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The distribution of the ratio of two correlated measured variables may not always be normal: Case studies related to meat quality and animal nutrition

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ABSTRACT

Ratios of measured normal variables associated with each other are commonly used in the animal sciences as an indicator of quality or some other meaningful outcome. For this purpose,the study of relative performance among two correlated outcomes is facilitated by the ratio of one to the other, *e.g.* omega-6 to omega-3 fatty acids ratio indicating meat quality for human health, closer to 1:1 the better. Similarly, feed constituent indigestibility (complement of digestibility) is the ratio of faecal output to intake. In practice, the tendency is to assume that such ratios are normally distributed; however, that may not always be the case. The statistical distribution of these ratios is a five parameter distribution, *i.e.* μ and σ for the variable in the numerator of the ratio, μ and σ for the variable in the denominator and the correlation between both variables. The relative sizes of the coefficients of variation of the numerator and denominatorfor a given correlation will determine if the distribution is normal or not. In this communication, we demonstrate this phenomenon by analysing some meaningful ratios (*viz.* omega-6:omega-3, volatile fatty acid relative proportions and indigestibility) and outline some simple tests for samples and ratio normality evaluation.

Key words: Coefficient of variation, correlation, meat quality, omega-6, omega-3, ratio distribution, volatile fatty acids, digestibility

STATISTICAL BACKGROUND

In most areas of agricultural and environmental research, we come across measured or calculated variables that display pairwise correlation. Such correlations vary in strength. Study of the ratio of correlated normal variables is an integral part of experimental work, *e.g.* compositional data, digestibility, volatile fatty acid (VFA) ratios, omega-6: omega-3 in studies of polyunsaturated fatty acids, respiration quotients in animal energetics (CO_2 / O_2) etc. Another important case is when fitting a linear regression model (this may be the actual model or the limiting case of another model, *e.g.* limiting case of the simple exponential model is a straight line model) and we need inverse interpolation to find, say, x_0 at y_0 (Cedilnik et al., 2004), *viz.* Here x_0 may be interpreted as an estimate of, for example, lag-time or a much more meaningful quantity such as an estimate of the maintenance energy requirement in animals (Dhanoa et al., 2016a). However, there are situations when assumption that the distribution of such ratio data is normal can be erroneous.

For normally distributed variables X_i ($i = 1, 2$) with means μ_i, variances σ_i^2 and correlation coefficient ρ, the density function of the ratio $V = X_1/X_2$ was derived by Geary (1930), Fieller (1932) and later by Hinkley (1969). This joint density function, $f(v)$, is defined as follows with five parameters, μ_1, μ_2, σ_1, σ_2, ρ.

where, v denotes the argument of the density function, Φ is the cumulative distribution function of normal variable with mean 0 and variance 1, and

Other derivations of the ratio distribution and its applications have been described elsewhere (Marsaglia, 1965; Korhonen and Narula, 1989; Öksoy and Aroian, 1994; Pham-Gia et al., 2006; Koti, 2007; Pollastri and Tulli, 2015).

The tendency is to assume that the distribution of any ratio of correlated normal variables is also normal but this may not always be true. This

$$f(v) = \frac{hl}{\sqrt{2\pi}\sigma_1\sigma_2 g^3}\left[\Phi\left(\frac{h}{\sqrt{1-\rho^2}\,g}\right) - \Phi\left(-\frac{h}{\sqrt{1-\rho^2}\,g}\right)\right] + \frac{\sqrt{1-\rho^2}}{\pi\sigma_1\sigma_2 g^2}\exp\left(-\frac{k}{2\left(1-\rho^2\right)}\right)$$

problem was illustrated by Shanmugalingam (1982) using a Monte Carlo study. From standard normal variables (*i.e.* mean zero and variance one) Z_1 and

$$h = \left(\frac{\mu_1}{\sigma_1} - \frac{\rho\mu_2}{\sigma_2}\right)\frac{v}{\sigma_1} + \left(\frac{\mu_2}{\sigma_2} - \frac{\rho\mu_1}{\sigma_1}\right)\frac{1}{\sigma_2}$$

$$l = \exp\left[\frac{1}{2\left(1-\rho^2\right)}\left(\frac{h^2}{g^2} - k\right)\right]$$

$$g = \left(\frac{v^2}{\sigma_1^2} - \frac{2\rho v}{\sigma_1\sigma_2} + \frac{1}{\sigma_2^2}\right)^{\frac{1}{2}}$$

$$k = \frac{\mu_1^2}{\sigma_1^2} - \frac{2\rho\mu_1\mu_2}{\sigma_1\sigma_2} + \frac{\mu_2^2}{\sigma_2^2},$$

Z_2, two correlated variables (sample size =20) were generated following the procedures described by Kemp and Loukas (1978), *viz.*

Two possible cases were simulated, with correlations (ρ) between $X_1(=Y)$ and $X_2(=X)$ of either 0.30 or 0.75. This process was repeated 400 times. At each correlation, regions of normally distributed samples were identified in relation to the CV% of the ratio numerator X_1, ($CV_1 = \sigma_1/\mu_1$) and denominator, X_2 ($CV_2 = \sigma_2/\mu_2$). The maximum for CV_1 was 66.7% and CV_2 accounted up to 40%. The study showed that ratio samples were generally (> 50 %) normal if CV_1 was less than ≈25% at ρ = 0.30, whereas at ρ=0.75 the same applied if CV_1 was less than ≈20% across the whole range of CV_2.

$$X_1 = \mu_1 + Z_1\sigma_1$$

$$X_2 = \mu_2 + \sigma_2\left(\rho\, Z_1 + \sqrt{1-\rho^2}\; Z_2\right)$$

Unwarranted assumptions about normality can have unexpected implications. It is always prudent to check the ratio-numerator and ratio-denominator coefficients of variations and seek appropriate data transformation. The Box-Cox transformation (Box and Cox, 1964) system, which includes logarithmic transformation when λ=0 (see Dhanoa et al., 2016b for consequences of log-transformation), is a good starting point if the ratio sample is not normally distributed. The Geary-Hinkley transformation is an alternative and under suitable conditions, makes the distribution of the ($W=Y/X$) approximately N (0,1). Hayya et al. (1975) studied the performance of the Geary-Hinkley transformation and concluded that normal approximation is possible using :

Provided that the CV of the denominator, *X,* is <39% and the CV of the numerator, *Y,* is >5%. These authors also carried out a Monte Carlo simulation study and found that results were not affected by the size of correlation ρ. The confidence interval and standard error of ratio W may be calculated as described by Dunlap and Silver (1986).

Just like the ratio distribution, the product of independent normal variables may be normal under certain conditions only *e.g.* as the inverse of CV (μ/σ) becomes smaller (Seijas-Macías and Oliveira, 2012). Similarly, the sum of dependent normal variables can be non-normal sometimes (Holton, 2003).

$$z = \frac{W\mu_x - \mu_y}{\sqrt{\sigma_y^2 - 2W\rho\sigma_x\sigma_y + W^2\sigma_x^2}}$$

PRACTICAL APPLICATIONS

Possible ratios of measured quantities are too numerous to list here but to illustrate the general approach for analyses, we have used examples from the following three relatively important ratios in animal science:

1. Omega-6:omega-3 ratio of long-chain polyunsaturated fatty acids (LC-PUFA) in beef,
2. Nonglucogenic VFA ratio [(acetate + butyrate) / propionate] in rumen contents used in ruminant feed evaluation studies, and
3. Digestibility of animal feed components.

1. Omega-6: omega-3 LC-PUFA ratio in beef

Debate about saturated and unsaturated fats aside,essentiallong-chain polyunsaturated fatty acids (LC-PUFA), with 18 or more carbons are considered to be important in the nutrition and wellbeing of humans and animals. Important LC-PUFA in human diets are discussed by Abedi and Sahari (2014) such as α-linolenic acid (18:3*n*-3), docosahexaenoic acid (22:6*n*-3), eicosapentaenoic acid (20:5*n*-3) and docosahexanenoic acid (22:5*n*-3) and others. Simopoulos (2002) discussed the importance of reducing the ratio of omega-6 to omega-3 for medical benefits and Harris et al. (2009) detailed cardiovascular disease risks of omega-6 fatty acids. Trees, plants and their products do give us sufficient supply of PUFA for our needs. Even the products from animals living on humble grass add to the supply of PUFAs. GB Health Watch list sources of omega-3 and omega-6 fatty acids and their effects on chronic diseases and medical implications (http://www.gbhealthwatch.com/Science-Omega3-Omega6.php; 11 January 2018). Additional comprehensive description of PUFA functionality can be found in Abedi and Sahari (2014). As described above, the health and medical concerns surrounding the omega-6:omega-3 ratio require that meat produced in the UK contains as low a ratio as can be achieved through good animal nutrition. For an example of this ratio, we have taken meat quality data from beef animals either grazing or eating forage-based diets.

a) Grazing cattle

Marley et al. (2018) reported fatty acid concentrations in muscle *longissimus* from beef steers (n=18) after grazing a perennial ryegrass sward in a 2-year finishing system. Omega-6 was calculated as the sum of 18:2*n*-6, 20:3*n*-6, 20:4*n*-6 and 22:4*n*-6 and omega-3 as the sum of 18:3*n*-3, 20:4*n*-3, 20:5*n*-3, 22:5*n*-3 and 22:6*n*-3. Mean concentrations of omega-6 and omega-3 were 90.03 and 86.04 mg 100 g^{-1} tissue with CV's of 12.99 and 7.40%, respectively, and correlation 0.7239. There was no evidence of departure from normality in omega-6, omega-3 or the ratio (P = 0.066, 0.171 and 0.897, respectively).

b) Indoor-fed cattle

Warren et al. (2008) reported body composition data for animals fed either grass silage or a concentrate diet consisting of barley, molassed sugar beet pulp, molasses and full-fat soya. Effects of diet (concentrate versus silage), breed (Aberdeen Angus (AA) versus Hereford (HF)) and slaughter age (14, 19 or 24 months) were evaluated. There were eight steers on each of these 12 treatments. Along with all other carcass composition, long chain PUFAs were measured in the *longissimus* muscle and the omega-6 to omega-3 ratio calculated. Apart from one treatment (concentrate/AA/24 months), all other samples were normally distributed. With increasing age, omega-6 concentration increased relative to omega-3 and the omega-6: omega-3 ratio was very high for all concentrate treatments compared to the corresponding silage treatments (Fig. 1).

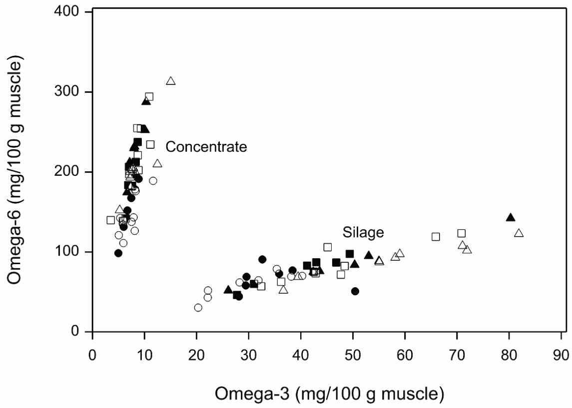

Fig. 1. Dichotomy between concentrate and silage diets when omega-6 versus omega-3 ratios in *M. longissimus* muscle of steers are compared (Warren et al., 2008). Aberdeen Angus and Hereford breeds are denoted by open and closed symbols and 14-, 19- and 24-month slaughter ages by circles, squares and triangles, respectively.

After visualising the data it was clear that overall analysis of the combined data set for all treatment combinations was not possible. Parameters relating to the distribution of the omega-6: omega-3 ratio within each of the 12 treatment combinations are given in Table 2. Both numerator and denominator were without exception normally distributed. With one exception the correlation was above 0.7. The ratio of omega-6: omega-3 was non-normal for only one treatment combination and based on data reported by Shanmugalingam (1982) between 50 and 80% of samples with similar CV% values to those observed for Concentrate/HF/24 months would give a normally distributed ratio.

Table 1. Ratio distribution of the PUFA omega-6 : omega-3 tested in experimental study (Warren et al., 2008) with two diets (concentrate, silage), two breeds (Aberdeen Angus (AA), Hereford (HF)) and three slaughter ages (14, 19, 24 months) (n = 8 per treatment combination)

Diet	Breed	Age	CV%		ρ	Normality test[#]probability		
			Omega-6	Omega-3		Omega-6	Omega-3	Ratio
Concentrate	AA	14	17.6	27.2	0.728	0.629	0.283	0.595
		19	25.4	22.6	0.910	0.612	0.311	0.649
		24	19.3	16.5	0.820	0.052	0.250	0.025
	HF	14	18.3	15.0	0.824	0.520	0.733	0.501
		19	16.9	13.2	0.868	0.688	0.615	0.884
		24	18.7	24.1	0.788	0.856	0.652	0.053
Silage	AA	14	27.1	26.8	0.890	0.289	0.680	0.716
		19	28.4	23.1	0.937	0.146	0.161	0.453
		24	23.4	21.6	0.972	0.445	0.594	0.740
	HF	14	25.9	25.5	0.249	0.966	0.552	0.472
		19	27.7	28.9	0.987	0.641	0.634	0.436
		24	31.0	29.5	0.993	0.137	0.407	0.658

[#] Shapiro-Wilk test of normality (Royston, 1993; 1995)

2. Nonglucogenic VFA ratio following *in vitro* fermentation

Ingested food by the ruminants undergoes microbial fermentation in the rumen and important products are VFAs which are a major source of energy for the animals. The relative pool sizes of the main VFAs (acetate, propionate, butyrate, valerate,etc.) depends on the type of feed substrate, microbial population and the rumen environment. The ratio of non-glucogenic VFAs (acetate and butyrate) to the glucogenic fatty acid (propionate) is important as it is associated with methane production, milk composition and energy balance (Morvay et al., 2011). Glucogenic VFA is the main source of glucose production in the animal whilst non-glucogenic VFAs are precursors for long-chain fatty acids in body fat depots. Similarly, VFA are end-products formed by microbial fermentation of carbohydrates in the hind-gut of herbivore animals (e.g., horses). VFA data from an in vitro gas production studyreported by Murray et al. (2009) using the methodology detailed by (France et al., 1993, Theodorou et al., 1994) was used to give examples of this ratio. Inoculum was derived from faeces from horses fed grass hay. Horses were either predisposed to laminitis or clinically normal (n = 7 per group) and three substrates, grass hay, starch and inulin, were compared. At the end of the in vitro incubation of substrates, VFA concentrations were assayed in the incubation medium. Barycentric triangle plots (Aitchison, 1986) (Fig. 2) allow us to visualise the relative proportions of the three main VFAs but the distribution of any ratio between specific VFAs needs to be examined prior to further statistical analyses. Table 2 shows the parameters needed to test the ratio distribution along with normal distribution test of the numerator and denominator parts of the nonglucogenic ratio, (Ac+Bu)/Pr.

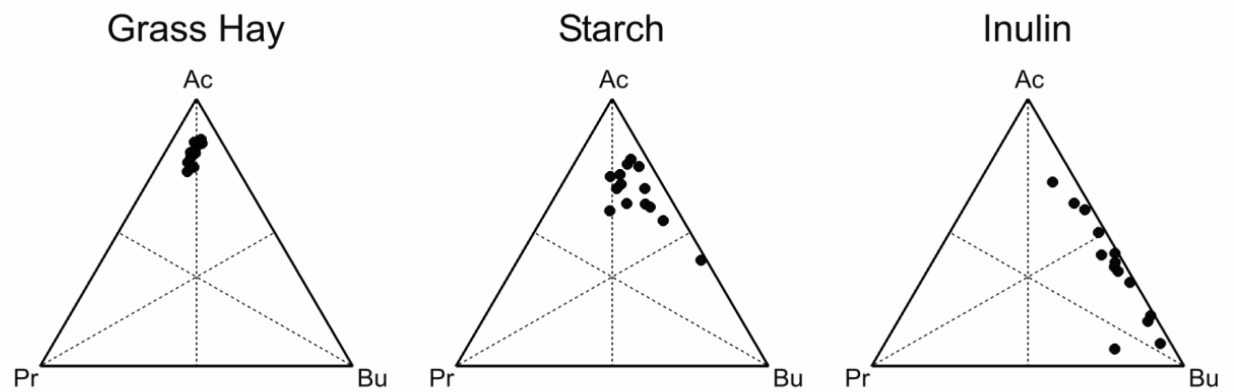

Fig. 2. Barycentric triangle display of the relative concentrations of the principal VFAs, acetate (Ac), butyrate (Bu) and propionate (Pr) following *in vitro* fermentation of grass hay, starch or inulin using inocula derived from faeces from either clinically normal horses or horses predisposed to laminitis.

As expected with either the numerator or denominator or both being non-normal and with the high CV% for propionate the nonglucogenic ratios are generally non-normal (Table 2). These results suggest that the distribution of the ratios should be examined and the data transformed appropriately before further analysis.

3. *In vivo* digestibility

For proper and target-driven animal nutrition it is necessary to establish the relevant quality of feedstuff (Blaxter et al., 1956; Raymond, 1969; Dhanoa et al., 2008). Feeds can be characterised *in vitro*, *in vivo* or *in situ* (Lopez et al., 2005), but the reference quality measurements only come from *in vivo* assessments. The *in vivo* digestibility procedure consists of determination of a) intake and b) corresponding voided faecal matter of a feed constituent of interest. Given this information, then the whole tract digestibility of a feed component, say DM, is the balance between intake and output as a proportion of intake i.e. (DM intake– Faecal DM output)/ DM Intake. The complement of this is indigestibility of a feed component and this is simply the ratio of Faecal DM output / Feed DM intake.The distribution of this indigestibility ratio will carry animal variability plus other uncertainty as *in vivo* assessment takes some time to complete.

Indigestibility coefficients for various components of silage-based diets offered to growing steers were calculated using data from a trial carried out by Sanderson et al. (1992) (Table 3). It is clearly prudent to examine the distribution of these indigestibility ratios.

Table 2. Variability and correlation associated with final concentrations of acetate + butyrate (Ac+Bu) and propionate (Pr) in incubation medium from an *in vitro* gas production study using inoculum derived from the faeces of normal horses and horses predisposed to laminitis (Murray et al., 2009)

Laminitis	Substrate	CV%		ρ	Normality test# probability		
		Ac+Bu	Pr		Ac+Bu	Pr	(Ac+Bu) / Pr
Normal	Grass hay	8.6	39.0	–0.056	0.323	0.038	0.002
	Starch	30.5	63.9	0.724	0.002	0.397	<0.001
	Inulin	38.5	81.1	0.070	0.014	0.014	<0.001
Predisposed	Grass hay	15.1	22.2	0.318	0.004	0.379	0.212
	Starch	15.0	74.1	–0.214	0.808	0.004	0.155
	Inulin	44.3	120.8	0.638	0.180	<0.001	0.003
Overall		32.8	79.2	0.522	0.015	<0.001	<0.001

Shapiro-Wilk test of normality (Royston, 1993; 1995)

Table 3. Indigestibility data for various constituents of silage-based diets fed to steers at a near maintenance feeding level (n=24) (Sanderson et al., 1992)

Feed component	CV%		ρ	Normality test# probability		
	Faeces	Intake		Faeces	Intake	Faeces/ Intake
Dry matter	15.9	15.1	0.653	0.035	0.817	<0.001
Organic matter	16.2	14.9	0.652	0.062	0.581	0.001
Neutral detergent fibre	14.8	14.1	0.560	0.015	0.074	0.007
Acid detergent fibre	16.5	13.3	0.652	0.052	0.141	0.018
Nitrogen	16.9	22.8	0.865	0.184	0.886	0.778
Gross energy	16.2	16.8	0.761	0.087	0.459	0.011

Shapiro-Wilk test of normality (Royston, 1993; 1995)

CONCLUSION

In most scientific disciplines, ratios of measured quantities feature prominently. The distribution of the ratio of correlated quantities depends on its five parameters, the mean and variance of both the variables in the numerator and in the denominator, and their correlation. Shapiro-Wilk test of normality (Royston, 1993, 1995) or some other test may be used to test the normality of ratio samples. If departure from normality is indicated, then some suitable transformation can be identified using,for example, the Box and Cox (1964) transformation system. Examination of the three important ratios within this communication underlines the need for scrutiny of ratio sample distribution in order to ensure further analyses are appropriate.

Study of ratios is a means by which two interacting measured quantities are convoluted into a meaningful entity. However, distributional aspects need to be kept in mind when undertaking statistical analyses and modelling of the ratio data. Growth of components of whole organism generally are modelled using the allometric relationship or condensed into ratios. Another option would be the part-whole relationships using some appropriate regression model (Dhanoa et al. 2016). Untested distributional assumptions may lead to problems in statistical analyses. The outcome of this research is of relevance because in scientific pursuit one should always ask questions and seek to provide answers with evidence.

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