THE DERIVATION OF CHEMICAL PREDICTION EQUATIONS FOR MONITORING ENERGY DECLARATIONS

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SUMMARY

Proposals for the introduction of energy declarations on mixed feeds have raised questions about monitoring and verifying the values. The use of direct bioassays or of in vitro simulation of digestion for this purpose is briefly discussed, but chemical prediction equations are most likely to be used in Europe.

Prediction equations are derived from experimental data on the measured ME values of feeds of varying chemical composition. The selection of interpretative models is briefly discussed, the main distinction being between purely empirical statistical descriptions of data and models which incorporate known biological concepts. Equations which are determined empirically but are subsequently found to agree with theoretical expectations find widespread support.

The derivation of equations in the U.K. and Europe is briefly described, The resulting range of alternative equations offers a choice between accuracy of prediction and complexity, and cost, of the chemical analyses. Selection of an equation should take account both of its predictive properties and the reproducibility of the analyses involved. Several equations are available with residual standard deviations, incorporating both these sources of variation, between 0.30 and 0.40 MJ/kg. By comparison the standard error of a bioassay result is 0.15 to 0.20 MJ/kg.

A ring-test of one equation is described. Within-laboratory repeatability was good (s.d. = 0.40% of mean) but between-laboratory reproducibility was much poorer (s.d. = 4.48% of mean). Better standardisation of analytical methods might improve the latter value. The correlation between observed and predicted ME values obtained with this equation was high (r = 0.98) but there was a large bias which varied significantly between cockerels and young chicks.

INTRODUCTION

In most countries of the world trade in compound animal feeds is governed by legal regulations. In particular these define the information on composition and nutritional value which must be provided by the seller and also means by which the values given can be monitored and verified. The regulations differ in detail and the impact they have on the pattern of trade will vary according to the organisation of the industry e.g. the importance of integration, co-operative trading etc. , However there are also a lot of elements which are common to most countries.

In the U.K. the main points of the regulations are a) that ingredients should be "wholesome suitable for their purpose and free fran associated hazards", b) declarations of oil, crude protein, fibre, ash levels; total vitamin A, E and D contents plus indications of

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storage life: copper (if >50 mg/kg), magnesium (>0.5%), molybdenum and selenium (if added), urea, biuret, urea phosphate and IBDU (expressed as protein equivalent), uric acid (expressed as protein equivalent if greater than 1%); antioxidants, colourants, preservatives and medicaments. These regulations have evolved over time, the recent developments being an interpretation for use in the U.K. of various directives from the European Economic Community. It is of course an eventual. purpose of the EEC #at uniform regulations should govern trade throughout the community.

Although this information is obviously useful it does not define the most important nutritional factors which determine the econanic value of a feed - energy and amino acid levels, Furthermore there is a view held by sane farmers that the declarations do not advise them sufficiently about the use of unusual ingredients, especially industrial by-products. It is not the purpose of this paper to reflect in any way the political arguments in the U.K. about declarations nor to represent the views of the compounders or their customers. Suffice it to say that a strong appeal to government from the Farmers Unions to legislate for open declarations (i.e. a listing of each formula) has led, not to agreement on this point, but to an undertaking that the amount of nutritionally useful information should be increased. In the first place energy declarations will be introduced and this runs in parallel with a similar decision in the EEC.

The introduction of energy declarations is not uncontroversial and since no legislation has been announced I will outline briefly how the topic has developed from a technical point of view. I should also stress that the views expressed here are personal ones. Once agreement was reached to introduce energy declarations then important questions arose as to how the values were to be defined and how they could be monitored and verified. Most of the discussion was about chemical prediction equations since this is the method of control most likely to be used. Alternatives such as rapid bioassays and in vitro digestion methods do exist but at this stage it is clear that legislation in Europe will be based on equations which relate the AME value of a feed (corrected to zero N-retention, AME_n) to readily defineable and measurable chemical components. Existing equations (Table 1) seemed insufficient for the purpose of verification for two main reasons. Firstly they did not consider a very wide range of chemical variables and therefore the potential pay-off between the complexity of equations and their accuracy could not be fully explored. Secondly, they did not take account of recent developments in ME systems, in particular the introduction of TME. The Poultry Research Centre were therefore asked to undertake new experiments and the results have been published (Fisher, 1982a). At about the same time technical discussions were taking place in Europe to establish a basis for legislation in the EEC. In this wider forum there was naturally a range of opinions and, to try and reconcile these, experimental data fran four (more recently five) laboratories were analysed. The 'best' equation from this combined analysis was similar to that suggested by Härtel et al. (1977) and it has nw been adopted as the basis of an EEC directive. The results of a ring-test to establish its reproducibility and some evaluation of its predictive properties have been published (Fisher, 1983).

At present these issues are being debated by government and by the interested parties. The eventual outcome is not certain but the

equation finally adopted within the EEC will probably be used throughout Europe and some alternative equations suggested in the work of Fisher (1982a), although more efficient and cheaper are likely to be overlooked. There are still some technical matters to be resolved.

PREDICTION EQUATIONS FOR METABOLISABLE ENERGY

The calculation of energy values fran the chemically defined constituents of a feed is well established. It is nearly 100 years since Atwater defined his 'factors' stating that protein, fat and carbohydrate, when digested and absorb&,' yield 4, 9 and 4 kcal/g 'available* energy. From this starting point a variety of chemical prediction equations for poultry have been proposed (Table 1) and widely used in practical feed formulation.

TABLE 1 Prediction equations for AME values

Equ.1. AME (kcal/g) = 53 + 38 (%CP + 2.25%FAT + 1.1%STC + %SUG)

Equ.2. AME (kcal/g) = 40.8 (0.87%CP + 0.87*2.25%FAT + AV.CHO + 4.09) [2] [3] [4]

Equ.3. AME_n (kcal/g) = 78.5%FAT + 35.2%CP + 41.0%STC + 35.5%SUG

Equ.4. AME_n (kcal/g) = 76.9%FAT + 36.2%CP + 40.6%STC + 26.1%SUG

Equ.5. AME_n (kJ/g) = -2.13 + 0.90 (0.235%CP + 0.395%FAT + 0.175%CF + 0.175%NFE) -27.9CF/(CF + NFE)

Transformed equations $\hat{y}$ = AME or AME_n, MJ/kg DM

Equ	$\hat{y}$	a	b_1	b_2	b_3	b_4	b_5	b_6
1	AME	0.247	35.8	15.9	17.5	15.9		
2	AME	0.836	33.4	14.9	17.1	15.5		
3	AME_n	-	32.8	14.7	17.2	14.9		
4	AME_n	-	32.2	15.1	17.0	10.9		
5	AME_n	-2.13	35.6	21.2		-	15.8	-27.9

x_1 = FAT, g/g; x_2 = CP, crude protein, g/g; x_3 = STC, starch, g/g; x_4 = SUG, sugar, g/g; x_5 = NFE + CF, NFE + crude fibre, g/g; x_6 = CF/(NFE + CF), g/g.

1/0.9 DM basis 2/AV.CHO = "available carbohydrate = starch + 0.91*sugar 3/constant = 4.9 for adults, = 2.5 for chicks 4/moisture content on which constant was computed is not known 5/DM basis

Equ. 1. Carpenter and Clegg (1956); Equ. 2. Bolton (1962)
Equ. 3. Sibbald et al. (1963); Equ. 4. Härtel et al. (1977);
Equ. 5. Moir et al. (1980).

It is interesting that most of these equations, although derived independantly and in different ways, are extremely similar when recalculated on a comparable basis. The coefficients for fats, proteins and carbohydrates are also realistic if it is assumed that, when digested, these nutrients yield 38.5, 18.5 and 17.2 W/g respectively (Härtel et al. 1977).

The derivation of prediction equations has been reviewed elsewhere (Fisher 1982b) but two points should be stressed. Having assembled data on the ME values of a range of feeds of varying composition they can be interpreted in different ways and judicious selection of an interpretative model can overcome some of the inherent limitations of the experimental approach. An equation may be judged to be more "robust" for practical use if, in addition to being an effective empirical descriptor of the data from which it was derived, it is also consistent with the external evidence and expectations about the underlying relationships. Thus, for example, we can consider whether equations should contain constant terms, whether they should contain negative predictors and whether the coefficients agree with theoretical energy values.

Models which are a summation of the energy yielding components of a feed are attractive. If they include all such components a constant term should not be required, and if each component can reasonably be represented by a single coefficient for all feeds, then other dietary characteristics and interactions should not be required. Such arguments led Härtel (1979) to propose an equation with fat, protein, starch and sugar as energy-yielding variables and with no constant. Obviously this argument has limitations. If the energy value of a digested nutrient is constant, then the use of single coefficients to describe crude nutrients is equivalent to assuming constant digestibility for all feeds, This is clearly untrue and a factor such as fibre level my feature in a prediction equation as an empirical index of digestibility rather than an energy source per se.

The second general issue is the implication for prediction equations of Sibbald's ideas about true and apparent ME. Sibbald (1976) and elsewhere has argued that the constant excretion of endogenous energy from birds in ME experiments leads to the observed AME being reduced as food intake declines. The result is that AME values may be systematically underestimated in some feeds and if these effects are correlated with any chemical variable e.g. high fibre levels, then this will lead to a spurious relationship between energy values and chemical canposition and the true relationship will be concealed, This problem can be overcome in several ways e.g. by controlling intake (Fisher 1982a; Härtel et al. 1977) but it should not be ignored.

POULTRY RESEARCH CENTRE EXPERIMENTS

These have been reported by Fisher (1982a). Twenty eight feeds made fran practical feed ingredients and varying in fat (20-160 g/kg), crude protein (120-250 g/kg) and calculated AME (9-15 kJ/g) were used. Each was tested both as a meal and pelleted but as extrusion had little effect the data were combined to give 56 estimates of ME for feeds of known canposition. Each feed was analysed for a range of chemical variables in at least three laboratories to provide an estimate of reproducibility of the chemical analyses.

Metabolisable energy determinations were made with adult cockerels using-, a modification of Sibbald's (1976) TME assay. Six replicate birds were given 30 g of each test feed by intubation after a 40 h starvation period. Excreta were collected for 48 h. Endogenous energy losses were determined in birds treated similarly but given 30 g

TABLE 2. Derived prediction equations for AME_n values, MJ/kg (Fisher, 1982a)

Equation	x_1	x_2	x_3	x_4	x_5	x_6	x_7	a	b_1	b_2	b_3	b_4	b_5	b_6	b_7
6	FAT	STC	NDF	CP	USR			7.42	26.22	9.76	-9.30	7.93	0.069		
7	FAT	STC	NDF	CP				8.33	25.72	8.97	-10.59	7.18			
8	FAT	STC	CF	CP				6.21	26.92	10.84	-16.10	10.49			
9	FAT	STC_2	CP	SUG				-	34.25	17.86	16.66	18.47			
10	FAT	FAT^2	CF	CP	ASH			18.10	34.66	-83.25	-26.23	1.17	-66.14		
11	FAT	FAT*CF	CP	CP*CF	NFE	NFE*CF	CF	-20.11	54.23	-177.8	38.85	-201.9	38.01	-188.4	147.0

	r^2	s	s'	s"
6	.985	.242	.331	.194
7	.983	.260	.342	.190
8	.981	.277	.355	.204
9	.975	.311	.473	.306
10	.956	.421	.482	.198
11	.980	.292	.475	.322

STC - starch enzymatic method; USR - unsaturated:saturated fatty acid ratio.
FAT*CF - fat x crude fibre etc.
Equations predict AME_n in MJ/kg when nutrient concentrations are expressed as g/g, except USR which is dimensionless.
Coefficients underlined are less than 2x their standard error.

. Over a series of experiments endogenous losses of energy, corrected to zero N- balance, were found to be very constant and a single value 32.5 kJ/48 h was used in all calculations, Starting with the observed AME_n values for birds receiving 30 g food, values corresponding with an intake of 80 g were calculated via TME as follows (McNab and Fisher, 1982).

$$TME_n = AME_n(30) + EEL_n\ (48h)/30$$

$$AME_n(80) = TME_n - EEL_n\ (24h)/80$$

EEL_n (24 h) was calculated as 0.55 EEL_n (48 h), the factor 0.55 being derived from a number of published and unpublished experiments.

The rationale of these procedures was to use the rapid and accurate Sibbald method and to avoid bias due to variations in food intake, With the benefit of hindsight and of recent developments in our technique we would now use a higher intake, 50 or 60 g, with a consequent reduction in inherent errors.

The data fran this experiment consisted of 56 sets of AME_n(80) values and corresponding results for 14 analytical variables; the proximate components, neutral and acid detergent fibre, acid detergent lignin, Christian lignin, starch (by enzymic hydrolysis and polarimetry), sugar, fatty acid ratios, gross energy and a measure of in vitro 'digestibility. These were analysed by conventional regression methods which produce an estimate of residual standard deviation (s) by which equations can be assessed. However in a related study on ruminant feeds (Wainman et al. 1981) it was pointed out that the reproducibility of the chemical analyses used should also be considered when assessing each equation. Thus of two equations with the same s value, the one with more reproducible analyses will be preferred for practical use. The cost of the analyses will also be important but this has not been formally incorporated into the assessment of equations.

Therefore the 'accuracy' of equations can be looked at in three ways .

1. by s, the conventional residual standard deviation. This measures how well an equation described the observed variation in AME.

2. by s', a standard deviation which includes both the unexplained variation and the analytical variability. The derivation of this will be found in Wainman et al. (1981) or Fisher (1982a).

3. by s", a standard deviation which includes only the analytical variability.

The selection of an equation should be based on s, and, in particular, on s'. Once selected, reproducibility is a function only of s", and this forms the basis, for example, of tolerance limits.

Several thousand prediction equations were computed during this work but only the six shown in Table 2 will be discussed. Equation 6 is the best descriptor of these data that was found; best in the sense that it had the lowest s value and all of the regression coefficients were individually significant (t>2). It explained 98.5% of the observed variation in the observed AME values and has an s value of

0.24 MJ//kg, increasing to 0.33 MJ/kg when the variability of the analytical methods is considered (s'). This is a fairly complex and costly equation. The calculated energy value for fat (33.6 kJ/g), starch (17.2 kJ/g) and protein (15.3 kJ/g) are realistic but there is a highly significant negative effect of NDF which is assumed to be acting as an index of digestibility. The inclusion of this negative term for a "fibre" fraction necessitates the positive and significant constant term. The unsaturated to saturated fatty acid ratio has a small, but statistically significant, effect on goodness of fit.

Equation 7 is the same as equ. 6 except that the expensive fatty acid analysis is omitted. This has only a minor effect and gives a slight improvement in the s" value. Equation 8 is the same as 7 except that CF is substituted for NDF. A general finding in this work was that NDF was a more effective predictor of ME than CF. Equation 9 contains the four major energy sources, fat, protein, starch and sugar as predictors. The constant term was not significant in this equation confirming the absence of other energy sources. This very straightforward model was proposed by Härtel (1979) and is also used in the EEC-equation discussed below. It is a very effective predictor of ME values but is neither the best nor the cheapest.

Equations 10 and 11 are both based on the proximate components and could therefore be implemented in the UK without additional analytical costs . Equ. 10 combines the canponents in a conventional way and accounts, in these data as in those of Härtel et al. (1977) for about 95% of the total variation. In this case the quadratic term for fat is significant but the effect of CP is not. The rather complicated re-arrangement of the proximate components in equ. 11 stems from the hypothesis that the effect of fibre is to reduce the energy value of the other canponents of the feed. This is supported by the significant interactions between fibre and fat, protein and NFE but the equation is empirical because there is still a highly significant positive coefficient for fibre and a large negative intercept. However this equation has a smaller s value than equation 9 which requires starch and sugar analyses although it falls down on the theoretical variance of the analytic methods because of the interaction terms. The advantage of NDF wer CF could 'also be shwn in this type of equation.

Three main conclusions were drawn from these experiments. Firstly that, within the range of 'normal' feed ingredients used, chemical prediction equations could effectively predict the ME values of compound poultry feeds. The residual standard deviation of the 'best' equation was 0.24 MJ/kg whilst a mean determined value based on six replicates had a standard error of 0.15 MJ/kg. Prediction was therefore nearly as good as direct measurement. The final conclusion was that selection of an equation for practical use would have to reflect the balance of accuracy and cost and that such a balance should take account of analytical variability.

DERIVATION OF EEC EQUATION

Initially five sets of results totalling 177 observations were analysed. However one of these, the only one based on young birds, showed much higher variability than the remaining four and was therefore omitted. The selected equation was based on 141 observations

on adult birds and from the following sources: 1) Statens Husdyrbrugsforsøg, Copenhagen. Dr V.E. Petersen (29 feeds, cockerels) 2) Institute for Poultry Research. Beekbergen, The Netherlands. Dr C.A. Kan (18 feeds, cockerels) 3) Härtel et al. (1977), University of Hohenheim (39 feeds, hens) 4) Fisher (1982a), as discussed above. [Note; whilst this paper is in preparation further data have become available from SRA, Nouzilly, France, Dr B. Leclercq and inclusion of these in the final. analysis will probably lead to slightly different equations from those shown here].

The test feeds used in the different laboratories varied widely, both in composition and in the range of variables covered. The analytical methods also differed slightly but, of necessity, this had to be ignored, Analytical results were available for FAT, CP, CF, ASH, STC and SUG with derived values for NFE and what Härtel et al. (1977) call residual NFE (RNF = NFE - SIC - SUG). Preliminary analyses revealed little evidence of significant differences in regression slopes between laboratories and therefore "parallel—line" regression models were fitted. There were significant differences in intercept values for different laboratories which were combined into a single average figure.

In this combined analysis the 'best' equation contained, like Härtel's (1979) equation and equation 9 (Table 2), FAT, CP, STC and SUG as predictors. The intercept values ranged from -0.22 to +0.72 MJ/kg, the average 0.077 MJ/kg being combined into the coeff icients to yield the equation.

AME (MJ/kg) = 33.6 FAT + 15.5 CP + 16.8 STC + 11.1 SUG

It is this equation that has been adopted provisionally within the EEC.

Unlike the PRC data these pooled results yielded no equations which are superior either as predictors or on the basis of cost. Data were not available for NDF but there was no benefit in adding CF to the equation above. A combined starch and sugar figure was about as effective as the separate analyses which might reduce analytical costs but no combinations of the proximate components were found which had any promise. It is not clear why these results differed in this respect fran those found at PRC.

RING-TEST EVALUATION OF EEC EQUATION

As already pointed out, once an equation has been selected its "accuracy" or reproducibility is a reflection only of the analytical. methods employed. To determine this for the proposed EEC equation four feeds were made up and circulated to 21 laboratories throughout Europe for analysis for FAT, CP, SIC and SUG. AME values were also determined on the same feeds using both tube-fed cockerels and young chicks.

The results of this exercise showed that repeatability of the chemical determinations, and therefore of the predicted AME values, was excellent within a laboratory whilst the reproducibility between laboratories, was much poorer. The repeatability and reproducibility standard deviations (Steiner 1975) illustrate this quite clearly (Table 3).

TABLE 3 Repeatability and reproducibility of chemical analyses

		Repeatability		Reproducibility	
		s.d.	% mean	s.d.	% mean
Moisture,	g/100g	0.053	0.49	0.573	5.31
FAT	g/100g d.m.	0.085	1.35	0.692	10.94
CP	"	0.118	0.57	0.802	3.82
STC	"	0.289	0.67	2.284	5.41
SUG	"	0.155	4.61	0.778	23.20

repeatability s.d. = $\sqrt{\sigma^2/2}$. reproducibility s.d. = $\sqrt{\sigma^2_l + \sigma^2_{fl} + \sigma^2/2}$

σ^2, σ^2_l, σ^2_{fl} = error (within lab.), laboratory and lab. x feed components of variance. Duplicate determinations assumed.

When expressed as a percentage of the mean values the repeatability s.d.'s for duplicate determinations range fran 0.49% for moisture to 4.6% for sugar. The reproducibility s.d.'s, again for duplicate determinations in two randomly selected laboratories, range fran 3.8% for crude protein to 23.2% for sugar. The 95% confidence intervals for pairs of duplicate determinations made in two laboratories on identical feeds are 1.6% moisture, 2.0% fat, 2.3% protein, 6.5% starch and 2.2% sugar (all on a d.m. basis).

These laboratories were all asked to use the same EEC analytical procedures but even so, there is reason to argue that better standardisation of methods would probably reduce the estimates of reproducibility. In a more limited study in which each analysis was done in three out of 8 laboratories, all within the U.K., the reproducibility limits were 0.85% fat, 1.3% protein, 4.3% starch and 0.7% sugar (Fisher 1982a). Nevertheless over the whole of Europe it is clear that interlaboratory differences in analytical results are going to be an important source of variability in predicted ME values.

When these ring-test data were used to calculate AME values with the proposed EEC equation the repeatability s.d. was 0.052 MJ/kg d.m. or 0.40% of the mean. The reproducibility s.d. was 0.582 MJ/kg d.m. or 4.48% of the mean. Thus if a sample of feed were to be analysed in duplicate at two randomly selected laboratories it is expected, with 95% probability, that the two mean predicted AME values would not differ by more than 1.65 MJ/kg or 12.7% of the mean. The average difference for many such comparisons is 0.56 MJ/kg. It is on the basis of results such as these that tolerance limits for ME declarations have to be fixed.

The comparison of the predicted and determined AME values is summarised in Fig. 1. When interpreted by a parallel line statistical model, which was not significantly different from the two separate models, there was a very high correlation between the predictions and observations ($r = 0.98$), but a considerable bias. The values for cockerels are underestimated by 0.57 MJ/kg and those for chicks overestimated by 2.24 MJ/kg. Thus the equation is predicting relative AME values effectively but corrections must be made to yield accurate absolute values. Since the equation was derived only with data for adult birds the relative magnitude of the bias at the two ages is as expected.

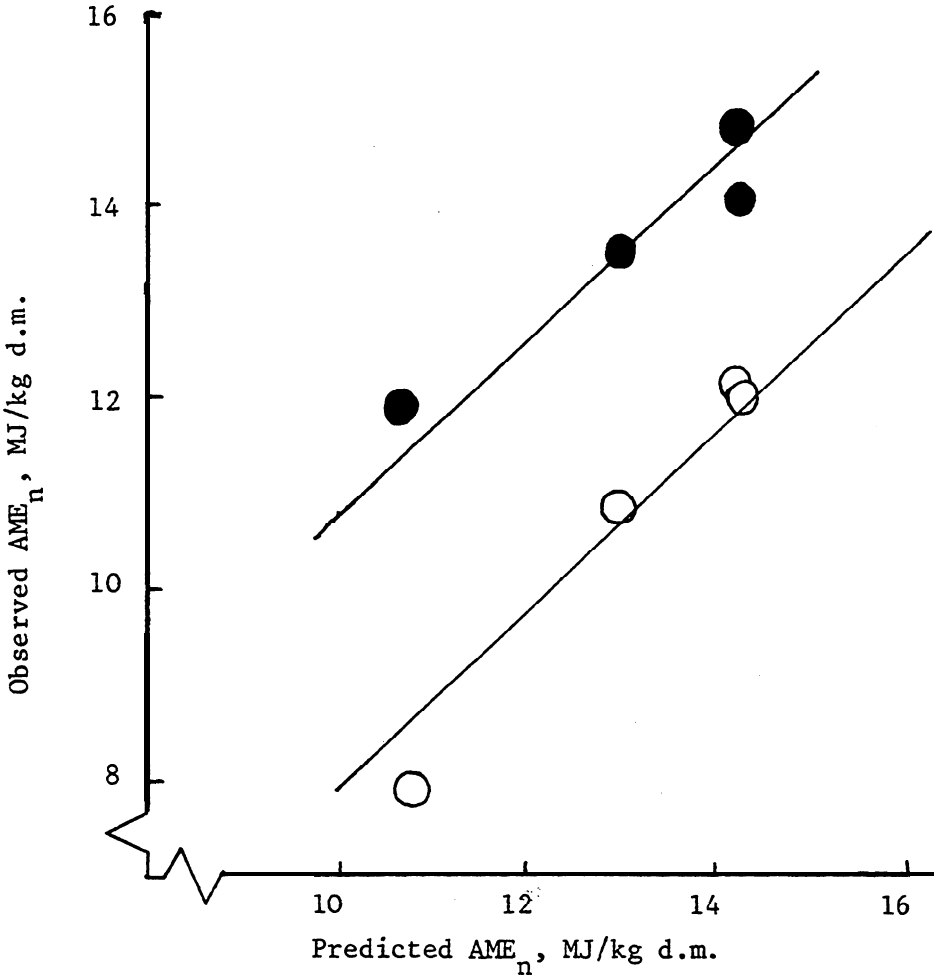

Fig. 1. The relationship between AME$_n$ values predicted by the EEC equation and observed with adult cockerels (●) and young chicks (○). The lines are parallel regression lines with the formula y = 0.567 (cockerels) - 2.236 (chicks) + 0.999x. (r = 0.98, rsd = 0.51 MJ/kg).

DISCUSSION

Taken as a whole these various results show that the prediction of ME values from four or five chemical variables is reasonably accurate. At best, in the PRC work we obtained a standard deviation, including the variability of the analytical procedures of about 0.33 MJ/kg and for several. equations the s.d. was below 0.4 MJ/kg or about 3% of the mean. Tolerance limits on declared values depend only on the reproducibility of the chemical analyses. In the PRC experiments it appeared that the appropriate s.d. might be as low as 0.20-0.25 MJ/kg but such an encouraging result was not obtained in the ring-test over the whole of Europe. Standardisation of analytical procedures is obviously a critically important question.

By comparison these various estimates of s.d. may be compared with the standard error of a mean determined AME value, using Six replicates, 'Of 0.15 MJ/kg. In routine work rather higher values would probably be found, depending on the technique used. It is also interesting to note 'that when the ME values of the test diets used in the PRC experiments was calculated fran four sets of table values the residual s.d.'s

ranged from 0.22 to 0.59 MJ/kg. Thus providing the problems of analytical variability can be reduced the chemical prediction equations are virtually as good as direct bioassays and somewhat better than complete knowledge of the formulations.

Dissatisfactions with prediction equations stem mainly fran the inherent weakness of the whole approach rather than fran the present state of development of the equations. Within the constraints of a legal declaration system it does not seem likely that new equations will be found by further experimentation. It would be foolish to rule out further developments in analytical chemistry but again, within the cost constraints of a routine declaration scheme, it seems unlikely that more general and robust predictors of ME values will be found. The one possible exception is Near IR spectrophotometry but even in this case some early promising work with ruminant feeds could not be reproduced with our poultry feeds (A. Hall, private communication).

An obvious limitation of the approach is that the results cannot be extrapolated beyond the type and range of feeds tested. It has been assumed that the feeds used in the experiments are a satisfactory sample of the population about which predictions are to be made; assuming of course that such a population is homogenous with respect to the relationships under investigation. A wide range of practical feed ingredients were used in the trials but it is inevitable that some feeds will not be well described by any one, reasonably simple, equation. Variations in anti-nutritive factors or the use of feeds containing very high levels of single ingredients are the most likely sources of systematic error and it is only by the use of a direct bioassay that such possibilities can be completely avoided. Recent developments in the techniques for rapid bioassays probably make them quite competitive on a cost basis with even relatively simple arrays of chemical analyses but there would be enormous organisational costs and problems in using such a bioassay as the basis of a declaration scheme. An alternative arrangement would be to use the bioassay as a final check and arbiter in cases of dispute.

The most obvious theoretical deficiency of prediction equations is that they imply that the energy yield of crude nutrients is constant i.e. that digestibility is constant. Thus in the EEC equation the coefficient for sugar, 11.1 kJ/g, is considered to be too low by some commentators. This figure implies a digestibility of the energy fran sugar of 0.71 if digested sugar yields 15.6 kJ/g (Härtel et al. 1977). Certainly feeds containing sucrose will be underestimated since this sugar has a digestibility of 0.99 (Härtel et al.. 1977), whilst feeds with milk sugars, digestibility = ca. 0.6, will be overestimated. Such problems can be resolved if they are anticipated, what seems to be impossible is to guard against them in general.

The energy value of fat is influenced by several known factors and may differ quite widely from an average assumed figure. In the PRC work this was reflected in the significant effect of fatty acid ratios but this is an expensive parameter for routine measurement. For feeds containing 1 to 2% of vegetable fat from ingredients and up to 5% of a reasonable quality feed fat variations in fat composition may not have very large effects. To deal with the question in any general way again threatens the simplicity of the scheme. A general proposition is that the ME of fat declines with level of inclusion and in the PRC work the quadratic effect of fat level was quite often significant, especially

in the data for pelleted feeds. This effect of pelletting is attributed entirely to chance, In the four sets of data analysed to derive the EEC equations non-linear effects of fat level could not be demonstrated.

Variations in ME values between different classes of poultry remain as an unresolved problem. Most of the development work has been done with adult fowls and the application of the equations to young birds or to other species may or may not be justified. The direct evidence on this topic is extremely confused and will not be reviewed here. At present there is no clear-cut quantitative answer to the problem and further experimental work is required. The question as to how such work should be done to give unequivocal answers is also complex.

In the very limited test of the EEC equation the interpretation shown in Fig. 1 implies that the relative values of the feeds were the same for chicks and cockerels. Whilst this is a statistically justif ied conclusion it will be seen that, in detail, the low energy feed tended to be underestimated for the cockerels and considerably overestimated for the chicks. The situation may therefore not be so simple. The magnitude of the bias between the two types of bird used in this experiment was unexpectedly large and this may reflect, to some extent, the very different experimental techniques that were used. Particular care is required to obtain valid comparisons of energy availability and the measurement of nutrient digestibility rather than of ME might be preferred,

The development and standardisation of accurate analytical methods is an obvious requirement for an energy declaration scheme based on chemical prediction equations. Although it has been argued that equations should be assessed on the basis of their chemical reproducibility there is of course a danger that their relative merits will be changed by future developments in analytical technique. This topic cannot be discussed in detail but a few points should be noted.

Firstly, we found no benefit in the ring-test from adjusting the results to a dry matter basis, although we assume that this would always be done. The standard EEC procedures for crude fat analysis specify ether extract for most feeds and acid ether extract for a range of materials for which ether extract is incomplete, including compound feeds with added fat. In the ring-test three laboratories analysed the feeds by both methods and found an average, 0.82, 0.91 and 0.69% more fat following acid hydrolysis. For the prediction equation it is assumed that the acid ether extract is always used although this is not theoretically appropriate in ail cases. Starch is determined by polarimetry after solubilisation in dilute HCl and correction for sugars extracted in 40% ethanol. In the PRC experiments this gave, on average, 2.1%' more starch than a method using amyloglucosidase/glucose oxidase, but the two could not be distinguished by their effectivess in predicting ME values. . In some recent data from France a wider difference, 6.14% starch, was found over 48 feeds (B. Leclercq, private communication). Sugars are extracted in 40% alcohol and determined, after inversion, as saccharose using the Luff-Schoorl method.

Amongst possible alternatives to chemical prediction equations the use of direct bioassays has already been mentioned, The other main alternative is to replace chemical analyses with an in vitro simulation

of digestion and to measure the disappearance of energy in the system used. Furuya et al. (1979) described a two-stage assay using pepsin/HCl and an extract of porcine intestinal fluid. Sakamoto et al. (1980) showed that this assay accurately predicted both dry matter and crude protein digestibility in the hen. We have tested a similar system but using a commercial pancreatic enzyme preparation in the second stage. The solubilisation of energy was assessed to give a measure of in vitro DE (IVDE). For 28 feeds used in the ME experiments the mean IVDE was 14.69 MJ/kg which compared well with the observed value of 14.20 MJ/kg. The correlation was 0.87 with a residual standard deviation in the observed values after regression on IVDE of 1.00 MJ/kg. This compared with an r.s.d. for the better chemical equations of 0.30 MJ/kg or less, Combination of the in vitro results with chemical analyses did not produce any improvement over the chemical analyses alone. At this stage of development therefore the in vitro method, although reasonably effective, does not look like an alternative to chemical prediction. Whether it can be improved and whether it would better detect feeds which were poorly described by a prediction equation will have to await further development.

It is concluded that if energy declaration schemes are introduced for commercial and political reasons then reasonable solutions can be found to the technical problems raised, by the use of chemical prediction 'equations. However completely robust equations are not available and within the constraints of a simple and econanical practical system further significant improvements do not seem very likely. If a completely 'safe' scheme is required it will have to be based on a direct bioassay.

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