Part 2. Biotechnology

UDC 60:619:616.71-007.23:636.22/.28.034.085.22:577.128

BONE MINERAL DENSITY IN EVALUATION THE PRODUCTIVE TRAITS AND REPRODUCTIVE HEALTH OF DAIRY COWS

Fedota O. M. [1], Babalian V. O. [2], Mitiohlo L. V. [3], Mazniakov S. M. [2], Valilshchikov M. V. [2], Tyzhnenko T. V. [1], Ruban S. Yu. [4]

[1] V. N. Karazin Kharkiv National University, Kharkiv, Ukraine, e-mail: amsfedota@gmail.com
[2] Kharkiv Medical Academy of Postgraduate Education
[3] SE RF 'Nyva', Khrystynivka, Ukraine
[4] LLC 'MPK Ekaterinoslavsky', Dnipro, Ukraine

Summary. Importance of studying osteoporosis and bone mineral density is due to the search for understanding on the regulation of bone mineral density, finding the signaling pathways and novel therapeutic targets, clarification of the gene network of osteoporosis. Since it is not always possible to assess the influence of various factors on the normal variation in bone mineral density, it is advisable to use animals as models. The aim of this study was evaluation of the bone mineral density and its relation with reproductive and productive characteristics of lactating dairy cows. Statistical methods included Pearson's chi-squared and *t* criteria, Pearson's and Spearmen correlation coefficient *r* and ANOVA. We analyzed the bone tissue indicators, productive and reproductive traits of dairy cows. The relation between health and reproduction traits of dairy cows — somatic cell count in milk, the open day, efficiency of insemination and the bone mineral density of cows was demonstrated. Also we found that bone mineral density was in a negative correlation with between somatic cell count in milk ($r = -0.67$), duration of open day ($r = -0.50$). More insemination was required for animals with a lower level of bone mineral density ($r = -0.67$). The level of bone mineral density is notified as independent from age, number of lactations and pregnancy, and the productivity of cows — milk yield, milk fat, milk protein. In the absence of the influence of chemical agents on metabolism, the processes of pregnancy and lactation are not a reason for reducing the bone mineral density in a balanced and high-grade diet.

Keywords: bone mineral density, dairy cows, milk traits, osteoporosis, reproduction of cows, somatic cell count

Introduction. Bone mineral density (BMD) is a quantitative trait used as a surrogate phenotype for the diagnosis of osteoporosis, the common metabolic disorder characterized by increased fracture risk as a result of a decreased bone mass and deterioration of the microarchitecture of the bone. Normal variation in BMD is determined by both environmental and genetic factors.

Whereas traditional pathophysiological concepts emphasize endocrine mechanisms, it has been recognized that also components of the immune system have a significant impact on bone (Boudin et al., 2016; Zhang et al., 2015; Özbaş, Onrat and Özdamar, 2012; Alam et al., 2014; Pietschmann et al., 2016).

The pathogenesis of osteoporosis is multifactorial. The genetic component is represented by a number of genes for osteoporosis susceptibility (Rocha-Braz and Ferraz-de-Souza, 2016; Li et al., 2016; Boudin et al., 2016). The environmental component includes such risk factors as corticosteroid therapy, sedative medication, low physical activity, unbalanced nutrition, lack of ultraviolet light. The study of the influence of environmental factors on the development of osteoporosis in man, variation in BMD and the interpretation of the results of different authors could, nevertheless, present considerable difficulties due to differences in ethnos, food culture, physical activity, other pathologies and their pharmacological support and other influences on the patient's organism. It is not always possible to assess the ratio of the contribution of these or other factors to the genesis of pathology. In this regard, it is advisable to evaluate the signs, using animals as model objects.

It was presented by Zimin et al. (2009), that cows and humans have sufficient DNA sequence similarity to enable to map the human genome almost entirely onto cow. Authors were able to demonstrate large majority, approximately 91%, of the genome has been placed onto the 30 *Bos taurus* chromosomes. Researchers identified 25,710 RefSeq proteins representing 18,019 distinct human genes, and aligned these to the cow genome. Of the 18,019 human genes, 17,253 (95.7%) mapped to cow (Zimin et al., 2009).

In our opinion, the advantages of the *Bos taurus* as a model include: the living of animals in a controlled environment, the possibility of monitoring at all stages of development, the conditions for targeted selection for the analysis of important signs, ongoing veterinary supervision. In this connection, cows can be a suitable model for studying the bone mineral density and its interaction with other traits. Actually, in cows, changes in BMD with exercise and parity have been reported, but the number of reports of cows is less than the number in humans and horses (Maetani et al., 2016; Hiney et al., 2004; Keene et al., 2004).

According to a number of authors, dairy cows are often affected milk fever at parturient, because of the decrease in serum calcium concentration. When serum *Calcium* range is decreased, it is compensated by intestinal absorption and bone resorption. In addition, it is known that signs of osteoporosis is observed in beef cattle that were fed low amounts of phosphorus diets for a long period and that bone mineral content of cattle has changed with diet and calcium-phosphorus metabolism (Maetani et al., 2016; Shupe et al., 1988; Williams et al., 1991).

In recent years, the researchers have presented a number of papers devoted to the methodologies for studying BMD in cows. The study of Maetani et al. (2016) is devoted to assess the measurement of BMD by quantitative computed tomography (QCT), comparing the relationships of BMD between QCT and dual-energy X-ray absorptiometry (DXA) and between QCT and radiographic absorptiometry (RA) in the metacarpal bone of Holstein dairy cows. In the work of Coates et al. (2016) the effects of animal weight, age and maturity on tailbone BMD of P-adequate animals, and the different responses to P deficiency observed in young growing steers, first-calf cows and mature breeders are discussed in relation to the use of single photon absorptiometry. It was noted that rib-bone and tailbone BMD at the end of experiment were closely correlated (r = 0.93) (Coates et al., 2016).

The same results were presented after investigation the relation between chemical measures and imaging estimates (radiographic photometry and dual-energy x-ray absorptiometry) of bone mineral content in dairy cows and evaluating the effects of parity, stage of lactation, and site of measurement (fused third and fourth metacarpal bone vs. caudal vertebrae 14 and 15) on bone mineral content (Keene et al., 2004).

The aim of the study was the evaluation of bone mineral density and its relationship with reproductive and productive characteristics of lactating dairy cows.

Materials and methods. A study was conducted at the State Enterprise Research Farm 'Nyva' of Institute of Animal Breeding and Genetics named after M. V. Zubets of the National Academy of Agrarian Sciencies of Ukraine and V. N. Karazin Kharkiv National University.

The type of production in SE RF 'Nyva' is organic. In this farm, cows had milk production (6,514 l), milk fat (3.65%) and milk protein (3.20%) concentration during 2016. The base total mixed ration (TMR) was alfalfa/haylage based with corn and other silage. Part of soybean meal is 3–5% of the grain mixture. The system of keeping cows is traditional. Part of time animals spent in the walking areas.

To estimate the bone mineral density and other traits, 35 cows Ukrainian red-spotted milk and Ukrainian black-spotted milk breeds at the age of 2–7th lactation after calving were tested in the study.

All animals had the same light/dark schedule, humidity and temperature. The air temperature at this time of year in the daytime was 22–34 °C outside, and 18–26 °C — in the animal facilities.

Cows were milked from 6 to 8 °a.m., and from 5 to 7 p.m. daily. Samples of milk were individually stored and analyzed by Ecomilk-Standart ('Bulteh 2000' Ltd., Bulgaria) for fat, protein and somatic cell score.

Bone mineral density (BMD) of cows was estimated by ultrasonic densitometry of the middle third of 12 pair rib-bone by Sunlight Omni 7000 ('Sunlight Medical' Ltd., Israel). Traditional production and reproduction parameters were analyzed.

Statistical analysis was performed with the Shapiro-Wilk and Kolmogorov-Smirnov tests for normality and hypotheses — criteria t and χ^2. The relationship between traits was estimated by the Spearman and Pearson correlation analysis. Means for two groups were compared by ANOVA (Atramentova and Utevskaya, 2008).

Results. The analysis of the results obtained in the present study indicated that cows have the same pedigree — on average, every animal has 79.5±1.9% of Holstein breed, 16.3±1.92% of Simmental, 2.89±0.82% of Montbeliarde. They do not receive hormonal and other medications, even to stimulate ovulation.

Lactating cows are characterized by an absence of alcohol and tobacco smoking influence. They have a balanced diet of plant origin, intake of calcium and other elements with food, there is no digestive disorder. Free content and walking areas provide good motor activity and access to ultraviolet light. Risk factors for excretion of calcium from the organism are a continuous activity of the reproductive system, pregnancy and lactations.

In our research animals with the different values of BMD had the same milk yield (17.9±1.15 l) milk fat (3.37±0.39%), milk protein (3.00±0.09%).

Study found no association between bone mineral density and age of an animals, r = –0.29 (p = 0.29), and number of lactation r = 0.22 (p = 0.38) (Table 1, Fig. 1).

The same results were received by Keene et al. (2004). It was demonstrated no effects of stage of lactation were observed on bone mineral in the caudal vertebrae. Total bone mineral content was not affected by parity in the metacarpal or caudal vertebra. Changes in bone mineral observed with stage of lactation and parity were insignificant (Keene et al., 2004).

Table 1 — Relationship between the bone mineral density and the signs of cow

Signs of cows	Parameters of study		
	Bone mineral density, m/sec	**r**	**p**
Age of an animals, 4–10 years	2,920.0–3,947.0	–0.29	0.29
Number of lactation, 2–7	2,920.0–3,947.0	0.24	0.38
Open day, 43–230 days	2,920.0–3,947.0	–0.50	0.05
Number of the last successful insemination, 1–3	2,920.0–3,947.0	–0.46	0.08
Milk yield, 11–23 kg /d	2,920.0–3,947.0	0.10	0.72
Somatic cell count in milk, 90,000–944,000 cells/ml	2,920.0–3,947.0	–0.67	0.006

Notes: r — correlation coefficient, p — significance level.

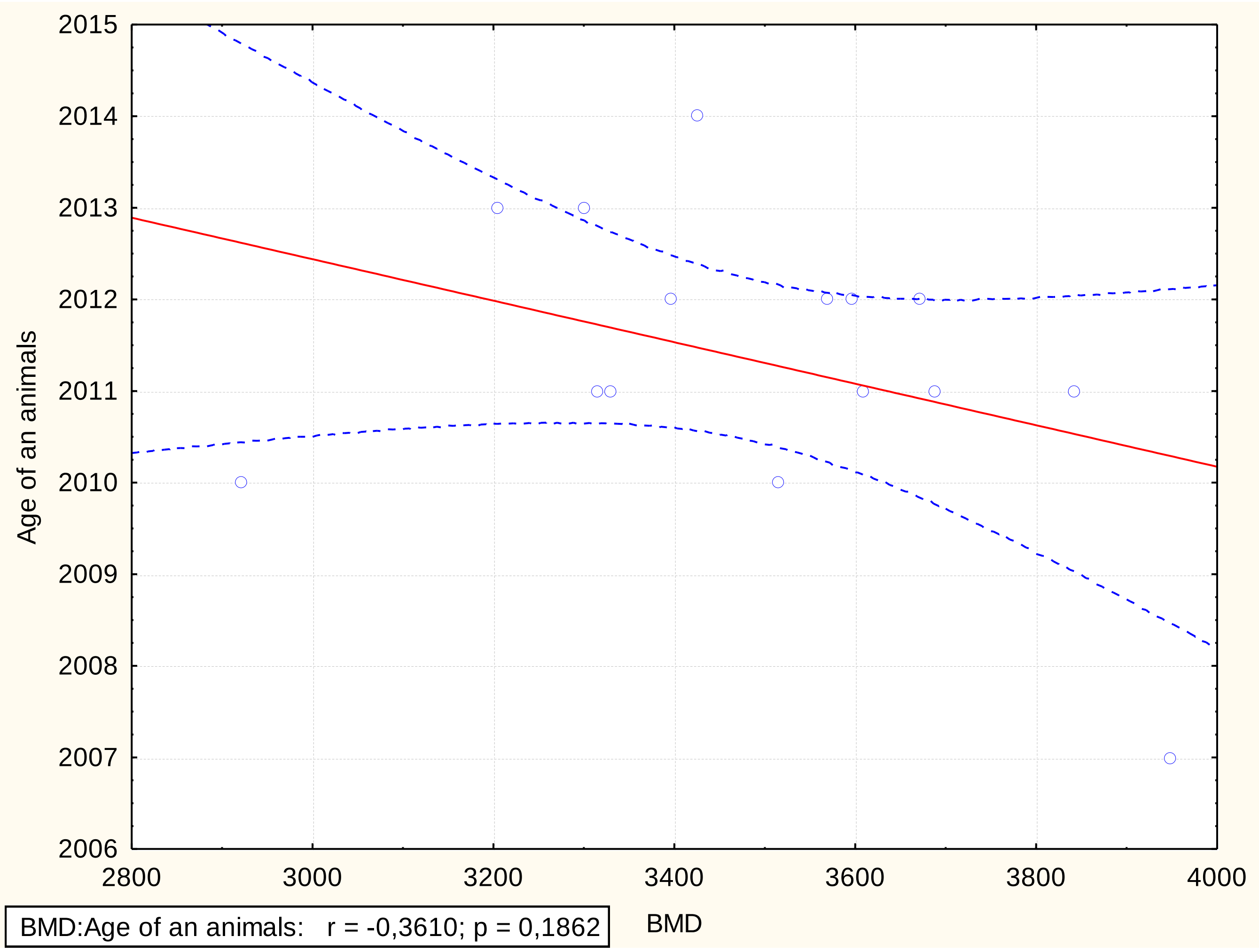

Figure 1. Dependence of bone mineral density on the age of cows

Coates et al. (2016) demonstrated, that BMD was estimated using single photon absorptiometry and in steers increased with live weight and age from ~ 0.25–0.27 g/cc (8 months, 200 kg LW) to ~ 0.34 g/cc (32 months, 490 kg LW). The authors concluded that BMD was initially 0.33 g/cc (~ 25 months, 400 kg LW) and did not change through pregnancy and lactation in cows (Coates et al., 2016).

It is important to note that we found negative correlation within bone mineral density and duration of open day of cows — r = –0.50 (p = 0.05), and, respectively, the number of inseminations carried out before successful fertilization and development of pregnancy of cows — r = –0.46 (p = 0.08). More insemination was required for animals with a lower level of bone mineral density (Table 1, Fig. 2).

Also we observed a statistically significant negative correlation between somatic cell count in milk and bone mineral density of cows — r = –0.67 (p = 0.006) (Table 1, Fig. 3).

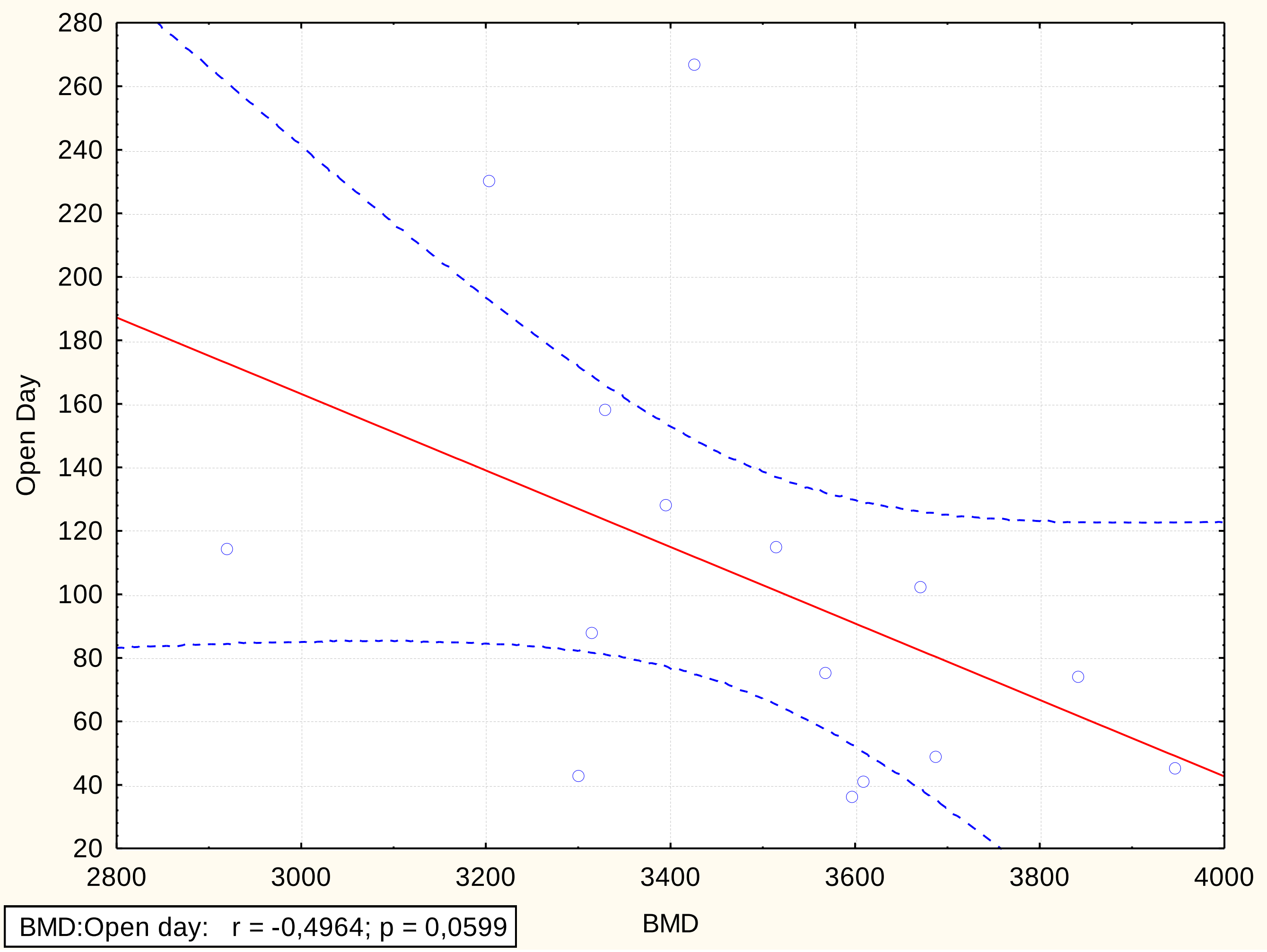

Figure 2. Dependence of open day of cows on bone mineral density

The data could testify to the relation of bone health indicators and the state of the reproductive and immune systems. Lactogenesis and synthesis of prolactin accompanies a large period of life of dairy cows. Prolactin, produced by anterior pituitary gland and various extrapituitary sites, is known to be associated with of bone metabolism (Li et al., 2017).

Many papers describe, that in addition to its classic function in mammary gland development and lactation, as prolactin receptor is a member of cytokine receptor superfamily, prolactin has been proposed to play multifunctional cytokine role, synthesized and secreted by peripheral blood mononuclear cells and has been reported to promote lymphocytes proliferation, interacts with cytokines and functions as co-activator (Pellegrini et al., 1992; Stevens et al., 2001).

According to literature, polymorphisms of prolactin gene have been reported to regulate prolactin mRNA expression in the T lymphocytes (Gellersen et al., 1992). It is worth noting that immune cells exert an indispensable role in regulating the activity of bone cells (Lee et al., 2009; Mori et al., 2013; Wythe et al., 2014; Thomas, 2010; Ray, 2014).

Different researchers established that arguments about the role of inflammatory cytokines influencing the fine-tuned balance between bone resorption and bone formation have helped to explain the occurrence of osteoporosis in conjunction with chronic inflammatory reactions. Given the tight anatomical and physiological coexistence of B cells and the bone-forming units in the bone marrow, a role of B cells in osteoimmunological interactions has long been suspected (Pietschmann et al., 2016).

We hypothesized that an increasing of bone mineral density is an indicator of body health and its effect contributed to successful inseminations, good fertility of cows and health pregnancy.

The absence of a decrease in BMD with age, lactation and pregnancy (Tables 1 and 2) is probably due to genetic factors, the absence of exposure to risk factors, known to humans, but mainly by the structure and balance of the diet — vegetable food, which contains in the required proportions fats, proteins, carbohydrates, vitamins and minerals.

Calves receive dairy products only up to 4 months, until the final formation of a complex rumen.

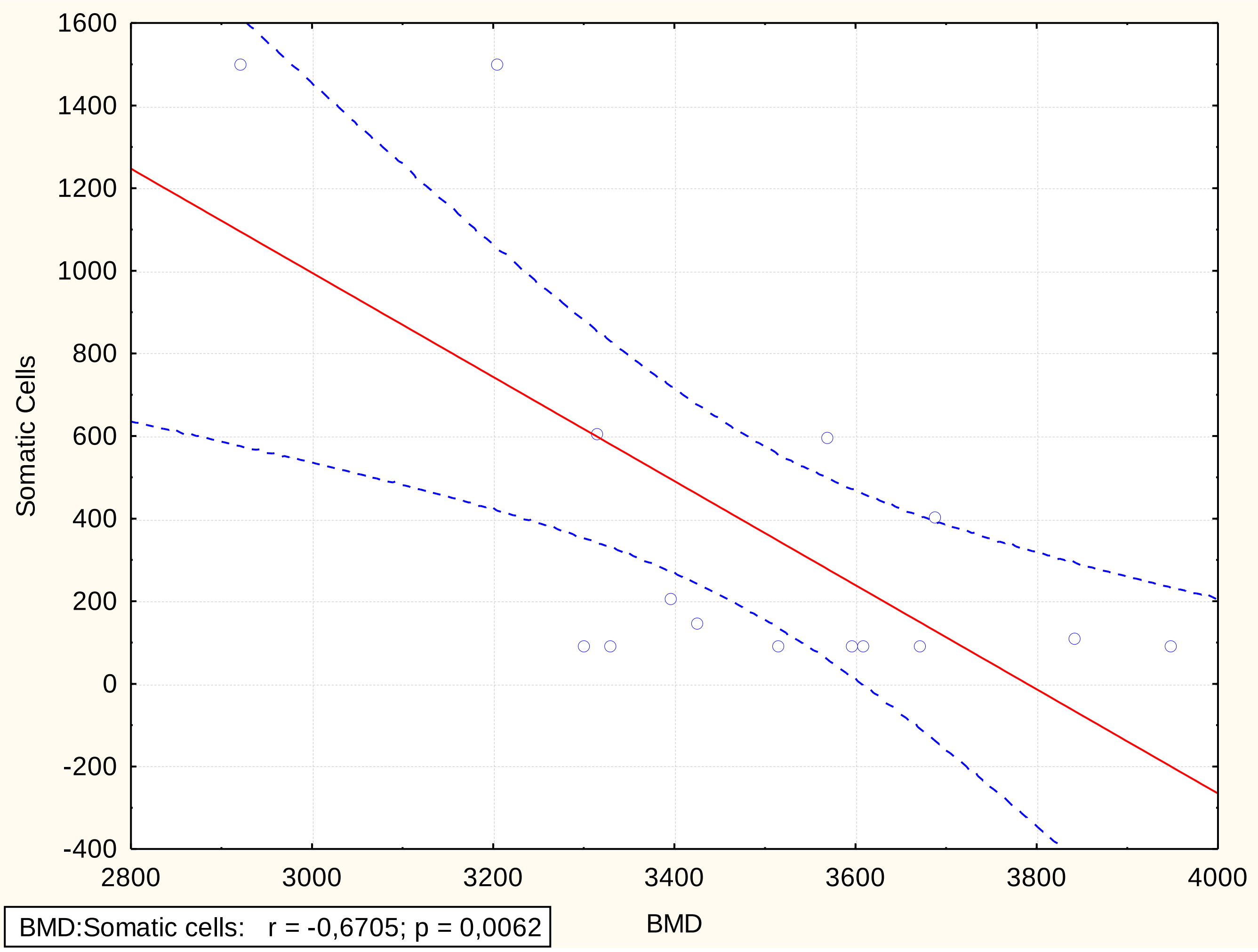

Figure 3. Correlation between bone mineral density and somatic cell count in milk

Table 2 — Parameters of animals in groups with different bone mineral density

Signs of cows	Bone mineral density (BMD)		F	p
	< 3,500	> 3,500		
Number of animals, heads	7	8		
Average value of BMD, m/sec	3,269.9±64.2	3,679.0±51.7	7.14	0.02
Open day, days	146.9±21.8	67.1±20.4	7.14	0.02
Number of lactation	2.6±0.6	3.6±0.5	1.7	0.21
Milk yield, kg/d	17.6±1.6	18.3±1.8	0.08	0.78

Notes: F — Fisher test, p — significance level.

The literature data demonstrate that the human Western diet compared to the semi-vegetarian diet in some Asian countries may alter hormone production, metabolism or action at the cellular level by some biochemical mechanisms and researchers have been focused on two groups of hormone-like diphenolic phyto-oestrogens of dietary origin, the lignans and isoflavonoids abundant in plasma.

According to the researchers, the precursors of the biologically active compounds detected in man are found in soybean products, whole-grain cereal food, seeds, and berries.

The plant lignan and isoflavonoid glycosides are converted by intestinal bacteria to hormone-like compounds. The weakly oestrogenic diphenols formed influence sex-hormone production, metabolism and biological activity, intracellular enzymes, protein synthesis, growth factor action, malignant cell proliferation, differentiation, cell adhesion and angiogenesis, bone resorption such a way as to make them strong candidates for a role as natural protective compounds. Owing to their oestrogenic activity they demonstrate some degree of osteoporosis inhibition (Adlercreutz and Mazur, 1997).

Other epidemiological studies presented those women who have high soy food consumption have a lower risk of osteoporosis than women who consume a typical Western diet (Somekawa et al., 2001; Zhang et al., 2005).

A role for soy isoflavones in modifying human bone mass has been suggested by Bone et al. (2000) — they argued that soy protein supplements enriched in isoflavones attenuated bone loss in postmenopausal and perimenopausal women.

Besides, the meta-analysis presented by Wei et al. (2012) demonstrate the effect of soy isoflavones on prevention of osteoporosis in human, the effective dosage of soy isoflavones and its duration and that significant effect of soy isoflavones on BMD is relative to menopausal status, supplement type, isoflavone dose and intervention duration.

It should be noted that proteins of vegetable origin make up to 20% of the basis of the diet of cows, and soy and its derivatives can reach 15–20% of the grains mix in the diet of highly productive cows.

Conclusions. We analyzed the bone tissue indicators, productive and reproductive traits of dairy cows without the chemical factor influence, with a balanced high-grade diet and physical activity of cows.

The relationship between health and reproduction traits of dairy cows — somatic cell count in milk, the open day, efficiency of insemination and the bone mineral density of cows was shown.

Bone mineral density was in a negative correlation with between somatic cell count in milk ($r = -0.67$), and duration of open day ($r = -0.50$). More insemination was required for animals with a lower level of bone mineral density ($r = -0.67$).

The level of BMD was independent of age, number of lactations and pregnancy, and the productivity of cows — milk yield, milk fat, milk protein, which could be due to the structure and balance of the diet.

Thus, the obtained results show the prospects for further research of dairy cows as a suitable model for studying of normal and pathological signs and processes in the other animals and humans. Such investigations make it possible to identify factors explaining normal variation in BMD, its regulation and osteoporosis susceptibility, could be the basis for future research a genes involved in the pathways.

References

Adlercreutz, H. and Mazur, W. (1997) 'Phyto-oestrogens and Western Diseases', *Annals of Medicine*, 29(2), pp. 95–120. doi: 10.3109/07853899709113696.

Alam, I., Padgett, L. R., Ichikawa, S., Alkhouli, M., Koller, D. L., Lai, D., Peacock, M., Xuei, X., Foroud, T., Edenberg, H. J. and Econs, M. J. (2014) 'SIBLING family genes and bone mineral density: Association and allele-specific expression in humans', *Bone*, 64, pp. 166–172. doi: 10.1016/j.bone.2014.04.013.

Atramentova, L. A. and Utevskaya, A. M. (2008) *Statistical methods in biology [Statisticheskie metody v biologii]*. Gorlovka: Likhtar. ISBN 9789662129267. [in Russian].

Bone, H. G., Greenspan, S. L., McKeever, C., Bell, N., Davidson, M., Downs, R. W., Emkey, R., Meunier, P. J., Miller, S. S., Mulloy, A. L., Recker, R. R., Weiss, S. R., Heyden, N., Musliner, T., Suryawanshi, S., Yates, A. J. and Lombardi, A. (2000) 'Alendronate and estrogen effects in postmenopausal women with low bone mineral density 1', *The Journal of Clinical Endocrinology & Metabolism*, 85(2), pp. 720–726. doi: 10.1210/jcem.85.2.6393.

Boudin, E., Fijalkowski, I., Hendrickx, G. and Van Hul, W. (2016) 'Genetic control of bone mass', *Molecular and Cellular Endocrinology*, 432, pp. 3–13. doi: 10.1016/j.mce.2015.12.021.

Coates, D. B., Dixon, R. M., Murray, R. M., Mayer, R. J. and Miller, C. P. (2016) 'Bone mineral density in the tail-bones of cattle: effect of dietary phosphorus status, liveweight, age and physiological status', *Animal Production Science*, 56(12), pp. 2054–2059. doi: 10.1071/AN16376.

Gellersen, B., Kempf, R., Telgmann, R. and DiMattia, G. E. (1994) 'Nonpituitary human prolactin gene transcription is independent of Pit-1 and differentially controlled in lymphocytes and in endometrial stroma', *Molecular Endocrinology*, 8(3), pp. 356–373. doi: 10.1210/mend.8.3.8015553.

Hiney, K. M., Nielsen, B. D., Rosenstein, D., Orth, M. W. and Marks, B. P. (2004) 'High-intensity exercise of short duration alters bovine bone density and shape', *Journal of Animal Science*, 82(6), pp. 1612–1620. doi: 10.2527/2004.8261612x.

Keene, B. E., Knowlton, K. F., McGilliard, M. L., Lawrence, L. A., Nickols-Richardson, S. M., Wilson, J. H., Rutledge, A. M., McDowell, L. R. and Van Amburgh, M. E. (2004) 'Measures of bone mineral content in mature dairy cows', *Journal of Dairy Science*, 87(11), pp. 3816–3825. doi: 10.3168/jds.S0022-0302(04)73521-3.

Lee, Y. C., Raychaudhuri, S., Cui, J., de Vivo, I., Ding, B., Alfredsson, L., Padyukov, L., Costenbader, K. H., Seielstad, M., Graham, R. R., Klareskog, L., Gregersen, P. K., Plenge, R. M. and Karlson, E. W. (2009) 'The PRL −1149 G/T polymorphism and rheumatoid arthritis susceptibility', *Arthritis and Rheumatism*, 60(5), pp. 1250–1254. doi: 10.1002/art.24468.

Li, B. Y., Yang, Y. M., Liu, Y., Sun, J., Ye, Y., Liu, X. N., Liu, H. X., Sun, Z. Q., Li, M., Cui, J., Sun, D. J. and Gao, Y. H. (2017) 'Prolactin rs1341239 T allele may have protective role against the brick tea type skeletal fluorosis', *PLoS ONE*, 12(2), p. e0171011. doi: 10.1371/journal.pone.0171011.

Li, J. J., Wang, B. Q., Fei, Q., Yang, Y. and Li, D. (2016) 'Identification of candidate genes in osteoporosis by integrated microarray analysis', *Bone and Joint Research*, 5(12), pp. 594–601. doi: 10.1302/2046-3758.512.BJR-2016-0073.R1.

Maetani, A., Itoh, M., Nishihara, K., Aoki, T., Ohtani, M., Shibano, K., Kayano, M. and Yamada, K. (2016) 'Experimental assessment of bone mineral density using quantitative computed tomography in Holstein dairy cows', *Journal of Veterinary Medical Science*, 78(7), pp. 1209–1211. doi: 10.1292/jvms.15-0642.

Mori, G., D'Amelio, P., Faccio, R. and Brunetti, G. (2013) 'The interplay between the bone and the immune system', *Clinical and Developmental Immunology*, 2013(720504), pp. 1–16. doi: 10.1155/2013/720504.

Özbaş, H., Onrat, S. T. and Özdamar, K. (2012) 'Genetic and environmental factors in human osteoporosis', *Molecular Biology Reports*, 39(12), pp. 11289–11296. doi: 10.1007/s11033-012-2038-5.

Pellegrini, I., Lebrun, J. J., Ali, S. and Kelly, P. A. (1992) 'Expression of prolactin and its receptor in human lymphoid cells', *Molecular Endocrinology*, 6(7), pp. 1023–1031. doi: 10.1210/mend.6.7.1508218.

Pietschmann, P., Mechtcheriakova, D., Meshcheryakova, A., Föger-Samwald, U. and Ellinger, I. (2016) 'Immunology of osteoporosis: A mini-review', *Gerontology*, 62(2), pp. 128–137. doi: 10.1159/000431091.

Ray, K. (2014) 'Bone: The immune system takes control of bone homeostasis', *Nature Reviews Rheumatology*, 10(7), pp. 382–382. doi: 10.1038/nrrheum.2014.83.

Rocha-Braz, M. G. M. and Ferraz-de-Souza, B. (2016) 'Genetics of osteoporosis: Searching for candidate genes for bone fragility', *Archives of Endocrinology and Metabolism*, 60(4), pp. 391–401. doi: 10.1590/2359-3997000000178.

Shupe, J. L., Butcher, J. E., Call, J. W., Olson, A. E. and Blake, J. T. (1988) 'Clinical signs and bone changes associated with phosphorus deficiency in beef cattle', *American Journal of Veterinary Research*, 49(9), pp. 1629–1636. PMID: 3223676.

Somekawa, Y., Chiguchi, M., Ishibashi, T. and Aso, T. (2001) 'Soy intake related to menopausal symptoms, serum lipids, and bone mineral density in postmenopausal Japanese women', *Obstetrics and Gynecology*, 97(1), pp. 109–115. doi: 10.1016/S0029-7844(00)01080-2.

Stevens, A., Ray, D., Alansari, A., Hajeer, A., Thomson, W., Donn, R., Ollier, W. E. R., Worthington, J. and Davis, J. R. E. (2001) 'Characterization of a prolactin gene polymorphism and its associations with systemic lupus erythematosus', *Arthritis and Rheumatism*, 44(10), pp. 2358–2366. doi: 10.1002/1529-0131(200110)44:10<2358::AID-ART399>3.0.CO;2-K.

Thomas, T. (2010) 'Nouveaux acteurs du remodelage osseux, rôle du système immunitaire', *Bulletin de l'Academie Nationale de Medecine*, 194(8), pp. 1493-4503. PMID: 22046712. Available at: http://www.academie-medecine.fr/wp-content/uploads/2013/03/2010.8.pdf. [in French].

Wei, P., Liu, M., Chen, Y. and Chen, D.-C. (2012) 'Systematic review of soy isoflavone supplements on osteoporosis in women', *Asian Pacific Journal of Tropical Medicine*, 5(3), pp. 243–248. doi: 10.1016/S1995-7645(12)60033-9.

Williams, S. N., Lawrence, L. A., McDowell, L. R., Wilkinson, N. S., Ferguson, P. W. and Warnick, A. C. (1991) 'Criteria to evaluate bone mineralization in cattle: I. Effect of dietary phosphorus on chemical, physical, and mechanical properties', *Journal of Animal Science*, 69(3), pp. 1232–1242. doi: 10.2527/1991.6931232x.

Wythe, S. E., Nicolaidou, V. and Horwood, N. J. (2014) 'Cells of the immune system orchestrate changes in bone cell function', *Calcified Tissue International*, 94(1), pp. 98–111. doi: 10.1007/s00223-013-9764-0.

Zhang, X., Shu, X.-O., Li, H., Yang, G., Li, Q., Gao, Y.-T. and Zheng, W. (2005) 'Prospective cohort study of soy food consumption and risk of bone fracture among postmenopausal women', *Archives of Internal Medicine*, 165(16), pp. 1890–1895. doi: 10.1001/archinte.165.16.1890.

Zhang, Y., Liu, H., Zhang, C., Zhang, T., Zhang, B., Li, L., Chen, G., Fu, D. and Wang, K. (2015) 'Endochondral ossification pathway genes and postmenopausal osteoporosis: Association and specific allele related serum bone sialoprotein levels in Han Chinese', *Scientific Reports*, 5(1), p. 16783. doi: 10.1038/srep16783.

Zimin, A. V, Delcher, A. L., Florea, L., Kelley, D. R., Schatz, M. C., Puiu, D., Hanrahan, F., Pertea, G., Van Tassell, C. P., Sonstegard, T. S., Marçais, G., Roberts, M., Subramanian, P., Yorke, J. A. and Salzberg, S. L. (2009) 'A whole-genome assembly of the domestic cow, *Bos taurus*', *Genome Biology*, 10(4), p. R42. doi: 10.1186/gb-2009-10-4-r42.