A note on the estimation of nitrogen retention in chicks by body analysis and balance methods

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1. In a balance study with young chicks estimates of nitrogen retention were made by a balance method and by a body analysis method.

2. N retention appeared to be about 15 % greater by the balance method when no allowance was made for N in alimentary tract contents. Allowance for tract contents reduced the difference to about 13 %.

3. Only about one-third of the discrepancy could be accounted for in terms of the possible sources of N loss investigated.

4. About a quarter of the difference was due to loss of N on drying the droppings before analysis and another tenth to loss of N on freeze-drying minced chick tissues.

5. No appreciable loss of ammonia could be detected from chickens and excreta under conditions prevailing in the balance trial. The efficiency of digestion of feathers, uric acid and droppings from birds appeared to be complete by the Kjeldahl method employed.

6. A difference of about 8 % could therefore not be explained.

There is still controversy regarding discrepancies encountered between values for body N obtained indirectly by balance work and directly by carcass analysis. A critical assessment of the present position with references has been given recently by Duncan (1966) in a review of the limitations of the balance trial in nutrition work. The following short study comparing results obtained for N retention in young cockerels by direct and indirect methods was carried out after an observation made during other studies had indicated that gain in body N as measured by direct analysis was about one-fifth less than that measured by the indirect method.

The results of this study indicate that at least part of the discrepancy may arise from an accumulation of errors each relatively small in itself.

EXPERIMENTAL

Animals and diets

Two hundred day-old White Leghorn × Rhode Island Red cockerels were fed cracked grain (1 part oats + 1 part maize) for 3 days and then a commercial chick starter diet for 4 days. They were then weighed, arranged in ascending weight order and forty-eight birds in the middle weight range were randomized to four lots of equal weight each containing twelve birds. Two lots representing the experimental groups at the start of the test period were killed and analysed immediately and two lots were given a starter diet. All birds were individually caged.

Each group was given 6000 g diet over 3 weeks, the food being sampled for analysis at the beginning of the experiment.

All the birds were killed by chloroform to avoid the possibility of losing blood and were stored at −20° until required for analysis.

Methods of analysis

Each lot of twelve birds killed at the start of the experiment was halved to give duplicate analyses. Birds in the first lot were analysed without separating gut contents whilst in the second lot alimentary tract contents were analysed separately. The birds were minced in a hand mincer, transferred to weighed aluminium trays, freeze-dried, and again minced in the dried state and stored in an air-tight bottle before analysis. Samples were taken for dry-matter determinations at all stages of preparation.

At the end of the experiment all the birds were processed individually although the final results were combined for presentation in groups.

The droppings which included spilled food, scales and feathers, were collected every 2nd day and stored in bottles at $-20°$ until required for drying in an oven at $80-85°$ before being milled to pass a 1 mm sieve. Dried and milled droppings were stored in air-tight bottles until analysed. As will be shown later, such drying treatment can lead to losses of N. Care was taken to collect all excreta and cast body tissues from the cage structure at the end of the experiment. Contents of water pots were emptied each day into a bottle containing dilute acid for subsequent determination of N.

N was measured by a Kjeldahl method which was essentially that of the Association of Official Agricultural Chemists (1960). To minimize frothing during digestion of body tissues approximately 1 g freeze-dried chick mince plus 15 g K_2SO_4, 0.75 g HgO and 35 ml H_2SO_4 with 35 ml H_2O were mixed and left overnight in the Kjeldahl flasks before digestion was started.

Supplementary tests

Loss of ammonia from expired air and droppings. Week-old chicks were housed in an air-tight Perspex cage for 3 days with sufficient food and water for the period. Heat was supplied by an external source (100 W light bulb) to give an internal temperature of around 28° at that end of the cage away from the source and a slow stream of acid-scrubbed air was drawn through the cage by a water pump. The air leaving the cage passed through two acid traps in series. All the traps contained 0.1 N-H_2SO_4 with a few drops of methyl red solution. At the end of 3 days the absorbing acid solutions were analysed for ammonia.

N loss on drying droppings. Moist droppings from the Perspex cage were placed in a large wash-bottle in an air oven at 80–85°. The inlet and outlet of the bottle were connected by means of polythene tubing passing through the vent at the top of the oven to an acid scrubbing system. A slow stream of ammonia-free air was passed over the droppings for 3 days by which time they were dry and the ammonia in the scrubbing bottles was estimated.

N loss on freeze-drying minced chick tissues. A young cockerel weighing around 250 g was killed and thoroughly minced before six samples, each of about 4 g were weighed into Kjeldahl flasks for immediate analysis. A further six samples were freeze-dried overnight before analysis. Two large samples of tissue were freeze-dried and used as a source of material for assessing the effect of storage up to 56 days at room temperature on the N recovery from freeze-dried samples.

Nitrate N. In view of a possibility that nitrate N might be present in tissues and not recovered during analysis several samples of chick mince were given preliminary treatments with 2 g salicylic acid and sodium thiosulphate (Association of Official Agricultural Chemists, 1960) or 2 g sucrose and thiosulphate (Bradstreet, 1960) before digestion.

Value of adding starch to high-protein material before digestion. Because of the possibility that materials rich in protein and low in carbohydrate might not be efficiently digested owing to a lack of a readily available source of carbon, a sample of white fish meal was digested with and without the addition of 1 g starch and N was subsequently determined in the usual way.

RESULTS

The N concentrations in each half of the two initial lots of birds killed at the beginning of the experimental period showed good agreement differing only by 1·3 and 0·3 %. Only the mean results are given in Table 1 which shows that the difference

Table 1. *Measurements made in calculating nitrogen retention in two groups of chicks by body analysis at the beginning and end of, and by balance during, an experimental period of 3 weeks*

Group no. and when killed	By body analysis (g N)				By normal balance (g N)			Difference (%)	
	Total body content including digesta	Digesta content	Carcass content excluding digesta content	Reten-tion	Food eaten	Droppings spillage etc.	Reten-tion	Referred to body analysis value	After allowing for gut fill
1 A, at beginning	—	0·4	15·8	—	—	—	—	—	—
2 A, at beginning	16·0	—	—	—	—	—	—	—	—
1 B, at end	—	1·8	79·7	63·9	177·2	103·2	74·0	16	14
2 B, at end	81·6	—	—	64·2*	177·2	104·4	72·8	13*	11*

* On the assumption that gut fills were the same as in groups 1 A and 1 B.

between N retention as measured by balance and by body analysis methods was 13-16 % using the techniques here employed. This was reduced to 11-14 % when gut fill was taken into consideration. Differences arising from neglecting 'gut fill' and spillage into water pots were small compared with the unexplained difference.

Volatile bases lost in expired air and from droppings. After 3 days in the air-tight cage ammonia found in the acid traps was equivalent to a loss of 1 mg N per chick in 3 days or around 0·1 g N for twelve chicks in 21 days. This was a negligible amount, being about 0·2 % of the gain in body N.

Volatile bases lost from droppings dried at 80–85°. Most of the volatile basic material was found in the first trap, which showed that about 50 g wet droppings (23 g dry matter) gave off the equivalent of 29 mg ammonia N during drying. From this value it was calculated that in the balance trial about 2·3 g N may have been lost during drying of the droppings. This would be about 3·5 % of the gain in body N.

Recovery of N from uric acid. By our Kjeldahl procedure 99% of the theoretical quantity of N was recovered from a sample of uric acid (guaranteed purity 98%) and so it is unlikely that the N concentrations found in chick droppings would be low on account of inefficient digestion of this component.

Volatile loss on freeze-drying chick mince. Table 2 shows that there may well have been a loss of around 2% during freeze-drying but no loss thereafter during storage at room temperature.

Efficiency of digestion of feather N. In one trial with twelve chicks the crude protein ($N \times 6 \cdot 25$) accounted for 99 ± 3% of the feather dry matter and ash accounted for 2%. It seems unlikely that an underestimation of body N arose from underestimation of feather N.

Table 2. *Effect of freeze-drying tissues of chicks on estimates of nitrogen content*

Tissues	Storage time (days)	N in dry matter (%)	Significance of difference
Fresh (mean of 6)	—	3·22⎫	
Freeze-dried (mean of 6)	—	3·16⎭	$P < 0 \cdot 01$
Stored freeze-dried	1	3·18	
	7	3·17	
	21	3·14	
	28	3·17	
	56	3·15	

Nitrate N. There was no increase in the N concentration measured by the Kjeldahl method when chick mince was given a preliminary treatment with salicylic acid–thiosulphate or sucrose–thiosulphate before digestion.

Value of adding starch before digestion of high-protein material. There was no appreciable difference in the concentration of N found in white fish meal whether or not starch was present in the digestion mixture. Concentrations found ranged from 10·26 to 10·34% with starch added and from 10·28 to 10·39% without starch.

DISCUSSION

A possible cause of discrepancy may have arisen from the loss of volatile bases during the trial or on drying droppings in the oven at 80–85°. Our results indicate that of the 11–14% discrepancy between the two techniques only about 0·2% may have arisen from volatile losses during the trial, but that around $3\frac{1}{2}$%, or a quarter of the total discrepancy, may have arisen from volatile losses during drying of the droppings.

Possible sources of inaccuracy in the estimation based on body analyses were a loss of volatile matter when tissues were freeze-dried or inefficient recovery of N in body tissues. This study has shown that a loss of 2% from the N in minced tissue was possible during freeze-drying but that no significant loss was likely to occur thereafter. Nitrate N was not a problem, probably because it was not present in appreciable amounts. Feather N in these young birds was efficiently digested by the normal method.

The Kjeldahl method itself has been under suspicion from time to time and has been compared with the Dumas method. However the latter is not free from criticism and the former in our hands, with mercury as the catalyst in the digestion mixture, has not yet been found to give erroneous values for feeds, animal tissues or excreta. However, even if the Kjeldahl method does give results some 2–4 % lower than the Dumas method, as has been reported by Becker & Harnish (1958) who used copper as the catalyst, this would not account for more than one-tenth of the differences encountered in the present study. To do so there would have to be an appreciable error in one or two but not all the tissues.

Our conclusion has been that, of the large difference of 11–14 % found between estimates of N retention by direct and indirect methods, less than half can be explained in terms of volatile losses when droppings are dried in an oven at 80–85° and when body tissues are freeze-dried. No appreciable loss appeared to arise from loss of volatile materials during the balance experiment, or by inefficiency of digestion and estimation of N by the Kjeldahl method. An unexplained difference of the order of 8 % remains.

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