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EFFECT OF PECTIN AND CELLULOSE ON THE CONTENT OF MINERALS IN THE FEMUR OF RATS

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Male Buffalo rats were fed a fiber-free diet (FF) or diets containing 5% of pectin (PEC5) or cellulose (CEL5), or 2.5% of pectin and 2.5% of cellulose combined (PEC2.5+CEL2.5) for over 6 weeks. Determinations were conducted for: diet intake, body weight, and femur weight in rats, as well as for femur contents of Ca, Mg, Fe, Zn and P and the activity of alkaline phosphatase (ALP). An addition of pectin and/or cellulose to the diet led to a lower ALP activity and femur Zn content in rats. The ingestion of PEC5 and PEC2.5+CEL2.5 diets caused a lower femur P content as compared to the FF diet and also a decrease in diet intake as compared to FF and CEL5 diets. A significant decrease in Mg accumulation in the femur has also been observed in rats fed PEC5 in comparison to those fed with CEL5 and FF diets, *i.e.* 29% and 25%, respectively.

INTRODUCTION

Pectin and cellulose are plant-derived fiber that is resistant to digestion by endogenous enzymes in the small intestine, but may be degraded by microbial fermentation in the large intestine. Physiochemical properties of fiber – solubility, viscosity, fermentability – have profound effects on fiber functionality [Spiller, 2001].

The lower intake of dietary soluble and insoluble fibers is thought to be associated with coronary heart disease, gastrointestinal tract diseases and some cancers [Cade *et al.,* 2007; Schneeman, 1998; Pereira *et al.,* 2004; Theuwissen & Mensink, 2008]. Therefore, recently attention has been increasingly focussed on fiber isolates, including pectin and cellulose, for their physiological and health promoting properties and thus for their potential to represent candidates for supplements and functional foods.

Only a few studies have tried to determine the effects of pectin and/or cellulose on mineral absorbability [Gralak *et al.,* 1996; El-Zoghbi & Sitohy, 2001; Kim *et al.,* 1996] and bone mineral content [Isai & Lei, 1979]. The comparison of the influences of cellulose and pectin on bone mineral composition has received scant attention.

Bioavailability of bone-related minerals (Ca, P, Mg, F, Zn, Fe, Cu) has a critical impact on bone matrix formation and mineral concentration [Ilich *et al*., 2000; Hermann *et al*., 1997]. Pectin may decrease mineral absorption by forming gels and binding mainly bivalent ions to its free carboxyl groups [El-Zoghbi & Sitohy, 2001]. Pectin may also affect mineral absorption by stimulating bacterial production of short chain fatty acids (SCFA) and luminal acidification [Stark & Madar, 1993]. Cellulose, through accelerating digestive transit, leads to diminished nutrient absorption [Spiller, 2001].

The present paper describes the effects of dietary cellulose and/or pectin on the content of minerals in the femur of rats. The expected effects of two types of dietary fiber used in this experiment are based on different mechanisms of action. The presented results are part of wider studies concerning the influence of fiber ingestion on macro- and micronutrient balance in rats.

MATERIAL AND METHODS

Animals, diets and experimental conditions

The experiment lasted 6 weeks and was performed on forty male Buffalo rats weighing 125±10 g, divided into four groups of 10 animals each, and fed the experimental diets: fiber-free (FF), with addition of 5% of cellulose (CEL5) or 5% of pectin (PEC5) or 2.5% of cellulose + 2.5% of pectin (CEL2.5+PEC2.5), (Table 1). The diets were prepared according to Reeves [1997] with some modification: cholesterol and lard were added to the diets due to the fact that in the same experiment the influence of pectin and cellulose on lipid metabolism was investigated. We used apple pectin WEJ-3F produced by Pektowin, Sp. z o.o. and α-cellulose from Sigma-Aldrich (C6429). The contents of minerals in all experimental diets were similar and the diets contained (per g diet), respectively: 3.17±0.2 mg Ca, 1.67±0.08 mg P, 0.59±0.03 mg Mg, 43.2±2.3 μg Zn, and 50.2±3.6 μg Fe. The animals were allowed free access to diet and distilled water during the experiment. The rats were kept in cages under a 12-h light cycle, a temperature of 22±1°C and humidity of 55-60%. The study was approved by the First Local Ethics Commission in Wroclaw.

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TABLE 1. Composition of the experimental diets.

Components (g/kg diet)	Experimental diets			
	FF	CEL5	PEC5	CEL2.5+PEC2.5
Wheat starch	615.7	565.7	565.7	565.7
Casein	145	145	145	145
Sucrose	88	88	88	88
Soybean oil	10	10	10	10
Lard	90	90	90	90
Mineral mixture	35	35	35	35
Vitamin mixture	10	10	10	10
L-cystine	1.8	1.8	1.8	1.8
Choline chloride	2.5	2.5	2.5	2.5
Cholesterol	2.0	2.0	2.0	2.0
Cellulose	-	50	-	25
Pectin	-	-	50	25

FF – the fiber free diet, CEL5, PEC5, CEL2.5+PEC2.5 – the diets with 5% cellulose, 5% pectin and 2.5% cellulose + 2.5% pectin.

Chemical analyses

After 6 weeks on the experimental diets, the rats were feed deprived for 12 h and then anesthetized with bioketan. Femurs were separated, cleaned, weighed and then frozen at -20°C. Samples of femurs destined for mineral determination were mineralized with 4 mL of HNO_3 (65% Suprapur; Merck) in a microwave digestion system Plazmatronika according to the producer's recommendations. Digested solutions were diluted with deionised water to 10 mL after removing nitrogen dioxide fumes and cooling. The blank digests were carried out in the same way. The Perkin Elmer Model 3110 flame atomic absorption spectrometer was used for Ca, Mg, Fe, and Zn determination. Phosphorus was determined spectrophotometrically using Scheel's method [Rutkowska, 1981]. Similarly, the contents of Ca, Mg, Fe, Zn and P were measured in the experimental diets. The accuracy and precision were assessed by the determination of Ca, Mg, Fe and Zn in the certified reference material (NCS ZC730016 Chicken), which was digested analogously to the femur samples. The recovery of the certified Ca, Mg, Fe, Zn levels were: 89%, 91%, 99% and 101%, respectively. For the determination of alkaline phosphatase (ALP) activity in 0.1mol/L Tris bone extracts, a kinetic photometric test (ELTECH DIAGNOSTICS) was used after a protein determination with the Bradford's method [Bradford, 1976].

Statistical analysis

Results were subjected to statistical analyses using ANOVA, and significant differences between groups were estimated with the Tukey's test (Statistica Ver. 6.0). Differences were considered significant at $p \leq 0.05$.

RESULTS

Diet intake, body weight gain, femur weight and alkaline phosphatase activity

Diet intake, body weight gain, femur weight and bone ALP activity were shown in Table 2. The diet intake differed significantly between rats fed diets with pectin and cellulose, while body weight gain and femur weight did not differ between all experimental groups. The mean diet intake was significantly lower among rats fed the PEC5 and CEL2.5+PEC2.5 diets as compared to those fed the FF and CEL5 diets.

ALP activity was significantly lower in rats fed the CEL5, PEC5 and CEL2.5+PEC2.5 diets as compared to the FF group. There were no significant differences in ALP activity between rats fed diets with pectin and/or cellulose.

Content of calcium, zinc, iron, magnesium and phosphorus in the femur of experimental rats

The mean contents of minerals in the femur analysed in this study were reported in Table 3. The rats fed the CEL5 diet were observed to have a lower Zn femur content in comparison with the FF group. The CEL5 diet did not significantly influence the content of the other investigated minerals in the femur of rats. However, in rats fed the PEC5 and CEL2.5+PEC2.5 diets, we observed a lower content of P and Zn in the femur as compared to the animals administered the FF diet. The content of Mg in the femur of the PEC5 groups was also lower in respect of the FF and CEL5 groups. The PEC5 and CEL2.5+PEC2.5 diets did not substantially affect Ca nor Fe content in the femur of rats.

DISCUSSION

The observed reduction of a diet intake after PEC5 and CEL2.5+PEC2.5 feeding in relation to the FF and CEL5 diets, indicates that the ingestion of pectin is responsible for changes of this parameter. Gralak *et al.* [1996] also showed a lower diet intake in the rats fed citrus pectin in comparison with other fiber sources. There were no significant differences in body weight gain between groups of rats, despite different

TABLE 2. Effect of experimental diet on diet intake, body weight gain, femur weight and alkaline phosphatase activity (ALP) in the femur of rats (mean ± SD).

Specification	Experimental diets			
	FF	CEL5	PEC5	CEL2.5+PEC2.5
Diet intake (g/day)	15.4 ± 2.3^a	16.1 ± 1.9^a	12.8 ± 1.1^b	12.6 ± 1.1^b
Body weight gain (g)	100.0 ± 22.0^a	103.0 ± 12.5^a	94.0 ± 19.0^a	122.0 ± 28.6^a
Femur weight (g)	0.76 ± 0.08^a	0.87 ± 0.08^a	0.82 ± 0.18^a	0.73 ± 0.12^a
ALP activity (U/min/g protein)	43.6 ± 9.5^b	30.5 ± 9.4^a	28.0 ± 8.0^a	22.0 ± 2.3^a

FF – the fiber free diet, CEL5, PEC5, CEL2.5+PEC2.5 – the diets with 5% cellulose, 5% pectin and 2.5% cellulose + 2.5% pectin, respectively; values with different superscript letter are statistically different at $p \leq 0.05$.

TABLE 3. Effect of experimental diet on calcium, zinc, iron, magnesium and phosphorus content in the femur of rats (mean ± SD).

Mineral per g of femur	Experimental diets			
	FF	CEL5	PEC5	CEL2.5+PEC2.5
Ca (mg/g)	158.4±20.5^{a}	142.6±27.0^{a}	141.3±14.0^{a}	160.7±20.2^{a}
P (mg/g)	82.3±8.2^{a}	76.6±9.2^{ab}	68.0±6.5^{b}	65.9±8.6^{b}
Mg (mg/g)	4.94±0.87^{a}	5.19±0.99^{ab}	3.68±1.02^{c}	4.35±0.93^{abc}
Zn (μg/g)	135.2±8.14^{a}	124.5±6.18^{b}	123.2±6.7^{b}	121.2±7.45^{b}
Fe (μg/g)	59.59±8.14^{a}	62.90±6.8^{a}	66.00±7.62^{a}	69.30±9.24^{a}

FF – the fiber free diet, CEL5, PEC5, CEL2.5+PEC2.5 – the diets with 5% cellulose, 5% pectin and 2.5% cellulose + 2.5% pectin, respectively; values with different superscript letter are statistically different at $p \leq 0.05$.

diet intake. In contrast to our findings, Farenss *et al.* [1982] demonstrated a higher diet intake but no differences in weight gain in rats fed a 20% cellulose-containing diet as compared to the animals from the FF group. In the same study, a diet with 5% pectin did not affect the weight gain of animals at the identical diet intake, in comparison with the group fed a fiber-free diet.

In this study, the lower intake of diet with pectin might have an effect on the lower P, Mg and Zn contents in femur of rats. We have also observed that after six weeks the apparent absorption of Mg and Zn was significantly lower in rats fed the diet with pectin than in those administered the FF or CEL5 diets, and that pectin had an insignificant effect on Ca and Fe balances [Krzysik, Department of Food Science and Dietetics, Wroclaw Medical University, unpublished data]. As shown by other studies, the effect of dietary fiber on mineral absorption may change in time of experiment. Gralak *et al*. [1996] observed changes in the apparent absorption of minerals after six weeks of feeding rats diets with different fibers and that the time effect depended on the type of fiber: citrus pectin in a diet caused an increase in the apparent absorption of Ca, Mg and Zn and a decrease in that of Fe after six weeks as compared to three weeks of feeding. The improvement of absorption could be attributed to adaptation mechanisms which include modification in the intestinal and colon morphology, SCFA production and pH reduction [Paczkowska & Kunachowicz, 2006]. However, such a mechanism does not seem to reveal in time period of our experiment.

Bone isoenzyme of ALP is responsible for normal skeletal mineralization and is one of the important indicators of bone formation. It had previously been reported that an increase in ALP activity in the serum was connected with a diminished mineral density [Rahnama *et al*., 2002]. An increased activity of the ALP reflects an acceleration of osteogenesis, which is connected with increased bone resorption. Reduced ALP in serum, which is mainly bone originated, suggests a decrease in osteoblastic function. The lower activity of bone ALP connected with lower Mg content in femur observed in the PEC5 and CEL2.5+PEC2.5 groups (*vs*. CEL5 and FF) is in agreement with the results of a study by Rude *et al.* [2005] on the effect of an Mg-restricted diet on a decrease in serum ALP activity.

The effect of fiber fraction on mineral absorption is difficult to characterize because of the inconsistency of published reports. In general, fiber has been found to reduce the apparent absorption of minerals (such as Ca, Mg, Zn and Mn) [Greger, 1999]. In turn, Van der Aar *et al.* [1983] reported that fiber did not affect tissue Ca and Fe status; whereas El-Zoghbi & Sitohy [2001] observed that pectin administration reduced the level of bivalent minerals (Zn, Cu, Ca, Fe, Mg) in the serum of albino rats. Other studies have shown pectin to have no effect on mineral absorption [Gralak *et al*., 1996; Harrington *et al.,* 2001]. On the contrary, Coudray *et al.* [1997] reported that soluble fermentable fiber significantly increased the absorption of Ca and its balance in humans. According to a number of studies, the addition of cellulose to a diet caused a negative balance of Ca, Mg, P and Zn [Ismail-Beigi *et al.,* 1977; Jiang, 1986; Kelsay *et al.,* 1979].

The observed different effects of cellulose and pectin on the mineral absorption depend largely on the nature and amount of fiber, and also on the homeostasis of minerals concerned [Coudray *et al.* 2003]. Pectin appears to have the highest affinity for binding mineral cations. Bivalent ions are capable of cross-linking pectin chains through the reaction with free carboxyl groups and thus avoid absorption as being embedded between the stacked chains of pectin [El-Zoghbi & Sitohy, 2001].

The observed effects of fiber on Zn, P and Mg contents in femurs may result in the mineral deficiency of bone and consequently impair bone health. A deficiency of Mg invokes a decrease of bone strength and volume, and also contributes to poor bone development, uncoupling of bone formation and resorption [Rude *et al.,* 2005]. Zn deficiency results in impaired DNA synthesis and protein metabolism, which leads to negative effects on bone formation [Ilich *et al*., 2000]. A decrease in bone P may be an indicator of an imbalance between the Ca/P ratio. Sharpio *et al*. [2003] reported that equivalent levels of Ca and P supplementation promoted significantly greater body weight gain, femur weight, tensile strength, bone ash, bone mineralization, bone density, Ca and P deposition, and Ca utilization in comparison with a diet without phosphorus salts.

CONCLUSIONS

1. The results obtained indicated that cellulose in the experimental diet affected only femur Zn content; however, pectin and pectin in a mixture with cellulose in diets influenced bone Zn, Mg and P contents, without any effect on Ca and Fe accumulation.

2. Our study showed also that the addition of 5% of fiber to the diet significantly affected the ALP activity, and thus influenced skeletal mineralization.

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