, when estimating the optimal SID-TRP:SID-LYS based on average estimated SID-TRP requirements of 3.0 mg per g of EM (based on results presented in Table 2) and SID-LYS requirements as estimated based on CVB Documentation report nr. 69 an average SID-TRP:SID-LYS requirement ratio of 22 is found (Table 4).

Table 4. Estimated optimal SID-TRP 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 SID-TRP requirement of 3.0 mg per g of EM as presented in Table 2. The calculated feed intake required for an average egg weight of 60 g and at egg production rates of 85, 90 and 95% are based on the assumptions presented as a footnote (*) underneath this Table.

BW (kg)	Feed intake (g/d)		Egg mass (g/d)		SID-TRP (mg/d)		Dietary SID-TRP (%)		SID-TRP:SID-LYS ratio**	
	Egg production rate (%)									
	90	95	90	95	90	95	90	95	90	95
1.5	112	115	54	57	162	171	0.145	0.149	22	22
1.6	114	117	54	57	162	171	0.142	0.146	22	22
1.7	117	120	54	57	162	171	0.138	0.142	22	22
1.8	120	123	54	57	162	171	0.135	0.139	22	22
1.9	122	125	54	57	162	171	0.132	0.136	22	22
2.0	125	128	54	57	162	171	0.130	0.134	22	22

*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-TRP:SID-LYS ratio for maximum EM is calculated based on the ratio between SID-TRP intake (SID-TRP requirements calculated based on a SID-TRP requirement of 3.0 mg per g of EM as presented in Table 2) and SID-LYS intake which is based on formula [F8] described in CVB Documentation report nr. 69.

4   Conclusions

It is concluded that a SID-TRP supply of 3.0 mg per gram of EM produced is sufficient for optimal performance. A SID-TRP supply of 3.0 mg per gram of EM produced combined with a SID-LYS requirement estimated using formula [F8] in CVB documentation report number 69 results in a SID-TRP : SID-LYS requirement ratio of 22%.
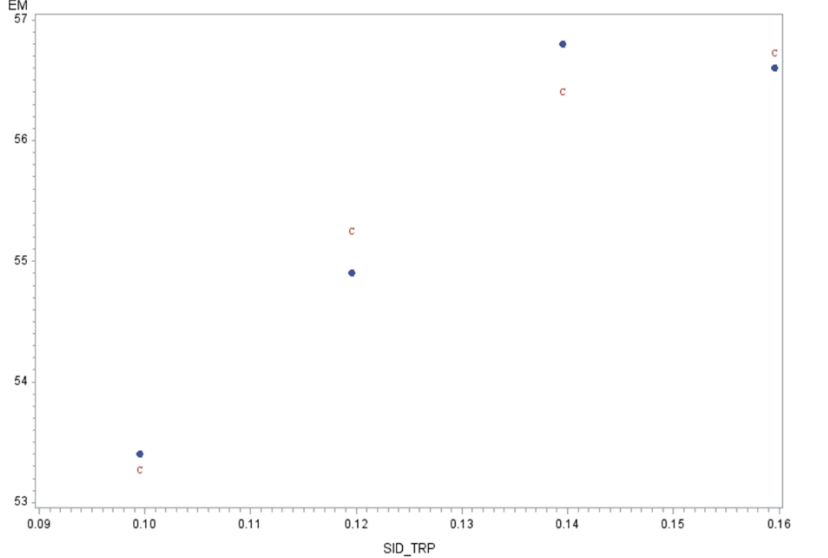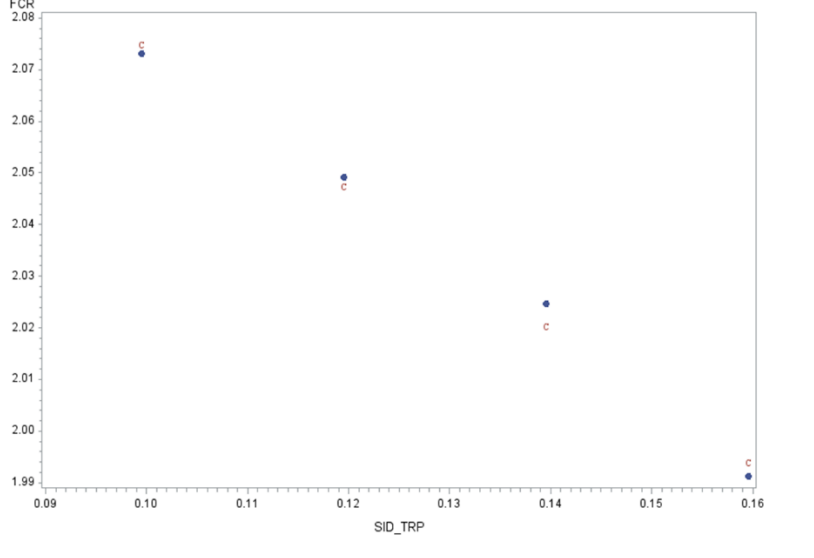
List of studies included in the meta-analysis

Calderano, A. A. 2011. Relacao triptofano:Lisina em racoes para poedeiras level. Thesis, Univeristy of Vicosa, Brasil, pp 1-115.

Calderano, A. A., P. C. Gomes, J. L. Donzele, S. L. D. Barreto, C. R. Vellasco, H. H. D. Mello, G. R. Lelis, and T. C. da Rocha. 2012. Digestible tryptophan: Digestible lysine ratio in diets for laying hens from 24 to 40 weeks of age. Rev. Bras. Zootecn. 41(10):2176-2182.

de Lima, M. R., Costa, F. G. P., Guerra, R. R., da Silva, J. H. V., Rabello, C. B. V., Miglino, M. A., Nogueira, E. T. & Pinheiro, S. G. 2012. Digestible tryptophan: Lysine ratio for laying hens. *Revista Brasileira de Zootecnia,* 41**,** 2203-2210.

Deponti, B. J., De Faria, D. E., De Faria Filho, D. E., Rombola, L. G., Araujo, L. F. & Junqueira, O. M. 2007. Tryptophan requirements and recovery performance pattern of commercial laying hens after feeding tryptophan-deficient diets. *Revista Brasileira de Zootecnia,* 36**,** 1324-1330.

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Mousavi, S. N., Afsar, A., Khalaji, S. & Abbasi, M. 2017. Estimation of digestible tryptophan:lysine ratios for maximum performance, egg quality and welfare of white-egg-laying hens by fitting the different non-linear models. *Journal of Applied Animal Research***,** 1-6.

Peganova, S., Hirche, F. & Eder, K. 2003. Requirement of tryptophan in relation to the supply of large neutral amino acids in laying hens. *Poultry Science,* 82**,** 815-822.

Russell, G. B. & Harms, R. H. 1999. Tryptophan requirement of the commercial hen. *Poultry Science,* 78**,** 1283-1285.

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Spek, J. W. 2018. Standardized ileal digestible lysine requirement for laying hens. CVB Documentation report nr. 69.
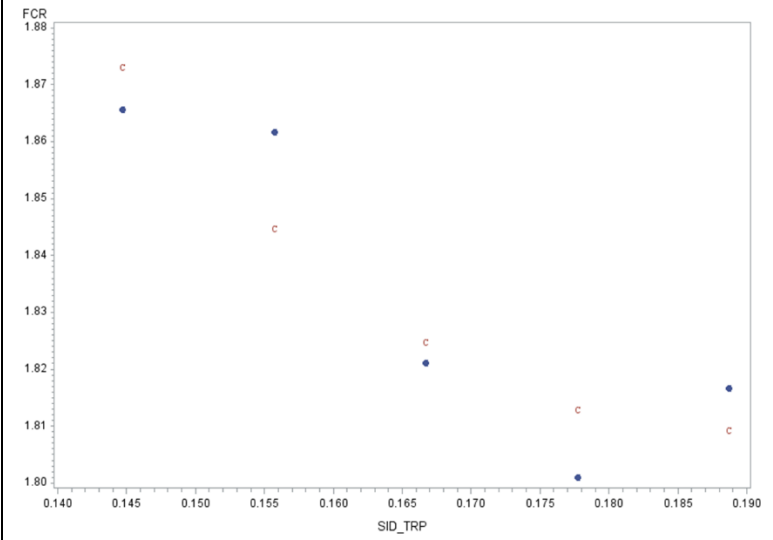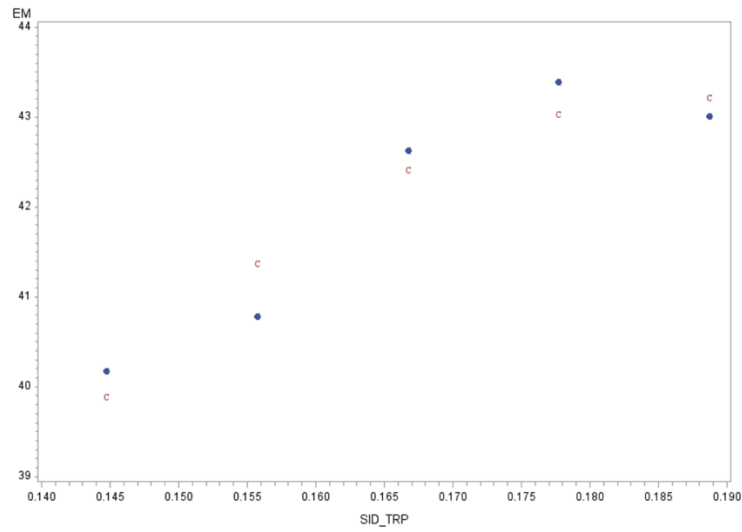
Appendix A. Relationship between dietary SID-TRP supply and performance parameters FCR and EM for the various titration trials including the estimated SID-TRP requirements based on the quadratic broken-line model

Study	Relationship between SID-TRP (%) and EM (g/hen/day)	Relationship between SID-TRP (%) and FCR (g feed/g EM)
1. Esteve-Garcia et al. (2001) SID-TRP req. EM. (%) 0.157 SID-TRP req. FCR. (%) 1.029 (extrapolated value)		

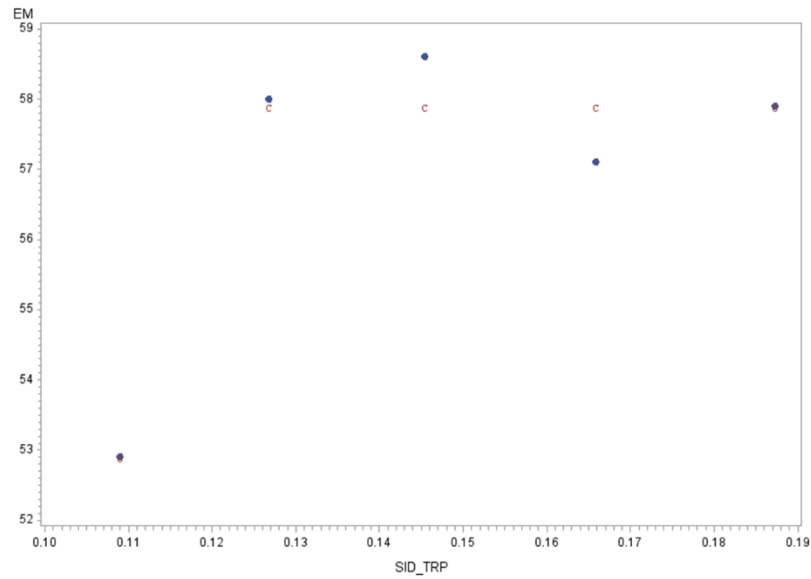|		
2. Calderano et al. (2012) SID-TRP req. EM. (%) 0.188 SID-TRP req. FCR. (%) 0.188		
3. Calderano et al. (2011) SID-TRP req. EM. (%) 0.153 SID-TRP req. FCR. (%) 0.155		
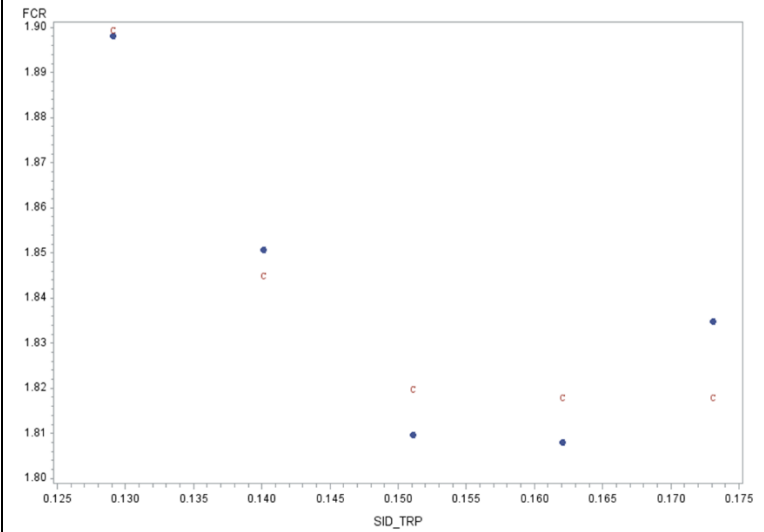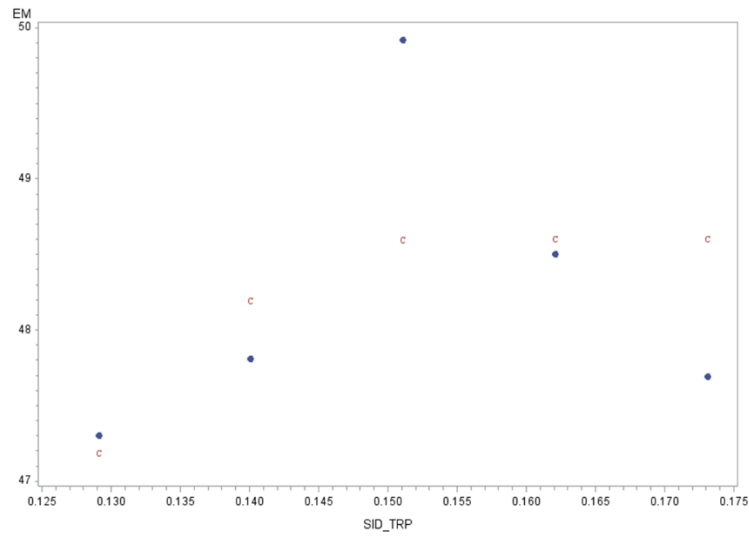
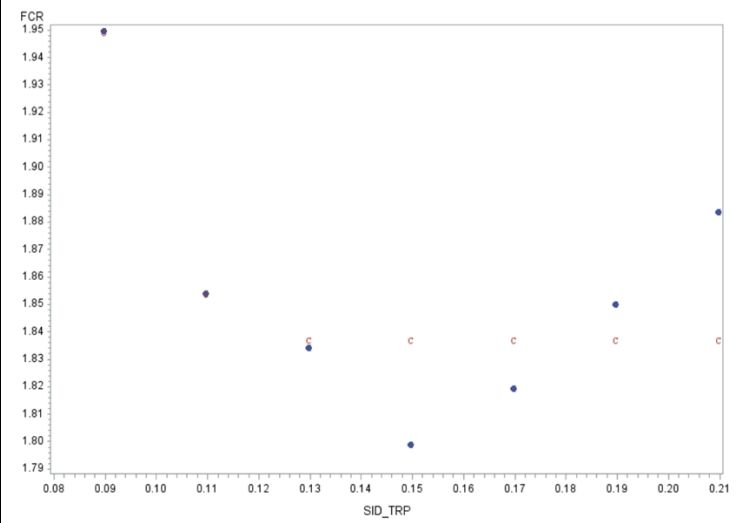| 4. Deponti et al. (2007) SID-TRP req. EM. (%) 0.127 (no unique estimate possible) SID-TRP req. FCR. (%) 0.153	EM chart	FCR chart

4.
Deponti et al. (2007)

SID-TRP req. EM. (%) 0.127 (no unique estimate possible)

SID-TRP req. FCR. (%) 0.153

EM — SID_TRP

FCR — SID_TRP
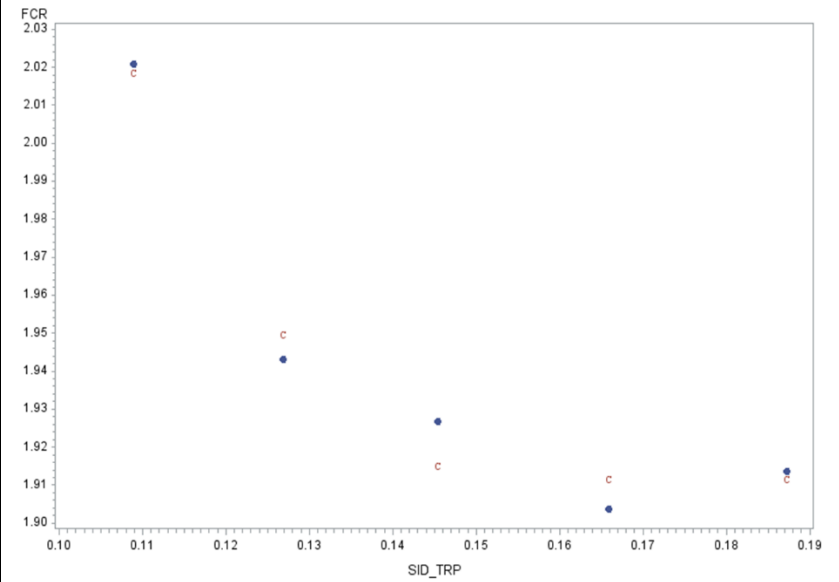
5.
Russel and Harms (1999)

SID-TRP req. EM. (%) 0.146

SID-TRP req. FCR. (%) 0.122

EM — SID_TRP

FCR — SID_TRP
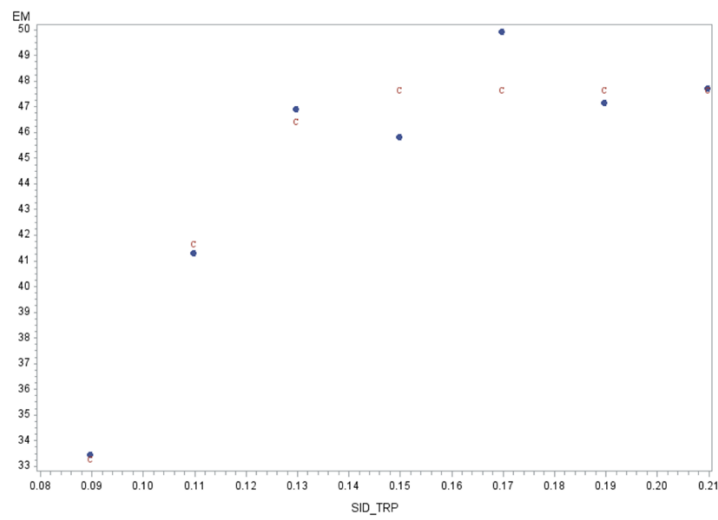
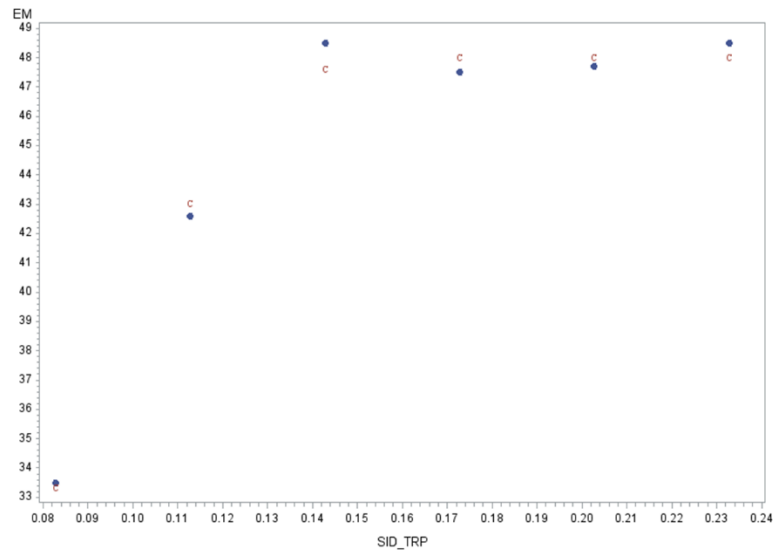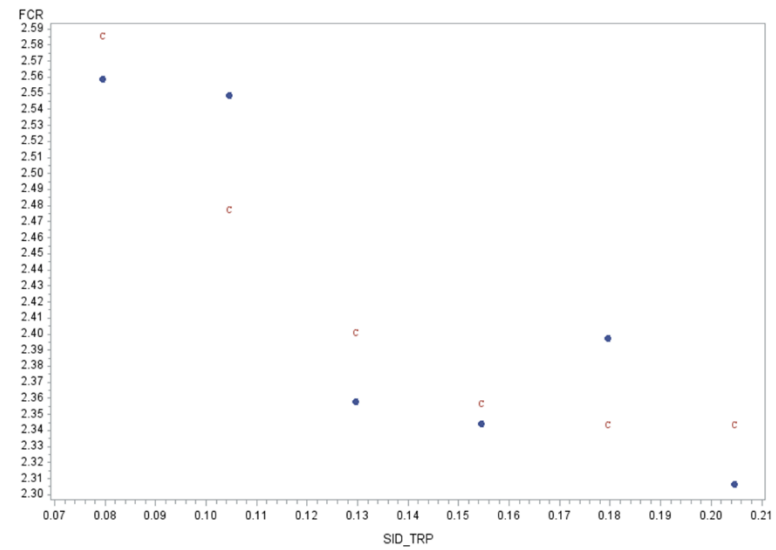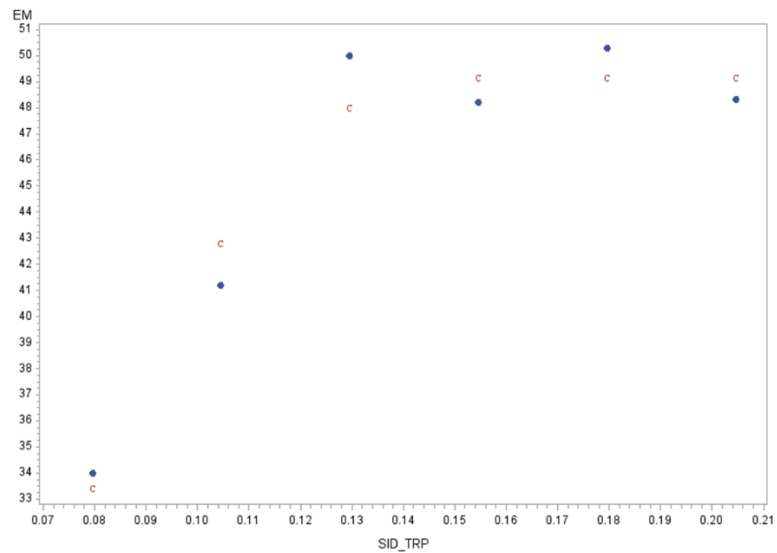| 6. de Lima et al. (2012) SID-TRP req. EM. (%) 0.172 SID-TRP req. FCR. (%) 0.167		
7. Mousavi et al. (2017) SID-TRP req. EM. (%) 0.155 SID-TRP req. FCR. (%) 0.135		
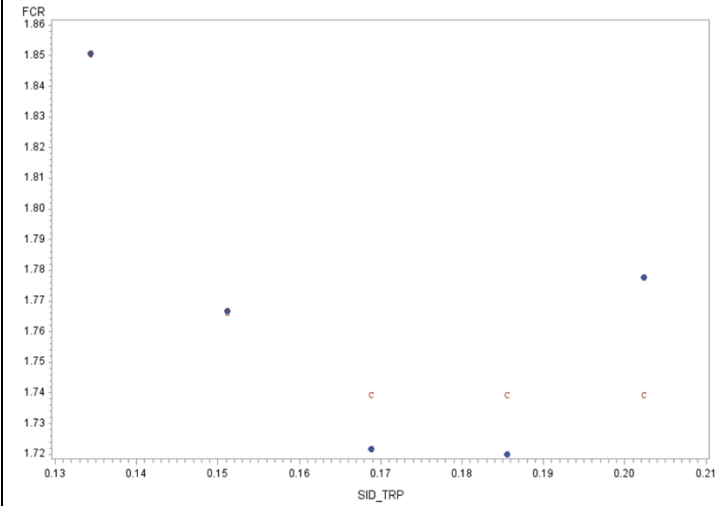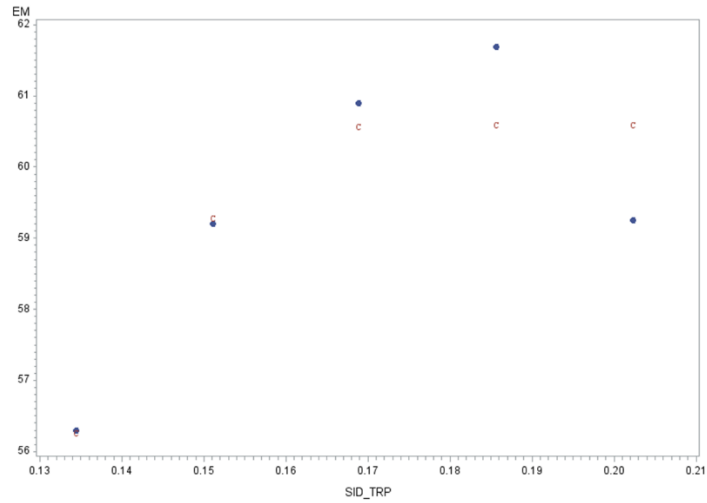
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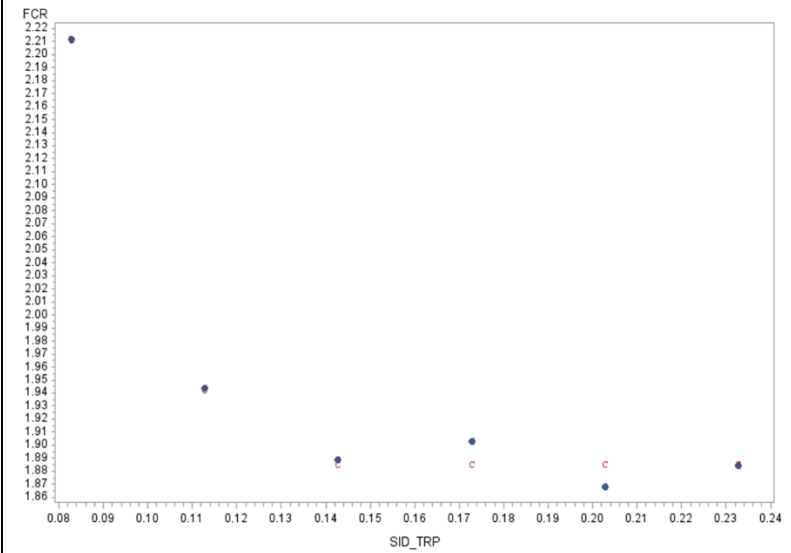
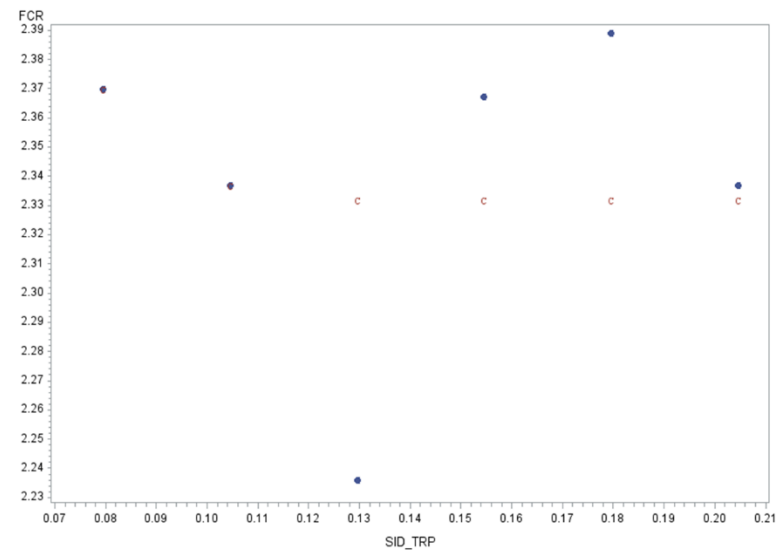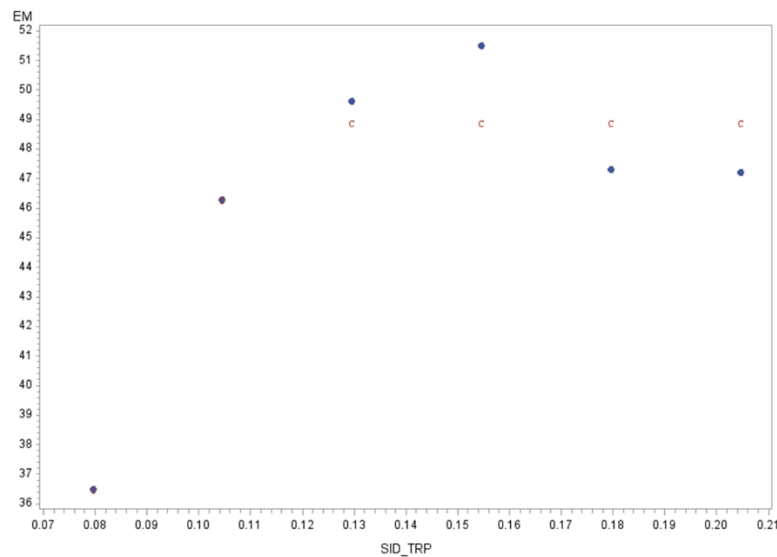| 8. Peganova et al. (2003) Trial 1

SID-TRP req. EM. (%) 0.148

SID-TRP req. FCR. (%) 0.177 | EM plot vs SID_TRP | FCR plot vs SID_TRP |
| 9. Peganova et al. (2003) Trial 2

SID-TRP req. EM. (%) 0.126

SID-TRP req. FCR. (%) 0.118 | EM plot vs SID_TRP | FCR plot vs SID_TRP |

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

SID-TRP model estimates for minimum FCR. The estimated R values shown in bold were excluded in the statistical analysis of SID-TRP requirements.

Trial nr.	Estimate L	Std. Err. L	Estimate R	Std. Err. R	Estimate U	Std. Err. U	R^2
1	1.43	5.777	**1.029**	8.5666	-1	7	0.991
2	1.81	0.016	0.188	0.0253	-34	38	0.834
3	1.82	0.011	0.155	0.0117	-121	110	0.905
4	1.91	0.007	0.153	0.0105	-54	26	0.971
5	1.84	0.014	0.122	0.0212	-106	141	0.719
6	1.74	0.019	0.167	0.0218	-103	140	0.811
7	1.89	0.007	0.135	0.0052	-122	25	0.993
8	2.34	0.043	0.177	0.0497	-25	26	0.795
9	2.33	0.034	**0.118**	0.1670	-25	218	0.077

SID-TRP model estimates for maximum EM. The estimated R values shown in bold were excluded in the statistical analysis of SID-TRP requirements.

Trial nr	Estimate L	Std. Err. L	Estimate R	Std. Err. R	Estimate U	Std. Err. U	R^2
1	57	0.5	0.157	0.0223	1037	765	0.959
2	43	0.5	0.188	0.0165	1778	1303	0.922
3	49	0.7	0.153	0.0461	2479	9719	0.352
4	58	0.3	**0.127**	.	15657	2158	0.946
5	48	0.8	0.146	0.0115	4500	1940	0.951
6	61	0.8	0.172	0.0251	3119	4294	0.818
7	48	0.4	0.155	0.0077	2846	625	0.991
8	49	1.0	0.148	0.0158	3349	1612	0.953
9	49	1.0	**0.126**	0.0169	5830	4415	0.906