*Physiol. Res. 50: 197-204, 2001*

# Different Influence of Hypodynamy on Calcium and Phosphorus Levels in Bones of Male and Female Japanese Quails

J. ANTALÍKOVÁ, M. BARANOVSKÁ, I. MRAVCOVÁ, V. SABO, P. ŠKROBÁNEK

*Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences, Ivanka pri Dunaji, Slovak Republic*

*Received October 25, 1999*
*Accepted September 4, 2000*

## Summary

The influence of long-term hypodynamy on the calcium and phosphorus levels was studied in the bones of Japanese quails. The hypodynamy evoked different changes in the calcium and phosphorus content in males and females. The calcium content in the marrow of femurs was only changed in the hens, while in cockerels it was significantly decreased in the upper part and marrow of the tibia. Furthermore, changes in the phosphorus content were observed only in the tibia of cockerels.

**Key words**
Japanese quail • Bone • Calcium content • Hypodynamy

## Introduction

The formation and destruction of bone material in the skeleton is carried out by two specialized types of cells – osteoclasts and osteoblasts. The osteoclasts are responsible for bone resorption and release of calcium and phosphorus from the skeleton – the reservoir of these minerals for body mobilization. The ratio of calcium to phosphorus is practically constant and somewhat greater than 2:1 (McDowel 1992). The composition and the constitution of poultry bones (Taylor *et al.* 1960) as well as their ontogenetic determinants (Field *et al.* 1974) had been studied previously. Birds are able to maintain calcium and/or phosphorus levels in the plasma by mobilizing the calcium reserves in the skeleton. Alternatively, by depositing more bone in specific sites of the skeleton, birds can adapt their bone conformation to physical challenges (Gross and Bain 1993).

Living bones are continually undergoing processes of reinforcement and resorption, partially but not totally driven by changes in their mechanical load environment (Cowin 1998). While the Japanese quail is assumed to be part of a closed ecological system in space flying, in our study we were interested in bone mineral metabolism of the quail under conditions similar to those in space. As the hypodynamy can serve a model for weightlessness, the distribution of calcium and phosphorus in Japanese quail bones was studied under long-term hypodynamy.



Fax+4202 24920590
E-mail physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

**Table 1.** Mass of the long bone parts (g).

| BONE/Part | Group | 28th day | | 56th day | | 84th day | |
|---|---|---|---|---|---|---|---|
| | | **Males** | **Females** | **Males** | **Females** | **Males** | **Females** |
| *FEMUR* | *Experimental* | 0.042 | 0.039 | 0.042 | 0.060 | 0.045 | 0.052 |
| *Upper end* | | ±0.005 | ±0.012 | ±0.010 | ±0.010 | ±0.010 | ±0.001 |
| | *Control* | 0.033 | 0.048 | 0.039 | 0.066 | 0.045 | 0.068 |
| | | ±0.009 | 0.017 | ±0.004 | ±0.007 | ±0.004 | ±0.011 |
| | *Experimental* | 0.048 | 0.053 | 0.048 | 0.074 | 0.057 | 0.064 |
| *Lower end* | | ±0.005 | ±0.010 | ±0.003 | ±0.007 | ±0.008 | ±0.003 |
| | *Control* | 0.050 | 0.063 | 0.052 | 0.074 | 0.057 | 0.072 |
| | | ±0.004 | ±0.020 | ±0.007 | ±0.006 | ±0.006 | ±0.005 |
| | *Experimental* | 0.112 | 0.105 | 0.120 | 0.120 | 0.123 | 0.114 |
| *Cortical* | | ±0.009 | ±0.019 | ±0.008 | ±0.013 | ±0.008 | ±0.020 |
| | *Control* | 0.116 | 0.117 | 0.113 | 0.107 | 0.125 | 0.117 |
| | | ±0.012 | ±0.033 | ±0.008 | ±0.004 | ±0.009 | ±0.016 |
| | *Experimental* | 0.024 | 0.063 | 0.027 | 0.058 | 0.036 | 0.033 |
| *Marrow* | | ±0.008 | ±0.021 | ±0.011 | ±0.016 | ±0.009 | × |
| | *Control* | 0.024 | 0.048 | 0.015 | 0.063 | 0.024 | 0.058 |
| | | ±0.008 | ±0.006 | ±0.006 | ±0.009 | ±0.016 | × |
| *TIBIA* | *Experimental* | 0.055 | 0.079 | 0.058 | 0.102 | 0.072 | 0.076 |
| *Upper end* | | ±0.009 | ±0.013 | ±0.009 | ±0.017 | ±0.005 | ±0.004 |
| | *Control* | 0.062 | 0.089 | 0.061 | 0.096 | 0.065 | 0.074 |
| | | ±0.004 | ±0.019 | ±0.004 | ±0.014 | ±0.010 | × |
| | *Experimental* | 0.047 | 0.051 | 0.036 | 0.063 | 0.044 | 0.05 |
| *Lower end* | | ±0.019 | ±0.008 | ±0.013 | ±0.006 | ±0.008 | ±0.005 |
| | *Control* | 0.043 | 0.053 | 0.046 | 0.054 | 0.047 | 0.046 |
| | | ±0.002 | ±0.009 | ±0.004 | ±0.007 | ±0.006 | ±0.013 |
| | *Experimental* | 0.141 | 0.162 | 0.168 | 0.136 | 0.159 | 0.156 |
| *Cortical* | | ±0.027 | ±0.008 | ±0.022 | ±0.012 | ±0.013 | ±0.022 |
| | *Control* | 0.155 | 0.164 | 0.154 | 0.135 | 0.161 | 0.17 |
| | | ±0.013 | ±0.027 | ±0.036 | ±0.011 | ±0.010 | × |
| | *Experimental* | 0.027 | 0.081 | 0.021 | 0.077 | 0.024 | 0.07 |
| *Marrow* | | ±0.006 | ±0.017 | ±0.006 | ±0.011 | ±0.011 | ±0.036 |
| | *Control* | 0.017 | 0.062 | 0.014 | 0.059 | 0.016 | 0.072 |
| | | ±0.007 | ±0.015 | ±0.005 | ±0.017 | ±0.005 | ±0.025 |

Data are means ± SEM.

Females are often supposed to have a lighter skeleton than males, even in avian domestic species. However, in broiler chickens, females are less susceptible to bone deformities than males (Rose *et al*. 1996). We therefore also compared the sex differences in bone mineral metabolism during hypodynamy.

## Material and Methods

*Experimental design*

Hens and cockerels of Japanese quails (*Coturnix coturnix japonica*) aged 66 days were divided into two groups. The birds in the experimental groups were sustained in jackets to immobilize their movement for 28, 56 and 84 days according Juráni *et al.* (1983). Water and food (calcium 26.91 g/kg, phosphorus 7.69 g/kg) were given *ad libitum* to both the control and experimental groups. On day 28, 56 or 84 of the experiment, six birds from each group were sacrificed and the skull, rib, femur and tibia were removed as in previous experiments (Jankela *et al.* 1998). The bones were weighed, autoclaved for 2 hours, defatted with ether and the muscular tissues were excised. The femur and tibia were divided into four parts: the upper and lower ends, the cortical segment and the bone marrow. The bones were ashed at 550 $^{o}$C for 7-8 hours, dissolved in HCl (water solution 1:1) and made up to the same volume. The calcium content was measured chelatometrically by a modified method of Davídek (1977) and inorganic phosphorus using the BioLa test (Lachema Diagnostika).

*Statistical analysis*

Differences between the experimental groups were evaluated using Student's paired t-test ($P<0.05$ or $P<0.001$).

## Results

The body weight was not changed in either the hens or cockerels. There were also no differences in the weight of bones between control and hypodynamy groups of birds (Table 1). However, some changes were observed in the content of calcium in the ribs and skull of the males and females.

Long bones, e.g. the femur or tibia, were divided into four parts (upper and lower ends, the cortical segment and bone marrow). It seems that only the tibia of males is more sensitive to hypodynamy. We observed a significant fall ($P<0.05$) in the content of calcium in the proximal portion on day 28, 56 and 84 of hypodynamy and an increase ($P<0.05$) in the phosphorus content by the 28th day (Fig 1). In the marrow, we observed a decreased content of both phosphorus and calcium ($P<0.001$) on the 56th and 84th day of hypodynamy (Fig. 2). The content of these minerals in the tibia of females was unchanged during hypodynamy. In contrast, significant changes of the calcium content in the femur marrow were observed in female birds on the 28th and 56th day ($P<0.001$ and $P<0.05$, respectively) (Fig 3). The calcium content in the marrow of cockerels was only about 20 % of the calcium content in the marrow of long bones in the hens (Figs 2 and 3). The content of calcium and phosphorus in various parts of long bones are shown in Tables 2 and 3. The laying of eggs and the content of calcium and phosphorus in the egg shells were without any changes.

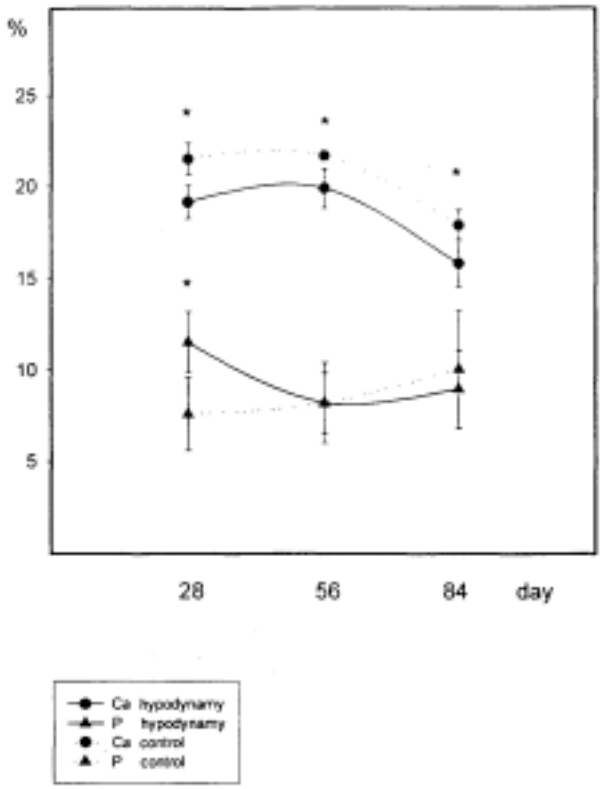


**Fig. 1.** The content of calcium and phosphorus in the upper end of the tibia of cockerels on the 28th, 56th and 84th day of hypodynamy. *$P<0.05$ vs. control group.

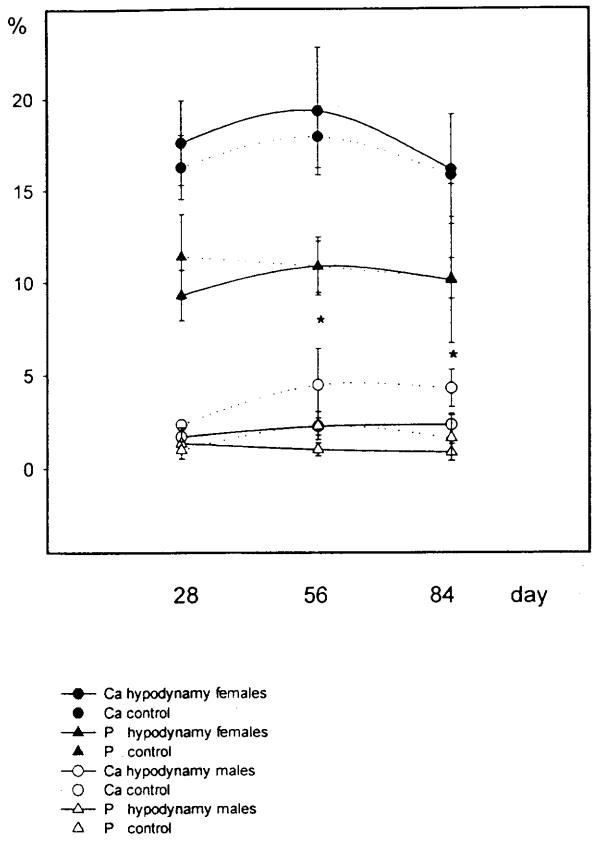


**Fig. 2.** The contents of calcium and phosphorus in the marrow of tibia of hens and cockerels on the 28th, 56th and 84th day of hypodynamy. *P<0.001 vs. control group.

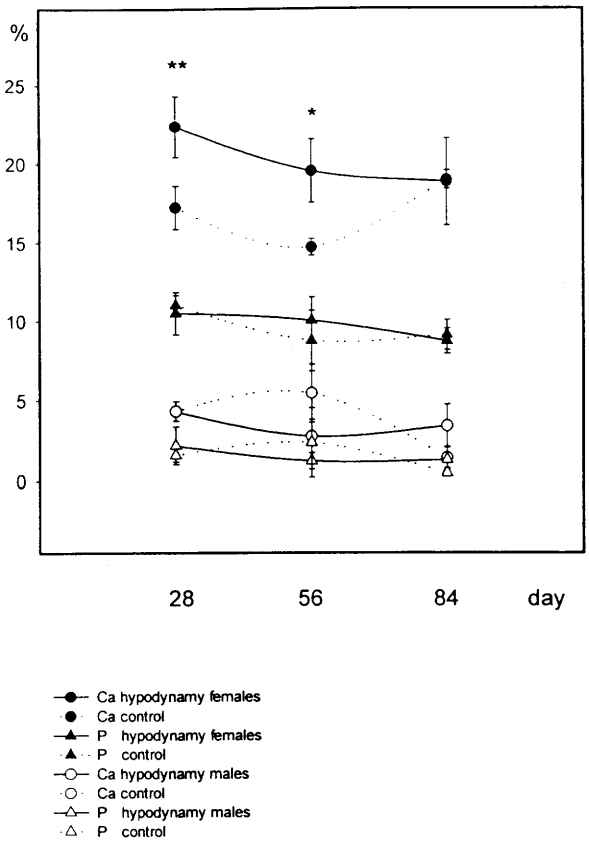


**Fig. 3.** The contents of calcium and phosphorus in the marrow of femur of the hens and cockerels on the 28th, 56th and 84th day of hypodynamy. *P<0.05, **P<0.001 vs. control group.

## Discussion

In general terms, the response of bones to external physical stimulation (exercise or mechanical loading of some kind) is well documented and has been termed adaptive bone remodeling (Rubin and Lanyon 1987). When functional loading is suppressed in mammalian subjects, e.g. during space flight or prolonged bed rest, loss of bone invariably follows (Simmons *et al.* 1986, Leblanc *et al.* 1990). In avian bone biology, excessive activity or functional strain have been shown to be either detrimental or of little consequence in terms of skeletal mass in immature bones (Matsuda *et al.* 1986, Biewener and Bertram 1991). In agreement with this conclusion is our observation that prolonged hypodynamy did not have any negative effect on the bone mass of immobilized birds.

When the birds are subjected to treatments, such as loading or feeding a diet low in calcium, their bones undergo intensive remodeling. Changes in the rate of bone resorption or deposition can be brought about by either mechanical or hormonal stimulation. Especially in laying hens, the need for calcium balance is obvious. The actual processes of bone remodeling is still poorly understood (Price and Russel 1992).

There is also little information concerning calcium turnover in bird bones under conditions of weightlessness. Hypodynamy appears to be a promising model of weightlessness.

The results obtained in our experiment show that there are no changes in the content of calcium or phosphorus in the ribs and skull of male and female immobilized birds. In the long bones – the femur and tibia – we observed significant differences evoked by hypodynamy between males and females especially in the

**Table 2.** The content of calcium and phosphorus (%) in parts of femur

| Bone part | Group | 28th day | | | | 56th day | | | | 84th day | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | % Ca | | % P | | % Ca | | % P | | % Ca | | % P | |
| | | Males | Females | Males | Females | Males | Females | Males | Females | Males | Females | Males | Females |
| *Upper end* | *Experimental* | 20.29 ±1.55 | 31.75 * 0.85 | 11.20 ±1.41 | 12.83 0.76 | 22.80 ±0.77 | 24.63 ±1.67 | 12.08 ±1.45 | 10.68 ±1.06 | 20.33 ±1.43 | 23.22 ±0.22 | 11.05 ±1.36 | 11.09 ±0.82 |
| | *Control* | 21.78 ±2.34 | 25.89 ±1.56 | 12.49 ±1.01 | 11.44 1.55 | 23.34 ±1.58 | 24.14 ±1.10 | 11.85 ±1.29 | 10.46 ±0.69 | 19.82 ±1.51 | 23.55 ±1.51 | 10.69 ±0.50 | 10.05 ±0.66 |
| *Lower end* | *Experimental* | 19.13 ±1.06 | 29.27 ±2.47 | 10.58 ±0.58 | 11.80 1.24 | 20.34 ±3.14 | 24.62 ±1.17 | 11.26 ±1.23 | 10.46 ±0.46 | 20.33 ±1.43 | 22.18 ±0.39 | 9.24 ±1.08 | 9.97 ±0.22 |
| | *Control* | 21.62 ±2.22 | 27.69 ±2.58 | 10.72 ±1.00 | 10.85 1.45 | 21.55 ±1.54 | 23.23 ±2.41 | 10.42 ±1.13 | 9.85 ±0.81 | 19.82 ±1.51 | 23.44 ±1.68 | 9.28 ±0.86 | 10.24 ±0.65 |
| *Cortical* | *Experimental* | 26.25 ±0.63 | 33.02 ±1.06 | 8.86 ±0.51 | 10.35 0.57 | 24.64 ±1.40 | 27.75 ±1.36 | 8.81 ±0.86 | 9.51 ±0.55 | 25.17 ±1.57 | 27.30 ±0.75 | 8.56 ±0.43 | 8.75 ±0.50 |
| | *Control* | 24.85 ±1.64 | 29.68 ±2.63 | 8.86 ±0.77 | 9.85 1.13 | 24.66 ±1.14 | 27.00 ±0.54 | 8.28 ±0.96 | 10.12 ±0.75 | 25.28 ±0.77 | 27.23 ±1.01 | 8.54 ±0.49 | 9.31 ±1.34 |
| *Marrow* | *Experimental* | 4.29 ±0.10 | 22.37 ** ±1.94 | 2.18 ±1.17 | 10.50 1.37 | 2.75 ±1.04 | 19.53 ±2.02 | 1.21 ±0.51 | 10.04 ±1.49 | 3.39 ±1.35 | 18.85 ±2.75 | 1.27 ±0.83 | 8.73 ±0.80 |
| | *Control* | 4.32 ±0.61 | 17.23 ±1.36 | 1.58 ±0.42 | 11.02 0.63 | 5.45 ±1.83 | 14.71 * ±0.53 | 2.36 ±2.16 | 8.76 ±1.91 | 1.41 ±0.64 | 18.97 ±0.59 | 0.46 ±0.23 | 9.12 ±0.96 |

*$P<0.05$, **$P<0.001$ *vs. control group.*

**Table 3.** The content of calcium and phosphorus (%) in parts of tibia.

| Bone part | Group | 28th day | | | | 56th day | | | | 84th day | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | % Ca | | % P | | % Ca | | % P | | % Ca | | % P | |
| | | Males | Females | Males | Females | Males | Females | Males | Females | Males | Females | Males | Females |
| *Upper end* | *Experimental* | 19.14 * | 21.48 ** | 11.65 * | 9.63 | 19.89 * | 24.20 | 8.21 | 5.54 | 15.80 * | 25.85 | 8.93 | 12.44 |
| | | ±0.91 | ±2.74 | ±1.54 | ±1.18 | ±1.06 | ±0.97 | ±2.21 | ±1.79 | ±1.27 | ±3.23 | ±2.11 | ±3.20 |
| | *Control* | 21.48 | 22.28 | 7.58 | 9.58 | 21.66 | 24.05 | 8.18 | 6.71 | 17.99 | 26.74 | 10.04 | 13.41 |
| | | ±0.88 | ±1.88 | ±2.00 | ±1.23 | ±0.28 | ±0.64 | ±1.64 | ±1.85 | ±0.75 | ±1.43 | ±3.23 | × |
| *Lower end* | *Experimental* | 22.73 | 23.40 | 10.51 | 11.76 | 23.37 | 25.17 | 12.03 | 7.21 | 19.75 | 24.19 | 13.65 | 11.38 |
| | | ±3.70 | ±1.47 | ±2.73 | ±0.84 | ±2.46 | ±1.79 | ±1.41 | ±0.67 | ±1.49 | ±0.55 | ±2.50 | ±2.49 |
| | *Control* | 24.01 | 23.42 | 9.75 | 11.70 | 24.23 | 25.75 | 9.72 | 9.07 | 21.69 | 24.03 | 15.54 | 19.33 |
| | | ±1.20 | ±1.00 | ±2.89 | ±0.53 | ±0.64 | ±1.42 | ±2.22 | ±1.42 | ±1.71 | ±1.34 | ±2.91 | ±1.02 |
| *Cortical* | *Experimental* | 26.24 | 26.25 | 6.97 | 8.57 | 23.50 | 26.09 | 5.83 | 6.45 | 23.68 | 26.83 | 15.80 | 8.04 * |
| | | ±0.41 | ±0.63 | ±1.65 | ±0.34 | ±1.01 | ±2.46 | ±1.09 | ±1.19 | ±1.29 | ±0.94 | ±1.06 | ±1.78 |
| | *Control* | 24.16 | 26.91 | 5.94 | 9.19 | 24.06 | 26.55 | 7.13 | 6.84 | 24.53 | 26.57 | 15.60 | 15.59 |
| | | ±1.79 | ±0.65 | ±0.65 | ±0.55 | ±1.04 | ±2.04 | ±1.73 | ±0.82 | ±0.88 | ±2.48 | ±1.39 | ±0.35 |
| *Marrow* | *Experimental* | 1.72 | 17.63 | 1.36 | 9.31 | 2.25 * | 19.33 | 1.02 | 10.89 | 2.34 ** | 16.14 | 0.87 | 10.13 |
| | | ±0.82 | ±2.31 | ±0.85 | ±1.38 | ±0.46 | ±3.47 | ±0.35 | ±1.59 | ±0.59 | ±2.97 | ±0.46 | ±3.43 |
| | *Control* | 2.35 | 16.30 | 1.01 | 11.41 | 4.49 | 17.92 | 2.29 | 10.86 | 4.30 | 15.84 | 1.64 | 10.21 |
| | | ±0.15 | ±1.75 | ±0.48 | ±2.33 | ±1.94 | ±1.69 | ±0.75 | ±1.41 | ±1.01 | ±0.51 | ±1.20 | ±1.10 |

**P<0.05, **P<0.001 vs. control group.*

content of calcium. The calcium content in the bones of hens was only changed in the marrow of femurs, while it was also significantly decreased in the upper end and marrow of the tibia in cockerels. Furthermore, changes in the phosphorus content were only observed in the tibia of cockerels. Hurwitz (1965) studied calcium turnover in different segments of the femur and tibia of hens. The maximal turnover rate of calcium was observed in the tibia and femur medullary segments. In our experiment, hypodynamy affected the calcium content only in the bone marrow of the femur (part of medullary segment). Although the medullary bones play no significant role in the mechanical properties of the skeleton, it is extremely important component in the calcium metabolism of the birds (Clunies *et al*. 1992). It seems that under conditions of hypodynamy the marrow of long bones of hens deposited certain calcium reserves (Jankela *et al.* 1998). This assumption is also supported by the observation that the content of calcium in the marrow of long bones of hens is at the level of calcium content of cortical and end segments, while in cockerels it is composed of only approximately 20 % of the calcium content of other bone in both femur and tibia.

It seems that hens are less sensitive to hypodynamy than cockerels. Rose *et al*. (1996) also suggested that female broiler chickens are less susceptible to bone deformities than males. The reason why bones of females are more adapted to physiological stress is not clear. The *in situ* identification of estrogen target cells in bones suggests that the combination of estrogen actions with those of microstrains induced by mechanical loading will produce a cellular signal for the osteogenic pathways (Braidman *et al.* 1995). Cheng *et al.* (1995) demonstrated in rats that the osteogenic response to a load combined with estradiol stimulation is higher in females than in males.

## Acknowledgements

The experiment was supported by the project VEGA 2/4014.

## References

BIEWENER AA, BERTRAM JEA: Bone growth modeling in relation to functional strain. *Abstr 37th Annu Mtg Orthoped Res Soc, Anaheim,* 1991, p 9.

BRAIDMAN IP, DAVENPORT LK, CARTER DH, SELBY PL, MAWER EB, FREEMONT AJ: Preliminary in situ identification of estrogen target cells in bone. *J Bone Min Res* **10**: 74-80, 1995.

CHENG MZ, ZAMAN G, RAWLINSON SCF, SUSWILLO RFL, LANYON LE: Estrogen amplifies bone's osteogenic responses to load bearing in female rat ulna in vitro. *Calcif Tissue Int* **56**: 428, 1995.

CLUNIES M, EMSLIE J, LEESON S: Effect of dietary calcium level on medullary bone calcium reserves and shell weight of Leghorn hens. *Poult Sci* **71**: 1348-1356, 1992.

COWIN SC: On mechanosensation in bone under microgravity. *Bone* **22** (Suppl): 119S-125S, 1998.

DAVÍDEK J: *Reference Book for Laboratory Analysis of Foodstuff* (in Czech) Publishing House SNTL, Prague, 1977, pp 141-143.

FIELD RA, RILEY ML, MELLO FC, CORBRIDGE MH, KOTULA AW: Bone composition in cattle, pigs, sheep and poultry. *J Anim Sci* **39**: 493-499, 1974.

GROSS TS, BAIN SD: Skeletal adaptation to functional stimuli. In: *Current Issues in Biomechanics,* MD GRABINER (ed), Human Kinetics Books, Champaign, IL, 1993, pp 265-277.

HURWITZ S: Calcium turnover in different bone segments of laying fowl. *Am J Physiol* **208**: 203-207, 1965.

JANKELA J, BARANOVSKÁ M, MRAVCOVÁ I, ANTALÍKOVÁ J: The influence of hypodynamy on the calcium and phosphorus contents in Japanese quail's bones. *Folia Vet* **42**: S29-S52, 1998.

JURÁNI M, VÝBOH P, LAMOŠOVÁ D, BAROŠKOVÁ Ž, SOMOGYIOVÁ E, BOĎA K, GAŽO M: The effect of 90 day hypodynamy on the neurohumoral system, egg laying and metabolism of proteins in Japanese quail. *Physiologist* **26**: 145-148, 1983.

LEBLANC AD, SCHNEIDER VS, EVANS HJ, ENGELBRETSON DA, KREBS JM : Bone mineral loss and recovery after 17 weeks of bed rest. *J Bone Miner Res* **5:** 843-850, 1990.

MATSUDA JJ, ZERNICKE RF, VAILAS AC, PEDRINI VA, PEDRINI-MILLE A, MAYNARD JA: Structural and mechanical adaptation of immature bone to strenuous exercise. *J Orthop Res* **60**: 2028-2034, 1986.

McDOWELL LR: In: *Minerals and Animal and Human Nutrition,* TJ CUNHA (ed.), Academic Press, New York, 1992, pp 26-77.

PRICE JS, RUSSEL RGG: Bone remodelling: regulation by systemic and local factors. In: *Poultry Science Symposium 23: Bone Biology and Skeletal Disorders in Poultry*, Carfax Publishing Company, 1992.

ROSE N, CONSTANTIN P, LETERRIER C: Sex differences in bone growth of broiler chicken. *Growth Dev Aging* **60**: 49-59, 1996.

RUBIN CT, LANYON LE: Osteoregulatory nature of mechanical stimuli: function as a determinant for adaptive remodeling in bone. *J Orthop Res* **5**: 300-310, 1987.

SIMMONS DJ, RUSSELL JE, GRYNPAS MD: Bone maturation and quality of bone material in rats flown on the space shuttle ´Spacelab-3 Mission´. *Bone Miner* **1**: 485-493, 1986.

TAYLOR TG, MOORE JH, HERTELENDY F: Variations in the mineral composition of individual bones of the skeleton of the domestic fowl. *Br J Nutr* **14**: 49, 1960.

**Reprint requests**
J. Antalíková, Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences, 900 28 Ivanka pri Dunaji, Slovak Republic. Fax: + 421 7 45943 932, E-mail: antalikova@ubgz.savba.sk