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EFFECT OF PROTEIN QUALITY AND DIETARY LEVEL OF IRON, ZINC AND COPPER ON THE UTILIZATION OF THESE ELEMENTS BY GROWING RATS.*) PART I. GENERAL DEVELOPMENT OF ANIMALS AND APPARENT ABSORPTION OF IRON, ZINC AND COPPER**)

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The effect of protein quality and dietary level of Fe, Zn and Cu on feed efficiency ratio and apparent absorption of these elements was studied in growing male Wistar rats during a six-week experiment. When the protein source was the limiting factor for animal growth and metal absorption, diet supplementation with trace elements was of questionable efficiency. The apparent absorption of trace elements depends on both their dietary level and the source of protein. No interactions during intestinal absorption between Fe, Zn and Cu were observed.

INTRODUCTION

Interactions between elements of similar physico-chemical properties, such as iron, zinc and copper, may take place in various parts of the organism [3]. Many studies, involving both humans and animals, confirm that either a low or a high intake of one of these elements may affect the utilization and metabolism of the other two [3, 10, 11]. These interactions are yet to be clarified fully, because there are many factors, among them diet composition, which may modify them [10, 11, 18, 19].

It is suggested that a simultaneous increase of the supply of iron, zinc and copper in proportion to their requirements reduces the antogonistic interactions between them. [8] but there is no experimental evidence to confirm this.

In our research we investigated the effect of proportional increases of the contents of iron, zinc and copper in diets on the utilization of these minerals in rats. An additional variable factor in our experiments was the source of protein in the diet. In the first part of our report we present results concerning the general development of animals and the absorption of iron, zinc and copper. The second part will demonstrate the distribution of the examined elements in the animals tissues.

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MATERIALS AND METHODS

The six-week experiment was performed with growing male Wistar rats initially weighing 102 ± 3 g. The animals were kept individually in plexiglass cages; in room with controlled temperature (21 + 2°C), humidity (50%), and light cycle (12 -h). The experimental semisynthetic diets and redistilled water were provided ad libitum.

Table 1. Composition of the experimental diets

Dietary component	Diet C	Diet G	Diet M
		g/kg	
Casein[1]	222	—	111
Wheat gluten[2]	—	270	135
Wheat starch[3]	628	580	604
Soybean oil[4]	100	100	100
Cellulose[5]	10	10	10
Mineral mix[6]	30	30	30
Vitamin mix[7]	10	10	10

1) Zakład Mleczarski, Łuków, Poland.
2) Raision Tehtaat Oy Raisio, Finland.
3) Meelunie B. V., Amsterdam, Holland.
4) Purchased locally.
5) Wood cellulose, Huta Baildon, Poland.
6) According to AOAC, 1975/20. Mineral mix was modified by excluding iron, zinc and copper salts. This mix supplied per kg of diet (in g): $CaHPO_4$, 22.05; K_2HPO_4, 2.43; K_2SO_4, 2.04; NaCl, 0.92; $CaCO_3$, 0.63; Na_2HPO_4, 0.64; MgO, 0.75; $MnCO_3$, 0.126; KI, 0.0002.
7) According to AOAC, 1975/20. Vitamin mix supplied (mg/kg diet): choline chloride, 2000; inisitol, 100; p-aminobenzoic acid, 100; niacin, 40; calcium pantothenate, 40; riboflavin, 8; thiamin·HCl, 5; pyridoxine·HCl, 5; folic acid, 2; biotin, 0.4; vitamin B_{12}, 0.03; menadione, 5; as well as vitamin A, 20.000 IU vitamin D_3, 2.000 IU; vitamin E, 100 IU.

The experimental diets (Table 1) contained 20% of protein. The sources of protein were casein (C), wheat gluten (G) or a 1 : 1 mixture thereof (M). To obtain diets with low level of minerals, casein and gluten were purified with disodium versenate and redistilled water [15].

The content of minerals was adjusted by suitable additions of iron citrate, zinc acetate, and copper carbonate to obtain in the diets either 60% (DM — deficiency of microelements), 100% (SM — standard content) or 300% (high content) of level recommended for rats [1] i.e. 35 mg of iron, 12 mg of zinc, and 5 mg of copper per 1 kg of diet. The analytically determined contents of protein and elements in experimental diets are given in Table 2.

Diet consumption was recorded daily, and body weight gain weekly. In the second, fourth and sixth weeks of experiment, the faeces of each rat were collected for four days. The apparent absorption of the elements, expressed in per cent, was calculated from the formula

$$\frac{\text{intake} - \text{excretion in faeces}}{\text{intake}} \times 100$$

Table 2. Content of protein as well as iron, zinc and copper in air-dry experimental diets (mean ± standard error of mean)

Diet	Protein %	Iron mg/kg	Zinc mg/kg	Cooper mg/kg
CDM	19.99±0.61	21.05±0.28	8.45±0.21	1.33±0.07
CSM	19.95±0.21	34.55±0.67	11.87±0.69	3.85±0.20
CHM	20.08±0.45	98.11±1.95	34.86±0.27	11.28±0.20
GDM	20.89±0.42	21.30±1.45	8.10±0.22	3.02±0.12
GSM	20.56±0.29	33.82±0.80	13.19±0.45	4.44±0.25
GHM	21.14±0.16	97.24±2.28	36.82±0.54	12.71±0.08
MDM	20.94±0.53	18.96±0.28	7.26±0.48	1.94±0.09
MSM	20.36±0.23	32.91±0.81	11.27±0.29	4.38±0.21
MHM	20.94±0.46	110.10±3.56	33.78±1.14	15.53±0.43

The results are mean figures from the three test periods.

The contents of iron, zinc and copper in the diets and in faeces were determined by atomic absorption spectrophotometry in a Perkin-Elmer model 300 apparatus after dry ashing at 450°C and dissolving in hydrochloric acid.

The results were analysed statistically by two way analysis of variance and the Duncan test [21].

RESULTS

Regardless of the iron, zinc and copper contents in the diets, all the animals fed the gluten diets achieved low weight gain (Table 3). This was partly the result

Table 3. Consumption of diet, body weight gain and FER after six weeks (mean ± standard error of mean)

Diet	Diet consumption (g/rat/6 weeks)	Body weight gain (g/rat/6 weeks)	FER*)
CDM (7)**)	548±26	133±10	0.250±0.007
CSM (16)	618±15	157± 3	0.254±0.008
CHM (6)	641±11	176±12	0.273±0.014
GDM (6)	443±23	45± 6	0.101±0.010
GSM (15)	514±14	51± 3	0.099±0.005
GHM (6)	476±13	54± 2	0.114±0.005
MDM (10)	604±16	150± 3	0.247±0.007
MSM (27)	648±13	169± 6	0.259±0.008
MHM (10)	711±16	191± 9	0.268±0.009
	Analysis of variance (p values)		
Protein	0.0001	0.0001	0.0001
Mineral	0.0001	0.0003	0.0170
P×M	NS***)	NS	NS

*) FER — Foof Efficiency Ratio (body weight gain in relation to diet consumption)

**) Number of animals

***) Not significant ($p>0.05$)

of a lower diet consumption than in the case of rats fed casein (C) and mixed (M) diets. The animals fed M diets ate more food and gained more weight than those fed C diets with the same contents of minerals, but the differences were not statistically significant.

Diet comsumption and body weight gain were lower in the case of DM diets than in the case of HM diets, with the differences being statistically significant (according to Duncan's test) only for the C and M diets. There were no differences in these parameters between animals fed DM and SM diets, or between SM and HM diets for all sources of protein.

The apparent absorption of the studied elements depended significantly on the source of protein in the diet, but this was more pronounced in the case of zinc and copper than for iron (Table 4). For G and M diets, the mean per cent apparent absorption of iron and zinc was lower than for C diets, whereas mean copper absorption was significantly lower for G diets than for the C and M ones.

During the experiment, the rats absorbed iron less efficiently (39.6% on average) than copper (48.8% on average) and zinc (51.2% on average). Apparent absorption is inversely correlated with the content of the elements in the diet. The greatest differences in absorption between DM and HM diets were in the case of copper from C and M diets. This was predictable since the copper content in CDM and MDM diets was lower than assumed (1.33 and 1.99 mg/kg respectively).

Table 4. Apparent absorption of the investigated elements (mean ± standard error of mean)

Diet	Apparent absorption		
	iron	zinc	cooper
CDM	49.5±4.3	68.8±3.1	75.5±1.7
CSM	45.2±1.8	60.4±3.0	48.1±1.8
CHM	40.1±4.2	49.9±4.0	42.0±5.2
GDM	43.1±3.7	52.3±2.1	45.5±1.4
GSM	37.6±4.6	46.8±1.6	42.3±4.0
GHM	26.4±3.0	37.8±2.4	33.7±3.5
MDM	45.9±6.7	57.4±2.5	68.4±1.8
MSM	41.4±3.0	47.7±2.7	44.2±1.3
MHM	27.3±3.6	40.3±2.0	39.5±2.2
	Analysis of variance (p values)		
Protein	0.0137	0.0001	0.0001
Minerals	0.0001	0.0001	0.0001
P × M	NS	NS	0.0002

*) Not significant (p > 0.05)

DISCUSSION

The utilization of diet for growth (FER) depended mainly of protein quality and to a lesser extent on the content of elements in the diet. Significant deficiency of essential amino acids, mainly lysine and threonine in gluten diets, and low consumption of these diets restricted the growth rate of animals. The mixed diets were utilized for growth as efficiently as the casein ones thanks to the mutual complementation of amino acids, a phenomenon observed in other experiments as well [4, 7, 12].

When iron, zinc and copper contents were low (DM), the animals consumed less food, and this led to lower body weight gain. The differences in weight gain were clearly apparent because the rats were fed ad libitum, but Johnson and Evans [11] observed similar tendencies when animals were pair-fed with diets containing high and low amounts of iron, zinc and copper.

Reduced growth rate was also observed when the diets contained small quantities of only one of the studied elements. For example, diet consumption and weight gain of rats fed diets deficient in zinc (< 3 ppm) were always lower than that control group 7, but at marginal zinc level (6-10 mg Zn/kg) the response of the animals depended on the quality of protein in the diet [4, 12]. Zinc supplementation affected the rats' weight only when the amino acids composition of the protein was good. This observationas confirmed by our research: Duncan's test failed to demonstrate differences in weight gain of rats fed gluten diets.

When the animals were pair-fed, diet consumption and body weight gain were not lover in rats eating diets deficient in copper [10]. In the case of ad libitum feeding, however, there was a gradual decrease in the growth rate of animals fed diets with a low copper content (2.5 mg/kg), and statistically significant differences appeared after 60 days [22].

Although the iron, zinc and copper contents in diets used in our experiment were not very low, we observed a decrease of diet consumption and weight gain due to the cumulative effect of the moderate deficits of the three elements. The differences in diet utilization for growth may be accounted for by reduced rates of the synthesis of nucleic acids or protein [4] and by the increased catabolism of proteins in animals suffering from a deficiency of trace elements [5].

A small excess (not exceeding physiological doses) of iron, zinc and copper in the diet did not lead to greater diet consumption or body weight of the animals than in the case of diets with standard contents of these elements.

As already mentioned, the coefficients of apparent absorption for iron were lower than for copper and zinc. This in turn led to changes in mutual proportions of these elements absorbed from the intestine in relation to these proportions in the diets. The mean iron-to-copper ratio descreased from 9.0 in the diet to 7.2 after absorption, the mean iron-to-zinc ratio from 2.8 to 2.2, while the mean zinc-to-copper ratio remained practically unchanged (3.3 and 3.4). Neither the amount of elements in the diet nor the source of protein affected these relations.

The apparent absorption of microelements depends on many factors, one of the most important among them is their content in the diet. According to other authors [10, 17, 19, 23], regardless of the protein source, the apparent absorption of the studied elements expressed as per cent of the consumed dose was lower for higher contents of the elements in the diet. At the same time, the amount of the absorbed elements was higher.

The mean iron-to copper and zinc-to-copper ratio after absorption were lower in HM diets and higher in DM diets, while the mean iron-to-zinc proportion was similar irrespective of the content of these elements in the diets. Since analogous relations between the investigated components were determined in the diets, it may be surmised that to anatogonistic interraction occur in intestinal absorption. Davies [8] and Solomons [20] also suggested that when the considered elements are consumed in amounts corresponding to the daily requirements, the binding sities of intestinal absorption are not fully saturated, and that it is only after the point of saturation is overstepped that competition could begin. However, in the available literature there are no experimental data that can be directly compared with the findings of our present research.

The absorption of iron, zinc and copper also depends to a significant extent on the source of protein. This problem is widely discussed in the literatue. In general, absorption from diets containing animal proteins (e.g. milk powder, fish meal [24], casein [17-19] or protein mixtures [6, 17, 23]) is higher than from diets with plant proteins (such as soya flour [6, 24], wheat gluten [17, 18] and proteins of vegetable products [23]). The lower absorption of mineral components from plant proteins is seen by many authors as due to the effect of nonnutritive substances like cellulose, phytates, etc. [6, 23]. The gluten used in our experiment had a very low phytates content, and so other factors may have played a significant role. The form of protein precipitate in the stomach, the ability to bind mineral components [16] as well as the buffer capacity of proteins [14] affect the releasing and rebinding as well as the intragastric distribution of elements, all of which is important in absorption processes in the intestine.

Moreover, the specific break-down products generated from proteins during the gastrointestinal passage may also enhance or inhibit the absorption of trace elements [2]. However, there are no data that would enable a comparsion of gluten and casein in this respect.

Some authors suggest that the action of amino acids such as methionine [13], cysteine [9], histidine and lysine in the intestine canal consists in reducing iron to forms that dissolve more easily [2] or in chelating metals. The casein, gluten and mixed diets used in our experiment differed significantly as regards lysine and cysteine contents, and this could have been one of the reasons for the observed differences in absorption coefficients for the examined elements.

Since the effect of protein kind was practically the same in the case of all the considered elements, this factor did not significantly alter the proportion of iron to zinc and copper after absorption in comparison to the same ratios in the diets.

To conclude, it must be pointed out that the growth rate of the animals and trace elements absorption influenced the tissue saturation with the studied elements. In this experiment both investigated factors*), were important. The rats fed DM diets absorbed, on average, about 30% less of each of the studied mineral per 1 g of body weight gain than rats fed SM diets, while those eating HM diets

*) i.e. level of trace elements in the diets and source of protein.

absorbed about two times the amount of trace elements absorbed by the latter, irrespective of the source of protein. Moreover, in rats fed gluten diets the absorption of each of the investigated element per 1 g of body weight gain was on average about twice higher that in animals fed casein and mixed diets.

CONCLUSIONS

1. Iron, zinc and copper additions enhance the diet utilization for growth if the protein in the diet is of good quality.
2. As in the content of iron, zinc and copper in the diets was nigher the per cent coefficients of absorption the absorbed amount of these elements increased regardless of the quality of protein in the diet.
3. The source of protein in the diet significantly affected the per cent coefficients of apparent absorption of iron, zinc and copper irrespective of their content in the diet. When protein quality was poor (wheat gluten), the amounts of elements absorbed per 1 g of body weight gain were highest despite the lowest absorption.
4. For the doses of iron, zinc and copper applied in this experiment there was no interaction between these elements in the stage of intestinal absorption.

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WPŁYW JAKOŚCI BIAŁKA ORAZ ZAWARTOŚCI ŻELAZA, CYNKU I MIEDZI W DIECIE NA ICH WYKORZYSTANIE PRZEZ ROSNĄCE SZCZURY*. I. OGÓLNY ROZWÓJ ZWIERZĄT I OBSORPCJA POZORNA ŻELAZA, CYNKU I MIEDZI**

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Streszczenie

Rosnące szczury samce były karmione przez 6 tygodni dietami zawierającymi 20% białka, którego źródłem była kazeina, gluten pszenny lub ich mieszanina (1 : 1). Zawartość żelaza, cynku i miedzi była zróżnicowana następująco: 60, 100 lub 300% zaleceń dla szczurów, przy zachowaniu stałego stosunku tych pierwiastków. Stwierdzono, że wykorzystanie diety glutenowej na cele wzrostowe było najniższe i nie zależało od ilości mikroelementów w diecie. Dodatek żelaza, cynku i miedzi do diet kazeinowych i mieszanych spowodował lepsze ich wykorzystanie na cele wzrostowe. Absorpcja pozorna Fe, Zn i Cu (w %) była niższa przy wyższej zawartości tych pierwiastków w diecie, ale ilość zaabsorbowanych pierwiastków większa. Dla każdego z pierwiastków, niezależnie od ich ilości w diecie, stwierdzono najniższą absorpcję z diet glutenowych i najwyższą z diet kazeinowych. Zaabsorbowana ilość żelaza, cynku i miedzi w przeliczeniu na 1 g przyrostu masy ciała była największa na dietach glutenowych. Przy zastosowanych w niniejszej pracy dawkach żelaza, cynku i miedzi nie stwiedzono interakcji między tymi pierwiastkami na etapie absorpcji jelitowej.

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