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Experimental Analysis of the Bending Resistance of the Long Cortical Bovine Bone under Controlled Conditions

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Abstract

The value spectrum for the Module of Elasticity of the long cortical bovine bone is ample, according to the consulted specialized bibliography. The studies made to reach those values have been developed under non controlled conditions, and that fact could have affected the biological and structural conditions of the observed samples. In addition, the methodology used to perform the testing has not been mentioned, either. In the present study, a mechanical-experimental analysis of the cortical bovine bone tissue under controlled conditions is exposed (destructive tests of flexure in three points), which guarantees a significant conservation degree of the biological characteristics of the bone. Additionally, the speed of the improvement of the load is controlled to study the behavior of the long bones as structural elements. Tests in six angles of the action of the load were performed, and a value of 2.23 GPa was obtained for the Module of Elasticity.

Keywords: cortical bone tissue, flexure tests, Module of Elasticity

1. Introduction

As a biological system, the bone inside the organism has some mechanical characteristics. Once it is separated from its “natural environment,” the bone tissue initiates a transformation process (decomposition) that implies a modification of its mechanical characteristics; it means the gradual loss of liquids and fat, which cause an increase on its rigidity, and elasticity module.

The mechanical behavior of the bone is complex; however, its structure has been simplified into different forms to propose models, which are similar to the real performance conditions, sacrificing with this process, accuracy in the results obtained. In the same way, one of the parameters that is always present in such models is the bone resistance. “It is considered that the bone resistance, understood as the necessary strength to trigger the biomechanical failure of a bone, is the result of the integration of two variables, the quantity and bone quality, dependent factors of the modeled bone” [4].

Jämsä et al. [10] and Arndt et al. [2] showed in their studies that for determining the mechanical properties of the long bones, the most common method is the destructive flexure test. Thus, several research has been made about the resistance of the flexure of the long cortical bovine bone, but in the experimental tests made, the real conditions of the bone “work” have not been generally considered. The lack of standardize norms, the conditions under which the tests are made, and the little clarity in the procedures constitute an observable problem in the reviewed bibliography. For the elasticity module of the bovine tibia reports of values that go from 9.6 GPa to 21.6 GPa are found, all of them obtained from a flexure test, and in most of the cases without specifying the conditions under which it was made. In some others, the experiment conditions have varied. For further consult, the following studies are recommended: [16], [6], [3], [7], [14], [11], [8], [13], [15], [12] and [5].

Focusing on long bones (length predominates over width and thickness) leads to the application of mechanical concepts for study; its tubular shape highlights the high technology that meets the skeleton within the concept of a good mechanical machine. The bone tissue includes hardness and strength with the minimum possible weight. The quasi-cylindrical area of a long bone, called the diaphysis, is made up of more compact tissue, thickened in the middle part and with a curvature that provides resistance to the bone; while the extremities (epiphysis) are constituted by spongy tissue. The tubular shape of the diaphysis is suitable to withstand torsional, bending and axial forces efficiently with small cross sections, while the large amount of cancellous bone in the epiphysis allows a smooth graduation in stress transfer.

If a sample is taken from the hard bone, we find collagen fibers reinforced with hydroxyapatite [9]. While Cowin in [7] provides that the collagen fibers, which form a calcified organic matrix, have a presence of 22%, and within this matrix are small amounts of fluids and fats responsible for giving a 20% elasticity to the bone. For that reason, it is not considered a fragile material, apart from being light. Hydroxyapatite is a hard compound based on calcium, present in 69% of the bone, which can exhibit some order, but branches are seen in most of the bone. This distribution of such compound is not arbitrary, but it obeys to factors due to the charges. Evolution has fostered biological solutions more suited to mechanical demands; for that reason, the bones are anisotropic, that is, the mechanical properties depend on the direction of the charge [17].

The objective of this work is to perform the same type of bending test to determine the value of the modulus of elasticity of the bovine tibia; but under controlled conditions of wetting and bone conservation temperature; age and weight of the animal supplier; and speed of advance of the load.

2. Materials and Methods

For the realization of the tests, samples of bovine cattle between 3 and 4 years were obtained, these are ages of maturity of the animal and therefore the high rate of daily sacrifice in the region, which facilitates the obtaining of the samples. And the bending tests are at three points, that is, the efforts are supported by the epiphysis (cortical bone).

The procedure begins with obtaining the specimens: rear tibia of female bovine. Table 1 shows the data provided by the Municipal Public Company of Trace and Livestock Squares (EMURPLAG EP) of the city of Cuenca, about the slaughter of bovine breeds.

Table 1. Bovine breeds normally sacrificed in the city of Cuenca, Ecuador

Breed	*Percentage*
Criolla	50 %
Holstein	20 %
Brahman	10 %
Others	20 %

The weight of the cattle that is slaughtered ranges between 350 and 400 pounds, and the daily average of sacrifices is 230 heads (EMURPLAG EP). Thus, considering the greater quantity of the *Criolla* breed and its easy recognition of the extremities, due to the notable difference in the tonality of its meat and the shape of the bone, in comparison with the other breeds present in the area; it was possible to guarantee the homogeneity of the sample. Thus, for the execution of the tests, tibias of this breed were used. The selection of the tibia specimens was made directly in the market, always to the same supplier. In addition, taking into account that in the chain slaughter-distribution-sale, the time elapsed from the death of the animal until the arrival of the bone to the laboratory, for its preparation, did not exceed 24 hours. Prior to the test, each specimen was verified with the help of radiographs, to guarantee the integrity of the specimen (Fig. 1). In case of observing any fissure, the test piece is dismissed.

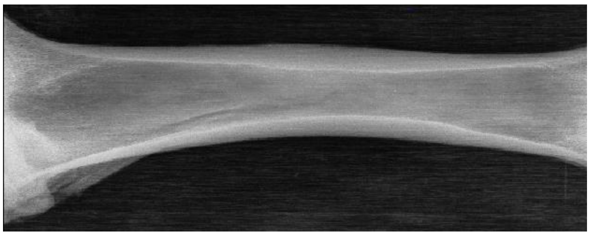

Figure 1. X-ray of a bovine tibia before performing the destructive test. **Source:** Authors, 2018

Since the animal is slaughtered, and then to the initial sectioning process, at a temperature of 10 ° C. This temperature is kept in the refrigerated cold transport in which the product is transferred to the market. The acquisition of the tests was carried out right at the start of the sales, prior coordination with the retailer, and its transfer to the laboratory was carried out in an isothermal box. Once in the laboratory and the X-ray was done, each proof was prepared for the test: manually, all the material was removed with the help of a scalpel, maintaining a constant humidification with saline solution.

Table 2 shows the factors that have been controlled, and that they could alter the mechanical properties of the bone, these characteristics are included in the following study:

Table 2. Factors that affect the bone’s mechanical properties

Factor	*Detail*
Breed	*Criolla*
Sex	Female
Age	3 – 4 (years)

Table 3. (Continued): Factors that affect the bone’s mechanical properties

Preservation	Isothermic box
Storage time	Less than 12 hours
Hydration	Saline solution
Preservation temperature	18° - 20°
Humidity	56 %

After this initial preparation, the specimen is placed on hexagonal supports specially designed for this purpose (Fig. 2)

Figure 2. Test-tubes ready to perform the destructive test of flexion at 3 points.

As it was shown in the previous figure, to ensure the fixation of each specimen in the supports, a mixture of dental plaster - portland cement was used as a filling material and the whole is left to rest for two hours, time required for the correct setting of the mixture [1]. In such process an exothermic chemical reaction takes place, however, Abad and his collaborators showed that the maximum temperature of the reaction reaches 37.12 ° C in a maximum time of 28.8 minutes. Table 3 shows the mechanical properties of the aforementioned mixture.

Table 3. Mechanical properties of the filling material based on Dental Plaster and Portland cement

Mechanical Properties	*Value*
Mixture Density	$\rho = 2.67$ g/cm^3
Elasticity module	20.5 GPa
Poisson´s ratio	$\nu = 0.4$
Material	Isotrópic

Thus, an hour and a half later the mixture recovers the ambient temperature, and Cowin [7] assures that carrying out tests with bones, at room temperature (23 degrees Celsius) would cause an increase in its Young's modulus; but not in a considerable amount. Taking into account that during the first half hour, after the pouring of the mixture into the molds, surrounding the extremities of the test tubes, the temperature reaches 37 degrees Celsius, and that from that moment begins to descend to match that of the environment; We consider that these affections do not reach to modify the biological properties and the structure of the epiphysis, the area where the load is applied.

Taking the tibial crest as reference, the geometry of the molds that can be used in view of the load, can be used for different angles. The 0 ° angle is established by placing the crest perpendicular to the direction of action. Next, in Fig. 3 it can be seen the assembly of the probe in the tests destructive machine to the flexion in 3 points, the schematization can be seen in Fig. 4.

Figure 3. Assembly of the test piece in the test machine.

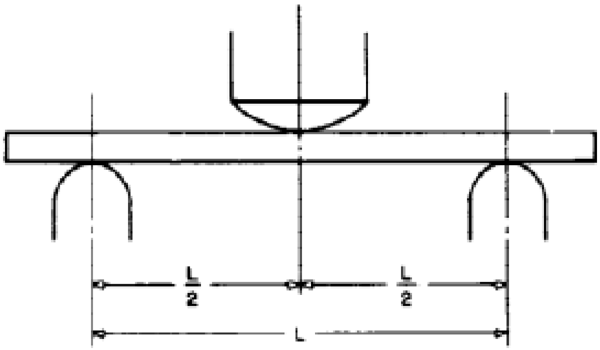

Figure 4. Three point bending test (ASTM D790-03 norm)

If the four-point bending test is performed, the shear stress is reduced; but the validity of the results is guaranteed only for constant cross sections, not present in complex bone structures. In this case, due to the short distance between the points of support, the shear stress is greater than the flexural stress, being the most advisable method the bending of three points for irregular geometries (ASTM Standard - D143). Taking into account the viscoelastic properties of the material, it was tested under ASTM D790-03 standards. Five samples were tested for each angle, at a feed rate of 2mm / min.

3. Analysis of results

Figures 5-10 show the force-deformation diagrams of the tested specimens at angles of 0 °; 60 °; 120 °; 180 °; 240 ° and 300 ° respectively. In all cases, from these diagrams, a linear regression adjustment was made in the region of elastic behavior, to obtain the value of the corresponding modulus of elasticity. Tables 4-9 show the results obtained for the tests and the corresponding rotation angles.

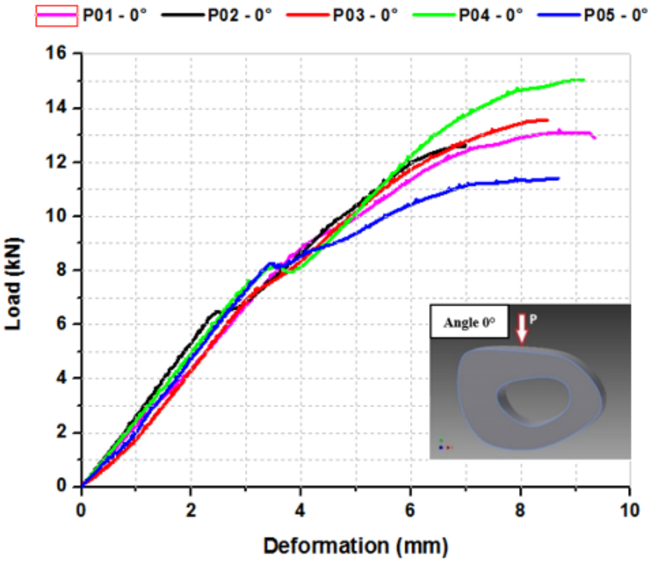

Figure 5. Test-tubes experimentally tested at 0°

Table 4. Properties of the test-tubes experimentally tested at 0°

Test-tube	*E (GPa)*	*Greater radial distance (mm)*
P01 – 0°	2.23	25.8
P02 – 0°	2.63	25
P03 – 0°	2.36	24.3
P04 – 0°	2.49	24.8
P05 – 0°	2.49	25
	Mean=2.44	

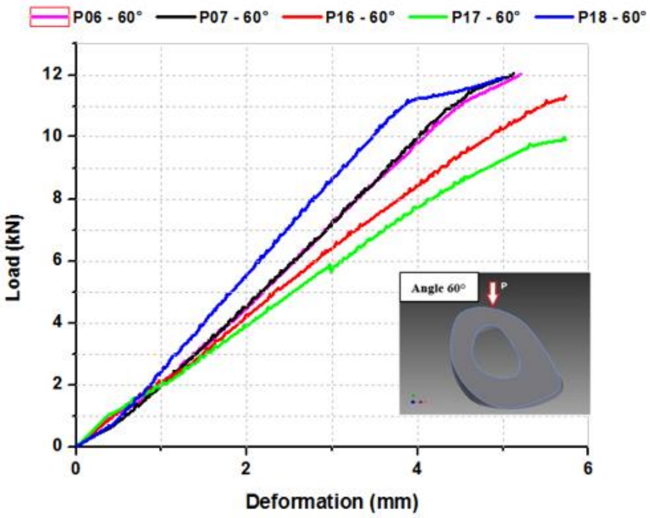

Figure 6. Test-tubes experimentally tested at 60°

Table 5. Properties of the test-tubes experimentally tested at 60°

Test-tube	*E (GPa)*	*Greater radial distance (mm)*
P06 – 60°	2.61	26.15
P07 – 60°	2.63	26.2
P16 – 60°	2.12	25.85
P17 – 60°	1.86	25.39
P18 – 60°	3.08	27.8
	mean=2.46	

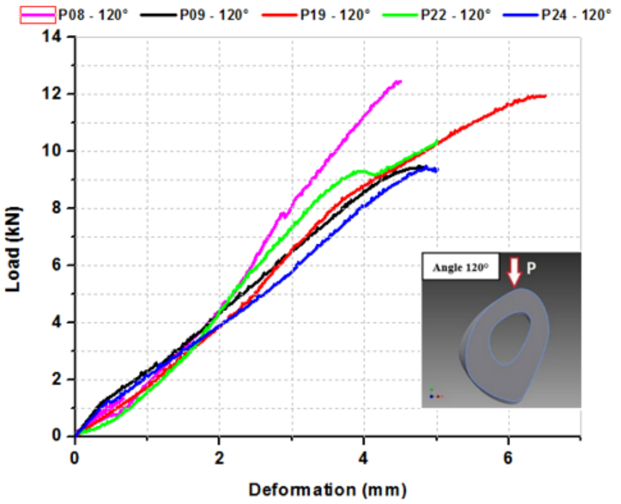

Figure 7. Test-tubes experimentally tested at 120°

Table 6. Properties of the test-tubes experimentally tested at 120°

Test-tube	*E (GPa)*	*Greater radial distance (mm)*
P08 – 120°	2.98	26.6
P09 – 120°	2.07	25.21
P19 – 120°	2.07	25.5
P22 – 120°	2.86	26
P24 – 120°	1.96	24.6
	mean=2.38	

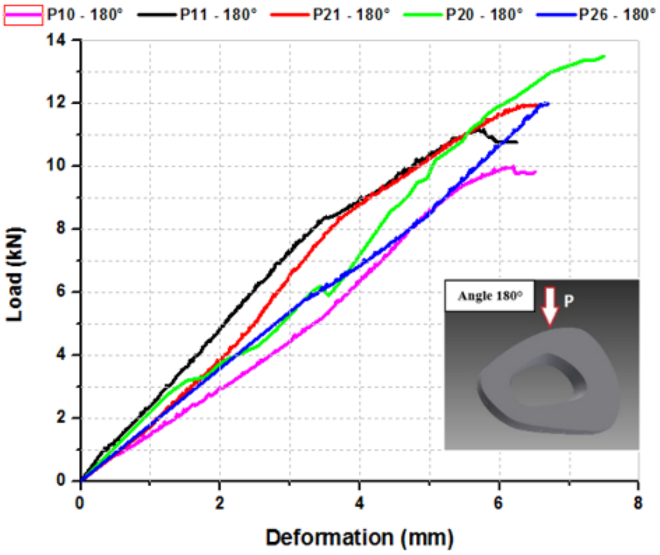

Figure 8. Test-tubes experimentally tested at 180°

Table 7. Properties of the test-tubes experimentally tested at 180°

Test-tube	*E (GPa)*	*Greater radial distance (mm)*
P10 – 180°	1.74	22.076
P11 – 180°	2.42	25.4
P20 – 180°	2.15	23
P21 – 180°	2.07	24.43
P26 – 180°	1.8	23.75
	mean = 2.03	

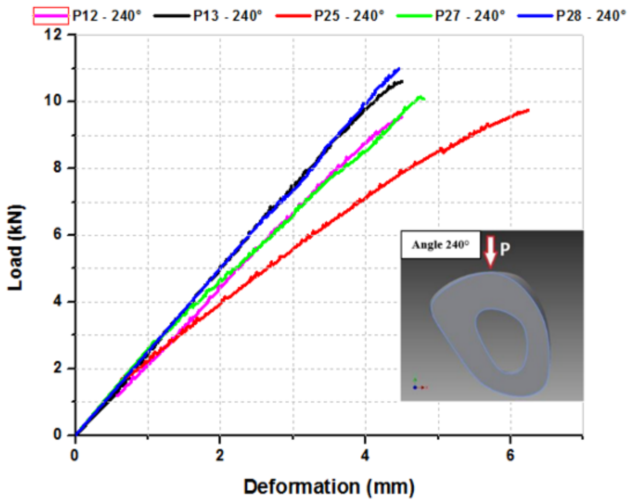

Figure 9. Test-tubes experimentally tested at 240°

Table 8. Properties of the test-tubes experimentally tested at 240°

Test-tube	*E (GPa)*	*Greater radial distance (mm)*
P12 - 240	2.19	23.8
P13 – 240	2.48	24.98
P25 - 240	1.54	20.4
P27 – 240	1.98	24.4
P28 - 240	2.27	24.91
	mean=2.09	

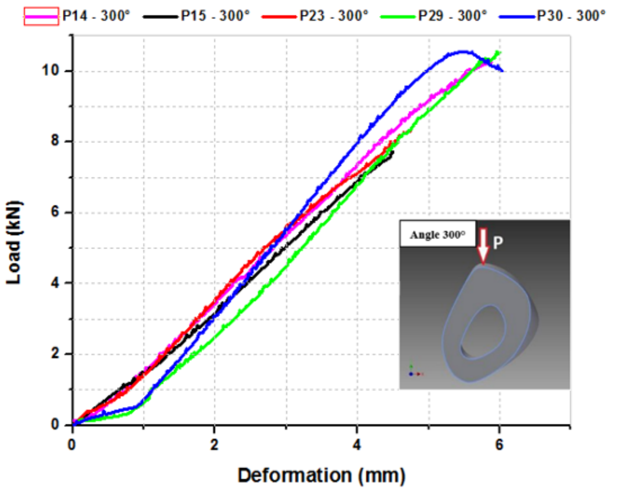

Figure 10. Test-tubes experimentally tested at 300°

Table 9. Properties of the test-tubes experimentally tested at 300°

Test-tube	*E (GPa)*	*Greater radial distance (mm)*
P14 – 300	1.94	26.3
P15 – 300	1.83	25.15
P23 – 300	1.92	25.91
P29 – 300	2.06	24.5
P30 - 300	2.1	25.7
	mean=1.97	

The means of the elasticity modules obtained experimentally are shown below, in table 10.

Table 10. Average modulus of elasticity of the specimens at different position angles

Angle	*Modulus of Elasticity (GPa)*
0 Degrees	2.44
60 Degrees	2.46
120 Degrees	2.38
180 Degrees	2.03
240 Degrees	2.09
300 Degrees	1.97
Mean	2.23

The general average value for the elasticity module of bovine tibia of the *Criolla* breed between 3 to 4 years of age, by controlling several other conditions, is 2.23 GPa.

4. Conclusions

The control of various conditions (age and breed of animals, temperature and humidity in the preservation of the specimens, speed of advance on the applied load, supports and mixture of filler to facilitate the placement of the specimens in 6 different angular positions) allowed to develop the study of the flexural strength of long bovine cortical bone. With the technique of destructive tests at three-point flexion, it was possible to determine the resistance of this type of bone (2.23 GPa); value that could be considered for future research in which this long bone is taken into account to receive a perpendicular impact in its central area. In addition, if the three-point bending test is considered, under given conditions, it is possible to avoid selecting the appropriate value within the broad spectrum of values for this elasticity module reported by other authors.

The results of the experimental tests show that the bovine cortical bone tissue behaves like an elastic material before presenting the fault. However, the complex geometry of the ends in this bone type and its relationship with the supports used to apply the load at different angles of rotation, introduce some level of noise in them. In addition, the reference angle for bone placement and subsequent application of the load influences the value of the elasticity module (see table 10), with a greater percentage variation of the order of 20%. Then one could surmise that the maximum value (that is, taking as reference the 2.46 GPa, see table 10) of the elasticity modulus of the bovine tibia of the *Criolla* breed, between 3 to 4 years of age, when the speed of advance of the load is controlled, the wetting of the specimens from the death of the animal to the preliminary preparation for the performance of the test and if carried out at room temperature, it is of around 3 GPa. Thus, the next challenges in analogous investigations would be done to refine the methodology applied to carry out the tests to arrive at conclusions about the conjecture.

References

[1] G. Abad-Farfán, T. F. Muñoz-Cuenca, P. B. Torres-Jara and E. Vázquez-Silva, Obtaining a Gypsum-Cement Blend, to be used as filling, with low hardening temperature, *Advances in Materials*, **6** (2017), no. 6, 115-121. https://doi.org/10.11648/j.am.20170606.12

[2] A. Arndt, P. Westblad, I. Ekenman, K. Halvorsen and A. Lundberg, An in vitro comparison of bone deformation measured with surface and staple mounted strain gauges, *Journal of Biomechanics*, **32** (1999), no. 12, 1359-1363. https://doi.org/10.1016/s0021-9290(99)00129-3

[3] A. H. Burstein, J. D. Currey, V. H. Frankel and D. T. Reilly, The ultimate properties of bone tissue: The effects of yielding, *Journal of Biomechanics*, **5** (1972), no. 1, 35-42. https://doi.org/10.1016/0021-9290(72)90017-6

[4] J. R. Caeiro-Rey, S. Dapía-Robleda, E. Vaquero-Cervino, L. Roca-Ruiz and M. A. Blanco-Ramos, Factores determinantes de la resistencia ósea, *Revista Española de Enfermedades Metabólicas Óseas*, **14** (2005), no. 14, 67-74. https://doi.org/10.1016/s1132-8460(05)72686-6

[5] Y. Chen and B. A. Alman, Wnt pathway, an essential role in bone regeneration, *Journal of cellular biochemistry*, **106** (2009), no. 3, 353-362. https://doi.org/10.1002/jcb.22020

[6] P. C. Chou, M. M. Porter, J. McKittrick and P. Chen, Vapor deposition polymerization as an alternative method to enhance the mechanical properties of bio-inspired scaffolds, in *Advances in Bioceramics and Biotechnologies II: Ceramic Transactions,* Vol. 247, (2014), 3-12.

https://doi.org/10.1002/9781118771587.ch1

[7] S. C. Cowin, *Bone Mechanics Handbook*, second edition, CRC Press Book, NY, 2001. https://doi.org/10.1201/b14263

[8] D. M. Cullinane and T. A. Einhorn, *Principles of Bone Biology*, Bilezikian J. P., Raisz L. G., Rodan G. A. (Eds.), Academic Press, Vol. II, 2002.

[9] I. S. Hage and R. F. Hamade, Structural micro processing of haversian systems of a cortical bovine femur using optical stereomicroscope and MATLAB, In *Proceedings of the ASME International Mechanical Engineering Congress & Exposition*, Vol. 2, Biomedical and Biotechnology, Houston, Texas, USA, 2012. https://doi.org/10.1115/imece2012-87423

[10] T. Jämsä, P. Jalovaara, Z. Peng, H. K. Väänänen and J. Tuukkanen, Comparison of three point bending test and peripheral quantitative computed tomography analysis in the evaluation of the strength of mouse femur and tibia, Bone, **23** (1998), no. 2, 155-161. https://doi.org/10.1016/s8756-3282(98)00076-3

[11] W. Kim, A. S. Voloshin, S. H. Johnson and A. Simkin, Measurement of the impulsive bone motion by skin-mounted accelerometers, *Journal Biomechanical Engineering*, **115** (1993), no. 1, 47-52. https://doi.org/10.1115/1.2895470

[12] M. D. Martínez, G. J. Schmid, J. A. MacKenzie, D. M. Ornitz and M. J. Silva, Healing of non-displaced fractures produced by fatigue loading of the mouse ulna, Bone, **46** (2010), no. 6, 1604-1612. https://doi.org/10.1016/j.bone.2010.02.030

[13] E. I. Ramírez-Díaz and A. Ortiz-Prado, Metodología para el modelado del comportamiento mecánico de hueso esponjoso a partir de sus microestructuras, *Ingeniería, Investigación y Tecnología*, **11** (2010), no. 2, 199-216. https://doi.org/10.22201/fi.25940732e.2010.11n2.017

[14] D. T. Reilly and A. H. Burstein, The mechanical properties of cortical bone, *Journal of Bone and Joint Surgery*, **56** (1974), no. 5, 1001-1022. https://doi.org/10.2106/00004623-197456050-00012

[15] E. D. Sedlin and C. Hirsch, Factors affecting the determination of the physical properties of femoral cortical bone, *Acta Orthopaedica Scandinavica*, **37** (1966), no. 1, 29-48. https://doi.org/10.3109/17453676608989401

[16] A. Simkin and G. Robin, The mechanical testing of bone in bending, *Journal of Biomechanics*, **6** (1973), no. 1, 31-36. https://doi.org/10.1016/0021-9290(73)90035-3

[17] H. An. Yuehuei and A. D. Robert, *Mechanical Testing of Bone and the Bone-Implant Interface*, Yuehuei H. An, Robert A. Draughn Eds., CRC Press. NY, 1999. https://doi.org/10.1201/9781420073560

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