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THE EFFECT OF NA-BUTYRATE AND *YUCCA SCHIDIGERA* EXTRACT ON BONE QUALITY IN DEVELOPING PIGS

DJELOVANJE NA-BUTIRATA I EKSTRAKTA *YUCCA SCHIDIGERA-E* NA KAKVOĆU KOSTI SVINJA U RAZVOJU

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SUMMARY

The aim of this study was to investigate the effects of Na-butyrate and *Yucca schidigera* extract treatment on skeletal development of pigs. The study was conducted from the 14th until the 56th day of postnatal life. Piglets were randomly assigned to control group fed on a standard diet supplemented with a coated blend of organic acids (fumaric, malic and citric - 1.5 kg/t feed), and an experimental group fed on a similar diet with the addition of coated Na-butyrate (900g/t feed) and extract of *Yucca schidigera* (75g/t feed). At age of the 28th, 35th and 56th days of life the piglets were slaughtered and the humera were isolated for further analyses. The bone formation and quality were measured based on weight, length, bone mineral content (BMC), bone mineral density (BMD), physical (ultimate strength, maximum elastic strength) and geometrical parameters. The bone weight and geometrical parameters such as cross-sectional area, second moment of inertia, mean relative wall thickness were significantly increased in experimental 56 d old piglets. Furthermore, experimental diet induced significantly higher BMC and BMD, and improved the mechanical endurance of bones in terms of the moments of maximum elastic strength and maximum strength. It is concluded that early postnatal treatment of piglets with Na-butyrate and extract of *Yucca schidigera* positively affects the development and function of the bones during growth.

Key words: butyrate, bone, piglets, SCFA

INTRODUCTION

The short-chain fatty acids (SCFA) have become an alternative of choice to replace antibiotics in piglet. Butyrate, one of the short-chain fatty acids, is a trophic factor for the intestinal epithelium (Bach Knudsen et al., 2003). It is the main end-product of anaerobic bacterial fermentation of carbohydrates (including dietary fiber) in the rumen of forestomach of animal species and in the colon of omnivorous species involving humans. Butyrate has diverse and

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apparently paradoxical effects on cellular proliferation, apoptosis and differentiation that may be either pro-neoplastic or antineoplastic, depending upon factors such as the level of exposure, availability of other metabolic substrates and the intracellular milieu (Miller, 2004; Sengupta et al., 2006).

Galfi and Bokori (1990) were the first to show positive influence of Na-butyrate on the body weight gain, feed conversion rate and composition of intestinal microflora in fattening pigs. They also indicated some trophic effects on the intestinal epithelium, like increase in the villi length and crypt depth.

It has been observed that piglets fed on the diet with other organic acids show higher body weight gain and feed utilization (Partanen and Mroz, 1999; Manzanilla et al., 2006; Radcliffe, 1998). However, Øverland et al. (2007) showed that dietary addition of organic acids had no significant effect on daily weight gain or feed intake, but tended to improve the feed conversion ratio of pigs. Organic acids also influenced phosphorus and calcium utilization, and bone mineralization. Recently, coated butyrate salts became available which led to normal intake by the animals, whereas uncoated butyrate is usually refused. However, the role of butyric acid on bone growth and development has not been investigated.

Beneficial effects of *Yucca schidigera* extract in swine production include increased growth rate and improved feed conversion efficiency, reduction in ammonia in animal facilities, inhibition of Gram-positive bacteria and reductions in numbers of pigs born dead. In poultry production a reduction in egg and tissue cholesterol contents was found. Moreover *Yucca schidigera* extract has anti-arthritic activity in horses and dogs (Cheeke, 2000).

The aim of this study was to investigate the effects of coated Na-butyrate and *Yucca schidigera* extract treatment on skeletal development of pigs.

MATERIAL AND METHODS

The experimental procedures used throughout this study were approved by The 2nd Local Committee of Animal Experimentation of the University of Life Sciences in Lublin, Poland.

The study was performed on Polish Large White x Petrain breed pigs. During the whole period of the study sows and piglets were housed indoors in pens under identical environmental conditions. During pregnancy and lactation sows were fed *ad libitum* a commercial diet, according to the stage of the production cycle. The study was conducted on pigs from the 14th until 56th day of their postnatal life. On the 14th day of age pigs were randomly divided into two weight-matched groups. 15 pigs were assigned to the control group (C) and 15 pigs to the experimental groups (E). Piglets from the control group were fed on the standard diet (14-28 - day protein 19,2%, energy 15,4 MJ, ash 4%, fat 10,3%, Ca 1%, P 0,6%; 29-42 day - protein 16,5%, energy 13 MJ, ash 7%, fat 3%, Ca 0,65%, P 0,5%; 43-56 day - protein 16%, energy 13 MJ, ash 6%, fat 30%, Ca 0,65%, P 0,5%) supplemented with a coated blend of organic acids (fumaric, malic and citric – 1,5 kg/t feed), while experimental animals obtained a similar diet with the addition of coated Na-butyrate (900g/t feed) and extract of *Yucca schidigera* (75g/t feed). Piglets were weaned on the 28th day of life.

Five piglets from each group were slaughtered on 28th, 35th and 56th days of life. After slaughter the humera were isolated, and their weight and length were measured. The bone samples were frozen at -25 ºC and maintained for further analysis.

Bone mineral density (BMD) and bone mineral content (BMC) were measured (dual energy X-ray absorptiometry method). Mechanical properties of bones, such as maximum elastic strength (Wy) and ultimate strength (Wf), were measured using three-point bending test and an INSTRON 4302 apparatus (Instron Corp., USA) linked to a computer, registering the relationship between the perpendicular force acting to the longitudinal axis of the bone and the resulting displacement (Ferretti et al. 1993). The distance between supports was set at 40% of the humerus length. On the basis of vertical and horizontal diameters of the bone mid-shaft cross section, the geometrical parameters such as the cross-sectional area (Ix), second moment of inertia (A) and mean relative wall thickness (MRWT) were estimated.

Data are presented as mean ± SD. Differences between means were tested with the use of a one-way Anova with the aid of Statistica 5.0 software. Differences showing a $P<0.05$ were statistically significant.

RESULTS

The bone formation and development was determined based on the weight, length, BMC, BMD, physical and geometrical parameters. The results are shown in Table 1. In 28 d old pigs no significant differences between the groups were observed in the analyzed parameters of bones. However, at the age of 35 d the ultimate strength, maximum elastic strength, cross-sectional area and mean relative wall thickness showed a tendency towards increased values in experimental pigs.

On the 56th day of age, humerus parameters were higher in the experimental pigs than in controls. After 42 days of experimental feeding pigs had higher bone mass, BMC and BMD by 11.1 %, 14.5% and 8.2%, respectively. The values of maximum strength were significantly higher by 13.7% and maximum elastic strength by 17% as compared to controls. Na-butyrate and *Yucca schidigera* treatment significantly increased the second moment of inertia, cross-sectional area and mean relative wall thickness, as well. Furthermore, no significant differences were noted for humerus length between control and experimental animals.

Table 1. Effect of experimental diet on the features of humera of pigs at the age of 28th, 35th and 56th days (mean ± SD).

Tablica 1. Djelovanje pokusnih obroka na na značajke humera svinja u dobi od 28, 35 i 56 dana (prosjek ± SD)

Parameters - Parametri	28 d		35 d		56 d	
	C	E	C	E	C	E
Body weight - Tjelesna težina (kg)	7.2±0.2	6.5±0.1	8.7±0.3	8.0±0.2	15.9±0.3	16.1±0.6
Bone weight - Težina kosti (g)	34 ±4.42	31 ±1.05	40 ±4.2	37 ±3,64	63 ^{a} ±5.88	70 ^{b} ±4.22
Bone length - Duljina kosti (mm)	85 ±3.9	83 ±1.62	88 ±5.39	85 ±4.44	103 ±1.95	106 ±3.67
BMC (g)	5.89 ±0.55	5.92 ±0.16	7.67 ±1.56	6.96 ±0.61	11.31^{a} ±1.11	12.96 ^{b} ±1.39
BMD (g/cm^2)	0.354 ±0.012	0.343 ±0.014	0.370 ±0.004	0.347 ±0.009	0.402 ^{a} ±0.029	0.435 ^{b} ±0.026
Wf (N·mm)	815 ±117	854 ±125	984 ±165	1041 ±127	1609 ^{a} ±117	1829 ^{b} ±204
Wy (N·mm)	511 ±72	538 ±122	570 ^{a} ±48	641 ^{b} ±5.8	854 ^{a} ±214	1000 ^{b} ±78
Ix (mm^4)	527 ±106	482 ±82	626 ±152	563 ±52	1114 ^{a} ±224	1178 ^{b} ±89
A (mm^2)	69 ±10.4	75 ±8.24	79 ±8.03	81 ±8.21	103 ^{a} ±9.85	115 ^{b} ±3.23
MRWT	0.876 ±0.197	1.21 ±0.163	0.921 ±0.127	1.08 ±0.201	0.897 ^{a} ±0.078	0.999 ^{b} ±0.050

a, b - means in rows with different superscripts differ significantly at P<0.05 at the respective pig's age 28, 35 and 56 day

a, b - u redovima s različitim natpisima razlikuju se značajno na P<0,05 u odnosnoj dobi praščića od 28, 35 i 56 dana

DISCUSSION

Numerous interrelated factors such as hormonal, genetic, nutritional, sex and physical activity may influence bone mineral mass and peak bone mass (PBM) acquired during skeletal maturity. PBM has been reported as an important factor in bone health in older organisms.

Mammals are born with poorly mineralized bones and, while they suckle, they do not receive enough calcium to fully mineralize them, however bone growth can be sustained by energy-rich milk. After weaning, there is normally a progressive increase in bone mineralization, stimulated by increased load-bearing, thus providing added strength and reserves of both calcium and phosphorus. Bone mineralization depends on minerals absorption from the gut, especially calcium and phosphorus, and their deposition in bone tissues in form of hydroxyapatite. Calcium and phosphorus absorption and retention are improved by the use of organic acids (Mroz et al., 2000; Radcliffe, 1998). In terms of low-phosphorus diet and other minerals, results are sometimes contradictory (Liesegang et al., 2002). On the other hand, bone retention of calcium and phosphorus can be diminished when pigs are fed very acidic feed (Partanen and Mroz, 1999). Massive water acidification could have the same negative effects.

The mechanical properties of bones depend on the amount of bone material, and its mechanical quality and architecture (Ferretti et al., 1993). In the present study the weight and geometrical parameters such as cross-sectional area, second moment of inertia, mean relative wall thickness were significantly increased in experimental 56 d old piglets. Furthermore, experimental diet induced significantly higher BMC and BMD, and improved the mechanical endurance of bones in terms of the moments of maximum elastic strength and maximum strength. This may be attributed to effects of Na-butyrate and *Yucca schidigera* extract on nutrients absorption in the gastro-intestinal tract. Application of Na-butyrate to piglets led either to an increase (Kotunia et al., 2004) or to a decrease (Piva et al., 2002) of villus length. By increasing the length of the intestinal villi, Na-butyrate probably enlarges the absorptive surface of the gut in particular in the proximal and distal jejunum and ileum thereby favorably influencing the absorption of nutrients from the gut lumen. Moreover, studies have demonstrated that saponins extracted from the *Yucca schidigera* plant can improve the absorption of nutrients by the intestinal mucosal surface as well (McAllister et al., 1998). On the other hand, recent research suggests another possible mode of action of yucca. Yucca contains anti-inflammatory polyphenolics such as resveratrol and yuccaols, which have antioxidant activity and free-radical scavenging effects (Oleszek et al, 2001; Piacente et al., 2004). Furthermore, these substances reduce the production of free radicals protecting chondrocytes during their maturation and differentiation, which improve chondrogenesis and bone growth (Seifert and Watkins, 1997). However, further molecular studies need to be done to verify hypothesis if and how polyphenolics of *Yucca schidigera* influence bone metabolism and growth.

CONCLUSIONS

Na- butyrate and *Yucca schidigera* extract may be used in feeding growing pigs. Early postnatal treatment of pigs with Na-butyrate and *Yucca schidigera* extract positively affects the development and function of the bones during growth, but further studies are recommended to investigate if this effect is the result of synergic or independent act.

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SAŽETAK

Cilj je rada bio istražiti djelovanje Na-butirata i tretmana s ekstraktom *Yucca schidigerae* na razvoj kralježnice svinja. Istraživanje je provedeno od 14. do 56. dana poslije rođenja. Praščići su nasumce podijeljeni u kontrolnu skupinu i hranjeni standardnim obrocima s dodatkom dražirane smjese organskih kiselina (fumarične, jabučne i limunske - 1,5 kg/t krmiva) i pokusnu skupinu hranjenu sličnim obrocima s dodatkom dražiranog Na-butirata (900 g/t krmiva) i ekstrakta Yucca schidigera-e (75 g/t krmiva). U dobi od 28, 35 i 56 dana praščići su zaklani te su izolirane nadlaktične kosti (HUMERA) za daljnje analize. Izmjereni su sastav i kakvoća kosti na temelju težine, duljine, sadržaja minerala u kostima (BMC), gustoće minerala u kostima (BMD), fizičke (konačna čvrstoća, maksimalna elastična čvrstoća) i geometrijskih parametara. Težina kosti i geometrijski parametri kao što su područje presjeka, drugi moment inercije, srednja relativna debljina stijenke značajno su porasli u pokusnoj skupini 56 dana starih praščića. Nadalje, pokusni obroci prouzročili su znatno više BMC-a i BMD-a i poboljšali mehaničku izdržljivost kosti u smislu momenata maksimalne elastične jakosti i maksimalne jakosti. Zaključuje se da rano tretiranje praščića nakon rođenja Na-butiratom i ekstraktom Yucca schidigera-e djeluje pozitivno na razvoj i funkciju kosti tijekom razvoja.

Ključne riječi: butirat, kost, praščići, SCFA

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