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## The relative value of three cereals as protein sources for growing chicken

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The prime consideration for the relative replacement values of the common cereals in poultry nutrition is their contribution of energy (either 'metabolizable' or 'productive'). However, they also contribute from one-third to two-thirds of the total protein in the diet. There have been suggestions that the usefulness of this nitrogen fraction differs significantly from one cereal to another and that there may be mutual supplementation within a mixture of several cereals. The published biological values of maize as a protein source for rats are lower than those for barley, oats and wheat (see review by Block & Mitchell, 1946-7).

Investigation of the protein value of cereals requires rigorous control if misleading results due to differences in the palatability and the energy content of the experimental rations are to be avoided. Also, in practice the cereal proteins are fed together with protein from one or more concentrates. Not every combination can be investigated—in the present work we have used two contrasting concentrates, one of high quality (white-fish meal) and one of low (groundnut meal). Nor was it possible to use more than a single sample each of barley, maize and oats.

We used individually caged growing chickens and measured their nitrogen retention on different rations in the same general way as described by Wilgus, Norris & Heuser (1935). Each bird was fed according to a scale based on live weight so as to provide the same constant daily intake of metabolizable energy, crude fibre and crude protein

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per kg body-weight from all the test rations. In other words, rations differed in their percentage content of protein but had a constant protein:calorie ratio.

With feeding below the *ad lib.* level of energy intake there is an increased tendency for protein to be used as an energy source; therefore, the protein level was kept low to ensure that it remained the critical factor restricting growth, measured here as nitrogen retention. The level fixed was 4.0 g crude protein ( $N \times 6.25$ )/100 metabolizable Cal., which corresponds to levels of 10.2–10.6% for the particular rations used.

The metabolizable energy of the ration ingredients was not determined directly, but estimated from the formula: metabolizable energy (Cal./kg) =  $53 + 38$  (crude protein (%)) +  $2.25 \times$  ether extract(%)) +  $1.1 \times$  starch(%)) + sugar(%)). In earlier work this formula was found to give a close estimate for the cereals concerned and for the mixed rations tested (Carpenter & Clegg, 1956).

## EXPERIMENTAL

Batches of individual components of the rations were analysed (Table 1) and then compounded for eight different mixes in the proportions set out in Table 2. The quantities shown there for each diet were designed to be the daily ration/kg body-weight for the experimental birds, and were calculated to contribute, in each diet,

Table 1. Percentage composition of individual feeding-stuffs

Ingredient	Moisture	Crude protein ( $N \times 6.25$ )	Ether extract	Crude fibre	Ash	Carbohydrate by difference	'Starch'*	'Sugars'*
Maize	14.6	9.1	4.8	1.8	1.2	68.5	53.3	1.1
Oats	14.0	8.5	5.3	8.4	2.0	61.8	41.2	1.2
Barley	14.0	10.8	2.1	4.0	2.1	67.0	49.5	2.8
Groundnut meal (decorticated, extracted)	8.9	50.8	1.7	4.5	5.1	29.0	5.9	9.3
Fish meal (white)	10.7	62.9	4.0	—	22.2	0.2	—	—
Dried yeast	16.8	46.9	0.4	1.0	3.5	31.4	3.8	1.6
Dried whey	8.3	12.3	0.5	—	8.2	70.7	—	55.9
Oat feed (ground oat husk)	4.0	3.3	1.6	27.6	2.9	60.6	9.9	0.7
Maize starch	14.9	—	—	—	—	85.1	85.1	—

\* Estimated by the method of Clegg (1956).

12 g crude protein (6.75 g from cereal, 3.75 g from either fish meal or groundnut meal, and 1.5 g from dried yeast and whey which were included as vitamin supplements), 1.05 g calcium and 0.46 g phosphorus. The contribution of crude fibre was adjusted, by the addition of oat feed (i.e. ground oat husk), to be 0.7 g, except for rations 2G and 2F, both containing oats, which contributed 0.78 g. Finally, each ration was adjusted by the addition of maize starch to contribute an estimated 300 metabolizable Cal. (calculated as described above).

The birds received a generous daily allowance of a vitamin A and D supplement in oil during the preliminary period. The rations were calculated to provide more than the allowance of the remaining individual vitamins and minerals recommended by the (U.S.A.) National Research Council: Committee on Animal Nutrition (1954).

Table 2. *Composition of rations, and weight of ingredients allocated to pullets (g/kg body-weight/day)*

Ingredient	Ration							
	Maize		Oats		Barley		Mixed cereals	
	1 G	1 F	2 G	2 F	3 G	3 F	4 G	4 F
Cereal:								
Maize	74.20	74.20	—	—	—	—	24.75	24.75
Oats	—	—	79.50	79.50	—	—	26.55	26.55
Barley	—	—	—	—	62.25	62.25	20.70	20.70
Protein concentrate:								
Groundnut meal	7.38	—	7.38	—	7.38	—	7.38	—
Fish meal	—	6.00	—	6.00	—	6.00	—	6.00
Vitamin supplements:								
Dried yeast	1.61	1.61	1.61	1.61	1.61	1.61	1.61	1.61
Dried whey	7.02	7.02	7.02	7.02	7.02	7.02	7.02	7.02
Balancing components:								
Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	0.75	—	0.75	—	0.98	0.23	0.83	—
CaCO <sub>3</sub>	1.80	1.50	1.65	1.35	1.58	1.20	1.68	1.50
NaCl	0.60	0.53	0.60	0.53	0.60	0.53	0.60	0.53
Oat feed (ground oat husk)	19.35	20.55	—	1.20	15.00	16.50	11.25	12.75
Maize starch	4.95	5.62	15.00	15.90	21.00	21.00	13.50	13.50
Total ration	117.7	117.0	113.5	113.1	117.4	116.3	115.9	114.9

The three cereals chosen for the investigation were one sample each of Scottish oats, Canadian barley and Argentinian maize.

The balance trials with fifty-six birds were carried out in seven successive replications. In each replication eight White Leghorn × Rhode Island Red pullets were caged individually at 45 days of age and continued to receive their standard diet for a further 5 days. They were transferred then to one of the eight experimental rations according to a randomizing procedure based on body-weight, which was done by ranking the birds 1 to 8 in order of body-weight, and allotting them to rations according to a previously prepared 'random' table for the seven replications. The table had been checked to ensure that over the seven replications none of the rations would receive an undue proportion of either light or heavy birds. The experimental rations were given for a period of 15 days, which was divided into three subperiods each of 5 days. The first 5 days were regarded as preliminary for adjustment to the ration. Separate collection and nitrogen analysis of excreta were made for the second and third subperiods of 5 days each. The size of the daily ration was based on the body-weight at the beginning of each subperiod as illustrated in Table 3. During the preliminary period the birds became accustomed to receiving less than their *ad lib.* intake of feed and spilt very little. The feeds were given in the morning, and any spilt was returned to the feed tins later in the day. Small amounts transferred to the water pots were also returned.

The excreta were collected daily and kept at 1° in stoppered containers with 2 ml. toluene and 5 ml. N-HCl, after the method of VanLandingham, Clark & Schneider (1942), until the excreta for the whole 5 days were available for analysis. The material from each bird was mixed thoroughly, dried and analysed for its nitrogen content.

The retention of nitrogen by the bird was obtained as the difference between the nitrogen fed and that excreted. Calculation of the results from a typical subperiod is

given in Table 3. Altogether fourteen subperiod collections were made for each ration over the seven replications. However, the two results within a trial were not independent since they were obtained from the same bird. Therefore, to determine the significance of differences of response on the various rations, the analysis of variance had to be based on the mean response from each bird in subperiods 2 and 3, and the error term for this comparison was obtained as the interaction of this factor with the replication variable.

Table 3. Results from a typical experimental period (replication 5, subperiod 3) to illustrate the allocation of rations according to initial live weight of pullets and the calculation of nitrogen retention

Ration	Initial body-weight of chicken (g)	Feed allocated for 5 days (g)	Feed not eaten (g)	Nitrogen eaten (g) (X)	Dry matter excreted (g)	Nitrogen excreted (g) (Y)	Percentage nitrogen retention $\left(\frac{(X - Y) \times 100}{X}\right)$
Maize: 1 G	533	315.0	0	5.418	90.90	3.109	42.6
1 F	621	363.5	0.5	6.291	103.53	3.307	47.4
Oats: 2 G	484	274.5	0	4.639	80.20	2.521	45.7
2 F	502	285.0	0	4.856	82.77	2.573	47.0
Barley: 3 G	462	271.0	0	4.596	77.85	2.894	37.0
3 F	538	313.0	0	5.406	94.41	2.923	45.9
Mixed: 4 G	456	264.0	2.0	4.480	76.10	2.300	48.7
4 F	544	312.5	0	5.403	91.47	2.642	51.1

#### RESULTS

The mean nitrogen retentions corresponding to the eight rations are set out in Table 4. Two results for a subperiod were lost. For the rations concerned, 2G and 2F, there were therefore only thirteen instead of fourteen results. Missing values were fitted in the course of the statistical analysis. The results of the analysis of variance are summarized in Table 5. The analysis is shown in two sections; as explained above, the error term for considering the significance of difference between the mean nitrogen retentions on different rations is not the residual but the term for the interaction, rations  $\times$  replications.

The retentions on the rations containing barley (3G and 3F) were significantly lower than on those containing the other cereals in both the fish-meal and groundnut-meal series (Table 4); and there was no significant difference between the results with

Table 4. Mean percentage nitrogen retention according to cereal and protein concentrate given to the pullets\*

Protein concentrate	Cereal used				Mean†
	Maize	Oats	Barley	Mixed cereals	
Groundnut meal	42.9	43.8	39.4	43.5	42.4
Fish meal	51.4	50.7	47.7	52.1	50.5
Mean‡	47.2	47.2	43.6	47.8	—

\* Standard error for figures in the body of the table  $\pm 0.77$ .

† Standard error  $\pm 0.38$ .

‡ Standard error  $\pm 0.54$ .

maize, oats or mixed cereals in either series. Retention was consistently greater with fish meal than with groundnut meal, but there was no significant protein  $\times$  cereal interaction.

Table 5. *Analysis of variance of percentage nitrogen retention*

	Source of variation	Degrees of freedom	Mean squares
A	Rations (mean value from subperiods 2 and 3)	7	309***
	Replications	6	36**
	Rations $\times$ replications†	42	8
B	Subperiods (2 and 3)	1	134***
	Rations $\times$ subperiods	7	7
	Replications $\times$ subperiods	6	5
	Residual	40	6

\*\* Significant at  $P < 0.01$ . \*\*\* Significant at  $P < 0.001$ .

† This interaction represents the error term for 'Rations' as a source of variation.

Of the other significant findings from the analysis of variance, the difference between replications was due principally to a linear decline for successive trials. For example, the overall mean retention for replications 1 and 2 was 47.9%, and for replications 6 and 7 was 45.3%. Retentions were higher also in the second subperiod (mean 47.5%) than in the first (mean 45.4%), but there was no interaction between this effect and the type of ration used.

#### DISCUSSION

The experiment just described was designed to measure the relative value of the protein fractions of different cereals under specific and strictly controlled sets of conditions. For a parameter of growth we chose increased nitrogen content. We stress this point to avoid confusion of our type of result with that of VanLandingham *et al.* (1942, 1945). Those authors used nitrogen-balance figures to calculate values equivalent to those of net protein utilization (biological value  $\times$  true digestibility  $\div$  100) obtained for rats in the classical Thomas-Mitchell method (cf. Block & Mitchell, 1946-7). To obtain these figures VanLandingham *et al.* had, of course, to make estimates of the proportion of nitrogen in the excreta that was of endogenous origin.

For simplicity our values for increased nitrogen content of the birds have been expressed as percentage nitrogen retention, i.e. as a percentage of the nitrogen fed. However, since the nitrogen fed was proportional to the body-weight of the bird (1.92 g N/kg body-weight/day), and spillage was insignificant, nitrogen storage/day/kg body-weight was simply (percentage nitrogen retention)  $\times$  0.0192.

The results have confirmed the previous findings of Wilgus *et al.* (1935) that nitrogen retention may vary significantly from one trial to another. Whatever may be the explanation—perhaps season of the year or undetected variables in the environment—it seems to emphasize the importance of using an experimental design in which within-experiment comparisons are made, and of regarding the results as giving only relative values for the experimental diets.

In the present trials, an unexpected finding was the inferiority of the barley rations; the daily nitrogen retention was about 8% lower than with maize or oats. On the

basis of amino-acid analysis maize has usually been considered as inferior. The crude protein of maize contains 0.8% tryptophan, compared with 1.3% for oats and 1.0% for barley. The corresponding analyses of maize, oats and barley for lysine are 2.8, 3.3 and 3.0%. For methionine, however, the values are 2.3, 1.7 and 1.6% (analytical data collected and summarized by De Man & Zwiep, 1955). The mean of published values for the net utilization of maize proteins, as the source of protein for rats, is also only 49, as compared with values of 61 and 58 for oats and barley respectively. (Cf. reviews by Block & Mitchell, 1946-7.)

It is of interest that the relative value for the cereals was the same whether fish meal or groundnut meal was used as the source of supplementary protein; though this in fact would be expected so long as the level of protein was still critical with the higher-quality supplement. With each supplement, the performance on a mixture of three cereals was significantly better than the mean performance on the three cereals fed separately, which indicated that the inferiority of the barley was being overcome by a mutual-supplementation effect. Such an effect would be expected only if it were not the same amino-acid that was limiting with each cereal. On the basis of the published amino-acid analyses quoted above, it is possible that methionine was the limiting factor in the barley rations, and lysine in the maize rations, but that can be decided only by further experiment.

Finally, we repeat that these relative values for the cereals apply only when used in conjunction with the particular supplements studied and in the proportions described. Nevertheless, biological evaluations provide an essential check on predictions of nutritional value that are based entirely on amino-acid analyses.

#### SUMMARY

1. Fifty-six individually caged growing pullets in a randomized block design were given rations designed to supply daily, per kg live weight, 12 g crude protein and 300 Cal. metabolizable energy.

2. Each of the eight experimental rations contributed 6.75 g protein from a cereal (maize, oats, barley or a mixture of the three) and 3.75 g from a protein concentrate (either fish meal or groundnut meal).

3. In seven complete replications, one 7-week-old pullet was allotted to each of the eight rations. After 5 days for adjustment to the ration a nitrogen-balance trial was conducted for two successive 5-day periods.

4. For each protein concentrate nitrogen retention was significantly lower when barley was the cereal. Nitrogen retentions with maize, oats and a mixture of the three cereals did not differ significantly.

5. The fish-meal sample gave better retention than groundnut meal regardless of the cereal used, and there was no evidence of cereal  $\times$  supplement interaction.

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## The gut flora of the chick

### 3\*. Differences in caecal flora between 'infected', 'uninfected' and penicillin-fed chicks

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The gut flora of domestic animals has been studied extensively in recent years, mainly in attempts to elucidate the mechanism of antibiotic stimulation of the growth of young animals. There is strong reason to believe that the effect is mediated through the gut flora (Jukes, 1955), but considerable confusion exists because of the contradictory results obtained by different groups of workers. For example, when they gave chlortetracycline to chicks Dixon & Thayer (1951) reported an increase in the numbers of lactobacilli, March & Biely (1952) reported a decrease, whereas Eisenstark & Sanford (1953) and Anderson, Cunningham & Slinger (1953) found no change.

Coates, Dickinson, Harrison, Kon, Porter, Cummins & Cuthbertson (1952) have shown that in old premises that have housed several generations of chicks a growth-depressing condition exists which is transmissible and is counteracted by penicillin in the diet. In this connexion, Elam, Jacobs, Fowler & Couch (1954) reported a decrease in 'clostridia' in penicillin-fed chicks, and that 'clostridia' fed to chicks in a clean environment depressed growth; however, according to Smyser, Cleverdon, Kulp & Materson (1952) the numbers of *Clostridium perfringens* increased in the presence of dietary penicillin after 4 weeks, but chlortetracycline was without effect, and Brown & Luther (1950), Romoser (1951) and Anderson, Cunningham & Slinger (1951) found no reduction in numbers of anaerobes, and sometimes an increase, with chlortetracycline.

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