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Effect of Dietary Supplementation with Grass Pea (Lathyrus sativus L.) and Evening Primrose Seeds (Oenothera biennis L.) on Biomechanical Parameters of the Limb Bones in Guinea Pigs

Wpływ dodatku nasion lędźwianu siewnego (*Lathyrus sativus* L.) i nasion wiesiołka dwuletniego (*Oenothera biennis* L.) w diecie na cechy biomechaniczne kości kończyn świnek morskich

High content of protein, in grass pea seeds reaching 32%, of valuable amino acid composition, gives ground for their use for animal nutrition. However, the content of lathyrogens, BAPN and β-ODAP which evokes disorders in bone growth processes and mineralization and cause osteolathyrism, reducing their further nutritional use (1,2,5).

Boiling, autoclaving or extrusion of grass pea seeds retards or highly limits, the activity of antinutritional factors and enables their more extensive use as a source of valuable protein in animal nutrition (2,10).

High content of unsaturated fatty acids such as linoleic, linolenic and dihomo-gammalinolenic acid in the oil of evening primrose seeds (*Oenothera biennis* L.) may constitute an important source of nutritive supply in polyunsaturated fatty acids, however, the presence of antinutritive substances as well as tannins and phytochelates in evening primrose seeds makes it necessary to inactivate them or eliminate then from the diet in order to improve their nutritive value (3,7,9).

Taking into consideration the lack of studies in on biomechanical characteristics of limb bones many species of domestic as well as laboratory animals on the influence of diet supplementation with grass pea and evening primrose seeds, work on the effect of supplementing 20% raw and extruded grass pea seeds and adding 10 to 20% to the diet of guinea pigs has been undertaken.

MATERIAL AND METHODS

The studies were carried out on 50 guinea pigs of both sexes, which were divided into 4 experimental groups and a control. Each group constitited of 10 guinea pigs of mean initial body weight 366g. Group I (control) was fed with standard LSK fodder. Group II was fed the same standard LSK fodder but supplemented with 20% raw grass pea seeds and group III was supplemented with 20% of extruded grass pea seeds at 14% moisture and at a temperature of 220°C. Group IV was fed on the same LSK fodder mixture suplemented with 10% evening primrose seeds and group V - was fed a mixture supplemented with 20% evening primrose seeds. The animals were fed ad libitum with constant access to water with the addition of vitamin C. The experiment lasted 6 weeks and afterwards the guinea pigs were etherized and drained of blood. From each guinea pig femur, tibia and humerus were isolated and then were subjected to a dynamic test, with the strain force operating perpendicularly to the axis of the long bone, in a tripoint defloction test, according to Ferretti (4). The determinations of mechanical parameters of the bones were conducted with the application of the INSTRON 4302 apparatus, which enabled the measurement of maximum or ultimate strength (load at fracture), maximum elastic strength (load at the yielding point), maximum elastic defleaction as well as stiffness. The geometrical characteristics determined were the cross-sectional area, the second moment of inertia of the cross section in relation to the horizontal axis and mean relative wall thickness (MRWT, wall-lumen ratio). The weight and lengths of bones were also measured. The received results were statistically analysed by calculating mean values and their standard errors. Statistical significance of the differences among the mean values were tested with t Duncan test.

RESULTS AND DISCUSION

The highest value of femur ultimate strength was characteristic of the control group of guinea pigs (Tab.1.). In the experimental groups these values were lower and in group V - lowest. Statistically relevant differences were found between the control group and group II, IV and V and also between group V and groups II, III and IV. Group I was characterized by the highest maximum elastic strength of femur (Tab.1.). Maximum elastic defloaction of femur was the highest in the control group of guinea pigs, and statistically significant differences were observed between the value of controls (group I) and group IV and group III and IV (Tab.1.).The value of stiffness was the highest in the control group while all the experimental groups presented lower values with lowest value in the guinea pigs fed on a diet with 20 % primrose seeds (Table 1.).

The largest femur cross-section area was noted in the control group, and was smallest in group V of guinea pigs and between this group and statistically significant differences were found betweeen the control and group II. (Tab. 2.). The cross-sectional moment of inertia of femur was the highest in group II and differences were observed between this group and group V as well as the controls (group I), (Tab.2.) The mean relative wall thickness (MRWT) of femur was also greatest in the control group and the lowest in group V. Statistically significant

Tab. 1. Mechanical parameters of limb bones ($x \pm SE$) of control guinea pigs (gr. I) fed on LSK standard diet and supplemented LSK diet (II - 20% of raw grass pea seeds; III - 20% of extruded grass pea seeds; IV - 10% of raw evening primrose seeds; V - 20% of raw evening primrose seeds)

Characteristic	Bone	Feeding groups				
		I	II	III	IV	V
Bone ultimate strength (N)	Femur	$205^{a} \pm 6.6$	$173^{b} \pm 4.0$	$189^{ab} \pm 5.6$	$170^{b} \pm 7.2$	$142^{c} \pm 6.0$
	Tibia	$143^{a} \pm 4.3$	$119^{b} \pm 6.0$	$129^{ab} \pm 6.0$	$113^{b} \pm 4.0$	$118^{b} \pm 3.0$
	Humerus	$109^{a} \pm 3.2$	$91^{b} \pm 4.8$	$85^{b} \pm 3.5$	$85^{b} \pm 4.3$	$86^{b} \pm 2.0$
Bone maximum elastic (N)	Femur	116 ± 7.4	113 ± 10.0	113 ± 4.1	113 ± 10.5	97 ± 6.7
	Tibia	101 ± 7.6	91 ± 6.7	94 ± 4.4	86 ± 5.2	83 ± 3.0
	Humerus	$79^{a} \pm 10.8$	$72^{ab} \pm 4.3$	$73^{ab} \pm 3.3$	$66^{b} \pm 2.4$	$74^{ab} \pm 4.3$
Bone stiffness (N/mm)	Femur	413 ± 23.5	395 ± 14.8	409 ± 22.3	389 ± 18.4	336 ± 28.8
	Tibia	$343^{a} \pm 26.0$	$327^{a} \pm 27.0$	$317^{a} \pm 24.4$	$283^{a} \pm 22.5$	$252^{b} \pm 12.7$
	Humerus	258 ± 18.0	249 ± 14.0	240 ± 8.2	235 ± 22.4	
Bone maximum elastic deflection (mm)	Femur	$0.80^{a} \pm 0.06$	$0.70^{b} \pm 0.06$	$0.78^{ab} \pm 0.07$	$0.58^{bc} \pm 0.04$	$0.65^{abc} \pm 0.04$
	Tibia	0.69 ± 0.04	0.63 ± 0.03	0.79 ± 0.07	0.68 ± 0.03	$0,68 \pm 0.04$
	Humerus	0.74 ± 0.04	0.66 ± 0.05	0.62 ± 0.05	0.65 ± 0.05	0.65 ± 0.03

a, b, c - means with different letters differ significantly ($p < 0.05$).

differences occurred between the mean value of group V and the mean values of groups II, III and IV (Tab. 2). The femur weight of the control guinea pigs was also the highest and differed significantly from the bone weight of groups II, III, IV and V. Statistically significant differences were also noted between groups III and II, IV and the control (Tab.2.).

Tibias of the control group (Group I) were characterized by the highest values of ultimate strength and differed significantly from the values found in groups II, IV and V. Significantly relevant differences were also observed between the groups II, III and IV (Tab.1.). Also in the control group the highest maximum elastic strength of tibia was noted (Tab.1.). The highest maximum defloaction occurred in group III, yet the lowest in group II, although no statistically significant differences were observed (Tab.1.). The cross-sectional area and its moment of inertia were the highest in the control group, however, lower values were noted in experimental groups. Statistically significant differences of the cross-section area were found between the control and groups II and III, and group V (Tab.2.).

The control groups and group IV were characterized by the greatest length of tibia and statistically significant differences were observed between these groups and group II, III and V (Tab.2.). The highest weight was that of tibia in guinea pigs from group V, the lowest, from groups II and III, with significant differences between these two groups and group IV and V as well as the controls (group I), (Tab.2.).

Tab. 2. Physical and geometrical characteristics of limb bones in guinea pigs (x ± SE). Same presentation as in Tab.1.

Characteristic	Bone	Feeding groups				
		I	II	III	IV	V
Length (cm)	Femur	$4.24^{a} \pm 0.03$	$4.30^{a} \pm 0.04$	$4.11^{b} \pm 0.03$	$4.30^{a} \pm 0.03$	$4.08^{b} \pm 0.03$
	Tibia	$4.50^{a} \pm 0.02$	$4.30^{b} \pm 0.05$	$4.30^{b} \pm 0.02$	$4.50^{ac} \pm 0.03$	$4.20^{b} \pm 0.07$
	Humerus	$3.60^{a} \pm 0.02$	$3.30^{b} \pm 0.03$	$3.40^{cd} \pm 0.02$	$3.40^{c} \pm 0.02$	$3.50^{ad} \pm 0.04$
Weight (g)	Femur	$1.70^{a} \pm 0.04$	$1.53^{b} \pm 0.03$	$1.48^{bc} \pm 0.02$	$1.16^{cd} \pm 0.15$	$1.40^{d} \pm 0.03$
	Tibia	$1.10^{a} \pm 0.03$	$0.93^{b} \pm 0.02$	$0.83^{c} \pm 0.02$	$1.14^{a} \pm 0.06$	$1.20^{a} \pm 0.04$
	Humerus	$1.04^{a} \pm 0.02$	$0.90^{b} \pm 0.02$	$0.77^{c} \pm 0.02$	$0.90^{b} \pm 0.03$	$0.90^{b} \pm 0.02$
Cross-sectional area (mm ^{2})	Femur	$9.80^{a} \pm 0.40$	$9.70^{a} \pm 0.40$	$9.10^{ab} \pm 0.50$	$8.90^{ab} \pm 0.30$	$7.70^{b} \pm 0.50$
	Tibia	$6.10^{a} \pm 0.40$	$5.00^{ab} \pm 0.20$	$4.90^{b} \pm 0.20$	$5.30^{ab} \pm 0.20$	$5.20^{ab} \pm 0.30$
	Humerus	$6.60^{a} \pm 0.25$	$5.50^{b} \pm 0.20$	$5.90^{ab} \pm 0.30$	$4.90^{b} \pm 0.40$	$5.60^{b} \pm 0.10$
Second moment of inertia (mm ^{4})	Femur	$8.59^{a} \pm 0.233.$	$10.1^{b} \pm 0.60$	$8.90^{ab} \pm 0.60$	$8.90^{ab} \pm 0.44$	$8.00^{ab} \pm 0.60$
	Tibia	$80^{a} \pm 0.30$	$3.30^{ab} \pm 0.20$	$3.20^{ab} \pm 0.20$	$3.20^{ab} \pm 0.20$	$2.80^{b} \pm 0.20$
	Humerus	$4.50^{a} \pm 0.20$	$3.71^{b} \pm 0.30$	$3.20^{b} \pm 0.14$	$3.30^{b} \pm 0.30$	$3.70^{b} \pm 0.20$
Mean relative wall thickness	Femur	$0.81^{a} \pm 0.06$	$0.73^{a} \pm 0.03$	$0.80^{a} \pm 0.05$	$0.72^{a} \pm 0.02$	$0.60^{b} \pm 0.03$
	Tibia	0.98 ± 0.05	0.84 ± 0.04	0.90 ± 0.07	0.95 ± 0.04	0.92 ± 0.07
	Humerus	$0.94^{a} \pm 0.06$	$0.70^{b} \pm 0.03$	$0.80^{ab} \pm 0.05$	$0.66^{b} \pm 0.06$	$0.85^{a} \pm 0.03$

a, b, c - means with different differ significantly at $p < 0.05$.

The humerus of the control guinea pigs was characterized by the highest values of ultimate strength and by maximum elastic strength (Tab. 1.). The differences were statistically significant and the ultimate strength was found between the controls (group I) and all experimental groups (II, III, IV, V). The maximum elastic strength differed significanth - between group IV and the controls (group I). (Tab. 1.). Similarly, the maximum elastic defloaction of humerus was the greatest in the controls (group I), the smallest in group III (Tab. 1.).

The cross-sectionional area of humerus and its moment of inertia in the control group were the highest and differences were noted between the control group and groups II, IV and V, however, differences in the values of the moment of inertia, were additionally noted between the control and group III (Tab. 2.). Similarly, the mean relative wall thickness of humerus was greatest in the control group, and differences were observed between these two groups and group V and the controls (group I), (Tab. 2.). The length and the weight of humerus were the highest at the controls (Tab. 2.). Group II was characterized by the lowest length of humerus and differed significantly from the values observed in groups III, IV and V as well as the controls (group I). The significant differences were also noted between the controls and groups III and IV (Tab. 2.) The humerus of guinea pigs of group III was characterized by the smallest weight and differed significantly from the values found in groups II, IV, V as well as the controls and also between the controls (group I) and groups II, IV and V (Tab.2.).

Various species of Lathyrus are cultivated throughout the world and in Poland the most frequently planted is grass pea. In the seeds of grass pea, there are anti-nutritive factors, such as protease inhibitors, neurotoxins, osteotoxins and phytates with unknown effects on the growth and development of young animals (1, 2, 5, 6, 8). Two toxic syndromes associated with the ingestion of Lathyrus seeds have been found out (10). The first of these, termed as neurolathyrism, is a desease of man which involves paralysis of the legs. The second, termed osteolathyrism,involvess skeletal changes and causes loss of elasticity of blood vessel walls (8, 10). The component of grass pea seeds proved to be an osteolathyrogen is eta β-aminopropionitrile (BAPN). It is now established that osteolathyrogens block the formation of cross -links in the collagen molecule (8,10) . It was shown as well that osteolathyrogens can also prevent the cross-link formation in elastin molecules. The major changes in osteolathyrism are induction of general weakness in mesenchymal tissues and disorders in the growth of bones (2,10).

Results of these studies confirm the effect of feeding a diet supplemented with grass pea seeds or evening primrose seeds on biomechanical characteristics of limb bones of guinea pigs, as was observed in previous studies on pigs and guinea pigs (2, 11). Feeding and extruded grass pea seeds results in lowering the value of ultimate strength, maximum elastic strength, maximum defloaction, weight, the cross-sectional area as well as relative wall thickness of the studied bones. Feeding a 10% and 20% supplement of evening primrose seeds lowers the ultimate strength, the cross-sectional area and its moment of and inertia the length and mean relative wall thickness of all the studied bones and also lowers the maximum elastic strength of tibia and humerus. It also lowers the weight of femur and humerus. The changes observed in the biomechanical characteristics of guinea pig limb bones show the effect of feeding grass pea seeds as well as feeding the mixtures with evening primrose seeds on the skeletal system of guinea pigs and its biomechanical parameters.

The results of our studies prove that supplementing the diet with raw and extruded grass pea seeds affects the mechanical properties of guinea pig bones -after 6 weeks of feeding by lowering the values of the analysed parameters. Simillar effects were observed in our experiments with dietary supplementation the of evening primrose seeds. Despite the absence of beta-aminopropionitrile (BAPN) in the seeds of evening primrose it has been reported that other antinutritional factors such as fitochelates protease inhibitors, tannins and phytates are present (3). The present findings emphasise the need to determine the concentrations not only of osteolathyrogens but the other anti-nutritive factors as well, which may be responsible for the observed inhibitory influences on the bone properties in guinea pigs.

CONCLUSIONS

1. The use of a diet supplemented with 20% raw or extruded grass pea seeds results in lowering geometric and mechanical properties of guinea pig limb bones such as maximum elastic strength,ultimate strength, weight, length, cross-sectional area as wall as mean relative wall thickness and cross-sectional inertia of the diaphyses of femora , tibiae and humeri.

2. Supplementing the diet with 10% or 20% evening primrose seeds affects geometrical and mechanical properties of limb bones in guinea pigs by lowering the values of maximum elastic strength, ultimate strength, weight, length, cross-sectional area, mean relative wall thickness and cross-sectional inertia of diaphysis of femora, tibiae and humeri.

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STRESZCZENIE

Badania przeprowadzono na 50 świnkach morskich, podzielonych na 4 grupy doświadczalne żywione paszą typu LSK z 20% dodatkiem surowych lub ekstrudowanych nasion lędźwianu siewnego, a także nasion wiesiołka dwuletniego, oraz 1 grupę kontrolną (LSK standard). Zwierzęta żywiono *ad libitum* z dodatkiem do wody wit.C. Badania trwały 6 tygodni i po tym czasie świnki poddawano narkozie, izolując kości udowe, piszczelowe i ramienne, a następnie poddawano badaniom dynamicznym z działającą prostopadle siłą do długiej osi kości z zastosowaniem aparatu INSTRON 4302. Badano następujące parametry geometryczne i fizyczne kości: maksymalną siłę elastyczną, sztywność, maksymalne odkształcenie elastyczne oraz pole przekroju poprzecznego, wtórny moment bezwładności i względną grubość ścian kości. Dieta z udziałem nasion lędźwianu siewnego surowego lub ekstrudowanego powodowała zmniejszenie maksymalnej siły elastycznej i wytrzymałości, maksymalnego odkształcenia, sztywności, masy, długości, pola przekroju poprzecznego i względnej grubości ścian kości oraz momentu bezwładności kości udowej, piszczelowej i ramiennej. Dieta z dodatkiem nasion wiesiołka powodowała również zmniejszenie wartości badanych parametrów fizycznych i geometrycznych kości świnek morskich. Uzyskane wyniki badań dowodzą hamującego wpływu antyżywieniowych substancji nasion lędźwianu siewnego i wiesiołka dwuletniego na układ kostny i wskazują na konieczność prowadzenia dalszych badań nad mechanizmami stwierdzonych efektów.