Non-additivity of feedstuffs examined *in vitro* and the influence of incubation medium pH

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Inroduction Non-additivity occurs when the nutritive value of a mixture of feedstuffs differs from that of the sum of its components. It is most commonly observed when one dietary constituent influences, either positively or negatively, the apparent digestibility of another under conditions where components such as nitrogen and sulphur are non-limiting. In general negative effects occur due to the depression of rumen pH or substrate competition, while positive effects have been identified when readily fermentable fibre sources such as sugar beet pulp have been included in rations containing poorly fermented forages such as cereal straw. With the increasing use of *in vitro* systems, not just to examine feed degradation characteristics but to derive parameters such as microbial protein yield, the following study was conducted to determine whether such interactions could be identified *in vitro*.

Materials and methods The fermentation characteristics of three feeds - grass hay (*H*), wheat (*W*) and molassed sugar-beet pulp (*SBP*) - were examined alone and with either *W* or *SBP* in combination with *H* (65:35 DM basis). The influence of incubation medium was assessed by the inclusion of citric acid (Mould *et al*., 2000) to provide initial pH values of 6.74, 6.56, 6.31 and 5.86. A 5 x 4 factorial design was used with three replicates, plus appropriate controls, for each treatment combination at each of the five fermentation periods. The Reading Pressure Technique (Mauricio *et al*., 1999) was applied to obtain gas production yields and to assess organic matter degradation (OMD) dynamics over the 96 h incubation period. Rumen fluid inoculum was obtained from a dry cow offered grass hay *ad libitum* plus 1.0 kg concentrate daily. Incubation pH of each flask was measured at the termination of fermentation. Non-additive effects were identified by comparing observed (*O*) values with those calculated (*C*) by proportional summation of values from the substrates examined alone. An estimate of fermentation efficiency (FE) was obtained by relating OMD to gas release at 96 h. SAS procedures were utilised to generate LS means and identify significant difference both between treatments and observed and calculated values. Only feed combination data are presented in this summary.

Results Decreasing the fermentation medium pH depressed OMD and the quantity of gas released, with the magnitude varying inversely with the pH and was greatest with mean pH values below 6.0. Observed and calculated cumulative gas values for *W-H* combinations were similar at 24, 48 and 96 h post-inoculation. However significant negative associative effects were identified at nearly all incubation interval x pH levels with OMD values depressed below those calculated by as much as 67 g/kg. In contrast while no differences were observed at the highest incubation pH levels there was an increasing tendency, as pH decreased, for the *SBP-H* combinations to produce significantly more gas than calculated. These positive associative effects were also identified with OMD and especially at shorter time intervals and lower pH levels (e.g. at 24 h and pH 5.65 calculated and observed OMD values were 355 and 463 g/kg respectively). The strong inverse relationship identified between FE and incubation pH may result from a shift in VFA production towards C_3 from C_2 and C_4 due to the adverse conditions existing for fibre degradation.

Table 1 *Non-additivity of feed combinations: cumulative gas production, OMD and fermentation efficiency (EF)*

Feed	Mean pH†	Gas production (ml / g OM)						OMD (g / kg)						FE
		24 h		48 h		96 h		24 h		48 h		96 h		96 h
		O	*C*	*O*	*C*	*O*	*C*	*O*	*C*	*O*	*C*	*O*	*C*	
W-H	6.57	223^{a}	218	254^{a}	250	259^{a}	259	798^{a§}	825§	845^{a}	853	880^{a}	881	3.34
	6.34	205^{a}	209	250^{a}	244	248^{a}	257	797a	808	848^{a}	851	875^{ab‡}	886‡	3.47
	5.87	166^{b}	161	182^{b}	191	208^{b}	204	704^{b‡}	756‡	803^{b¶}	837¶	860^{b}	849	4.23
	5.61	132^{c}	125	137^{c}	139	125^{c§}	155§	628^{c}	642	720^{c¶}	752¶	724^{c¶}	791¶	5.40
SBP-H	6.62	211^{a‡}	201‡	258^{a}	246	249^{a}	259	788^{a§}	812§	838^{b}	841	882^{a§}	868§	3.49
	6.40	203^{a‡}	185‡	237^{a}	234	259^{a}	254	806^{a§}	771§	867^{a¶}	850¶	876^{ab}	876	3.45
	5.95	170^{b‡}	142‡	211^{b}	192	239^{b‡}	212‡	679^{b¶}	599¶	831^{b‡}	817‡	865^{a¶}	838¶	3.77
	5.65	115^{c‡}	88‡	144^{c‡}	115‡	167^{c‡}	144‡	463^{c¶}	355¶	676^{c¶}	578¶	728^{c¶}	668¶	4.41

† Mean of pH estimates taken over entire 96 h incubation period.
‡§¶ Contrasting O / C values with these symbols are significantly different at *P<0.05*, *P<0.01* and *P<0.001*, respectively.
Means in columns within feeds without similar letters are significantly different (*P<0.05*)

Conclusions Positive and negative non-additive effects were readily identified, with their magnitude varying both with incubation interval and fermentation medium pH. The results suggests that the use of fermentation parameters such as gas production or OMD derived from single feeds to generate similar estimates, microbial protein yield or ATP production for feed combinations is incorrect.

References

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