The effect of available dietary zinc on the utilization of protein by the chick and Japanese quail*

By J. ATKINSON,† P. VOHRA AND F. H. KRATZER

Department of Avian Sciences, University of California, Davis, California 95616, *USA*

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1. Chicks and quail (*Coturnix coturnix japonica*) were used in the measurement of net protein utilization and true digestibility of nitrogen of isolated soya-bean protein and a mixture of casein and gelatin in zinc-deficient diets.

2. The net protein utilization values for both protein sources were increased when the diets were supplemented with Zn or with the disodium salt of ethylenediaminetetra-acetic acid, as was the true digestibility of nitrogen of the isolated soya-bean protein.

Before the report of Tucker & Salmon (1955) zinc deficiency had been produced experimentally only at extremely low dietary levels of Zn. However, these workers described symptoms of Zn deficiency in pigs given diets containing 34–44 parts/10^6 Zn. The diets included cottonseed meal or a mixture of soya-bean, meat-and-bone and fish meals as sources of protein.

O'Dell & Savage (1957) described a definite Zn deficiency of similar aetiology in the chick given a diet containing isolated soya-bean protein (ISP). Thereafter several workers furnished evidence of an increased Zn requirement when rations including ISP were given to chicks (Edwards, Young & Gillis, 1958; Moeller & Scott, 1958; Roberson & Schaible, 1958; Morrison & Sarett, 1958; O'Dell, Newberne & Savage, 1958; Zeigler, Leach, Norris & Scott, 1961). Summarizing these reports, it can be stated that, for chicks given casein or egg-white as a protein source, a basal dietary Zn level of 10 parts/10^6 was found to be adequate, whereas 30–35 parts/10^6 were required to obtain maximum growth when ISP was used as the source of protein. Fox & Harrison (1964) found that a dietary level of 10 parts/10^6 Zn was inadequate for quail fed on diets containing ISP. From the work of Kratzer, Allred, Davis, Marshall & Vohra (1959) it was deduced that ISP possessed a specific factor or factors which rendered some of the dietary Zn unavailable to the animal.

Zn is a functional component of several enzyme systems and many studies have been made on enzyme activity in Zn-deficient animals. Hsu, Anilane & Scanlan (1966) found a lower carboxypeptidase activity in Zn-deficient rats when compared with controls, suggesting a possible depressive effect of low dietary level of Zn on protein digestion.

In view of the known interference of ISP with Zn utilization and the involvement of Zn in enzyme systems, a study was made of the possible effects of the dietary levels of

* Some of these results have been communicated in a preliminary form (Atkinson & Kratzer, 1970).

† Present address: Société d'Assistance Technique pour Produits Nestlé, S.A., Department Labior 1350 Orbe, Vaud, Switzerland.

Table 1. *Percentage composition of basal diets*

	Expt 1 (chicks)	Expt 2 (quail)	Expt 3 (chicks)
Soya-bean oil, degummed	10·00	3·75	5·00
Cellulose powder*	10·00	5·00	3·00
Mineral mix†	2·31	2·31	2·31
$CaCO_3$	2·50	2·50	2·50
$CaHPO_4.2H_2O$	2·50	3·00	2·50
Vitamin mix‡	0·43	1·00	0·43
Choline chloride§	0·68	0·68	0·68
DL-methionine	0·50	0·45	0·50
Glycine	—	0·75	—
Chromic oxide	—	—	0·30
Maize starch, pearl	71·08	2·99	—
Glucose¶	77·57	82·78	—

* Solka-Floc, Fine White, 200 W (McKesson and Robbins, Oakland, Calif., USA).

† Contributed (% of diet): K_2HPO_4, 0·5; NaCl, 1; KCl, 0·3; $MnSO_4.H_2O$, 0·03; KI, 0·001; $MgSO_4.7H_2O$, 0·4; $FeSO_4.7H_2O$, 0·065; $CuSO_4.5H_2O$, 0·008; $Co(CH_3COO)_2.4H_2O$, 0·002; $Al_2(SO_4)_3.18H_2O$, 0·025; $Na_2MoO_4.2H_2O$, 0·001.

‡ Contributed (mg/kg diet, unless stated otherwise): riboflavin, 10; thiamin hydrochloride, 10; pyridoxine hydrochloride, 10; calcium pantothenate, 30; nicotinic acid, 100 (Expt 2, 120); butylated hydroxytoluene, 1000; vitamin A, 10000 i.u. (Expt 2, 5000); cholecalciferol, 2500 i.u. (Expt 2, 4500); vitamin E, 20 i.u. (Expt 2, 88); folic acid, 4 (Expt 2, 5); menaphthone, 1 (Expt 2, 10); biotin, 1 (Expt 2, 0·4); cyanocobalamin, 10 μg.

§ 50% in wheat middlings carrier.

¶ Anhydrous cerelose (Corn Products Sales Company, San Francisco, Calif., USA).

available Zn on protein utilization using both chicks and Japanese quail (*Coturnix coturnix japonica*). Net protein utilization (NPU) was determined in chicks (Expt 1) and in quail (Expt 2), and true digestibility was determined in chicks (Expt 3).

EXPERIMENTAL

Procedure

The birds were kept in stainless-steel batteries and they were given distilled water in glass bottles. Chicks were fed from stainless-steel hoppers and quail from Perspex feeders with expanded stainless-steel covers. At the start of the experiments the birds were weighed, birds of extreme weight were eliminated, and the remainder were randomized, after stratification on the basis of weight and weight gain, into experimental groups.

There were, for each treatment, two replicates of four chicks per cage in Expt 1, three of one chick per cage in Expt 3 and five of five quail per cage in Expt 2.

The birds in Expts 1 and 3 were Arbor Acre cockerels (Arbor Acre Farms, Nipoma, California) and those in Expt 2 were Japanese quail from the stock of this Department. All were given a standard diet from 1 d of age until the start of the experiment, when they were 10 d old in Expt 1, 7 d old in Expt 2 and 42 d old in Expt 3.

Diets

The composition of the basal diets is given in Table 1. Reagent-grade salts were used throughout. The calcium level of the basal diets (1·6 and 1·7 % for chicks and quail respectively) was higher than normal, with the aim of reducing Zn availability.

The supplements given in Tables 2–4 were added to the protein-free diet at the expense of maize starch in Expts 1 and 3, and of glucose in Expt 2.

NPU determination

To determine NPU, a group of birds was starved for 6 h and killed by dislocation of the neck at the start of the experiment; the remaining chicks were killed on the 21st day after 11 d on experiment and the quail on the 14th day after 7 d on experiment, again after being starved. Both lots of birds were immediately deep-frozen pending carcass analysis. The chicks for Expt 1 were passed through a meat-mincing machine in a deep-frozen state (with precautions to minimize water losses) and portions of the minced carcass were taken for nitrogen determination. The quail were analysed for N by digestion of the whole carcass. NPU was calculated from formula '3' of Bender & Doell (1957) (see also Atkinson & Carpenter, 1970):

$$\text{NPU} = \frac{\text{(gain in carcass N test group)} - \text{(change in carcass N protein-free group)}}{\text{(N intake test group)} - \text{(N intake protein-free group)}} \times 100.$$

Measurement of true digestibility of N

The cloacal openings of the chicks for Expt 3 were separated according to the procedure of Newberne, Laerdal & O'Dell (1957), but the faeces were collected from a tray underneath the mesh floor of the cage and not in a balloon. After a postoperative recovery period of 3–4 d, collections of excreta were made every 2 h from 08.00 to 18.00 h. As chromic oxide was used as an inert marker, a total collection was unnecessary. Excreta were deep-frozen before drying under reduced pressure for analysis. True digestibility of N was calculated as follows:

$$\text{true digestibility} = \frac{\text{(Ratio of \% N to \% } Cr_2O_3 \text{ in faeces of test group)} - \text{(Ratio of \% N to \% } Cr_2O_3 \text{ in faeces of protein-free group)}}{\text{(Ratio of \% N to \% } Cr_2O_3 \text{ in diet of test group)}} \times 100.$$

Analytical methods

Zn was determined by atomic absorption spectroscopy after digestion with a mixture of perchloric and nitric acids (Anonymous, 1966). Chromium was determined by an unpublished modification (C. K. Milner, private communication) of the procedure of Czarnocki, Sibbald & Evans (1961). N was determined as ammonia after micro- or macro-digestion with concentrated sulphuric acid according to the Kjeldahl procedure (Association of Official Agricultural Chemists, 1960). A semi-micro distillation into boric acid solution was used for determination of ammonia (Ma & Zuazaga, 1942).

The Zn and N contents of the diets are given in Tables 2–4.

Table 2. *Expt* 1. *Composition of experimental diets A–G, their contents of nitrogen and zinc, and the net protein utilization* (NPU) *of chicks given the diets*

	A*	B	C	D	E	F	G
Isolated soya-bean protein† (%)	—	12·12	12·12	12·12	—	—	—
Casein‡ (%)	—	—	—	—	8·32	8·32	8·32
Gelatin§ (%)	—	—	—	—	3·80	3·80	3·80
Basal mix	28·92	28·92	28·92	28·92	28·92	28·92	28·92
ZnO (parts/10^6)	—	—	38	—	—	38	—
Na_2EDTA¶ (parts/10^6)	—	—	—	200	—	—	200
N content (%)	0·21	1·99	1·99	1·99	2·06	2·06	2·06
Zn content (parts/10^6)	5·2	16·5	46·8	14·7	8·7	37·1	8·8
Mean NPU (±0·71‖)	—	65·9	73·5	75·9	63·3	68·9	68·0

* Protein-free diet.
† RP-100 (Ralston-Purina Co., St Louis, Missouri, USA).
‡ Vitamin-free (Nutritional Biochemicals Corp., Cleveland, Ohio, USA).
§ From Nutritional Biochemicals Corp.
¶ Disodium salt of ethylenediaminetetra-acetic acid (K and K Laboratories, Inc., Plainview, New York, USA).
‖ Standard error of means.

Table 3. *Expt* 2. *Composition of experimental diets A–E, their contents of nitrogen and zinc, and the net protein utilization* (NPU) *of Japanese quail given the diets*

	A*	B	C	D	E
Isolated soya-bean protein (%)	—	—	32·6	—	32·6
Washed isolated soya-bean protein† (%)	—	32·6	—	32·6	—
Basal mix (%)	22·43	22·43	22·43	22·43	22·43
ZnO (parts/10^6)	—	—	—	121	121
N content (%)	0·2	5·2	5·01	5·2	5·01
Zn content (parts/10^6)	2·8	9·1	28·3	106·1	126·3
Mean NPU (±1·00‡)	—	41·1	44·9	45·3	46·0

* Protein-free diet.
† RP-100 washed twice with Na_2EDTA according to the method of Davis, Norris & Kratzer (1962).
‡ Standard error of means.

RESULTS

The NPU results for Expt 1 are given in Table 2. The chicks in one of the diet G cages ate little and grew slowly for no apparent reason, but this did not significantly alter the NPU value. Addition of supplementary Zn (as ZnO) or addition of Na_2EDTA to the diet caused an increase in the NPU values obtained for both the ISP, and casein and gelatin mixture. With ISP in the diet the birds given the supplemented diets (C and D) grew better than those fed on the unsupplemented diet B. With the casein and gelatin mixture, growth on the supplemented diets (F and G) was poorer than on the unsupplemented diet E, but the food consumed was utilized more efficiently. The net effect in both instances was an increase in NPU by Zn or Na_2EDTA supplementation.

Preliminary experiments with quail given diets with a high content of unwashed ISP failed to reveal a significant difference between NPU values whether or not the diets were supplemented with Zn. For this reason the ISP was washed with EDTA

Table 4. *Expt* 3. *Composition of experimental diets A–E, their contents of nitrogen and zinc, and the true digestibility of nitrogen of ISP*

	A*	B	C	D	E
Isolated soya-bean protein (%)	—	19·40	19·40	19·40	—
Casein (%)	—	—	—	—	14·60
Gelatin (%)	—	—	—	—	4·80
Basal mix	17·22	17·22	17·22	17·22	17·22
ZnO (parts/10^6)	—	—	135	—	—
Na_2EDTA (parts/10^6)	—	—	—	200	—
N content (%)	0·24	3·10	3·10	3·10	3·18
Zn content (parts/10^6)	4·4	18·8	132·5	18·0	9·6
Mean true digestibility (±0·95†)	—	90·91	95·32	95·44	93·93

* Protein-free diet. † Standard error of means.

in order to reduce its content of Zn. The NPU obtained for washed ISP in a low-Zn diet was significantly lower than the values obtained for the same preparation supplemented with Zn or for an unwashed preparation (Table 3).

The results of the digestibility determinations are summarized in Table 4. Chicks in the protein-free group excreted 0·25 g N/100 g diet eaten and this value was used as an estimate of metabolic N. It was subtracted from the corresponding N excretion value for chicks receiving ISP-supplemented diets to provide estimates of the 'true' digestibility of the protein N. From Table 4 it can be seen that addition of supplementary Zn or Na_2EDTA to the control diet increased the true digestibility of the N of the ISP.

DISCUSSION

The results obtained for NPU are similar to those reported by Summers & Fisher (1961) for chicks. These authors found values for ISP of 66·9 when included in Zn-supplemented diets at a protein level of 13% and 47·9 at a 26% protein level. Summers & Fisher (1962) found values of 41·8 for casein and 22·4 for gelatin when included at a 13% protein level in the same basal diet. Their value for casein is lower than the value of 60·0 reported for rats by Miller & Bender (1955). It is interesting to note that the NPU value for a casein and gelatin mixture supplemented with methionine, found in the experiment reported here, is higher than the value for casein alone reported above.

With chicks the effect of Zn on NPU has been demonstrated at a protein level below requirement (National Research Council, 1971), whereas with quail the protein level used was above that found by Gropp & Zucker (1968) to give maximal growth.

There are few previous reports of an effect of dietary level of available Zn on protein utilization. Kfoury, Reinhold & Simonian (1968) have reviewed the conflicting evidence on the effect of Zn deficiency on enzyme systems. As stated on p. 461, Hsu *et al.* (1966) found a lower carboxypeptidase A activity in Zn-deficient rats. Mills, Quarterman, Williams, Dalgarno & Panić (1967) also found a lower carboxypeptidase activity in Zn-deficient rats. These workers could find, however, no evidence for a reduction in the rate of protein digestion or absorption in Zn-deficient rats. Barré (1956) found

that proteins complexed with phytic acid were resistant to digestion with proteolytic enzymes. Fox & Harrison (1966) published results suggesting that Zn plays a part in plasma protein metabolism in Japanese quail. However, perhaps most pertinent to this work is the report of Oberleas & Prasad (1969), who found poorer utilization of soya-bean protein, as judged by growth rate in rats, when supplementary Zn was omitted.

In view of the results reported here and those of other workers, it may be necessary to reconsider some of the values obtained in the early work on protein quality, especially those for plant proteins. Possible interactions of Zn and protein metabolism may also have to be considered when approaching problems of protein malnutrition and its treatment in human beings.

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