Intrinsic labelling of chicken meat with stable isotopes of zinc, for intended use in human feeding studies: feasibility and design considerations

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1. The feasibility of intrinsically labelling poultry meat with the stable isotopes ^{68}Zn and ^{70}Zn was investigated.
2. Chickens (1-d-old) were gavaged with solutions of ^{68}Zn and ^{70}Zn several times over a 42 d period.
3. Isotopic analysis of edible tissues has shown that they were significantly enriched with respect to the gavaged isotope and that the extent of isotopic enrichment achieved was sufficient to permit their use in human feeding experiments designed to investigate availability of native Zn from such food models.
4. The absolute efficiency of retention of the stable isotopes from gavage solutions in the edible tissues has been calculated to be 2–3% of the administered dose; methods have been suggested to enhance this value, based on considerations of utilization of dietary Zn.

The availability of zinc from foods is an important determinant of the requirement for the mineral (Sandstead, 1973). In addition to the absolute level of Zn in the diet, several dietary factors may influence the availability of the element (O'Dell, 1969). Thus, the nature of the particular food might be an important determinant of the extent of its Zn absorption. However, it has not yet been established conclusively whether all Zn in a composite meal behaves as a single exchangeable pool at the absorption site. Evans & Johnson (1977) compared absorption of ^{65}Zn-labelled maize and rat liver preparations in rats with that from a similar diet in which ^{65}Zn was added extrinsically and found that absorption of the two isotope preparations was similar. However, their studies involved comparison of results with different groups of animals and so a direct test was not performed to evaluate the exchangeability of different pools of Zn within the intestine. Furthermore, the extent to which their results can be extrapolated to man is uncertain. Therefore, no direct evidence yet exists in reference to the exchangeability of Zn in composite meals in man similar to that already established for dietary iron (Cook *et al.* 1972).

Direct measurement of food Zn absorption in man has been made with isotopic labelling of the diet, either with radio-Zn (Sandstrom *et al.* 1980) or, more recently, with stable isotopes of the mineral (Janghorbani & Young, 1980). In this method it is now assumed that an extrinsically-added isotopic solution of Zn exchanges isotopically with the food Zn before absorption, and this provides an accurate measure of absorption of the native Zn from the food, an assumption whose validity has not yet been tested in man.

The need for establishing the validity of the extrinsic tag approach is especially crucial when stable isotopes are employed because these are used not at tracer levels but at levels approaching substrate values.

Preparation of foods biologically labelled with stable isotopes involves several issues not

associated with radio-labelling experiments, and their implications are different for different minerals and various foods (Janghorbani, Christensen *et al.* 1980). Specifically in regard to Zn, five stable isotopes are currently available as highly-enriched preparations and in theory lend themselves to biological labelling. This paper describes the biological labelling of poultry meat with stable isotopes of Zn for use in human feeding experiments.

METHODS

Stable isotopes

Stable isotopes ^{70}Zn (atomic enrichment 65·5%) and ^{68}Zn (atomic enrichment 99·0%) were purchased from Oak Ridge National Laboratory, Oak Ridge, TN, USA. The isotopes were dissolved in a small amount of concentrated hydrochloric acid and diluted to provide a zinc chloride solution for gavage.

Labelling procedure

Broiler type chicks (1-d-old) were obtained from a commercial hatchery and housed in electrically-heated, wire-bottom batteries at the Ralston Purina Research Laboratories. Temperature in the batteries was maintained at 32° for the first 2 weeks and then reduced to room temperature of 24°. The diet was a simplified maize–soya-bean-meal broiler starter ration which met all of the (US) National Research Council (1977) requirements for broiler chickens except Zn and contained 230 g protein (nitrogen × 6·25)/kg. The Zn content of the food was determined to be 18 mg/kg and was adjusted with inorganic Zn supplement to correspond to values given in Table 1.

Table 1. *Gavage schedule and zinc intake for chickens receiving ^{70}Zn and ^{68}Zn*

Age of chicken (d)	Dietary zinc content (mg/kg)	Gavage dose (mg ^{68}Zn or ^{70}Zn/dose)	No. of gavages/period
	^{70}Zn group		
0–21	40	0	0
22–28	25	1·26	3
29–35	25	2·20	3
36–43	20	2·69	3
	^{68}Zn group		
0–14	40	0	0
14–28	20	4·8	6
29–43	20	6·6	6

Two chickens were used in enrichment trials with ^{70}Zn and three with ^{68}Zn. Details of the gavage procedure for the two isotopes are given in Table 1. A total of 18·5 mg ^{70}Zn was gavaged per chicken while the corresponding value was 68·4 mg for ^{68}Zn.

At 43 d of age, the chickens were killed, bled, eviscerated, chilled in ice and placed in bags for shipment to Massachusetts Institute of Technology. Dressed weights of the ^{70}Zn-enriched chickens were 670 and 470 g and for the ^{68}Zn-enriched chickens the weights were 730, 740 and 755 g.

Isotopic analyses

On opening the bags, duplicate samples from each tissue (heart, gizzard, liver, breast meat, and leg meat) were taken with a clean stainless-steel blade, blotted with tissue paper and weighed accurately. Each sample was then wet ashed and analyzed for its ^{68}Zn and ^{70}Zn

isotopic composition using the method of Radiochemical Neutron Activation Analysis (RNAA) as described in detail previously (Janghorbani, Christensen *et al.* 1980). The results for each sample were then calculated as μg of isotope per g fresh weight of the tissue or mass isotopic ratio of ^{70}Zn to ^{68}Zn in the tissue.

RESULTS AND DISCUSSION

Selection of isotope for labelling. Five stable isotopes of Zn are currently available as highly-enriched preparations (Oak Ridge National Laboratory) and details are given in Fig. 1. As also indicated in Fig. 1 and discussed in detail elsewhere (Janghorbani & Young 1981), the analytical measurement method now available for routine application of stable isotopes of Zn to human nutrition is based on neutron-activation analysis. This method can measure only the three stable isotopes ^{64}Zn, ^{68}Zn and ^{70}Zn through the measurement of their neutron-activation products ^{65}Zn, ^{69m}Zn and ^{71m}Zn respectively. In a typical human feeding experiment designed to test the validity of the extrinsic tag approach, the diet is enriched intrinsically with one isotope, extrinsically with a second isotope, and a third isotope is also measured in the diet and the faeces in order to account for endogenous sources of the first two isotopes (see p. 400). Of the three isotopes which can be measured with neutron-activation analysis, ^{64}Zn has a relatively high natural abundance and thus will not permit enrichment in the chicken tissues by better than a factor of two. In addition, because of its relatively high natural abundance and its favourable neutron-activation characteristics, it is ideally suited as the isotope to be measured in order to account for endogenous Zn. Of the two remaining isotopes, i.e. ^{68}Zn and ^{70}Zn, both appear potentially useful for labelling experiments despite the apparent large differences in their natural isotopic abundances. For

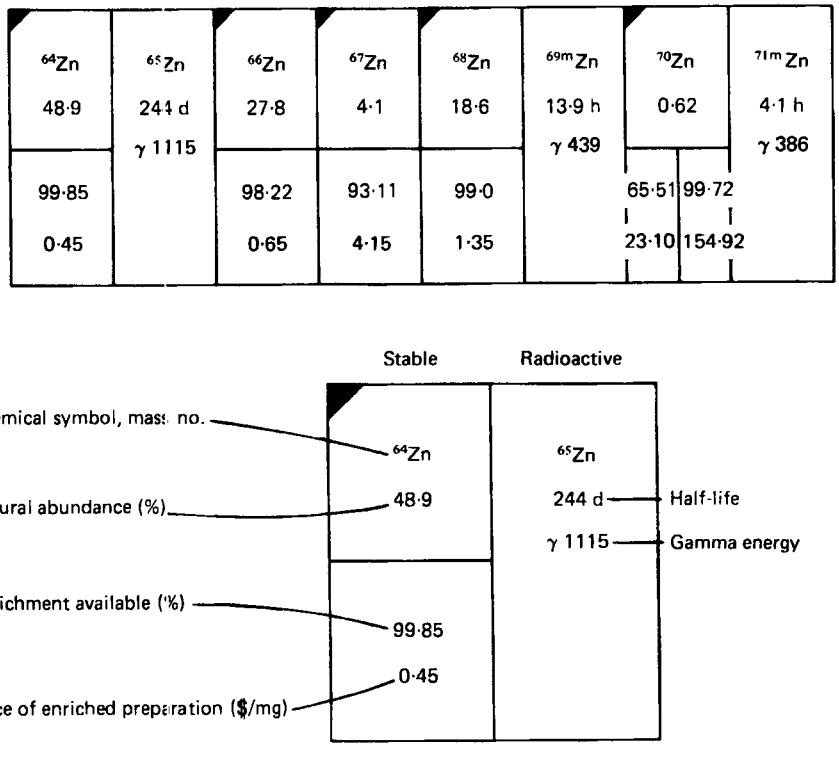

Fig. 1. Diagram showing isotopic constitution of zinc.

Table 2. *Typical values (μg/g fresh weight) for ^{68}Zn and ^{70}Zn in tissues of chickens from test groups*

(Mean values for duplicate analyses)

Chicken no.	Test Group	Tissue	^{68}Zn	^{70}Zn	Mass isotope ratio, ^{70}Zn:^{68}Zn*
927	^{70}Zn-enriched	Liver	7·33	11·2	1·53
		Gizzard	3·99	4·09	1·03
		Heart	3·89	4·59	1·18
		Skin	1·91	1·49	0·78
		Leg meat	3·05	2·35	0·77
		Breast meat	0·90	0·76	0·84
928	^{68}Zn-enriched	Liver	11·5	0·181	0·0157
		Gizzard	11·1	0·123	0·0111
		Heart	8·39	0·108	0·0129
		Skin	3·58	0·060	0·0168
		Leg meat	7·13	0·105	0·0147
		Breast meat	1·96	0·025	0·0128

* Natural mass isotopic ratio 0·0344.

Table 3. *Isotopic analyses of chickens enriched with ^{70}Zn and ^{68}Zn*

(Mean values for duplicate analyses, expressed as mass isotope ratio, ^{70}Zn:^{68}Zn*)

Chicken no.	Enriched isotope	Liver	Gizzard	Heart	Skin	Leg meat	Breast meat
926	^{70}Zn	0·651	0·555	0·577	0·401	0·434	0·429
927	^{70}Zn	1·53	1·03	1·18	0·780	0·771	0·844
927:926	—	2·4	1·9	2·0	2·0	1·8	2·0
	Mean 2·0 ± 0·2						
928	^{68}Zn	0·0157	0·0111	0·0129	0·0168	0·0147	0·0128
930	^{68}Zn	0·0109	0·0135	0·0112	0·0144	0·0123	0·0135
931	^{68}Zn	0·0133	0·0139	0·0138	0·0106	0·0130	0·0134

* Natural mass isotopic ratio, ^{70}Zn:^{68}Zn 0·0344.

these reasons, these two isotopes were selected for evaluation in labelling experiments reported here.

Accuracy of isotopic analyses. The accuracy and precision of the method used depends on the isotopic ratio for $^{70}Zn/^{68}Zn$ in the tissues (Janghorbani, Ting *et al.* 1980). In the present work, we anticipate the measured isotopic ratios to possess relative precisions of approximately 5% for the tissues enriched with ^{70}Zn and 10–20% for those enriched with ^{68}Zn.

Achieved extent of isotopic enrichment. The mass isotopic ratio, ^{70}Zn:^{68}Zn in unenriched tissues is 0·0344 (Lederer *et al.* 1967). Typical isotopic analysis of the various tissues of one chicken from each isotope group are summarized in Table 2. Examination of these values revealed that there was a parallel difference in the concentration of the Zn isotopes among the various tissues. Thus, liver contained the highest levels of both isotopes while breast meat was lowest in its concentration of both Zn isotopes. The expected Zn concentration difference between dark and light meat (Underwood, 1977) was readily observed for both isotopes from either group.

A summary of tissue isotopic enrichment values for all chickens from the two groups is given in Table 3. As evident, significant enrichment with ^{70}Zn or ^{68}Zn was achieved in both groups. For example, the mass isotopic ratio for ^{70}Zn:^{68}Zn in liver of chicken no. 927 was 1·53 compared with the unenriched isotope ratio of 0·0344, or a 45-fold enrichment with respect to ^{70}Zn. Similarly, the corresponding value in liver of chicken no. 931 was 0·0133, or a threefold enrichment with respect to ^{68}Zn. Furthermore, the extent of enrichment achieved with ^{70}Zn was several times greater than that with ^{68}Zn and this was a consequence of the much greater dietary enrichment for the ^{70}Zn group (Table 1). Comparing the two chickens in the ^{70}Zn group, it was clear that tissue enrichment was greater in chicken no. 927 than in chicken no. 926 by a factor of approximately 2 (Table 3). Similarly, native Zn concentrations were also higher in tissues of chicken no. 927 than for chicken no. 926. No such differences were observed among the ^{68}Zn-enriched chickens. Since the dressed weight of chicken no. 927 was significantly smaller than for chicken no. 926 (470 g *v.* 670 g) and no such differences were observed for the ^{68}Zn group, the observed differential enrichment between the ^{70}Zn-enriched chickens was in part due to differences in body size. Moreover, for the ^{70}Zn group, preferential tissue enrichment was observed in the following sequence: liver > heart > gizzard > skin ≈ leg meat ≈ breast meat. This was probably related to the lack of sufficient time for whole-body equilibration of the isotope before death and this effect would have been expected to be more pronounced for the ^{70}Zn group than for the ^{68}Zn group.

Due to the relatively high cost of isotopes associated with biological labelling of foods with Zn, it is important to estimate the efficiency of incorporation of the isotopes in the edible tissues. It was estimated that 1·6–3·2% of the gavaged dose was retained in the edible tissues of these chickens (meat + skin + heart + liver + gizzard). These results clearly showed that efficiency of retention of the label in this procedure was low, resulting in wastage of approximately 97–98% of the gavaged isotope. Average Zn retention from the food in normal broilers, weighing approximately 1500 g at 6 weeks and consuming 2500 g food with a Zn content of 30 mg/kg (Scott *et al.* 1969) was calculated to be approximately 8–10% of Zn intake in the edible tissues. Thus, it appeared that had the enriched isotope been incorporated into the food under normal conditions of food consumption and broiler growth, isotope retention would probably have been greater than achieved with the present gavage procedure. However, even under such conditions, about 90% of the dose would have been excreted. Since it is well established that retention of ^{65}Zn given to chicks with relatively low dietary Zn intake is higher than for birds receiving an adequate Zn intake (Scott *et al.* 1969), incorporation of the label into food low in native Zn would be expected to provide the most efficient vehicle for incorporation of the isotope. For example, the entire Zn requirement of a bird could be satisfied with the 68·4 mg ^{68}Zn which was gavaged in the ^{68}Zn group. This would correspond, on the average, to a food Zn concentration of approximately 30 mg/kg which is sufficient for good growth. However, such a procedure necessitates use of foods low in native Zn.

The levels of isotopic Zn gavaged in both groups combined with the food Zn concentrations (Table 1) corresponded to physiologically relevant intake levels for these birds. On average, the total Zn intake of these birds corresponded to 33–39 mg/kg for the ^{70}Zn group and approximately 55 mg/kg for the ^{68}Zn group so that incorporation of the isotope in various tissues was expected to have followed a pattern similar to that from normal levels of Zn supplementation.

Implications for studies in humans. Measurement of Zn absorption from the intrinsically-labelled food using the method of stable isotopes and faecal monitoring requires accurate estimation of the intake of enriched isotope and a second isotope from the enriched dietary pool, as well as both isotopes in the entire faecal pool (Janghorbani, Ting *et al.* 1980). These

concepts have been discussed previously and the expression used to calculate fractional absorption (F) of the enriched isotope from the enriched dietary pool (Janghorbani, Ting *et al.* 1980) is given as

$$F = \frac{A^*_{0,1} - A_{ft,1} + R_{1,2} \cdot A_{ft,2} - R_{1,2} \cdot A^*_{0,2}}{A^*_{0,1} - R_{1,2} \cdot A^*_{0,2}},$$

where $A^*_{0,1}$ and $A^*_{0,2}$ are the intake doses of the two isotopes of interest in the enriched diet pool, $A^*_{0,1}$ is intake dose of enriched isotope, $A_{ft,1}$ and $A_{ft,2}$ are the total isotope contents of faecal pool and $R_{1,2}$ is the natural isotope ratio, isotope 1:isotope 2.

In the instance of intrinsically-labelled foods $A^*_{0,2}$ was not likely to be negligible and could not be ignored in the equation. Furthermore, the value $A^*_{0,1}:A^*_{0,2}$ would by necessity be smaller than for extrinsic tag experiments. In addition, the total quantities of unabsorbed $A^*_{0,1}$ and $A^*_{0,2}$ would be further diluted in the faecal pool by the respective isotopes originating from the basal diet and via endogenous secretions. The consequences of this over-all isotopic dilution would result in increased contribution of the measurement uncertainty (σ_F/F; Janghorbani, Ting *et al.* 1980) to the over-all experimental uncertainty of the estimated value of F. The appropriate mathematical expression for estimating this measurement uncertainty has been given previously (Janghorbani, Ting *et al.* 1980) which allows realistic determination of the minimum measurement error involved under any given set of feeding and measurement conditions. In evaluating the adequacy of a given enrichment achieved for an intrinsically-labelled food, it is important to calculate this contribution to the over-all uncertainty of F, since the measurement uncertainty must be small compared to any biological variability.

The expected variability (σ_F/F) resulting from the over-all measurement precision for the labelled chickens whose isotopic compositions are typical of the values reported in the present paper is presented for various experimental conditions in Table 4. In Table 4, it has been assumed that either all meat or the white meat portion from a single chicken whose isotopic information conformed to the average values given in Tables 5 and 6 were given

Table 4. *Expected measurement error* (σ_F/F) *in the estimation of fractional absorption**

Case no.	Feeding regimen	Enriched isotope	Over-all measurement precision (%)	σ_F/F
1	All meat, 15 mg/d Zn supplement	^{70}Zn	5	0·26
2	All meat, 15 mg/d ^{64}Zn supplement	^{70}Zn	5	0·16
3	All meat, 15 mg/d ^{64}Zn supplement	^{70}Zn	1	0·03
4	White meat only, 15 mg/d ^{64}Zn supplement	^{70}Zn	5	0·29
5	White meat only, 15 mg/d ^{64}Zn supplement	^{70}Zn	1	0·06
6	All meat, 15 mg/d ^{64}Zn supplement	^{68}Zn	5	0·60
7	All meat, 15 mg/d ^{64}Zn supplement	^{68}Zn	1	0·12

* Assumptions: (1) Recommended daily allowance 15 mg (Sandstead, 1973), (2) Zn is in balance; daily faecal output = daily intake, (3) supplementation is such that total daily intake of Zn is 15 mg for the entire duration of the experiment, thus, on days of isotope administration, supplement is less than 15 mg by the amount of Zn supplied from the chicken.

Table 5. *Tissue zinc concentrations (μg Zn/g fresh tissue) of various chickens*

(For chickens nos. 926 and 927 the Zn content was calculated based on ^{68}Zn isotopic analyses, for the other chickens total Zn content was based on ^{70}Zn isotopic analyses)

Chicken no.	Liver	Leg meat	Breast meat
926	28·4	11·7	13·9
927	38·0	15·8	4·7
928	27·2	15·8	3·8
930	48·1	13·6	4·2
931	23·8	14·6	4·5
Mean ± 1 SD	33·1 ± 9·9	14·3 ± 1·7	6·2 ± 4·3

Table 6. *Isotopic contribution (μg) to daily intake of zinc**

	^{70}Zn-enriched			^{68}Zn-enriched		
Tissue	Zn	^{68}Zn	^{70}Zn	Zn	^{68}Zn	^{70}Zn
White meat	900	154	98	1050	400	5·3
Dark meat	3100	540	325	3700	1400	18·6
Liver	850	135	148	910	342	4·6
All meat	4000	694	423	4750	1800	23·9

* Assumptions: body-weight 1200 g, white meat 200 g, dark meat 200 g, liver 20 g; Zn concentrations (μg/g fresh weight): white meat 4, dark meat 14, liver 33; data based on analyses of this work for a single chicken.

to a subject in a single test meal. The expected value of σ_F/F under several realistic sets of intake conditions was calculated using the expression described previously (Janghorbani, Ting *et al.* 1980). For instance, all meat from a ^{70}Zn-enriched chicken may be used to provide a daily total Zn intake of approximately 4 mg (Table 6). If the diet were then supplemented with an additional 11 mg Zn on the day of isotope administration to bring the total Zn intake up to the recommended level and 15 mg Zn on other days and assuming that a faecal pool corresponding to approximately 3 d Zn intake would be sufficient to recover all unabsorbed isotope and accepting measurement precision of 5% (case no. 1, Table 4), the value for σ_F/F was 0·26. This value was unacceptably large and resulted partly from the dilution of the enriched food with the supplemented Zn. On the other hand, if the dilution of the enriched isotope was eliminated by supplementing the diet with ^{64}Zn (which can be purchased at 100% enrichment for $0·45/mg) instead of Zn under the same supplementation conditions as described previously, the value of σ_F/F could be expected to be reduced to 0·16 (case no. 2, Table 4). In addition, if measurement precision was approximately 1% (case no. 3, Table 4), there was a dramatic improvement in σ_F/F to 0·03. This indicated that in order to use successfully chickens intrinsically-labelled with ^{70}Zn, any dietary supplement with Zn must be made such that dilution of the enriched isotope is minimized and the isotopic analyses must be carried out with analytical precisions approaching 1%. Similar calculations indicate that for ^{70}Zn-enriched chickens it would be possible to use only part of the meat (case no. 5, Table 4) and so allow determination of the availability of Zn in different types of muscle, for example, but here the analytical measurement precision would have to reach 1%.

In contrast, the extent of enrichment achieved with ^{68}Zn in chickens nos. 928–930 was marginal for use in human studies (cases nos. 6 and 7, Table 4) unless either a higher portion

of the recommended Zn intake was supplied from the enriched chickens, a greater extent of ^{68}Zn enrichment was achieved through more efficient utilization of the enriched isotope, or the analytical measurement precision was close to 1%, all of which could, in principle, be achieved. This is, of course, due to the fact that if body Zn homoeostasis is maintained via secretion of the element into the intestinal lumen a significant fraction of total faecal ^{68}Zn arises from these secretions and elimination of ^{68}Zn from the dietary supplement does not effectively reduce dilution of the enriched isotope.

SUMMARY AND CONCLUSIONS

The feasibility experiments reported here indicate that: (1) intrinsic labelling of edible chicken tissues with stable isotopes of Zn can be realistically achieved on a scale sufficient to allow use in human metabolic studies; (2) the absolute isotopic utilization of the enriched isotope in the present experiments is low, resulting in high wastage of the administered isotope. However, improvements can be made by incorporation of the Zn isotope into the food, rather than using a gavage procedure; (3) based on cost analyses and other considerations, ^{68}Zn appears to be the prefered isotope for labelling purposes. Isotopic cost for labelling a single bird can be reduced to below $100; (4) measurement methodology now is available to permit routine investigation of double-labelling of diets with stable isotopes of Zn. This would allow resolution of issues concerned with exchangeability of Zn pools in the lumen of the intestine and this offers exciting opportunities for exploring the bioavailability of dietary Zn in human subjects under a wide range of nutritional conditions.

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