Article
https://doi.org/**10.61900/SPJVS.2023.03.02**

FACTORIAL ANALYSIS OF SOME INDICATORS IN CHRONIC OSTEO-ARTICULAR MODEL OF RABBITS

Bianca Cornelia LUNGU[1,2], Bratu Daniel[1], Spătaru Ioana-Irina, Torda Iuliu, Călin MIRCU[1,2*], Ioan HUTU [1,2]

* e-mail of corresponding author: bratu.daniel@usvt.ro

Abstract

The using the of animals in the procedure of research project are strictly regulated by EU and Romanian low. Currently, the assessment of pain, suffering or distress in animals used in procedures is based on the physiological responses and behavioral changes that the animal exhibits. In long time models is better to take into consideration more quantifiable variables. The aim of the paper was to study some variables such us body mass, feed conversion, average daily gain, feed intake or feed rests associated with chronic osteo-articular rabbit model (OA) in rabbits. A number of 30 (3-31/2 month old) rabbits in 4 groups (non OA, OC-control, OA-treatment 1 and OA-treatment 2) where observed for 8 weeks period. By the trial period, the initial (F=14.648 at P<0.000) and final body weight (F=17.141 at P<0.000) and average daily gain (F=3.596 at P=0.029) were associated with the OA, also group x weight interactions [F= 2.692 at p = 0.026] was found. The main effect of time was statistically significant (F=11.210 at p=0.000) on ADG and the interaction group x time was also effective (F=2.244 at p=0.009); the interaction was also significant for interaction group x feed consumption (F= 2.325 at p = 0.004). Generally, the results of the study were clearly influenced by treatments and sometimes by the environmental conditions and the interactions between factors in a multivariate analysis but repeated measuring of body mass (weekly) is enough for following the welfare of rabbits in chronically OA animal models.

Key words: osteo-articular, rabbit model, (OA) body mass.

The using of the animals in procedures of research project are strictly regulated by EU under Directive 2010/63/EU on the protection of animals used for scientific purposes, national rules (Low 43 / 2014 regarding the protection of animals used for scientific purposes and ANSVSA order no. 97/2015 for the approval of the Veterinary Sanitary Norm regarding the veterinary sanitary authorization procedure of units that use, breed and supply animals used for scientific purposes, for the approval of the Veterinary Sanitary Norm regarding the veterinary sanitary authorization procedure of projects involving the use of animals in procedures).

The Experimental Units of *Horia Cernescu* Research Unit are a authorised research infrastructure for using animal in the procedure of research projects under FELASA recommendations, SOPs, clinical observation or Welfare Committee controls together with principal investigator. Currently, the assessment of pain, suffering or distress in animals used in procedures is based on the physiological responses and behavioral changes that the animal exhibits. *(Hutu, 2018; Mota-Rojas et al, 2020, Benato et, al 2019).* Beside all of the SOP's, pain score charts, in long period procedures, some associated variables with less suffering have to be use.

The aim of paper is to study some variables (body mass, feed conversion, average daily gain, feed intake or feed rests) associated with chronic osteo-articular animal model (OA) in order to quantify the effects of time, treatments and housing on animals or to take the decision of end point of study in an irrefutable way for the study and principal investigator *(Yoshioka et al, 1996, Pelletier et al, 2015).*

[1] *Horia Cernescu* Research Unit – USV Timisoara, RO.

[2] University of Life Science "*The King Michael I*" – Faculty of Veterinary Medicine, 119th Calea Aradului, Timisoara, 300645, RO.

MATERIAL AND METHODS

A number of 30 New Zealand micro-chipped females rabbits, aged 3 - 31/2 months, were used in a osteoarthritis study. The study took place over a period of 8 weeks in the Experimental Units of the University of Life Science "King Michel I" from Timisoara under the Ethical Statement no. 87 07.05.2018 and Project authorization no. 002 25.06.2018.

The animals were divided into 4 groups: A non OA (3 rabbits), B –control OA (no treatments-9 rabbits), C – OA- treatment 1 (9 rabbits) and D – OA-treatment 2 (9 rabbits). After the accommodation period, in the first week of study the animal model was performed, followed in 2nd week by first intra-articular treatment and in 7th week by last treatment.

During the trial, the clinical signs, telemetry temperature *(Huțu et al, 2018)*, pain scoring *(Miller et. al 2022),* the pressure of legs was strictly monitored.

The rabbits were kept individual in four different types of cages (LxlxH): standard (S) cages (713×716×476 mm, Techniplast®) with plastic floor with holes, cats (C) and dog (D) stainless steel cages (1490x640x1580 mm) with steel floor with holes and Guinea Pig (GP) doubled cages (846×610 ×256+256, Techniplast®) with plastic floor with square holes, in three rooms: rabbits room (14.69 m^3) , guinea pig room (10.52 m^3) and rats room (11.35 m^3) .

The environment temperature and humidity were continuously monitored (every half an hour) by multi-functional wireless digital device Weather Station PCE FWS 20. The lighting program was 14 hours light /10 hours dark.

Each rabbit received daily 160 g of pelleted feed (*Davidson, 1975)* and water *ad libitum*. The fodder residues were weighed every week, on the same day being noted for each individual animal. The actual intake was calculated by multiplying the amount of feed administered daily (160 g) by 7 (days of the week), from which the remaining amount of feed was subtracted

To calculate the average daily gain (ADG), the rabbits were weighed every week *(Zawislak et al. 2015).*

The initial weight was subtracted from the final weight and thus the total week gain was obtained – for ADG, the total gain was divided by 7. The weekly feed consumption was established after eliminating the unconsumed feed from weekly consumption (regularly, 160 g intake x 7 days). The feed efficiency was calculated by dividing the feed consumption to body mass from each week during the trial period.

The statistical tests used were: ANOVA, *t*-test, GLM Analisys (Test of Equality of Covariance, Mauchly's Test, followed by Greenhouse-Geisser and / Huynh-Feldt) with reapeted measures using *SPSS Statistics for Windows, Version 17.0. (Chicago: SPSS Inc. USA).* A P-value of <0.05 was considered to be statistically significant.

RESULTS AND DISCUSSIONS

The treatments of the groups A-D, have had impact on the variables such as body mass (initial mass F=7.105 at P=0.000 and final F=7.790 at P=0.000), ADG (F=2.880 at P=0.037), rest of feed (F=3.277 at P=0.022) and feed consumption (F=6.169 at P=0.000).

The body mass was higher in group B (OA model without treatment - 3472.64±32.47g) and A (rabbits without OA - 3367.50±91.36 g) and lower in groups with treatments; C (3255±28.45 g) and D (3289.29±39.78 g). Also, the ADG was higher in group A (95.04±21.26) and B (54.03±11.18 g) and lower in groups C (50.69±11.18 g) and D (19.01±18.15 g).

During the trial period, which lasted for 8 weeks, the initial (F=14.648 at P<0.000) and final body weight (F=17.141 at P<0.000) and average daily gain (F=3.596 at P=0.029) were associated with the OA. The body mass had an increasing trend between initial 3,165.33±43.85 g to 3,690±43.01 g and final mass in week 8. The interactions of treatments (groups) with body mass, during 8 weeks (*Figure 1* by GLM method), was significant. The Mauchly's Test demonstrated that the sphericity assumption was not met (p=0,000). The repeated measure with Greenhouse-Geisser method of correction indicated that there were significant differences during the trial in weight [F= 105.077 at p = 0.000] and lot x weight interactions [F= 2.692 at p = 0.026].

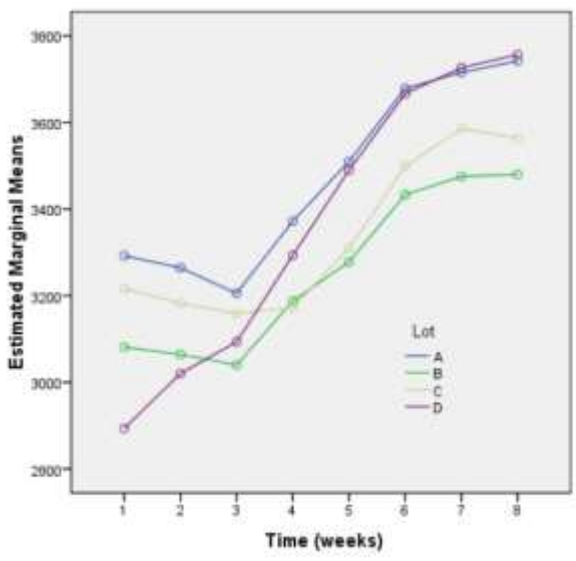

Figure 1. **Estimated marginal means of weight**

For entire study period the ADG was 46.97±7.55. The ADG was negative in the first two weeks *(Figure 2).* In the first week the OA model was perform and ADG was -7.30±17.74 g and in the second week the first intra-articular injection was perform and ADG was -25±15.83 g. From the third week it starts to grow (109.33±24.99 g in 3th week, 101.67±31.55 g in the 4th week, in 5th week, 58.73±22,152 g, in week 6 it was 54.83±12,354 g and 76.90±9.90 g in week 8). In the 7th week, the week of second intra-articular treatment the ADG was 6.55±12.47 g. The Box's test of equality of covariance indicates that the assumptions of homogeneity of covariance was met (p=0.103). The multivariate test demonstrated that the main effect of time was statistically significant Wilks' Lambda = 0.195, F(7,19)=11.210 at p=0.000 on ADG. This effect was qualified by any time x lot interactions, Wilks' Lambda = 0.170, F(21,55.1)=2.244 at p=0.009.

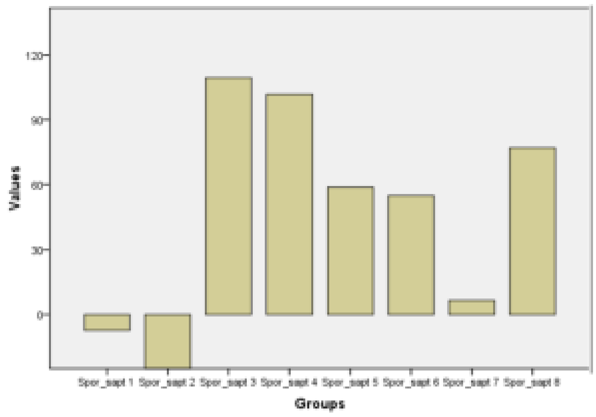

Figure 2. **Average daily gain distribution**

The cage type was associated with the body mass (F=9.716 at P=0.000 for initial body mass and F=11.315 at P=0.029 for final mass) and feed consumption (F=14.589 at P=0.000); the plastic floors of cages increased the food consumption (1178.06±19.28 g of pellets for S cages and 1248.33±28.36 g of pellets for GP cages).

Weekly feed consumption follows the same distribution like body mass: it was higher in group A (1248.33±28.36g) and B (1178.06±19.28 g) and lower in groups C (1135.42±17.01 g) and D (1087.68±26.63 g). The Mauchly's Test demonstrated that the sphericity assumption was not met (p=0,000). The repeated measure with Huynh-Feldt method (epsilon 0.776) of correction indicated that there were not significant differences in feed consumption during the 8 weeks of trial [F= 1236 at p = 0.294] but the interaction lot x feed consumption was significant [F= 2.325 at p = 0.004].

The rest of the feed accumulated in a week period was lowest in group A (-56.67±13.24 g) and highest in group D (-104.49±12.554 g) but the study was not powerful enough to find the significant factors associated.

One of the rabbits had to be euthanatized because of dramatically losses of body weight. In absence of any clinical signs of illness / Welfare Committee recommendation, when the body weight start do decrease, the lower action limit (LAL) by body weight was proposed and established at X±1.96x√2xSD for two consecutive weeks (measurements). After the necropsy we conclude that the peritonitis was the main cause of the death; the cause was not associated with the treatments which were intra-articular injections of tested products.

Generally, the results of the study were clearly influenced by treatments and sometimes by the environmental conditions and the interactions between factors in a multivariate analysis. Future statistics are need for establishing the end point of the study/animals for the cases which are outside of the limits in order to help the decision of Welfare Committee or the principal investigator.

CONCLUSIONS

Variation of the body mass is a good indicator reflecting the quality of life of animals in OA models. By the repeated measure design, the effect of time can be easily observed. Performing multivariate analysis, the effects of treatments or other variables can be measured in order to take the correct decision for welfare of animals.

ACKNOWLEGMENTS

The research was financed by Extension Unit, ONG, in Research Contract no 6584 from 19.10.2015 – *Const analysis and evaluation of needs in Horia Cernescu Reseach* and Contract no. 8359 of 26.06.2018 conducted under condition of Ethical Statement no. 87 07.05.2018 and Project authorization no. 002 25.06.2018.

REFERENCES

Benato L., Rooney N. J., & Murrell J. C., 2018 - Pain and analgesia in pet rabbits within the veterinary environment: a review. Veterinary Anaesthesia and Analgesia. doi:10.1016/j.vaa.2018.10.007

Davidson J. Spreadb d., 1975 - Nutrition of the New Zealand White rabbit. Proceedings of the Nutrition Society, 34(1), 75-83, doi: 10.1097/PNS19750013

Huțu I., 2018 – Manual de bune practice în unitățile experimentale. Editura Agroprint Timișoara, Vol. II, cap. VI, 207-234. ISBN: 978-606-785-036-9

Hutu, I., Patras,I Gherghel D., Lungu, B., Mircu, C, Application of infrared thermography in rabbit orthopaedic models , Lucrări științifice - Medicină veterinară, 2018, Vol. 61. Partea a 3-a.9-14, Editura "Ion Ionescu de la Brad".

Miller A.L., Clarkson J.M., Quigley C., Neville V., Krall C., Geijer-Simpson A., Flecknell P.A., Leach

M.C., 2022 - Evaluating Pain and Analgesia Effectiveness Following Routine Castration in Rabbits Using Behavior and Facial Expressions. Front Vet Sci., 9:782486. doi: 10.3389/fvets.2022.782486.

Mota-Rojas D, Olmos-Hernández A, Verduzco-Mendoza A, Hernández E, Martínez-Burnes J, Whittaker AL. The Utility of Grimace Scales for Practical Pain Assessment in Laboratory Animals. Animals (Basel). 2020 Oct 9;10(10):1838. doi: 10.3390/ani10101838. PMID: 33050267; PMCID: PMC7600890.

Pelletier J.P., Kapoor M., Martel-Pelletier J. 2015. 174 - Animal models of osteoarthritis. Rheumatology (Sixth Edition),Volume 2, Pages 1454-1461

Yoshioka M., Coutts R.D., Amiel D., Hacker S.A. Characterization of a model of osteoarthritis in the rabbit knee Osteoarthritis Cartilage. 1996 Jun;4(2):87-98. doi: 10.1016/s1063-4584(05)80318-8.

Zawislak J., SWIECICKA N., Surma D., BERNACKA H., 2015 - Analysis of factors affecting the final body weight in selected rabbit breeds. Journal of Central European Agriculture 16(2):28-37. DOI: 10.5513/JCEA01/16.2.1582

*****FELASA** working group on revision of guidelines for health monitoring of rodents and rabbits; Mähler Convenor M, Berard M, Feinstein R, Gallagher A, Illgen-Wilcke B, Pritchett-Corning K, Raspa M. FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. Lab Anim. 2014 Jul;48(3):178-192. doi: 10.1177/0023677213516312. Epub 2014 Feb 4. Erratum in: Lab Anim. 2015 Jan;49(1):88. PMID: 24496575.