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Reply – Simplified estimation of forage degradability in the rumen assuming zero-order kinetics and assumptions underlying the *in situ* polyester-bag technique

(Revised MS received 6 August 2009; First published online 9 September 2009)

Our paper (Dhanoa *et al.* 2009) attracts criticism in the Letter to The Editor published above. The comments are in our view misdirected. They are not so much about the content of our paper but the related issue of the suppositions underlying the analysis of *in situ* degradation curves. Our choice of a simple first-order model to compare with our zero-order procedure was not necessarily approval of the assumptions underlying the first-order model. In fact, we have identified and commented on those very assumptions for some time and tried to offer alternatives, as explained below.

We describe a new procedure in Dhanoa *et al.* (2009) for calculating extent of ruminal degradation (E), with an assumed rate of passage from the rumen and evaluate the new procedure using the discrete lag exponential (DL-EXP) model (Dhanoa 1988). While this model is very similar to the exponential (EXP) model of Ørskov & McDonald (1979), simultaneous estimation of discrete lag and exponential function parameters in the DL-EXP model mimics the sigmoidal features of *in situ* degradation curves. The simple EXP model has no inflexion point and hence is unable to approximate a sigmoidal shape. A logical alternative model would be either a double exponential or a classical growth function (e.g. Gompertz) (France *et al.* 1990; López *et al.* 1999). However, animal welfare considerations do not allow sufficiently frequent collection of data in the earlier stages of incubation to warrant general recommendation of these models. A new growth function (France *et al.* 1996) was used by Dhanoa *et al.* (1995) to improve on the DL-EXP model and was found to be superior in describing *in situ* curves. The impact on estimates of E depended on type and quality of the substrate. Further work on several other candidate models was undertaken by France *et al.* (1990) and López *et al.* (1999). Thus choice of a suitable model depends on the quality and nature of substrate (see Dhanoa *et al.* 1996). Dangers in interpreting computer-fitting solutions were pointed out by Sandoval-Castro (1996) and consequences of model choice

outlined by Dhanoa *et al.* (1996). Thus recommending and approving the use of a particular model is not sensible. However, for validation of our zero-order procedure in Dhanoa *et al.* (2009), we chose a first-order model and for that purpose the choice of the popular DL-EXP/EXP model was, we believe, appropriate.

The most contentious issue in relation to the EXP model has been treatment of the so-called soluble fraction (W). Firstly it is not *all* soluble. Small particles escape from the polyester bag in proportion to the pore sizes used. This effect was explored by Cockburn *et al.* (1993) using very small pore size filter paper. In that study the soluble fractions for grass and maize silages were 0.12 and 0.10 with filter paper, 0.16 and 0.14 with gentle hand washing of nylon bags, and 0.31 and 0.48 with bags when using a washing machine. This kind of soluble fraction over-estimation inevitably leads to an over-estimate of E because W in the EXP model is assumed to be rapidly and completely degraded. Further work was published by López *et al.* (1994) describing a correction procedure for particular matter loss from the *in situ* polyester-bag technique. In a detailed study, Dhanoa *et al.* (1999) consider various options for the fraction escaping from the polyester bags. The escaped fraction was partitioned into a soluble fraction (W_S) and a degradable particulate fraction (W_D). Clearly W_D will be subject to losses both due to out-flow and degradation. The effect of choice of relevant passage rate resulted in eqns (2)–(11) in Dhanoa *et al.* (1999) for the calculation of E . Four notable options for losses of W_D due to passage were (1) at the same rate as the remaining particulate fraction in the polyester bag, (2) at the rate of passage of liquid, (3) at the average of liquid and particulate passage rates and (4) assume there is no instantly and completely degradable fraction. We believe these suggestions mitigate some of the problems caused by assumptions implicit in the use of the EXP model. Also, solubility depends on the solvent characteristics. For example, de Jonge *et al.* (in press) showed decreasing the pH of

the solvent from 6.8 to 5.0 reduced N solubility for many feedstuffs. The effect was largest for untreated legume seeds, where N solubility decreased from 0.48 to 0.22 of total N upon a decline in pH from 6.8 to 5.0. This pH shift also affected the composition of the soluble N fraction by increasing the relative amount of non-protein-N and albumins and decreasing the relative amount of globulins. Moreover, solubility of N in water differed from that in buffers. With water, the final pH of the solution depended on the feedstuff and may be an undesirable source of variation among feedstuffs.

Thus, the soluble fraction is a methodological issue rather than a modelling problem. Our procedure, described in Dhanoa *et al.* (2009), can incorporate various kinetics of degradation of the soluble fraction. Actually two situations are examined in the paper (see the figures), one with a soluble fraction *W* that is completely and instantly degraded, and another with no *W* fraction, so that all the degradable fraction (soluble or insoluble) is degraded at the same rate, even though it has been shown that in this latter case *E* can be significantly underestimated (López *et al.* 2000). If a method is developed providing a more accurate measure of the soluble fraction (without particle loss) or of its rate of degradation, these values can be incorporated into our procedure to obtain more accurate estimates of effective degradability, but our procedure would be much the same, i.e. the assumptions about the kinetics of degradation of the *W* fraction do not invalidate our approach.

As far we are aware, most models describing passage of digesta through the gastro-intestinal tract consider at least two pools (e.g. Blaxter *et al.* 1956). Two-pool models were generalized to multi-compartmental models in Dhanoa *et al.* (1985) and France *et al.* (1985). Also concepts of diffusion and viscosity were incorporated by France *et al.* (1993) and Thornley *et al.* (1995) to examine the validity of two-pool and multi-pool models. The criticism by authors of this Letter to the Editor that 'another false assumption is the first-order one-compartment passage model' is not consistent with the literature. Perhaps they are thinking of die-back curves that can sometimes be analysed using the EXP model. Even here one must be mindful of the lag phase after infusion ceases and the declining phase commences. The assumption of first-order passage is perhaps not as influential in calculating *E* or protein absorbed from the intestine as the authors suggest. They do not demonstrate that this is so, but merely suggest it could be a reason for error when calculating *E*. There are many other factors that could be at least as influential as passage kinetics in estimating rumen outflow, such as marker used, sampling site (duodenum or faeces), or various methodological aspects.

The authors of the Letter base much of their case on a recent meta-analysis by Huhtanen & Hristov (2009),

which found rumen undegradable protein (RUP) was not correlated with milk production (MY). For various reasons, this cannot be the definitive argument. Firstly, the authors assume the dairy cow responds to the supply of additional metabolizable protein in producing more milk protein. However, current protein evaluation systems are requirement-based and do not predict responses. Thus, such protein evaluation systems, which usually include RUP calculations based on the *in situ* technique, can only predict the supply of metabolizable protein. The response of the animal though is not within the prediction framework of such evaluation systems, and indeed the marginal response to extra protein is often far smaller than assumed in using fixed efficiencies in various protein evaluation systems (see Dijkstra *et al.* 2007 for full discussion of requirement v. response systems). Milk production will depend finally on energy and protein supply. Energy is well correlated. As for protein, the important variable is not just RUP but the amount of protein reaching the duodenum and absorbed in the intestine. This is a mixture of RUP and microbial protein. Even if there is no RUP reaching the intestine, microbial amino acids will be absorbed and the cow will produce milk. Also, supply of RUP can be very high (low rumen degradation, supply of by-pass protein) and milk yield can be low because degradable protein and energy supply are imbalanced and microbial protein synthesis is not optimal. In this case, MY is not low because of low RUP supply but because of low microbial protein, and there may be problems associated with microbial fermentation in the rumen due to impaired supply of degradable protein or energy (as for instance with a high supply of fats). Evidence of this situation is actually provided by Ipharraguerre & Clark (2005). In a meta-analysis, they showed that various RUP sources indeed increased the observed non-ammonia, non-microbial N flow into the duodenum by 26% compared with soybean meal controlled diets. Although RUP increased, in another dataset with milk production data, the effect of various RUP sources compared with soybean meal on milk protein output was small or even negative. Given that an actually observed (not calculated based on the *in situ* technique) increase in RUP did not result in milk production response already shows that the *in situ* technique cannot be the only source of error or is of no error at all. Indeed, Ipharraguerre & Clark (2005) showed that the microbial N flow to the duodenum decreased upon comparing RUP sources with soybean meal control, and total non-ammonia N flow only increased by 6%. This further emphasizes the need to provide better predictions of response in microbial protein flow to changes in the diet, as discussed by Dijkstra *et al.* (2007), but cannot be indicative of an overvalue of RUP because of incorrect assumptions in the *in situ* technique. Thus, there

are situations (and they may be found under practical feeding conditions) where MY is not correlated with RUP. To justify lack of correlation between RUP and MY on the basis RUP is poorly estimated due to incorrect kinetic assumptions regarding degradation of *W* and passage is therefore highly speculative.

The *in situ* technique and its associated modelling have been accepted in most feeding systems (NRC, AFRC, INRA and CSIRO) as a means of obtaining values of degradability. Obviously the approach does not provide a direct measure and may be subject to several sources of error, which have been extensively

reviewed in the literature (López 2005). However, until a more accurate method is validated and accepted, this approach will be used and in most cases provides acceptable estimates of *E*, both at the academic (scientific) and farm levels. The approach can be questioned by authors when they do not obtain acceptable values, but there are hundreds of papers where the estimates have been considered acceptable. Under such circumstances, any modelling procedure to simplify the approach should be considered a useful tool. The criticism the authors levy is not of our procedure (Dhanao *et al.* 2009) but of assumptions underlying the *in situ* technique itself.

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