John E. Thomson, Ph.D., Evonik Corporation

Relative Bioavailability Values and Methionine Sources

The value of feed ingredients in formulation derives from the nutrients they deliver, the concentration of those nutrients and the ability of the animal to use those nutrients for maintenance or productive purposes. When making formulation and buying decisions on ingredients, it is critically important to know which ingredient delivers nutrients most economically.

Chemical assays identify which nutrients are present and in what concentration but provide no information on the availability of these nutrients to the animal. Digestibility trials are commonly used to determine amino acid and energy availability. Combined with corrections for endogenous losses, these techniques account for difference in digestibility but do not account for differences in metabolism of the nutrients that may occur after absorption.

Minerals, vitamins and feed grade amino acids typically serve as a concentrated source of one or two nutrients. Formulating diets with high enough concentrations of the single nutrient for use in a standard digestibility trial is usually impractical due to palatability or toxicity problems. Physiological responses may also be affected by the unusually high concentrations.

Requirements for nutrients from simple sources are often established using some common or "standard" additive. The availability of the nutrient from the standard source is assigned a value of 100 %. Other sources are compared to the "standard", and their availabilities are calculated relative to the standard. Indexing of test ingredients versus the standard ingredient is referred to as Relative Bioavailability Value (RBV) or Bioefficacy (BE). RBV is calculated as the amount of the standard ingredient (x_s) needed to achieve a given response divided by the amount of a test ingredient (x_t) needed to achieve the same level of response

$$\left(RBV = \frac{x_s}{x_t}\right)$$ (Littell et al., 1995).

Comparing actual responses to the nutrients accounts not only for any differences in digestibility but for differences in metabolism as well.

A linear response to a nutrient or ingredient can be expressed mathematically as $y = \alpha x + \beta$, where y is the response, α is the slope of the line, x is the amount of the nutrient or ingredient, and β is the response when none of the ingredient is added (y-intercept). For the standard ingredient, we could write this equation as $y_s = \alpha_s x_s + \beta_s$ and, for a test ingredient as $y_t = \alpha_t x_t + \beta_t$.

When comparing two source of the same nutrient, we are interested in the case where the responses are equal ($y_s = y_t$)

$$\alpha_s x_s + \beta_s = \alpha_t x_t + \beta_t$$

The response to no supplemental nutrient from either source is the same and so, $\beta_s = \beta_t$ and $\alpha_s x_s = \alpha_t x_t$.

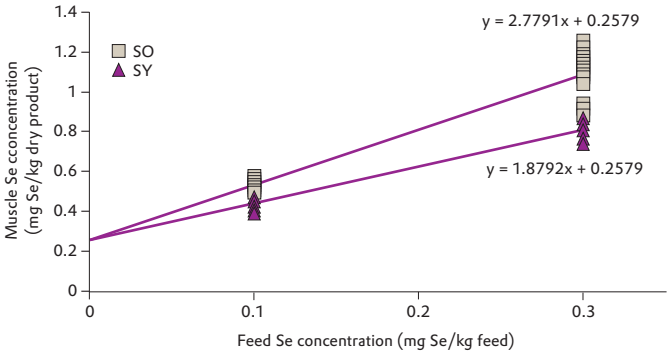
By rearranging the equations, we come to

$$RBV = \frac{x_s}{x_t} = \frac{\alpha_t}{\alpha_s}.$$

So, RBV is equal to the ratio of the slope of the response to the test ingredient to the slope of the response to the standard ingredient. We would set up a trial with a basal diet deficient in the nutrient of interest but at or above the requirement for all other nutrients. We would then supplement the basal diet with two or more levels of the nutrient from the standard ingredient and two or more levels of the test ingredient. As long as the response we measure is limited only by our nutrient, we should be able to plot two lines which extend from a common starting point (y-intercept). We would estimate the RBV as the ratio of the slopes of the lines. This type of trial is known as a slope-ratio assay.

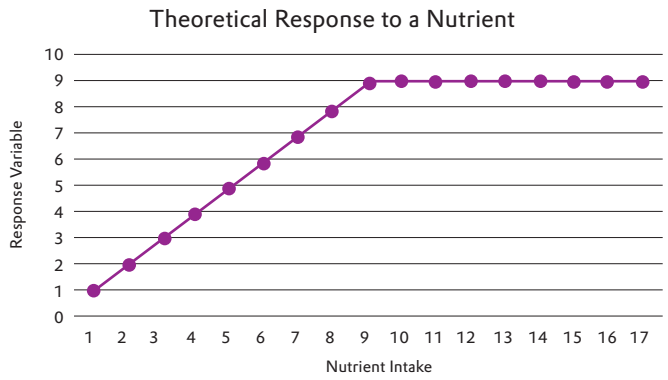
Briens et al. (2014) provides a good example of a slope-ratio assay from the literature. In this trial, male broiler chicks were fed either a basal diet with no added selenium or the basal diet supplemented with 0.1 or 0.3 ppm of selenium from either selenium yeast (SY) or 2-hydroxy-4-methylselenobutanoic acid (SO, Selisseo®) for 42 days. The response variable was muscle selenium concentration at day 42 **(Figure 1).**

FIGURE 1



Relative bioavailability of Se from Selenium Yeast (SY) versus 2-hydroxy-4-methylselenobutanoic acids (SO) (Briens et al., 2014)

FIGURE 2

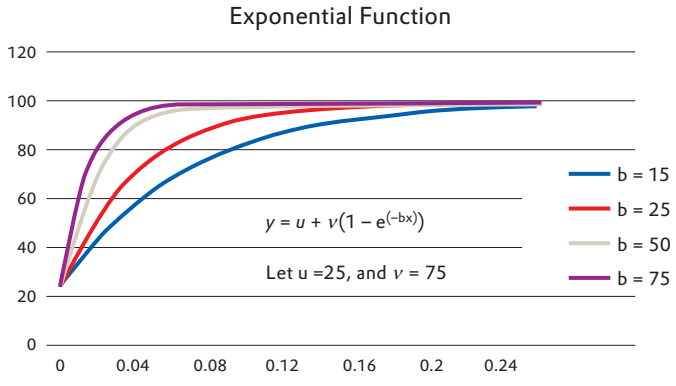


Taking SY as the standard, the RBV of SO in this experiment was calculated as 148 % (2.7791/1.8792 = 1.48). The reported 95 % confidence interval (95% CI) for this estimate was 138 % to 158 %, indicating a 95 % certainty that the true slope ratio lies within this range. Had the 95 % CI included 100 %, we would have to conclude that the sources were not significantly different.

Supplementing the basal diet with the deficient nutrient results in a linear increase in response. At some point, however, the maximum response is reached and further increases in the supplementation rate no longer results in an increased response **(Figure 2)**. The slope ($\Delta y/\Delta x$) at and above the maximum response is zero. In this range, RBV becomes meaningless since the response, y, is the same for every value of x. Consequently, slope-ratio assays are carried out with supplementation levels below the nutrient requirement.

There has been some criticism of using nutrient levels well below the requirement in slope-ratio assays. One solution to this issue is to use a response model that fits dose-response data covering the range from deficiency to the maximum response level. In this case, non-linear models give a better fit to responses. One example is the exponential model (Littell et al., 1997; Rodehutscord and Pack, 1999): $y = u + v(1 - e^{(-bx)})$ where y = the response variable (gain, feed conversion, etc.), x = the amount of the nutrient or ingredient in the feed, u = the response to the unsupplemented basal diet, v = the maximum response, e = the base of the natural log, and b = the steepness of the curve.

FIGURE 3



In addition to the experimental design requirements for the linear slope-ratio assay, at least three levels of supplementation above the basal diet are needed for each of the test and standard ingredients and the response for each source must reach the same plateau. One of the supplemented nutrient levels should be below the requirement, one near the requirement and one above the requirement. Any additional levels should be added below the requirement, since this is the portion of the response that is most important in comparing ingredients. The RBV is defined by comparing the steepness of the test and standard ingredient response curves. **Figure 3** shows how varying the value for b (steepness of the curve) affects the shape of the function. Setting the exponential equations for the standard and test ingredients equal, we can show that

$$\frac{b_t}{b_s} = \frac{x_s}{x_t} = RBV.$$

While there seems to be no dispute over the use of the slope-ratio assay in either the linear or exponential form for minerals, vitamins or other supplemental amino acids, there has been quite some controversy over applying the same approach to methionine sources. There are currently three feed grade methionine sources available for livestock and poultry feed: D,L-methionine (DL-Met), L-methionine (L-Met) and the hydroxy analog of methionine (D,L-2-hydoxy-4-methylthiobutanoic acid; DL-MHA). The first two sources are amino acids while the third has a hydroxyl group in place of the amino group. All three can be converted in the animal's body into L-Met, the biologically active form. D-Met and DL-MHA must be converted into L-Met, immediately raising a question as to the RBV of these different options.

It has been suggested that comparing efficacy of methionine sources should only be done at the standard industry supplementation rate. This supplementation rate includes some provision for a safety margin and falls in the plateau region of the response, where RBV is meaningless. Such an approach prevents any comparison of efficiency. The exponential slope-ratio assay only differentiates nutrient sources based on the supplementation rate at which a measured response reaches the maximum. The higher the RBV, the lower the supplementation rate needed to meet the animal's requirement.

Hoehler et al (2005) provides an example of determining RBV with a non-linear data set. A total of 2880 day-old chicks (Ross 208) were placed into floor pens of 30 birds/pen for a 42 day feeding trial. Pens were randomly assigned to one of 16 dietary treaments resulting in 6 replicates per treatment. A methionine deficient basal diet with no supplemental methionine source

FIGURE 4

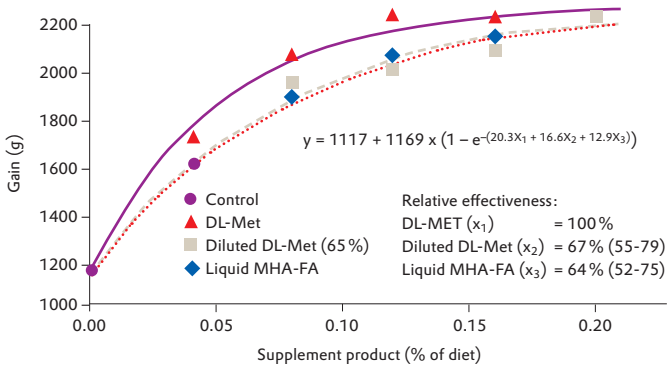
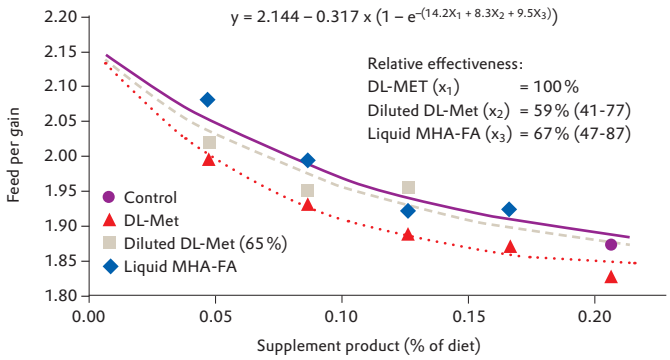


FIGURE 5



was formulated to contain 0.59 % and 0.52 % total sulfur amino acids in the starter (d1-21) and grower (d22-42) feeds, respectively. The treatments in each period comprised the basal diet and 3 series of diets containing graded levels (0.04, 0.08, 0.12 and 0.20) of DL-Met, D,L-Met diluted with glucose to 65 % of the original concentration (DL-Met 65%) and 88 % purity DL-MHA. DL-Met 65 % was included as an internal standard for the method. Results are shown in **Figures 4** and **5.**

Taking DL-Met as the standard, the RBVs for weight gain were 67 % and 64 % for DL-Met 65 % and DL-MHA, respectively. The 95 % Confidence Interval (CI) is shown in parenthesis

TABLE 1

Researcher	Institut	Country	published in	Relative Effectiveness (%) Weight gain	Feed conversion	Breast meat yleid
Mexican Integration		Mexico	Hoehler et al. 2005 b	63	73	
Brenman et al.	Shur-Gain Agresearch	Canada	Lemme et al., 2002	72	51	60
Ceylan et al.	Univ. of Ankara	Turkey	Trial report	63	60	
Esteve-Garda and Liaurado	Dept. of Animal Nutrition, Reus	Spain	Esteve-Garda and Llaurado, 1997	70	73	45
Gonzalo Matoo et al.	Univ. Madrid	Spain	Facts&Figure 1579, 2009	73	51	
Hashimoto et al.	Japan Scientific Feed Association	Japan	Payne et al., 2006	50	56	54
Jansman et al.	TNO Institute Wageningen	The Netherlands	Degussa Facts&Figures Poultry 6, 1999	57	58	–
Jensen et al.	Research Centre Foulum	Denmark	Hoehler et al., 2005b	64	67	–
Koreleski et al.	Research Institute of Animal Production	Poland	Payne et al., 2006	64	59	–
Mannion et al.	Queensland Poultry Research	Australia	Lemme et al., 2002	68	67	64
Marvil et al.	Perdue Farms, Inc., Salisbury	USA	Hoehler et al., 2005b	70	66	–
Qi and Wu	Chin. Acad. of Agr. Sci.	China	Xiao et al., 2007	53	66	–
Roemer and Abel	University of Göttingen	Germany	Roemer and Abel, 1999	46*		–
Rostagno and Barbosa	Univ. Federal de Vkosa	Brazil	Rostagno and Barbosa, 1995	73	59	–
Rostagno et al.	Univ. Federal de Vkosa	Brazil	Payne et al., 2006	68	–	–
Schutte e de Jong	TNO Institute Wageningen	The Netherlands	Schutte e de Jong, 1996	78	60	72
Vleira et al.	Univ. Rio Grand da Sul	Brazil	Hoehler et al., 2005b	52	82	56
Waldroup et al.	Univ. of Arkanasas	USA	Hoehler et al., 2005b	65	49	–
Wallis	SAC Dept. Biochem. & Nutr.	Scotland	Wallis, 1999	69	60	56
			Average	**64.1**	**62.2**	**58.1**
			n	**19**	**17**	**7**

* n-Resension

Relative effectiveness of liquid MHA-FA compared to DL-met for broilers determined from selected data sets published sine 1995 meeting requirements for simultaneous regression analysis

beside the RBV estimate for each source. The 95 % CI for DL-Met 65 % was from 55 % to 79 % and includes the expected value of 65 %. This is verification that the method was valid. In the case of DL-MHA, the 95 % CI was from 52 % to 75 % and does not include the expected value of 88 %. We would conclude that the RBV for DL-MHA is significantly ($P<0.05$) less than 88 % of DL-Met and not significantly different from DL-Met 65 %.

Similarly, using feed conversion as the response, the RBV estimate for DL-Met 65 % was 59 % and not significantly different from the expected value of 65 % (95 % CI = 41 % to 77 %). The RBV for DL-MHA was 67 %, which was significantly different from 88 % expected value but not significantly different from DL-Met 65 % value (95 % CI = 47 % to 87 %) .

Experimental evidence suggests that the actual methionine activity of DL-MHA is below 88 % on a product basis. RBV values for DL-MHA reported in the literature for pigs and poultry using average daily gain and feed conversion as the response criteria average below 70 % **(Table 1).**

The lower than expected performance of the analog may be related to partial polymerization (Nufer, 1966) resulting in dimers and oligomer with poor availability (Van Weerden et al., 1992; Mitchell and Lemme, 2008), lower effeciency of transporters (Maenz and Engele-Schaan, 1996a and b) and partial metabolism by intestinal microbes (Maenz and Engele-Schaan, 1996b).

While L-Met is the form used in metabolic processes of higher organisms, most species are able to convert the D form effciently to L and effectively use dietary sources of either (Lewis and Baker, 1995). Several RBV trials have recently been conducted using the approaches just outlined to compare L-Met to DL-Met.

Shen et al (2014) estimated the RBV of L-Met versus DL-Met in nursery pigs. A total of 168 cross bred pigs were alotted, three pigs per pen, to 56 pens. Eight replicate pens were assigned to one of seven dietary treatments: an unsupplemented methionine deficient basal diet or the basal diet suppemented with one of three graded levels of Met (0.048 %, 0.096 %, or 0.114 %) from either DL-Met or L-Met. The results for ADG were fitted to an exponential model **(Figure 6).**

The RBV estimate for L-Met from the slope ratio using ADG as the response variable was 159.1 %. The 95 % CI, however, spans the interval from 45.1 % to 272.8 %. The estmate, therefore, is not significantly different from 100 %.

A trial carried out with in Spain at PigCHAMP Pro Europa (Htoo and Morales, 2016) also estimated the RBV of L-Met in nursery pigs. A total of 252 8 kg pigs were allocated, 6 pigs per pen, to 42 pens. Six replicate pens were assigned to one of 7 dietary treatments: an unsupplemented methionine deficient basal diet or the basal diet with one of three levels (0.05, 0.10 or 0.15 %) of DL- or L-Met. The results for ADG were fit to a linear model **(Figure 7).**

The slopes of the lines were 103.8 and 103.4 for DL- and L-Met, respectively. This yields an RBV estimate of 100 % for L-Met. The 95 % CI was from 65 % to 134 %, indicating no significant differences in the methionine sources. Based on feed conversion, the RBV of L-Met was 89 % of DL-Met with a 95 % CI of 31 % to 147 %, also indicating no significant differences in the sources.

Kong et al (2016) estimated an RBV for DL-Met using L-Met as the standard in 13 kg pigs in a N balance study. The researchers calculated an RBV of 87.9 % for grams of N retained and 89.3 % for percent N retained. The 95 % CI (56 % to 120 %, g N; 57 % to 122 %, % N) indicate no significant difference between the sources for either response. These studies all support a 100 % RBV for L-Met versus DL-Met and are in agreement with other studies that have found high utilization rates of the D- form of methionine.

In conclusion, methionine supplements are best compared through simultaneous dose response trials. Data can be fit to

FIGURE 6

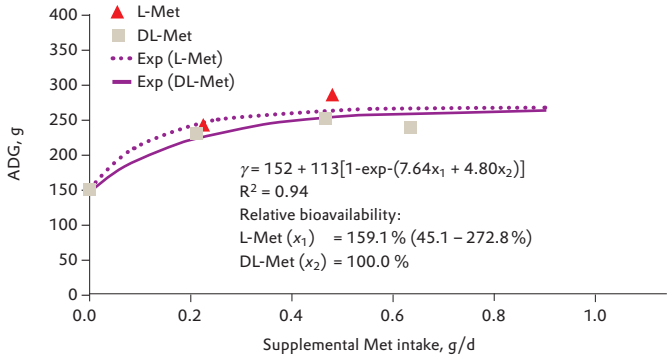


FIGURE 7

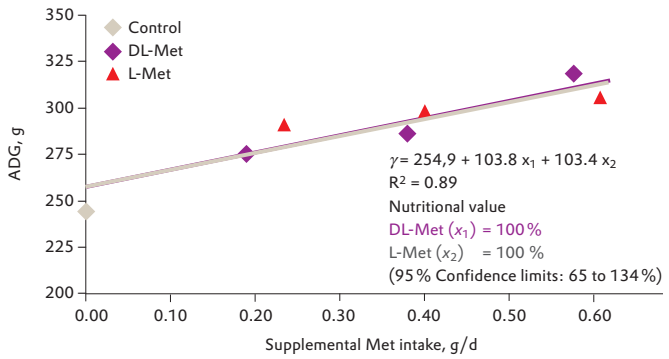
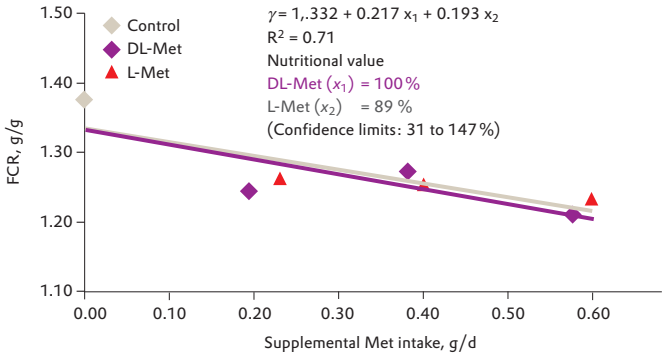


FIGURE 8



either linear or non-linear models successfully. The ratio of the slope of the test ingredient response to a standard ingredient provides an estimate of the RBV. Dosage levels should include the sensitive range below the nutrient requirement. Trials using diluted D,L-Met have validated the use of slope ratio to accurately predict the RBV of methionine sources. The estimate of the slope ratio must be compared to the 95% confidence interval or some other "fiduciary" limits to determine its significance. Finally, nutritionists and purchasing managers would benefit from a throrough understanding of RBV of the ingredients they have available. The consequences of not knowing the relative value of different sources of the same nutrient can have a huge financial impact on cost of production.

REFERENCES

Briens, M., Y. Mercier, F. Rouffineau, F. Mercerand, and P. Geraert. 2014. 2-Hydroxy-4-methyselenobutanoic acid induces additional tissue selenium enrichment in broiler chickens compared with other selenium sources. Poultry Sci. 93:85–93. doi: 10.3382/ps.2013–03182.

Hoehler, D., A. Lemme, S.K. Jensen, and S.L. Vieira. 2005. Relative effectiveness of methionine sources in diets for broiler chickens. J. Appl. Poult. Res. 14:679–693. doi: 10.1093/japr/14.4679.

Htoo, J.K. and J. Morales. 2016. Bioavailability of L-methionine relative to DL-methionine as a methionine source. J. Anim. Sci. 94:249252. doi: 10.2527/jas2015–9796.

Nufer, H.L., inventor. Monsanto Company, assignor. 1966. Preparation of methionine analogues. United States patent US 3,272,860.

Kong, C., C.S. Park, J.Y. Ahn, and B.G. Kim. 2016. Relative bioavailability of DL-methionine compared with L-methionine fed to nursery pigs. Anim. Feed Sci. Tech. 215:181–185. doi:10.1016/j.anifeedsci.2016.03.011.

Lewis, A.J. and D.H. Baker. 1995. Bioavailability of D-amino acids and DL-hydroxy methionine. In: C.B. Ammerman, D.H. Baker and A.J. Lewis, editors, Bioavailability of Nutrients for Animals: amino acids, minerals, and vitamins. Academic Press. San Diego, CA. p. 67–81.

Littell, R.C., A.J. Lewis, and P.R. Henry. 1995. Statistical evaluation of bioavailability assays. In: C.B. Ammerman, D.H. Baker and A.J. Lewis, editors, Bioavailability of Nutrients for Animals: amino acids, minerals, and vitamins. Academic Press. San Diego, CA. p. 5–9.

Littell, R.C., P.R. Henry, A.J. Lewis, and C.B. Ammerman. 1997. Estimation of relative bioavailability of nutrients using SAS Procedures. J. Anim. Sci. 75:2672–2683. doi:10.2527/1997.75102672x.

Maenz, D.D. and C.M. Engele-Schaan. 1996a. Methionine and 2-hydroxy-4-methylthiobutanoic acid are transported by distinct NA+-dependent and H+-dependent systems in the brush border membrane of the chick intestinal epithelium. J. Nutr. 126:529–536.

Maenz, D.D. and C.M. Engele-Schaan. 1996b. Methionine and 2-hydroxy-4-methylthiobutanoic acid are partially converted to nonabsorbed compounds during passage through the small intestine and heat exposure does not affect small intestinal absorption of methionine sources in broiler chicks. J. Nutr. 126:1438–1444.

Mitchell, M.A. and A. Lemme. 2008. Examination of the composition of the luminal fluids in the small intestine of broilers and absorption of amino acids under various ambient temperatures measured in vivo. Intl. J. Poult. Sci.

Rodehutscord, M. and M. Pack. 1999. Estimates of essential amino acid requirements from dose-response studies with rainbow trout and broiler chicken: Effects of mathematical model. Archiv für Tierernährung. 52:223–244. doi:10:1080/17450399909386164.

Shen, Y.B., A.C. Weaver, and S.W. Kim. 2014. Effect of feed grade L-methionine on growth gerformance and gut health in nursery pigs compared with conventional DL-methionine. J. Anim. Sci. 92:5530-5539. doi:10.2527/jas2014–7830.

Van Weerden, E.J., J.B. Schutte and H.L. Bertram. 1992. Utilization of polymers of methionine hydroxyl analogue free acid (MHA-FA) in broiler chicks. Archiv für Geflügelkunde. 56:63–68.