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The utilization of protein and mineral components of diets containing white lupin or casein supplemented with lupin hulls, manganese and sodium phytate*

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ABSTRACT

A high manganese content in a casein diet, similar to that in a lupin-containing diet (about 0.3 g/kg), had no adverse effects on the utilization of dietary protein and retention of Mn, Mg, Fe and P in rat body. The addition of 10% lupin hulls caused a significant decrease in the digestibility (89.5 vs. 92.8%) and protein efficiency ratio (2.8 vs. 3.03), and lowered the retention of mineral components in comparison with the control diet. Manganese retention declined from 3.84 to 1.22% of intake, phosphorus from 30.9 to 24.9%, magnesium from 24 to 12.8%, and iron from 26.6 to 22.1%. The addition of sodium phytate to the casein diet containing lupin hulls decreased only the bioavailability of manganese (from 1.22 to 0.64%) and phosphorus (from 24.9 to 23.0%). The utilization of P, Mg and Fe was the lowest in animals fed the diet containing white lupin seeds.

KEY WORDS: lupin, manganese, fibre , sodium phytate, protein digestibility, PER, minerals, rats

INTRODUCTION

Other wise as other legumes, white lupin is capable of storing manganese in seeds. Hung et al. (1987) found that the concentration of manganese in seeds of white lupin was twenty times higher than in a narrow-leafed variety cultivated in

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the same soil conditions. The high manganese content together with the seeds phytate and fibre, may decrease the feed value of white lupin (Cheeke and Kelly, 1989; Lantzsch,1990). The excess of manganese in the diet decreases the absorption of iron from the digestive tract and the blood haemoglobin level (Hurley and Keen,1987). Manganese may compete with iron and magnesium, reducing the activity of many enzymes in metabolic pathways (Miller, 1991). Such disorders were found at high manganese concentrations, exceeding 1 g/kg of pig diets (Batterham, 1979), but information on the metabolic consequences of lower levels of this element (4-6 times in excess of the nutritional requirements of animals), and on the concomitant presence of other factors reducing utilization of dietary minerals, e.g. fibre and phytates, is scarce.

The purpose of this study was to investigate the effect of high dietary levels of manganese, fibre and phytates, characteristic of white lupin seeds, on the consumption and utilization of diets and on retention of Mn, Mg, Fe and P by rats.

MATERIAL AND METHODS

The experiments were carried out on 40 Wistar rats with initial body weight 55.2 ± 3.8 g. The experimental groups comprised 8 males housed individually in cages of synthetic glass, at 21-22^{0}C, relative humidity of 50-70% and equal periods of dark and light.

The composition of diet is given in Table 1. The control diet did not contain fibre, and the Mn content was in agreement with the nutritional requirements of rats. The source of Mn was manganous carbonate that is one of the components of a standard feed mixture (NRC, 1976). The experimental groups were fed diets containing one, two or three of the additional components, i.e. manganese raised to about 0.3 g/kg (CMn diet), manganese and 10% lupin hulls (CMnF diet), or additional manganese, seed hulls and sodium phytate (CMnFPh diet).

The amount of manganese, fibre and phytate was increased to the levels corresponding to their content in a diet containing about 30% white lupin seeds. In diet L, the only source of protein were the seeds of the low-alkaloid variety of white lupin, Bardo. The Mn content in this diet (0.38 g/kg) was slightly higher than in CMn diet (0.3 g/kg). Due to the high Mg content in the lupin seeds, the magnesium content in L diet was twice the nutritional requirement of rats.

Protein efficiency ratio (PER) was determined during the 4 weeks of the experiment. True digestibility of protein (TD) was determined during a 5-day faeces collection in the last week of the experiment, the metabolic nitrogen was taken according to Rakowska et al. (1978), i.e. 46.54 mg/rat/5 days. After the growth experiment, the Mn, Mg, Fe and P contents were determined in homogenates of rat carcasses. The retention of elements was determined

TABLE 1

Composition of diets containing casein or lupin seeds, g/kg

Components	Group				
	C	CMn	CMnF	CMnFPh	L
Casein	119.5	119.5	112.5	112.5	–
Lupin seeds	–	–	–	–	300
Lupin hulls	–	–	100	100	–
Potato starch	100	100	–	–	–
Mineral mixture[1]	30	30	30	30	30
Vitamin mixture[2]	10	10	10	10	10
Soya oil	80	80	80	80	50
L–Lysine	–	–	–	–	0.9
DL–Methionine	1.5	1.5	1.5	1.5	1.0
$MnCO_3$	–	0.56	0.51	0.51	–
Phytate Na	–	–	–	2.26	–
Maize starch	659.0	658.44	665.49	663.23	608.1

[1] mineral mixture (NRC, 1976) containing in 100 g: 73.5 g $CaHPO_4$; 8.10 g K_2HPO_4; 6.80 g K_2SO_4; 3.06 g NaCl; 2.10 g $CaCO_3$; 2.14 g Na_2HPO_4; 2.50 g MgO; 558 mg ferric citrate; 81 mg $ZnCO_3$; 421 mg $MnCO_3$; 33.3 mg $CuCO_3$; 0.7 mg KJ and 705 mg citrate acid

[2] vitamin mixture (AOAC, 1975) containing in 1 g: 2 000 IU vitamin A; 200 IU vitamin D_3; 10 IU vitamin E; 0.5 mg vitamin K; 200 mg choline; 10 mg p–aminobenzoic acid; 10 mg inositol; 4 mg niacin; 4 mg calcium pantothenate; 0.8 mg riboflavin; 0.5 mg thiamin; 0.5 mg pyridoxine; 0.2 mg folic acid; 0.04 mg biotin; 0.003 mg cobalamine; sucrose (supplement to 1 g)

according to the method of Davies and Nightingale (1975), i.e. from the difference between content in rat carcasses at the end of the experiment and in the "zero" group comprising 8 rats with an average body weight of 55.2 g. The amount of P, Mg, Fe and Mn deposited in the rat carcasses during 4 weeks of the experiment was expressed in absolute terms as daily retention, and in relation to intake.

The mineral content of the diets and rat carcasses was determined by the AAS method using a Pye Unicam Solar 939 spectrophotometer. Before assay, the samples were ashed at 450°C.

The results of the experiments were subjected to statistical analysis in which the significance of differences was determined by the Duncan test.

RESULTS AND DISCUSSION

Feed intake and nutritional value of diets

Feed intake, body weight gain, protein digestibility and PER values are presented in Table 2. Increasing the manganese content of the diets did not reduce feed intake. This is in agreement with the results of Zduńczyk et al. (1996c)

TABLE 2

Influence of manganese level, dietary fibre and Na phytate on growth performance and protein utilization

Components	Group				
	C	CMn	CMnF	CMnFPh	L
Diet intake, g/28 days	316.4^{b}	335.2^{ab}	360.5^{a}	354.2^{a}	331.6^{ab}
Body weight gain, g/28 days	102.9^{A}	113.9^{A}	112.6^{A}	110.4^{A}	76.2^{B}
TD	92.8^{A}	91.8^{AB}	89.5^{C}	90.5^{BC}	85.9^{D}
PER	3.03^{A}	3.05^{A}	2.80^{B}	2.78^{B}	2.02^{C}

a, b – $P<0.05$
A, B, C, D – $P<0.01$

who found that the intake of diets containing white lupin seeds decreased only when the Mn content in the diet was 1000 mg/kg. The study by Przybill and Pallauf (1991) showed that a Mn deficiency (0.5 mg/kg) of the diet had not negative effect on feed intake by rats. The addition of lupin hulls, the equivalent of about 7.6% NDF and 8.1% DF, increased consumption of diets CMnF and CMnFPh in comparison with the control diet. The results of other authors also indicate that with an increased fibre content and lower dietary energy concentration, feed consumption usually rises (Lopez-Guisa et al., 1988; Smulikowska and Chibowska, 1993; Zduńczyk et al., 1994). The decrease in intake of the lupin containing diet (5.1 and 11% of NDF and DF, respectively) was insignificant.

True digestibility of protein in the control casein diet was 92.8%. Increasing the Mn content from 0.06 to about 0.3 g/kg did not affect negatively TD. Similar protein digestibility coefficients for the casein diet were found by Eggum (1973). The addition of lupin hulls to the casein diet (group CMnF) caused a significant decline in protein digestibility to 89.5%. The negative effect of fibre on protein digestibility of diets was also found by other authors (e.g. Eggum et al., 1984; Zhao et al., 1995). The addition of 2.3 g/kg of sodium phytate to the casein diet (group CMnFPh) did not affect TD. True digestibility of white lupin seeds protein (85.9%) was similar to that reported by Eggum et al. (1993) and Zduńczyk et al. (1996a,b) and was significantly lower than the TD of protein in the casein groups. Also, the weight gains of the rats in group L (about 76.2 g) were the lowest, and significantly lower than in the casein groups ($P<0.01$).

The additional amount of manganese in the diet of group CMn did not lower the protein efficiency ratio. PER values in groups C and CMn were 3.03 and 3.05, respectively. Zduńczyk et al. (1996c) also did not find any changes in PER when the Mn content was increased from about 0.3 to 0.45 g/kg in diets containing white lupin seeds. The addition of lupin seed hulls to the casein diet caused a significant ($P<0.01$) decrease in PER in groups CMnF and CMnFPh (by 2.80 and 2.78, respectively). Zduńczyk et al. (1996a) observed a similar tendency when they included whole and dehulled white lupin seeds in rat diets. No effect of adding sodium phytate on PER of the CMnFPh diet was found. The PER value was the lowest in the group receiving lupin, and was significantly lower than in the other groups. Similar PER values of seeds of Polish white lupin varieties were found by Zduńczyk et al. (1996b).

Retention of trace elements

The intake and retention of Mn, Mg, Fe and P are presented in Tables 3 to 6. Increasing the Mn dietary content from 0.06 to over 0.3 g/kg caused a significant rise in the intake of this element and a fourfold increase in the retention of manganese in the rat body (from about 33 to over 135 μg/day). At the same time, the utilization of manganese declined from 4.93 to 3.84% (Table 3). Reduced utilization of Mn occurs when the concentration of this element in the diet is high (Weigland et al., 1986). These authors found that along with the increase in the Mn concentration in the rat diets to over 0.2 g/kg, true absorption declined from about 30 to about 5% of Mn intake. The addition of white lupin hulls decreased the retention of manganese to levels equal to those in control group. The utilization of manganese from CMnF diets decreased to 1.22%. The addition of sodium phytate to CMnF diet decreased Mn retention from 44 to about 24 μg/day (nonsignificant difference), the utilization of this element was only 0.64%. In the experiment by Lantzsch (1990) a very strong negative effect ($P < 0.001$) of the addition of sodium phytate on the absorption of manganese in young rats was found. Davies and Nightingale (1975) also reported that phytates reduced manganese utilization. The utilization of manganese from diet L, containing with white lupin seeds, did not differ significantly from the utilization in groups CMnF or CMnFPh. This shows that the utilization of manganese from organic source (lupin) was similar to the utilization of manganous carbonate used in the casein diets.

TABLE 3

Influence of manganese level, dietary fibre and Na phytate on Mn utilization

Components	Group				
	C	CMn	CMnF	CMnFPh	L
Content in diet, mg/kg	59.6	287.5	275.9	306.4	382.5
Daily intake, mg	0.67^{C}	3.51^{B}	3.55^{B}	3.87^{B}	4.53^{A}
Content in body, mg/rat	1.16^{B}	3.99^{A}	1.42^{B}	1.02^{B}	1.56^{B}
Retention, μg/day	33.27^{Bbc}	135.46^{Aa}	44.1^{Bbc}	24.63^{Bc}	47.09^{Bb}
Retention,% of intake	4.93^{A}	3.84^{B}	1.22^{C}	0.64^{C}	1.04^{C}

a, b, c – $P < 0.05$
A, B, C – $P < 0.01$

TABLE 4

Influence of manganese level, dietary fibre and Na phytate on Fe utilization

Components	Group				
	C	CMn	CMnF	CMnFPh	L
Content in diet, mg/kg	44.9	41.6	42.6	42.3	40.2
Daily intake, mg	0.51[ab]	0.51[ab]	0.55[a]	0.54[a]	0.48[b]
Content in body, mg/rat	7.40[A]	7.38[A]	7.05[A]	6.76[A]	5.36[B]
Retention, P μg/day	134.29[Aa]	131.07[Aab]	121.87[Aab]	111.61[Ab]	66.67[Bc]
Retention,% of intake	26.47[A]	26.55[A]	22.12[B]	20.81[B]	13.78[C]

a, b, c – $P<0.05$
A, B, C – $P<0.01$

Daily retention and utilization of Fe in the control diet (C) and in the diet with the higher Mn content (CMn), were similar (Table 4). The Fe utilization coefficients in these groups were 26.5 and 26.6%, respectively. Also in the experiment of Zduńczyk at al. (1996c) no differences were found in the retention of iron from diets with the white lupin seeds and with Mn content of about 0.45 g/kg. The addition of white lupin seeds hulls to the diet caused a significant ($P<0.01$) decrease in Fe utilization from the casein diet from 26.6 to 22.1%. In the studies of other authors (Torre et al., 1991; Wing et al., 1995) lower iron availability was also found in diets with high fibre content. The lack of significant differences between groups CMnF (added hulls) and CMnFPh (added hulls and sodium phytate) may point to the minor effect of phytins on the utilization of iron from diets by growing rats. This is corroborated by the experiments of Lantzsch (1990) in which only a small, negative effect of sodium phytate was found on Fe absorption in young rats. However, a highly significant reduction of retention and utilization of iron, to levels half of those in control groups, were found in group L, in which the rats were fed diet containing lupin seeds. This is in agreement with the results of Kim et al. (1995) who found that intestinal bioavailability of iron in rats fed diets containing protein from soyabean meal was distingtly lower than in those fed a casein-containing diet. In vegetable products most of the iron is in the form of phytates, part of which (monoferrous phytates) are easily available, and part (di- and tetraferrous phytates) is not easily utilized by monogastric animals (Brzozowska, 1991).

Magnesium intake in the particular groups was proportional to the content of this element in the diets (Table 5). The magnesium utilization coefficient was the highest (24.1%) in the control group. Increasing the Mn content in the casein diet from 0.06 to over 0.3 g/kg did not adversely affect the retention of magnesium by rats, while the addition of lupin seed hulls decreased magnesium retention and utilization ($P<0.01$), despite increased uptake of this element. Torre et al. (1991) and De Schrijver and Conrad (1992) also reported the negative effect of dietary

TABLE 5

Influence of manganese level, dietary fibre and Na phytate on Mg utilization

Components	Group				
	C	CMn	CMnF	CMnFPh	L
Content in diet, g/kg	0.21	0.22	0.26	0.26	0.51
Daily intake, mg	2.39^{C}	2.67^{C}	3.31^{B}	3.34^{B}	6.09^{A}
Content in body, mg/rat	44.0^{ABab}	45.92^{Aa}	40.71^{BCc}	41.78^{Bbc}	37.66^{Cd}
Retention, μg/day	576.3^{Aab}	607.4^{Aa}	418.1^{ABc}	460.8^{ABbc}	350.2^{Bc}
Retention, % of intake	24.11^{A}	23.96^{A}	12.79^{B}	14.62^{B}	5.79^{C}

a, b, c, d – $P<0.05$
A, B, C – $P<0.01$

fibre on magnesium utilization. The addition of sodium phytate to the diet of group CMnFPh had not negative effect either on Mg retention or utilization. Different results were reported by Brink et al. (1991), who found that the addition of sodium phytate to a casein diet in amounts equal to those in the soyabean diet decreased Mg absorption to levels observed in the group of rats fed the soyabean diet. The lowest values of both daily and percentage retention of this element were found in group L, despite the highest daily Mg intake in this group, twice that in other groups. Because the fibre and phytate levels in the group L diet were similar to those in the CMnFPh diet, it can be postulated that other components of white lupin seeds affected dietary mineral utilization. From the studies of Brink et al. (1991) it results that the replacement of casein with vegetable protein (soya) decreases intestinal magnesium uptake and increases urinary excretion of this element. In vegetable products, a considerable proportion of magnesium is found in the phytate complexes that are less efficiently utilized (Torre et al., 1991).

The additional amount of manganese in the diet of group CMn did not lower the retention and utilization of phosphorus in comparison with the control diet

TABLE 6

Infuence of manganese level, dietary fibre and Na phytate on P utilization

Components	Group				
	C	CMn	CMnF	CMnFPh	L
Content in diet, g/kg	6.5	6.4	6.3	6.8	6.7
Daily intake, mg	73.4^{Bb}	78.01^{ABab}	81.69^{ABa}	86.03^{Aa}	78.98^{ABab}
Content in body, g/rat	1.00^{Aab}	1.08^{Aa}	0.98^{Abc}	0.98^{ABc}	0.89^{Bc}
Retention, μg/day	20.56^{ABb}	23.4^{Aa}	19.77^{ABb}	19.8^{ABb}	16.84^{Bc}
Retention,% of intake	28.92^{Ab}	30.91^{Aa}	24.9^{Bc}	23.01^{BCd}	21.33^{Ce}

a, b, c, d, e – $P<0.05$
A, B, C – $P<0.01$

(Table 6). As the result of adding lupin hulls to the CMn diet, a significant ($P < 0.05$) decrease in phosphorus retention from 23.4 mg/day in group CMn to 19.8 mg/day in group CMnF occurred, and the relative retention coefficient declined ($P < 0.01$) from 30.9 to 24.9%, respectively. De Schrijver and Conrad (1992) obtained different results, since they did not find significant changes in phosphorus retention due to increasing dietary fibre content in the diets of rats within the range of 6-57g/kg. The lowest P retention was found on the L diet, in which the source of protein was white lupin seeds. In this diet, part of the phosphorus was from lupin seeds where it is bound to phytic acid, which decreases the bioavailability of minerals (Torre et al., 1991).

CONCLUSIONS

The experiments did not show a negative effect of an increased level of manganese (over five times the amount in a standard casein diet) on the utilization of casein diets and retention of Mg, Fe and P in the body of rats. The addition of 10% lupin hulls to the casein diet caused a significant decrease in feed utilization and reduced the retention of the trace elements under study. The addition of sodium phytate (2.25 g/kg) did not affect the true digestibility and protein efficiency ratio, and did not lower the retention of Mg and Fe, but did decrease the utilization of Mn and P by rats. The bioavailability coefficients of Mg, Fe and P of diets containing white lupin seeds were significantly lower than for the casein diet containing manganese, fibre and phytate in amounts close to the level of these components in diets containing white lupin seeds. This indicates that other constituents of lupin seeds limit the bioavailability of minerals.

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STRESZCZENIE

Wykorzystanie białka i składników mineralnych diet zawierających nasiona łubinu białego lub kazeinę z dodatkiem okrywy nasiennej łubinu, manganu i fitynianu sodu

W doświadczeniach na szczurach badano, czy duża, charakterystyczna dla nasion łubinu białego, zawartość manganu, włókna i fitynianów wpływa na wykorzystanie białka i składników mineralnych. Wysoki poziom manganu w diecie kazeinowej, zbliżony do zawartości Mn w diecie zawierającej lubin (ok. 0,3 g/kg), nie miał ujemnego wpływu na wykorzystanie białka diety oraz retencję Mn, Mg, Fe i P w tuszy szczurów. Dodatek okrywy nasiennej łubinu w ilości 10% diety spowodował istotne pogorszenie strawności (89,5 vs 92,8%) i wydajności wzrostowej białka (2,8 vs 3,03) oraz zmniejszenie retencji składników mineralnych w porównaniu z dietą kontrolną. Retencja manganu zmalała z 3,84 do 1,22% ilości spożytej, fosforu z 30,9 do 24,9%, magnezu z 24 do 12,8% oraz żelaza z 26,6 do 22,1%. Dodatek fitynianu sodu do diety kazeinowej zawierającej okrywę nasienną zmniejszył jedynie biodostępność manganu (z 1,22 do 0,64%) i fosforu (z 24,9 do 23,0%). Wykorzystanie P, Mg i Fe było najmniejsze u zwierząt żywionych dietą zawierającą nasiona łubinu białego.