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Relative Bioavailability of DL and L-Methionine in Broilers

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Abstract

Studies on the relative bioavailability (RBV) of DL-Methionine (DL-Met) to L-Methionine (L-Met) have produced variable results. An experiment was conducted to determine the RBV of DL to L-Met. A total of 2268 1-day old male chicken were housed in 54 floor pens (42 bird/pen). There were 9 treatments (6 repetitions) including the basal diet (BD). The BD was deficient in Met content with 0.27, 0.26 and 0.25 in the starter, grower and finisher periods respectively. Four levels of experimental diets for each DL-Met and L-Met were created by supplementing 0.05%, 0.10%, 0.15% and 0.20% of DL- or L-Met to the BD. The feeding program consisted of starter (0-14 d, 21% CP and 2900 kcal ME/kg), grower (15 - 28 d, 20% CP and 3000 kcal ME/kg) and finisher period (29 - 37 d, 18.5% CP and 3050 kcal ME/kg). Chickens and feed were weighed at the end of each age period. Regression coefficients of a common plateau asymptotic regression were used to calculate RBV. Birds responded to gradual increase in Met levels, BW, FCR and ADG were significantly ($P < 0.05$) higher in treatment groups as compared to control. Through the study period (37 d), the RBVs of DL-Met for BW and FCR were 89 and 77 respectively.

Keywords

Bioavailability, DL-Methionine, L-Methionine, Broilers

1. Introduction

Better growth, economy and environment friendly commercial broiler production is limited to the right amount of available amino acids for efficient utilization in animal body. All of the crystalline amino acids supplemented in commercial poultry production are in their natural (L-isomer) form except methio-

nine (Met), which may be utilized in its synthetic (D- and L-isomers) form in poultry. However, birds have to transform the D-isomer form into L-isomer in order to make it available for protein synthesis and other essential metabolic functions. It is not clear whether this conversion process in 100% efficient and all of the supplemented D-isomer form is being converted into L-isomer for its further utilization in animal body.

Inefficient intestinal absorption of supplemented Met is one of the factors which may limit its availability to body metabolism. For instance, Esteve-Garcia and Austic [1] observed 1% higher recovery of DL-Met as compared to L-Met in terminal ileum.

A number of studies have been carried out about the bio-efficiency of D, L and DL-Met. However, their results remained indecisive. Some reports showed that D- and L-Methionine were equivalent [2] [3] [4]. While others showed D-Methionine to be inferior [5] [6]. With regard to L- and DL-Methioine, Grau and Almquist [2], Leveille *et al.* [4], Gutteridge and Lewis [7] and Dilger *et al.* [8] showed equal efficacy of L and DL-Met. In contrast, Marret *et al.* [9], Smith [6], Katz and Baker [10] suggested that L-Met is superior to DL-Met when biological efficacy is compared. Even two reports [9] [11] concluded that D and DL-Met have better bio-efficiency than L-Met alone. Marret *et al* [9] showed that diets containing large amounts of D-amino acids caused D-methionine to be less efficient, suggesting that the capacity of D-amino acid oxidase could be exceeded.

The goal of the present study was to determine the RBV of two sources of Met (DL vs. L-Met), supplemented at graded levels to practical broiler diets, using growth parameters as response criteria.

2. Materials and Methods

2.1. Animals and Diets

All animal housing and husbandry conformed to the European Union Guidelines [12] and the protocol was approved by the Ethical Animal Committee of IRTA. A total of 2268 one-day-old male broiler (Ross 308) chickens were distributed into 54 (4 m^2 each) pens. There were six replicates per treatment (42 birds/pen). Standard light and temperature plans were followed [13].

The basal diet was formulated according to nutrient recommendations of Ross 308 [13], however, Met (<60% of Ross requirement) was kept in limiting position. Dietary ingredient composition and analysed nutrient contents are described in **Table 1** and **Table 2**.

The feeding program was divided into three age periods; starter (0 - 14 d), grower (15 - 28 d) and finisher (29 - 37 d). Eight experimental diets were created by supplementing crystalline DL or L-Met to the BD in four graded levels, plus the un-supplemented diet. The calculated values for the experimental diets of DL-Met1-4 or L-Met1-4 were BD + 0.05, 0.10, 0.15 and 0.20% respectively, based on expected responses to methionine (Esteve-Garcia and Austic [1]; the laboratory analysed levels are shown in **Table 3**.

Dietary ingredients and experimental diets were analysed according to AOAC [15] for crude protein (method 968.06), ether extract (method 920.39) and crude ash (method 942.05). The amino acid content of the ingredients was analysed by ion exchange chromatography coupled with post column derivatization and photometric detection according to Neumann and Bassler [16].

Body weight and feed consumption were measured at 14, 28 and 37 d on a pen basis. Dead animals were not taken into account, and their weight was subtracted from the initial weight of the pen, according to the mean weight, or in case the animal was smaller than the initial weight due to disease, its weight at the time of death was subtracted from the initial weight of the pen. Corrected

Table 1. Ingredient composition of basal diet (g/kg as fed basis).

Ingredient	Starter	Grower	Finisher
Maize	550.2	480.4	503.4
Wheat	60	100	100
Soybean meal, 48% CP	280	224.7	200
Full fat extruded soybeans	-	45	38.1
Peas	-	40	52.5
Soy oil	29.7	-	-
Animal fat	-	42.8	55
Dicalcium phosphate	19.3	15	13.5
Calcium carbonate	7.7	7.9	7.9
Sodium chloride	4	3.5	3.5
L-Glu	30	30	15
L-Lys HCl	4.8	2.7	2.7
L-Arg HCl	2.3	0.5	0.8
L-Val	2.6	1.4	1.4
L-Thr	1.8	0.9	1
L-Ile	2.3	1.4	1.6
L-Trp	0.6	0.1	0.4
Choline chloride	1	-	-
Mineral and vitamin premix[1]	3	3	3
Maxiban G 160[2]	0.5	-	
Elancoban[3]	-	0.5	-
Ethoxyquin, 66%	0.2	0.2	0.2

[1]Provides per kg feed: vitamin A (E-672) 13500 IU; vitamin D_3 (E-671) 4800 IU; vitamin E (alfa-tocopherol) 45 mg; vitamin B_1 3 mg; vitamin B_2 9 mg; vitamin B_6 4.5 mg; vitamin B_{12} 16.5 µg; vitamin K_3 3 mg; calcium panthotenate 16.5 mg; nicotinic acid 51 mg; folic acid 1.8 mg; biotin 30 µg; Fe (E-1) (from $FeSO_4 \cdot 7H_2O$) 54 mg; I (E-2) (from $Ca(I_2O_3)_2$) 1.2 mg; Co (E-3) (from $2CoCO_3 \cdot 3Co(OH)_2 \cdot H_2O$) 0.6 mg; Cu (E-4) (from $CuSO_4 \cdot 5H_2O$) 12 mg; Mn (E-5) (from MnO) 90 mg; Zn (E-6) (from ZnO) 66 mg; Se (E-8) (from Na_2SeO_3) 0.18 mg; Mo (E-7) ($(NH_4)_6Mo_7O_{24}$) 1.2 mg. [2]Maxiban G 160: 80 g Narasin and 80 g Nicarbazin per kg of product. [3]Elancoban: 200 g Sodium Monensin per kg of product

Table 2. Analysed nutrient contents of basal diet (as fed basis).

Nutrient	Starter	Grower	Finisher
	0 - 14 d	15 - 28	29 - 37 d
ME MJ/kg[1]	12.13	12.55	12.55
CP g/kg	209.7	203.5	184.4
Ether extract g/kg	51.9	68.8	79.9
Crude ash g/kg	50.2	47.5	44.9
Amino acids g/kg			
Lys	13.2	11.4	10.6
Met[2]	2.70 (2.70)	2.50 (2.60)	2.30 (2.50)
Cys	3.2	3.3	2.8
Thr	8.1	7.6	7
Trp	2.6	2.4	2.1
Arg	12.9	11.8	11.5
Ile	9.7	9.1	8.5
Leu	15.6	14.6	13.8
Val	9.9	9.9	9
Glu	59.1	62.8	45.7
Phe	9.1	8.9	8.3
His	5	4.7	4.3
Asp	17.4	18.2	16.8
Gly	7.8	7.6	7.3
Ala	9	8.6	8.2
Pro	11.4	11.1	9.9

[1]ME contents were calculated based on WPSA [14]; [2]Met contents in parenthesis are based on dietary formulation.

Table 3. Analysed contents of Met in dietary treatments.

Trt.	Met source supplements (%)			
	Expected suppl. level	Starter 0 - 14 d	Grower 15 - 28 d	Finisher 29 - 37 d
Basal	-	-	-	-
L-Met1[1]	0.05	0.04	0.05	0.04
L-Met2	0.10	0.08	0.09	0.09
L-Met3	0.15	0.13	0.14	0.15
L-Met4	0.20	0.15	0.19	0.20
DL-Met1[2]	0.05	0.05	0.05	0.05
DL-Met2	0.10	0.09	0.09	0.10
DL-Met3	0.15	0.14	0.14	0.15
DL-Met4	0.20	0.19	0.19	0.20

[1]L-Met: Procured from CJ Europe GmbH, Germany; [2]DL-Met: Procured from Sumitomo Chemical Company, Japan.

feed conversion ratio (FCR) was calculated dividing the total feed consumed within the period to the weight gained by the live animals within the period plus the weight gain of the dead animals during the period. Average feed consumption was calculated as the product of weight gain (WG) and feed conversion ratio (FCR).

2.2. Statistical Evaluation

Results were analysed as a randomized complete block design by two way ANOVA with 6 blocks corresponding to location within the house and 9 treatments corresponding to the basal diet and each of the four levels of DL and L-Met. Treatment means were compared for significance ($P < 0.05$) using Tukey's test.

The RBV of DL and L-Met was calculated using the model of Littell *et al.* [17] to obtain the response coefficients by using SPSS (Version 24 for Windows; SPSS Inc., IBM, Chicago, IL).

$$Y = B1 + B2 \times \left(1 - e^{(B31 \times X1 + B32 \times X2)}\right) \quad (1)$$

where Y = response variable (Feed intake, BW, ADG and FCR,)

B1 = intercept (response of basal diet)

B1 + B2 = asymptote

B31 = Steepness coefficient for L-Met

B32 = Steepness coefficient for DL-Met

X1 = level of L-Met

X2 = level of DL-Met

The bioavailability for DL-Met relative to L-Met was calculated by the ratios of regression coefficients B31 and B32 according to Elwert *et al.* [18]. RBV = 100 * B31/B32

3. Results

The statistical means for BW, FI, ADG and FCR attributed by two way ANOVA did not reveal any significant ($P > 0.05$) difference depending on the source of Met, however, the growth parameters for the basal diet were significantly ($P < 0.05$) lower as compared to the experimental diets supplemented with Met (**Table 4(a)** and **Table 4(b)**). The body weight of birds increased gradually with the increase ($P < 0.05$) in supplementation of Met source, which indicates a clear deficiency of Met in the basal diet.

The growth response corresponding to L-Met supplementation in relation to DL-Met was numerically higher, but Tukey's test does not reveal significant ($P > 0.05$) difference due to the different Met sources.

A curvilinear response was observed from 0-37d for BW and FCR with graded Met supplementation (**Figure 1**) which was analysed by multi-exponential common plateau regression. The data of the present study appeared to fit well with the asymptotic nonlinear model and is considered to be adequate to compare the two sources.

Table 4. (a) Statistical means of the performance parameters depending on source and dietary Met levels (0 - 14 and 15 - 28 d); (b) Statistical means of the performance parameters depending on source and dietary Met levels (29 - 37 and 0 - 37 d).

(a)

Trt.	0 - 14 d					15 - 28 d				
	Met%	FI	BW	ADG	FCR	Met%	FI	BW	ADG	FCR
		g	g	g	g/g		g	g	g	g/g
BD	0	22.10[a]	252[a]	14.90[a]	1.48[a]	0	75.76[a]	742[a]	32.60[a]	2.32[a]
L-Met1	0.04	31.40[b]	392[b]	24.90[b]	1.26[b]	0.05	109.30[b]	1383[b]	66.00[b]	1.65[b]
L-Met2	0.08	35.30[c]	447[c]	28.80[c]	1.22[bc]	0.09	119.20[c]	1674[c]	81.80[c]	1.45[c]
L-Met3	0.13	35.70[c]	457[c]	29.50[c]	1.21[c]	0.14	123.30[c]	1749[d]	86.10[d]	1.43[c]
L-Met4	0.15	35.90[c]	464[c]	30.00[c]	1.19[c]	0.19	126.00[c]	1798[d]	88.90[d]	1.41[c]
DL-Met1	0.05	31.50[b]	400[b]	25.50[b]	1.24[bc]	0.05	112.50[b]	1375[b]	64.90[b]	1.73[b]
DL-Met2	0.09	34.60[c]	445[c]	28.70[c]	1.21[c]	0.09	122.40[c]	1675[c]	82.00[c]	1.49[c]
DL-Met3	0.14	35.60[c]	458[c]	29.60[c]	1.20[c]	0.14	122.70[c]	1747[d]	85.90[d]	1.42[c]
DL-Met4	0.19	36.10[c]	454[c]	29.30[c]	1.24[c]	0.19	123.40[c]	1767[d]	87.50[d]	1.41[c]

[a-c]Columns with different superscripts are statistically significant ($P < 0.05$); Trt = experimental treatment; Met% = supplemental methionine as a percent; FI = Average daily feed intake; BW = Live body weight; ADG = Average daily gain; FCR = Feed conversion ratio.

(b)

Trt.		29 - 37 d				0 - 37 d			
		FI	BW	ADG	FCR	FI	BW	ADG	FCR
		g	g	g	g/g	g	g	g	g/g
BD	0	113.00[a]	1137[a]	49.40[a]	2.28[a]	63.50[a]	1137[a]	29.50[a]	2.15[a]
L-Met1	0.04	177.90[b]	2168[b]	98.10[b]	1.81[b]	94.60[b]	2168[b]	57.40[b]	1.64[b]
L-Met2	0.09	196.60[c]	2552[c]	109.60[c]	1.79[bc]	104.10[c]	2552[c]	67.80[c]	1.53[c]
L-Met3	0.15	200.50[c]	2683[d]	116.70[d]	1.72[bc]	106.80[c]	2683[d]	71.30[d]	1.49[c]
L-Met4	0.2	201.80[c]	2747[d]	118.40[d]	1.70[c]	108.20[c]	2746[d]	73.00[d]	1.48[c]
DL-Met1	0.05	180.00[b]	2162[b]	98.40[b]	1.83[b]	96.40[b]	2162[b]	57.20[b]	1.68[b]
DL-Met2	0.1	199.10[c]	2562[c]	110.80[c]	1.79[bc]	105.80[c]	2562[c]	68.00[c]	1.55[c]
DL-Met3	0.15	200.50[c]	2672[d]	115.50[d]	1.74[bc]	106.40[c]	2672[d]	71.00[d]	1.49[c]
DL-Met4	0.2	199.00[c]	2698[d]	116.20[d]	1.71[c]	107.10[c]	2698[d]	71.70[d]	1.49[c]

[a-d]Columns with different superscripts are statistically significant ($P < 0.05$).

The RBV of DL to L-Met was calculated by the ratio of B32/B31 as described in Equation 1. The results of RBV of DL-Met through the study period are summarized in Table 5 and demonstrated in Figure 1. The parameter estimates are described in Table 6. RBV of DL-Met across the study period was for FI: 99, BW: 89, ADG: 99 and FCR: 77.

4. Discussion

Based on ANOVA, the statistical non-significant differences ($P > 0.05$) on performance parameters depending on Met sources may be attributed to the difference in calculated and analysed contents of the dietary treatments of L-Met, which were found lower than expected (Table 3). In contrast, test diets containing DL-Met, the expected and calculated values of Met were in good agreement.

It is difficult to ascertain the level of one source over the other when two different sources of the test product are supplemented at closer levels [19]. The

Table 5. Summary of relative bioavailability (RBV) of DL-Met according to various response criteria from 0 - 37 d (%, L-Met: 100) RBV= 100 * B31/B32.

Criterion of response	RBV	95% Confidence interval
Feed intake	99	71 - 128
Body weight	89	78 - 100
Average daily gain	89	78 - 100
Feed to gain	77	51 - 103

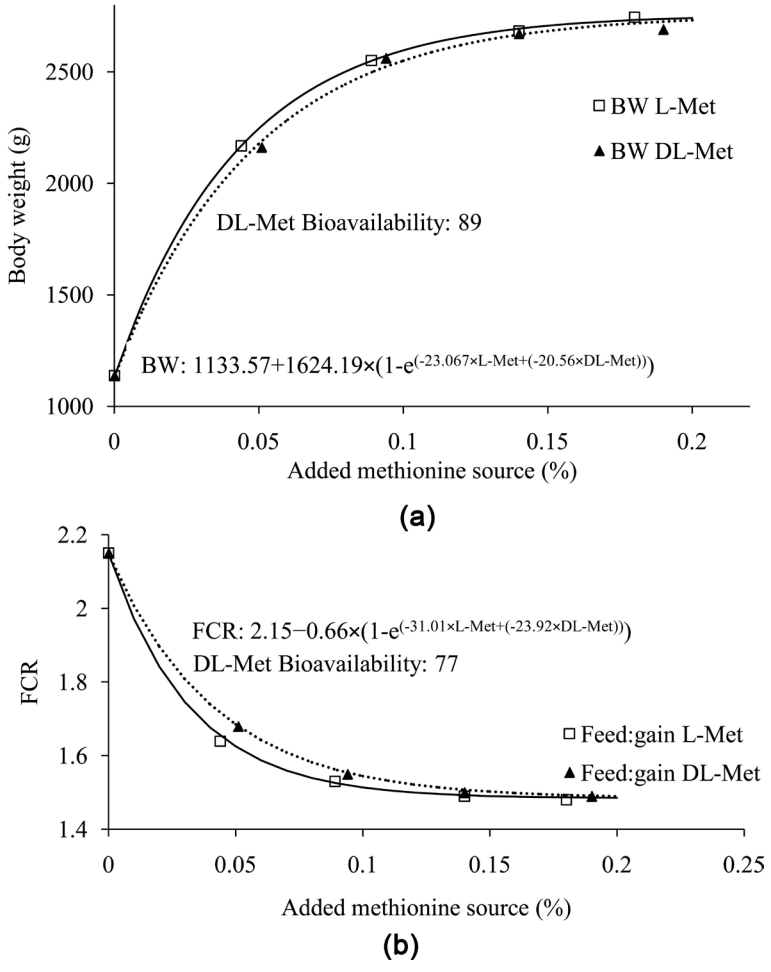

Figure 1. Body weight (a) and FCR (b) of chickens at 37 days of age feeding different levels of DL and L-Met.

Table 6. Parameters of nonlinear regression model describing relationship between response criteria at different supplementation levels of DL and L-Met (0 - 37).

Criteria of response	Parameter	Estimate	Standard error	95% confidence interval		R^2
				Lower	Upper	
Feed intake (g)	B1	63.442	1.864	59.697	67.187	0.90
	B2	44.504	2.077	40.332	48.677	
	B31	−27.477	3.503	−34.513	−20.441	
	B32	−27.4	3.68	−34.792	−20.009	
Body weight (g)	B1	1133.57	34.051	1065.18	1201.96	0.97
	B2	1624.19	38.776	1546.31	1702.07	
	B31	−23.067	1.493	−26.066	−20.069	
	B32	−20.56	1.297	−23.165	−17.955	
Average daily gain (g)	B1	29.467	0.92	27.618	31.315	0.93
	B2	43.897	1.048	41.792	46.002	
	B31	−23.067	1.493	−26.066	−20.069	
	B32	−20.56	1.297	−23.165	−17.955	
Feed to gain	B1	2.149	0.033	2.083	2.215	0.87
	B2	−0.665	0.037	−0.739	−0.591	
	B31	−31.018	4.97	−41.001	−21.034	
	B32	−23.926	3.513	−30.982	−16.869	

B1 = intercept (response of basal diet); B1 + B2 = asymptote; B31 = Steepness coefficient for L-Met; B32 = Steepness coefficient for DL-Met.

study remained indecisive to quantify one sources over the other without affecting the performance parameters.

Studies conducted by Zelenka *et al.* [20] about the performance parameters of broilers, revealed similar findings, as of present experiment, through the application of ANOVA. They compared the DL-Met with MHA with graded levels of Met dietary concentration. The data for whole experiment showed better FCR and body weight in DL-Met treatment groups, however, the results were statistically non-significant ($P > 0.05$) over the MHA counterparts.

In a metabolic experiment for 20 days in post weaned growing pigs, Shen *et al.* [21] used the mixed model of SAS with completely randomized design, and observed a nonlinear response for growth parameters like ADG ($P = 0.087$), FCR and reduced plasma urea nitrogen (PUN) in L-Met as compared to DL-Met.

Whereas, Kong *et al.* [22] compared the both Met isomers in a nitrogen balance experiment with weaned piglets. Final BW, N intake, faecal N, Urinary N and apparent N digestibility were observed in L-Met vs. DL-Met using orthogonal polynomial contrast analysis and observed no response ($P > 0.05$) in performance parameters.

ANOVA appears to be insufficient for the above mentioned as well as the present study to estimate the difference between the closely matching treat-

ments. Moreover, two-way ANOVA also suffers from some limitations related to the present study, for instance it considers the levels of Met as categorical, while in fact they are continuous.

Therefore, the non-linear model of Littell *et al.* [17] was adopted to estimate the RBV. This model was questioned by Rosen [23] who states that "nutrient response curves are inevitably quadratic". When the quadratic model was applied to our data, the curve shows a maxima below the 0.20% level of Met which the data do not justify, as there seems to be further response beyond the 0.15% level. Furthermore, the quadratic model was proposed by Kratzer and Littell [24] for DL-Met and DL-MHA, in which different maxima are achieved depending on the Met source which has later been criticized by Piepho [25] and Elwert *et al.* [18]. The hypothesis of different plateaus does not seem reasonable if one evaluates the response to the same nutrient, because the maximum should be the same for different sources. The data of the present study seemed to fit, in most cases, the asymptotic non-linear model was considered to be adequate to compare the two sources.

The RBV estimated in the present study is in agreement with the dose response studies of Noll *et al.* [26]. They conducted in total three experiments in large white turkeys from 7-28 days. L-Met (100%) and Met hydroxy analogue-free acid (88%) were evaluated for biopotency compared to DL-Met (99%) in a starter diet. The Met levels of supplementation were 0%, 0.04%, 0.10%, 0.16%, 0.28%, 0.44%, and 1.00%.

They observed that the level of Met effected the growth of the birds significantly ($P < 0.05$). Based on the three studies the biopotency (± SE) of L-Met was significantly superior to DL-Met (131% ± 10%); the biopotency of the analogue was not significantly different from DL-Met (96% ± 7%). In the present study, as a whole (37d), the RBV of DL- and L-Met was considerably diverse as compared to the observation of Noll *et al.* [26]. These dissimilarities in RBV can be attributed to difference in species of animals and age period of animals during the study.

The difference in exponential graphic curves (**Figure 1**) is widened at lower Met levels. As the Met supplementation approaches to requirement or above the two curves inclined to converge. The presented observation is in accordance to the study of Katz and Baker [10], who conducted four experiments in young growing chickens to determine the relative efficacy of DL, L- and D-Met. They observed that L-Met supported faster and more efficient gains than D- or DL-Met when fed at levels below the requirement. However, when different sources supplemented up the level of requirement, equal efficacy was attained. They concluded that at lower levels of supplementation, L-Met is a better source of sulphur amino acids than D-Met. They also concluded that L- and D-Met appear to have equal efficacy when incorporated into diets that are only marginally deficient in sulphur-bearing amino acids.

The RBV for body weight, DL-Met = 89, is in contrast with the observations

of Dilger *et al.* [10] who determined the relative bioefficacy of Met precursor compounds in young chicks. They compared the DL-Met with the L-Met precursor (2-keto-4-(methylthio) butyric acid) using Met-deficient diets of differing composition. Based on weight gain they concluded the relative bioefficacy values of 98.5% and 89.3% for DL-Met and keto-Met respectively. The difference in RBV for L-Met may attributed to the intermediated precursor (Keto-Met) which needs to be converted into L-Met through the transamination process in order to come in to the metabolic pathway. Moreover, the RBV of the L-Met concluded by Shen *et al.* [21] was higher than that of the present study. They reported the RBV of L-Met as 159% to 100% of DL-Met for AGD and 138.5% to 100% for FCR respectively in weaned piglets. Recently, Kong *et al.* [22] determined the bioavailability of D-Met relative to L-Met for nursery pigs using the slope-ratio assay. They concluded that the mean relative bioequivalence of D- to L-Met was 87.6% based on urinary N output or 89.6% based on N retention. These values closely matched to the present study in which the RBV for DL-Met = 89, although, the difference in the species of the animals cannot be ignored.

5. Conclusion

Statistically, when comparing if one source (DL-Met) "equivalent" to the reference (L-Met) ignoring the type II error could have important practical consequences. D-Met must be converted to L-Met in the body. The process requires different steps, and it is not clear that process is 100% efficient; this may be projected through performance parameters. In the present experiment the RBV of DL to L-Met was 89:100 for BW and 77:100 for FCR.

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