The digestion of heat-damaged protein*

BY M. C. NESHEIM† AND K. J. CARPENTER
School of Agriculture, University of Cambridge

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1. The apparent digestibilities for chicks, operated on so as to allow separate collection of urine and faeces, of the nitrogen in a heat-damaged cod flour (C35) and of a control, freeze-dried cod muscle (C23) were 77 and 90% respectively.

2. The differences are similar to those found for rats in earlier work and considerably smaller than the differences found in nutritional value of the materials as sources of either lysine or methionine for chicks.

3. Chicks killed 3 h after a test meal containing C23 showed little more N in their small intestine than did those on a N-free diet; other chicks receiving C35 showed much more N remaining in the gut.

4. It is hypothesized that significant quantities of heat-damaged protein may remain undigested in the small intestine, but may then be de-aminated by fermentation in the caecum so that values for the digestibility of N and of individual amino acids may be misleadingly high.

5. This hypothesis is supported by the finding that in caecectomized chicks the apparent digestibility of the N of C35 was only 68%, whereas the digestibility of C23 remained the same as in intact chicks.

It is known that the biological value of proteins may be reduced by heat damage to a greater extent than can be explained either by changes in amino acid composition of acid-hydrolysates made from these materials, or by changes in the overall digestibility of the materials.

The reactions which can bind the ϵ amino group of lysine seem to provide a satisfactory explanation for observed falls in the availability of this amino acid. However, in an earlier study with cod muscle (C23) heated in various ways (Miller, Carpenter & Milner, 1965) the results of growth assays with chicks suggested that the potency of the most severely heated material, C35, as a source of methionine was reduced by about 33%, i.e. to nearly the same extent as for lysine, even though the digestibility of both total protein and methionine (measured with rats) was much less reduced.

The question arises as to whether methionine also is involved in a specific reaction through its side-chain, or whether all amino acids, even those with a paraffin (and presumably unreactive side-chain) are similarly affected. For the proteolytic organism, *Streptococcus zymogenes*, the indication is that heat treatment of cod protein reduces its value as a source of leucine and isoleucine to approximately the same extent (Ford, 1965; Miller, Carpenter & Milner, 1965) as with methionine. If this were so for the chick also, then it might be expected that the overall nitrogen digestibility by chicks of the heat-damaged material would be similarly less than that of the control cod.

Although the results obtained with rats (Miller, Carpenter & Milner, 1965) indi-

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† Permanent address: Department of Poultry Science, Cornell University, Ithaca, New York.

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cated that the heated cod was still relatively well digested, it seemed essential to confirm this with the chick, i.e. the species used for the growth assays. Digestibility determinations were first made by indirect estimations with intact chicks and then, because of discrepancies depending on which of the common assumptions was used to estimate the partition of urinary and faecal N, the work was repeated with chicks that had been operated on so as to allow separate collections of urine and faeces. Also in these studies measurements have been made of the net changes in intestinal N after the ingestion of control and damaged protein, and of the effect of caecectomy on faecal N levels. This was done to give some indication of possible differences, both in the rate of intestinal absorption and in the significance of caecal fermentation for the two contrasting materials.

Finally an unsuccessful attempt was made, by growth assay with the two materials, to obtain direct values with the chick for the availability of isoleucine, chosen as an example of an amino acid with a paraffin side-chain.

EXPERIMENTAL

Protein sources

The freeze-dried cod muscle used as control was from the same batch (C23) as that used by Miller, Carpenter & Milner (1965); it contained 3 % moisture and 96·8 g crude protein/100 g dry matter. C35, from the same series of experiments, was chosen as the heat-damaged material; it had been prepared by adjusting C23 to 14 % moisture content and heating it for 27 h at 116°. When the C35 was exhausted, further batches of material (C35A and C35B) were prepared from C23 under the same conditions. With each batch the heated material had a determined value of 13·1 % moisture, and the protein content on a dry-matter basis was unchanged.

Diets

The basal N-free diet used consisted of arachis oil 5, ground oat husk 3, 'salts N' (Fox & Briggs, 1960) 6, vitamin mix (Miller, Carpenter, Morgan & Boyne, 1965) 1, 'chromium bread' (Kane, Jacobson & Moore, 1950) 1, choline chloride 0·3 and maize starch *ad* 100. The only variant from this was in Expts 4*b* and 4*c* in which maize oil replaced arachis oil, and cellulose powder (Solka-Floc; Brown Co., New York) replaced ground oat husk.

The chromium bread was designed to contain approximately 0·3 % Cr_2O_3 but the final concentration in each diet was determined by analysis. Each test protein source was included at the expense of starch, so as to contribute 16 % protein (N × 6·25) to each diet. In addition, test diet 1 (with C23) had 2 % water added (also at the expense of starch), to give the same total moisture content and gross energy value as test diet 2 (containing C35).

The chicks used were males from a light hybrid laying strain.

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Nitrogen levels in the intestinal tract

Expt 1 (*a*). Twelve chicks at 20 days of age were randomized into six cages (with two chicks each) and the chicks in each of three cages were fed on test diets 1 or 2 for a further 9 days. They were fed for two 2 h periods (8–10 am and 4–6 pm) each day to train them to eat their requirements quickly. On the final morning they were allowed access to food for only 1 h. An hour later the chicks were killed by dislocation of the neck, and the contents of the small intestine were expressed into a weighed centrifuge tube. Borate buffer (pH 8·0) was added, 4 ml for each g of contents; the borate solution and intestinal contents were mixed and the tubes centrifuged. The supernatant liquid was decanted and the total N content of the residue estimated in a single digestion. Two 0·5 ml samples of the decanted liquor were taken; one was immediately digested in a Kjeldahl flask and the other mixed with 0·5 ml 10% (w/v) trichloroacetic acid (TCA), the mixture left for 1 h and then centrifuged, and the whole supernatant liquid digested for the determination of its N content.

Expt 2. Chicks at 15–20 days old were placed on the basal, i.e. N-free, diet (made up without the chromium bread) for 24 h, and then they had their diet removed on the evening before the experiment. Next morning after 12 h without food, they were given a test meal of approximately 3 g of one of the experimental diets. This was prepared as a soft pellet (by compression in a machine used for the preparation of pellets for a bomb calorimeter) which was broken up and placed piece by piece at the back of the chick's tongue with tweezers. Each meal was given in approximately 10 min; water was given by dropper to assist the birds to swallow the meal.

At a given interval after the meal, each bird was killed by dislocation of the neck and the alimentary tract dissected out. The contents of the small intestine from the gizzard to the point just above the base of the caeca (i.e. including the duodenum) were then squeezed out into a graduated centrifuge tube, made up to 9 ml with water, shaken and poured into a Potter–Elvehjem tissue homogenizer. The tube was washed out with a further 1 ml water and the suspension homogenized. Two 1 ml portions were taken with blow-out pipettes for N determination and the whole residue was washed into a digestion flask for chromium determination.

The oesophagus, proventriculus and crop of each bird were washed out, the contents made up to 50 ml and homogenized. Two 5 ml samples were taken for N determination and the residue was used for a single Cr determination. Negligible amounts of material of any sort were found in the large intestine.

Determination of apparent digestibility of N

Expt 1 (*b*). As part of Expt 1 described above, samples of mixed excreta were taken over the last 5 days of the experimental period from each cage and stored at −20°. The material from each cage was diluted with water to allow homogenizing in a Waring Blendor. The homogenate was then dried at 65° for 6–8 h and re-ground, before samples were taken for analysis.

Expt 3. Chicks 29 days old were operated on to expose the ureters and allow separate collection of urine and faeces, by the procedure of Newberne, Laerdal &

O'Dell (1957), except that the faeces were allowed to fall into a tray beneath the cage for collection.

On recovery from the anaesthetic, six chicks were placed in individual cages; three were fed on diet 1 and three on diet 2 *ad lib.* for 7 days. A separator was prepared from polythene tubing (13 mm inner diameter, 1 mm wall thickness) and attached by tying it to the root of the tail, with thread and adhesive tape; urine and samples of faeces were collected, by means of the separator, over the last 4 days. When there was any suspicion of faeces having been contaminated with urine they were discarded. At the end of the experimental period faeces were dried at 65° for 6 h and ground in a mortar.

Expt 4. In Expt 4*a*, chicks, 14 days old, were anaesthetized and their caeca were ligated as close to their junction with the intestine as possible and removed. After 2 days their ureters were exposed as in Expt 3 and the chicks were then placed on either diet 1 or diet 2 under the same conditions as in Expt 1. After a further 30 h, collection of urine and faeces was begun and this was continued for 4½ days. Expts 4*b* and 4*c* followed a similar pattern but at ages and times of operation as shown in Table 5.

Analytical procedures

Chromium. Cr was determined in food and excreta by an unpublished modification (C. K. Milner, private communication) of the prodecure of Czarnocki, Sibbald & Evans (1961).

N determination. N was determined by the Kjeldahl method using a macro-digestion procedure for analyses of diets, dried excreta and urine and a micro-digestion procedure for gut contents (Association of Official Agricultural Chemists, 1960, sections 2.034 and 38.011 respectively) followed by semi-micro distillation of the ammonia produced into 1% (w/v) boric acid containing a mixed indicator for titration with 1/70 N-HCl (Ma & Zuazaga, 1942).

Uric acid. This was extracted from mixed excreta with hot lithium carbonate (Baker, 1946), and determined colorimetrically by the method of Benedict & Franke (1922). Preliminary runs with the iodometric procedure of Bose (1944) proved unsatisfactory because of a drifting end-point to the titrations.

Urine samples were homogenized with a few drops of capryl alcohol in a Waring Blendor and 5 ml portions pipetted immediately into a 250 ml volumetric flask containing 10 ml 0·5% (w/v) lithium carbonate and treated as described above for analysis of mixed excreta.

Urinary ammonia. This was determined by distillation of 1 ml urine with 2 ml saturated K_2CO_3 solution in a Markham still. The receiving flask contained 2% boric acid solution with bromcresol green indicator which was then titrated with 1/70 N-HCl.

'*Faecal protein*'. This was estimated in mixed excreta as 6·25 × N in the precipitate obtained with uranyl acetate after preliminary oxidation of the material with $KMnO_4$ to bring the uric acid present into solution (Ekman, Emanuelson & Fransson, 1949).

Gross energy of excreta. This was determined in an adiabatic bomb calorimeter and

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corrected to N equilibrium on the assumption that retained N would have the calorific value of uric acid, 8·22 kcal/g N, if it were excreted (cf. Hill & Anderson, 1958).

Microbiological assay for 'available isoleucine'. The procedure followed that described by Ford (1962) after the initial pretreatment with papain, in which the conditions used were those described by Rao, Sreenivas, Swaminathan, Carpenter & Morgan (1963), except that we used a new batch of papain (non-crystalline grade; British Drug Houses Ltd, Poole, Dorset) of which the strength has not been assayed.

Chick growth assay for 'available isoleucine'. We followed the general procedure previously described for methionine (Miller, Carpenter, Morgan & Boyne, 1965). The basal diet in Expt 5 was a modification of that used by Deshpande, Harper, Collins & Elvehjem (1957) with rats and consisted of blood flour 29·0, arachis oil 5·0, ground oat husk 3·0, L-isoleucine 0·1, L-arginine hydrochloride 0·6, DL-methionine 0·25, choline chloride 0·3, vitamin and mineral premixes as in Expt 1 and maize starch *ad* 100. For the experimental diets the additions were made at the expense of starch. Each of the eight experimental diets was fed to a total of eight groups of three chicks each, the whole experiment being done in two stages with four replicates at each stage. The chicks were randomized at 9 days of age and the experimental period was another 9 days. In further assays tryptophan was added to the basal diet but the results are not reported in detail.

The blood flour (X. 544) used for the assays was purchased as a commercial fertilizer. In preliminary trials with another batch of blood meal (X. 537) sold as a feedingstuff no isoleucine deficiency was obtained. Assay of the two samples gave the following results:

	X. 537	X. 544
Crude protein (N × 6·25) (%)	83·7	87·5
Available isoleucine, for *S. zymogenes* (g/16 g N)	1·86	0·48

Apparently the isoleucine content of blood preparations varies greatly according to the relative proportions of blood cells and serum that they contain (cf. Block & Weiss, 1956).

RESULTS

Nitrogen levels in the intestinal tract

Expt 1(*a*). The results of the experiment are summarized in Table 1. The chicks on the two treatments ate similar quantities for their final meal. However, the total quantity of N recovered from the intestinal contents of chicks receiving the heat-treated protein (diet 2) was nearly three times that recovered from those receiving freeze-dried protein (diet 1). While all three N fractions increased, the largest relative increase on diet 2 was for the fraction soluble in pH 8 buffer but insoluble in TCA. Since Cr was not determined, it was not possible to relate the N found to a given quantity of diet.

Expt 2. As is seen in Table 2, the total N in the contents of the small intestine was again nearly three times as great in chicks fed on diet 2 as in those fed on diet 1. The Cr values indicated that the higher N corresponded to a smaller quantity of diet present in the small intestine. As a consequence, the 'apparent' extent of digestion in these

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3 h samples of the material in the small intestine was only 43 % for the heat-damaged protein (diet 2) compared with 85 % for diet 1. Of course, not all the N found was of dietary origin, since the value for the chicks on the N-free diet was nearly equal to that for the chicks on diet 1. It is also recognized that dietary N in the small intestine represents proteins at different stages of digestion. However, it is difficult to explain the observed differences other than by the suggestion that the digestion of cod 35 proceeded at a slower rate or to a lesser extent or both.

Table 1. *Expt 1 (a). Partition of the nitrogen in contents of small intestine of chicks 1 h after they received freeze-dried or heat-damaged protein**

Treatment	Weight of chicks (g)	Food/chick in final meal (g)	Total N recovered from small intestine (mg)	Insoluble N (at pH 8) (mg)	Soluble N pre-cipitated by TCA (mg)	TCA-soluble N (mg)
Diet 1 (cod 23, freeze-dried)	226	6·5	13·7	3·7	2·2	7·8
	228	10·0	14·2	4·2	1·1	8·9
	267	8·0	13·6	4·3	1·4	7·9
Treatment mean	240	8·2	13·8	4·1	1·6	8·2
Diet 2 (cod 35, heat-damaged)	246	9·0	49·9†	15·6†	7·0	26·0
	218	7·5	34·8	7·9	5·8	21·1
	248	9·0	38·7	7·1	8·6	23·0
Treatment mean	237	8·5	39·4	9·1	7·1	23·4

* Each value is the mean of determinations on two chicks in a single cage.
† Represents a single determination; the other sample was lost.

Table 2. *Expt 2. Nitrogen and chromic oxide levels in the alimentary tract of chicks 3 h after they received test meals**

Treatment	N content of test meal (mg)	Crop, proventriculus and gizzard Cr_2O_3 (% of dose)	Small intestine: Cr_2O_3 (% of dose)	Small intestine: N (mg)	Small intestine: % of dietary N 'apparently' digested
Basal diet (N-free)	—	11	58	6 (5–7)	—
Test diet 1 (C23)	81	5	63	7 (6–10)	85
Test diet 2 (C35)	82	15†	41	19 (16–22)	43

* Three chicks per treatment, with individual analyses of which the mean is shown, together with the range for N only.
† Mean of only two values. The third value obtained indicated negligible Cr_2O_3 content and was considered suspect.

The total Cr recovered in the upper digestive tract suggests that the passage time was less for diet 2 than for the other two diets. However, in preliminary experiments it proved difficult to recover more than 85% of the Cr in a test dose from the alimentary tract even a few minutes after the meal was given.

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Determination of apparent digestibility of N

Expt 1 (*b*). As is seen in Table 3, the mean body-weights and food intakes of the chicks on the two diets were similar, as was the total quantity of N excreted. However, nearly twice as much uric acid was apparently excreted by the birds receiving diet 1; in contrast there was more uranyl acetate-precipitable N in the excreta of the chicks given diet 2, but not sufficient to account for all the N in the latter. On the common assumptions (*a*) that the whole of the faecal N is precipitable with uranyl acetate (Ekman *et al.* 1949), and (*b*) that total urinary N can be estimated as '1·25 × uric acid N' (Katayama, 1924; O'Dell, Woods, Laerdal, Jeffay & Savage, 1960), some 45 % of the excreted N from diet 2 was still unaccounted for. With diet 1, the corresponding figure was only 12%. It follows from this that the digestion coefficient for diet 2 calculated on assumption (*a*) was much higher than that calculated on assumption (*b*), 87 % as opposed to 62 %, since only on the second assumption was the unexplained N regarded as being of indigestible origin. For diet 1 the difference was in the same direction but considerably smaller.

One surprising observation is that the recorded retention of N was similar on the two diets although the quality of the protein as judged by other tests (Miller, Carpenter & Milner, 1965) was very different. However, the mean live-weight gains, 58 and 56 g on diets 1 and 2 respectively over the whole feeding period, were also similar. The explanation may be that the spaced feeding procedure reduced food intake so that energy rather than protein was the factor limiting growth.

The metabolizable energy value of diet 2 was significantly less than that of diet 1. If the difference in the gross energy excretion (0·2 kcal/g diet eaten) was entirely due to additional undigested protein from diet 2, this would correspond to approximately 50 mg protein/g diet, or nearly one-third of the dietary protein.

Expt 3. The chicks recovered rapidly from their operation and maintained normal food consumption. Urine and faeces were separated successfully, and satisfactory samples of faeces were collected to permit calculation of apparent digestibility of N in diets 1 and 2 (Table 4). The N digestibility of diet 2, calculated from N to Cr ratios in feed and faeces, was 11 units less than that observed for diet 1. The partition of urinary N of chicks receiving these diets showed that chicks on diet 2 had slightly less urinary N in the form of uric acid and correspondingly more in the form of ammonia. With each diet other forms of N apparently made up about 25 % of the urinary N.

It was observed that faeces of chicks given diet 2 contained a considerable amount of brown semi-liquid material, similar in appearance to caecal contents, which was voided separately from the main body of the more solid, chromic oxide-coloured, faecal material. Much less of this brown material was seen in faeces voided by chicks given diet 1.

Expts 4(*a*), (*b*), (*c*). The caecectomy proved to be a feasible operation and chicks so modified were also successfully operated on to permit separation of faeces and urine. The birds, for which results are given in Table 5, were killed and examined when collections were ended to check that the caecectomy had been complete.

The apparent digestibility of the N in diet 1 by caecectomized chicks was 89 %,

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Table 3. *Expt* 1 (*b*). *Indirect determinations of apparent nitrogen digestibility with chicks*

Treatment	Weight/chick at mid-point of collection period (g)	Food/chick (g/day)	N excreted in different forms (mg N/100 mg N eaten)				Calculated coefficient for the apparent digestion of N*		Gross energy of excreta (N corrected) (kcal/g diet fed†)
			Total N	Uric acid N × 1·25	N precipitated by uranyl acetate	Unexplained residual N	(A)	(B)	
Diet 1 (C23, freeze-dried)	214	18·4	42·7	32·0	6·4	4·3	93·6	89·4	0·46
	226	22·5	57·0	39·9	9·1	8·0	90·9	82·8	0·59
	256	27·4	44·0	31·0	7·6	5·4	92·4	87·0	0·54
Treatment mean	232	22·8	47·9	34·3	7·7	5·9	92·3	86·4	0·53
Diet 2 (C35, heat-damaged)	239	27·5	52·8	18·6	12·8	21·4	87·2	62·8	0·77
	212	22·0	52·7	21·1	12·4	19·2	87·6	65·5	0·72
	234	26·5	54·2	15·1	15·0	24·1	85·0	58·0	0·77
Treatment mean	228	25·3	53·2	18·3	13·4	21·6	86·6	62·1	0·75

* (A) Calculated on the assumption that the uranyl-precipitable N represented the total undigestible fraction of dietary protein (after Ekman *et al.* 1949); (B) calculated on the assumption that the uric acid nitrogen represented 80 % of the total urinary N (after O'Dell *et al.* 1960).

† The values shown were corrected to N equilibrium by the procedure of Hill & Anderson (1958). The mean adjustments were +0·07 and +0·06 kcal/g for diets 1 and 2 respectively.

essentially the same as for intact chicks, but caecectomy significantly reduced the digestibility of the protein in diet 2. The faeces from chicks given diet 2 still contained a proportion of brown liquid material which seemed to coat all the ordinary faecal particles and which ran on to the collection trays and dried in a sticky film. Very little material of this type was seen in the faeces from chicks on diet 1.

Table 4. *Expt 3. Direct determinations of apparent nitrogen digestibility*

Treatment	Chick no.	Final body-weight (g)	Average amount of food digested (g/day)	Dietary N apparently eaten (%)	Partition of urinary N: Uric acid N (%)	NH_3-N (%)	Residual N (%)
Diet 1 (C23)	1	296	21	88	65	11	24
	2	327	27	91	69	9	22
	3	323	36	88	63	11	26
Treatment mean		315	27	89	66	10	24
Diet 2 (C35)	4	280	24	76	55	15	30
	5	283	25	80	63	14	23
	6	300	34	78	60	17	23
Treatment mean		288	28	78	59	15	25

Table 5. *Direct determinations of the apparent digestibility of cod proteins in intact and caecectomized chicks*

Expt no	Age at beginning of collection (weeks)	Digestibility: C23 (diet 1) Intact chicks	C23 (diet 1) Caecectomized chicks	C35 (diet 2) Intact chicks	C35 (diet 2) Caecectomized chicks
3	4½	88, 91, 88		76, 80, 79	.
4(*a*)	2½	.	91, 89, 86, 89	.	73, 67, 70
4(*b*)*	3	90, 93	86, 91, 90	83, 86, 84	71, 73, 69
4(*c*)†	4	91, 89	91, 91	71, 61	50, 70
Mean of individual values		90	89	77	68

* Caecectomized (where stated) at 7 days, ureters exposed at 18 days of age.
† Caecectomized (where stated) at 21 days, ureters exposed at 25 days of age.

Isoleucine assays

The results of Expt 5 are shown in Table 6. A good response was obtained by addition of isoleucine to the basal diet, although there were diminished increments in food conversion efficiency with the higher levels of isoleucine.

At the higher dose level, the cod samples appeared to be supplying a limiting factor in addition to isoleucine, since the response was not parallel to the isoleucine response curve. An estimate of potency obtained by reading off only the response to the lower level of test materials against the standard curve is given in Table 7. In further tests, supplementary tryptophan was included in the basal diet, and a higher response to the top level of isoleucine on the standard curve was obtained. However, in each of three successive assays, the responses to the two cod preparations were inconsistent. The graph of weight gain *v.* level of protein showed crossovers for responses to the two

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preparations. We have not, therefore, obtained any valid measure of the relative potency of the two materials as a source of isoleucine.

The results of microbiological assays of the samples are also shown in Table 7. A single assay was run using replicate acid hydrolysates as well as papain predigests of each material. Satisfactory response curves were obtained. C35A showed the same isoleucine content as C23 by acid hydrolysis. However, as judged by the papain–*S. zymogenes* assay, the heat treatment of the cod had reduced its potency as a source of isoleucine by approximately 45%. The result for chicks given the low level of supplementation in Expt 5 suggested that the potency was reduced by about 25%; but, as explained on p. 407, it was not possible to obtain a statistically valid estimate in any of the chick assays.

Table 6. *Expt 5. Mean response of isoleucine-deficient chicks to test materials and pure isoleucine*

Supplement to basal diet	Weight gain per chick per day (g)	Food conversion efficiency
None	0·17	0·020
L-isoleucine, 0·08%	2·66	0·257
L-isoleucine, 0·16%	4·35	0·359
L-isoleucine, 0·24%	5·55	0·402
C23, 3·0% (2·83 parts crude protein)	3·79*	0·338*
C23, 5·0% (4·71 parts crude protein)	5·68	0·442
C35A, 3·37% (2·83 parts crude protein)	3·22	0·297
C35A, 5·59% (4·71 parts crude protein)	4·97	0·402
Standard error of means	±0·24	±0·013

* These figures are means from only seven groups as one chick in the eighth group failed to gain weight in the whole experiment. If the eighth group had been included the overall means for weight gain and for food conversion efficiency would have been 3·64 and 0·331 respectively.

Table 7. *Isoleucine in the test materials as estimated from microbiological and chick experiments (g/16 g N)*

	C23	C35A	C35A/C23
Microbiological assays on			
(*a*) Acid hydrolysate	5·3	5·3	1·00
(*b*) Pepsin predigest	5·0	2·7*	0·54
Chick assays†			
(*a*) Weight gain	4·9	3·7	0·76
(*b*) Food conversion efficiency	4·9	3·7	0·76

* C35 was included in the same assay and gave a value of 2·76 g/16 g N as opposed to 2·68 for C35A. The difference is within the experimental error.

† The response to the lower level only of each test material was read against the standard curve, since the response to the high level of cod 23 was outside the limits of the standard curve.

DISCUSSION

It would have been useful to have unequivocal results for the effect of heat treatment of a protein on the availability for chicks of one of its amino acids with a paraffin side-chain. However, our assays for isoleucine encountered the difficulties already reported by Ousterhout, Grau & Lundholm (1959) and by Harper & De Muelenaere

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(1961) and explained by these authors as the result of mutual imbalance effects from leucine and valine. Our only direct evidence with chicks of the effects of heat on different amino acids is therefore that methionine was apparently rendered unavailable to approximately the same extent as lysine by the heat treatment given to the control cod material (Miller, Carpenter & Milner, 1965).

Melnick & Oser (1949) pointed out that processing which reduces the nutritive value of proteins for animals also seems to have the effect of reducing the rate of proteolytic attack on the proteins in vitro. They suggested that the same phenomenon occurs in vivo and that a slow rate of attack is in itself sufficient to explain the lower nutritive value of the protein.

Ford (1965) has demonstrated that a heat treatment of cod fillets similar to that which we used for the production of C35 results in reduced proteolysis with pepsin followed by papain in vitro. Further, our in vivo results in Expts 1 and 2 are consistent with C35 being proteolysed and absorbed more slowly than was C23 after a test meal. However, this in itself does not explain why the protein that is digested should be of inferior quality, as is apparently the case with C35 where nutritional value fell considerably more than did digestibility (Miller, Carpenter & Milner 1965). Melnick & Oser (1949) originally suggested that this might be due to delayed release of particular amino acids so that mutual supplementation between the different essential amino acids was impaired. With a chick that eats at frequent intervals, and has food at all stages of digestion at any one time, that would not seem to apply. The in vitro experiments of Ford (1965) do indicate some differential release from heat-damaged cod fillets, with the residual large peptides from digestion with pepsin and papain showing a particularly high lysine content, but they give no evidence of any similar hindrance to the release of methionine.

A factor not previously considered with respect to heat-damaged proteins is that their amino acids may be neither absorbed as such through the gut wall nor recovered in the faeces. As discussed by Carroll, Hensley & Graham (1952) and by Barnes & Kwong (1964) in connexion with experiments on rats, proteins which reach the lower intestinal region may undergo reactions there that differ from simple proteolysis and result in the absorption of nitrogenous compounds from the gut in forms other than the useful amino acids. For example, bacterial fermentation can result in the deamination of amino acids immediately on their release from peptide linkages and the N being absorbed in the form of ammonia. In chicks most of any such fermentation would be expected to occur in the caeca. We repeated our determinations of C35 digestibility with caecectomized birds and did indeed find a greater excretion of faecal N compared with that observed with unoperated birds. With the control cod, the operation had no effect on the faecal N recovered, so that with caecectomized birds, the apparent digestibility of C35 was 21 units lower than with C23, compared with a difference of only 13 units in intact chicks.

With intact chicks (Expt 1) the heat-damaged protein resulted in an additional energy excretion equivalent to one-third of the dietary protein intake. However, the direct determination of faecal N (Expts 3 and 4) showed an additional N excretion equivalent to only 13 % of the dietary protein. This is at least consistent with protein

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being deaminated in the lower digestive tract and the remaining portions of the molecules being incompletely absorbed. It is interesting that the difference in protein utilization as estimated by energy excretion was similar to the fall in available methionine as found in growth assays, i.e. 33 % (Miller, Carpenter & Milner, 1965).

Our working hypothesis is therefore that a significant proportion of the protein and peptides which escape digestion and absorption in the small intestine, enters the caeca and is fermented in such a way that N is absorbed as ammonia or in some other form with no nutritional value. From this hypothesis it might be predicted that in germ-free chicks, where there is no possibility of fermentation in either the caeca or the large intestine, digestibility values even lower than those in caecetomized chicks would be obtained for heat-damaged proteins that were incompletely digested in the small intestine. If this did prove to be so, the paradox between low availability values for amino acids, determined by growth methods compared with relatively high values for their digestibility (i.e. 'availability' by faecal analysis), would be explained.

Our analysis of urine samples in Expt 3 showed a somewhat higher proportion of urinary N as ammonia with cod 35 as the protein source, but this is of doubtful significance. Most ammonia entering the blood stream of chicks is converted into uric acid in the liver so that, to obtain an estimate of ammonia absorption, it would be necessary to measure concentrations in the portal blood stream as has been done by Warren & Newton (1959) with guinea-pigs. These workers found over four times the concentration of ammonia when conventional guinea-pigs were compared with germ-free animals. Francois & Michel (1964) have reviewed other work indicating microbial deamination of amino acids by the gut flora of different species.

Techniques for measuring protein digestibility with chicks

The direct method used for determining apparent digestibility in these experiments proved satisfactory. The use of chromic oxide as an indicator for calculating digestibility was necessary because complete collection of excreta was somewhat difficult under these conditions; also it is essential to discard any faeces suspected of being contaminated with urine. The chicks surgically modified to permit separate collection of urine and faeces recovered very rapidly from the operation and consumed adequate quantities of the diets. We are satisfied that the results obtained with this method are reliable.

We do not have an estimate of the metabolic faecal N excreted by chicks but, if we apply the correction suggested by Meyer (1956) for rats, the values for apparent digestibility would be raised by 5 units, i.e. to give values for estimated true digestibility for non-caecectomized chicks of 95 % for C23 and 82 % for C35. These values are very close to the corresponding figures of 96 and 84 respectively obtained with rats (Miller, Carpenter & Milner, 1965).

Most of the values in the literature for the digestibility of proteins by chicks were obtained with intact chicks, estimates being made by assuming either that total urinary N was '1·25 × uric acid N' or that faecal N was 'N precipitated by uranyl acetate after the oxidation of uric acid with potassium permanganate'. As already seen (Expt 1) the two assumptions lead to widely different estimates for the digestibility of C35. When

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urine from chicks receiving C35 was collected separately (Expt 3), analysis showed that no more than 60 % of its N was in the form of uric acid, and that even urea NH_3-N and uric acid N together made up only 75 % of the total.

O'Dell *et al.* (1960), whose work is usually quoted as the justification for taking urinary N as 'uric acid N × 1·25', found that uric acid N formed 80 %, and 'uric acid N + NH_3-N' formed over 90 % of the total urinary N with the range of diets they used. Tasaki & Okumura (1964) have also reported similar values in a recent study. However, O'Dell *et al.* (1960) also quote a number of earlier workers who had found uric acid N to constitute only 60–65 % of total urinary N. It seems possible, therefore, that the partition depends on the type of protein being given and other dietary factors. Alternatively, the discrepancies may be shown to arise from differences in analytical technique. For the time being it seems unwise to rely on the accuracy of indirect estimates, particularly for poorly digested proteins.

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