Eximia Journal

(ISSN 2784-0735)

Vol. 4

2022

Eximia Journal
Vol. 4, 14-18, May, 2022
ISSN: 2784-0735
www.eximiajournal.com

Study of some factors associated with polyclonal antibody production in rabbit

Bianca Lungu[1,3*], Ovidiu Georgescu[1,3*†], Beatrice Tudor[1,3], Roxana Buzan[4], Manuela Grijincu[4], Monica Cotarca[4], Carmen Panaitescu[4,5], Kuan-Wei Chen[4], Iuliu Torda[2,3], Calin Mircu[2,3], Ioan Hutu[1,3]

[1]Animal Productions and Public Veterinary Health Department, [2]Clinical Department of Faculty of Veterinary Medicine, and [3]*Horia Cernescu* Research Experimental Units from Banat University of Agricultural Science and Veterinary Medicine *King Michael I of Romania* – Timisoara, Calea Aradului 119, 300645, TM-RO, [4]OncoGen Research Center, Clinic *Pius Brînzeu* County Emergency Hospital Timisoara, Bulevardul Liviu Rebreanu 156, Timișoara 300723, TM-RO, [5]*Victor Babes* University of Medicine and Pharmacy, Piața Eftimie Murgu 2, Timisoara 300041, TM-RO

[†]Corresponding author: ovidiu.georgesccu@fmvt.ro

Abstract. Obtaining polyclonal antibody (pAbs) is a well-known and the most used procedure is rabbit models. The study was carried out over a period of 2 ½ years for several antigens in White New Zeeland rabbits (regularly two animals for each inoculated antigen). The control variables of antibody production were antibodies' optical densities measured after inoculation of antigens in animals - both male and female, both young and mature rabbits in the same 59-day standard polyclonal antibody production schedule. In this study, the ages at starting the production did not have a significant impact on optical densities (OD) of pAbs with two exceptions: after the first (2.1726±0.1626 vs. 2.7850±0.3391 at p=0.003) and second immunization (2.1198±0.1266 vs. 2.9778±0.5301, p=0.001) both at 10^3 dilutions. In the first immunisation, a significant difference was found for 10^6 dilutions between females and males (mean OD was 0.1182±0.0577 in female vs. 0.1975±0.0885 in male, p=0.035). Additionally, the final body weight was significantly different between males and females (3,864±364 in female vs. 3,507±491 in male, p=0.037). In overall, polyclonal antibody production has an impact on final body weight, total gain, average daily gain and feed conversion depending on the antigen type (protocol) but the level of impact is tolerable for the animals.

Keywords. rabbit production model, polyclonal antibodies

General

Obtaining the polyclonal antibody in rabbits is considered to generate superficial levels of pain, suffering or distress by Directive 2010/63/EU, Romanian Law 43/2014 and Order 97/2015 of National Authority for Veterinary and Food Safety, but in this present study no NSAID treatment was used *(Fishback et. all, 2016).* The main objective of the antibody production in a rabbit model is obtaining antiserum (pAbs; antisera) with a high titre and a high degree of affinity for experimental use or in diagnostic tests such as ELISA-type assays,

Eximia Journal
Vol. 4, 14-18, May, 2022
ISSN: 2784-0735
www.eximiajournal.com

Western blots, immuno-histo-chemical procedures, immuno-fluorescence and immuno-electron-microscopy. The aim of the study was to compare the possible factors impacting variation in pABs production in rabbits during 2 ½ years of discontinued production in Horia Cernescu Research Unit.

Materials and methods

The rabbits are easy to handle, to bleed and produce an adequate volume of high-titre, high affinity of antiserum. During a terminal bleed using saline displacement, the yield is 1g of pAb *(Stills, 1994)* or higher (unpublished results).

Polyclonal antibody production schedule used in *Horia Cernescu* Research Unit is described in Figure no 1. The antigens (the immunogen foreign substance, proteins, peptides etc.) were derived from *Ambrosia artemisiifolia* pollen. The blood collection was performed at 10 days, following a booster injection for maximum results, depending on the titre response curve generated by an animal and antigen.

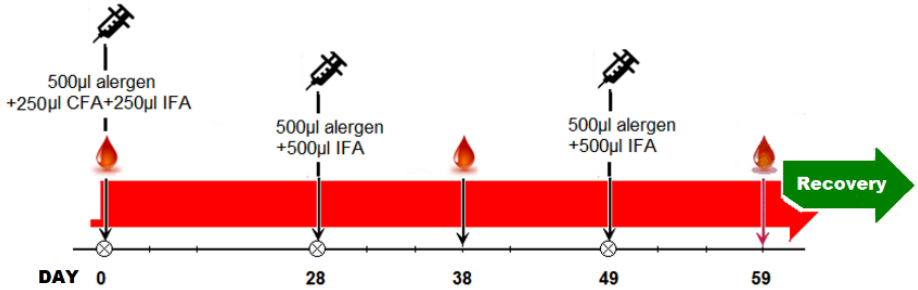

Figure no. 1: Standard polyclonal antibody production schedule

- Polyclonal antibody production in rabbits started at 4 and 6 months of age, scheduled for 59 days:
- Day 0 – pre-immunization bleed (preimmune serum) and a mix of antigen injection with Freund's complete (CFA) and incomplete adjuvant (IFA) in a mixture of 500 µl antigen + 250 µl CFA + 250 µl IFA divided in 8 serial s.c. inoculations in order to support the antibody production and avoid abscess formation (*Johnston et al. 1991 and Lai et al. 2012);*
- Day 28 - second antigen injection with Freund's incomplete adjuvant (500 µl antigen + 500µl IFA);
- Day 38 - bleed test;
- Day 49 - third antigen booster (allergen and Freund's incomplete adjuvant - (500 µl antigen + 500µl IFA). The third antigen inoculation and final bleeding depend on the titre response curve. The animals which were not able to produce a satisfactory titre after the booster were considered unsuitable for the antigen and excluded from the polyclonal antibody production group.
- Day 59 - blood collection.

Source: Experimantal Unit SOP, Hutu, 2017 and Hutu et al. 2019.

The ELISA-type assay was performed for obtaining optical densities of the produced antibodies – the optical densities at several dilutions was considered as an indicator of quality production control.

Sampled animals and data collection: a sample from 6 procedures (conducted during a 2 ½-year period) of 28 White New Zeeland rabbits was used, for measuring the relevant

factors associated with pAbs production such us: body weight at the beginning and at the end, total gain, the average daily gain and feed conversion. In the pre-immunisation day, all sampled animals were monitored and were clinically healthy (*Hutu et al, 2017)*; they were micro chipped and had normal growth parameters during the accommodation period.

Housing and feeding. The rabbits were kept individually in Tecniplast® X-type cage, L x l x h = 784 x 820 x 1.830 mm with 4.264 cm^2 space, walls and floors made of transparent (side panels) or opaque polycarbonate (rear panels, discontinuous floor and trash and purine trays). In the rabbits' room the environmental temperature and humidity were continuously monitored (every half an hour) by multi-functional wireless digital device *Weather Station PCE-FWS 20.* The environmental temperature was 24.40±1.25ºC (X±SD for all study period, with each ½ hour measurements) and significant differences between days of measurements were not observed. Air current speed in the rabbit room was 0.01 m/s (one measurement per day). The rabbit daily ration was 170±10 g of feed with digestibility up to 65%. The metabolic energy density of feed pellets was 1.980±45 kcal/kg. The energy was derived from proteins 23%, fat 10% and carbohydrates 67% *(Hutu et. al., 2018)*. During the production period, each cage had an elevated platform for welfare and environmental enrichment.

Statistical Analysis:

Pearson Correlation for association and *Mann-Whitney U* analysis were used to assess differences in variables. The study used *IBM® SPSS® Statistics* software for statistical analysis.

Results and discussion

All the animals were daily controlled and were clinically healthy during the production time. The polyclonal antibody production started in the rabbits' 4^{th} and 6^{th} month of age, after one month of accommodation. At the 4-5^{th} months, the average weights of sampled animals were 2.606±191 g and in the 6-7^{th} months 3.311±453 g.

A retroactive comparison between 4-5 month and 6-7 month rabbits were run after the control group ended its polyclonal antibody production two month later. In the study, the ages of starting the production did not have a significant impact on optical densities of pAbs with two exceptions: after first (2.1726±0.1626 vs. 2.7850±0.3391 at p=0.003) and second immunization (2.1198±0.1266 vs. 2.9778±0.5301 at p=0.001) both at 10^3 dilutions. In the sample of the study at 10^3 dilutions, older rabbits appear to obtain high optical densities, but with increasing dilutions the statistical significance disappears. The same trend was observed in a previous study (Hutu et al. 2019) – for the equivalent of more than 10^4 optical densities of pAbs no statistical differences were found. Although the results of the study did not support the hypothesis of association between age and level of pAbs production, the younger animals should be preferred, and animals not older than 6-7 months are recommended to be used in polyclonal antibody production.

The gender variability did not have an estimated impact either. In practice, female rabbits are more often used due to their docility; there are also suggestions that females are much more sensitive to lower doses to antigen, and many have significantly higher and more prolonged response to immunization than males *(Hutu, 2017)* but this study did not corroborate this hypothesis. The results of the study did not confirm the difference between body mass of males and females at the beginning (p=0.439 in Mann-Whitney U test); however, statistically

significant differences were observed at the end of the experiment (p=0.037). The result can be better explained if associated with normal differences in growth curve of males and females *(Masoud, 1986).* In the studied animals, the average daily gain, total gain, feed conversion and level of pAbs production quantified by ELISA assay in terms of optical densities (OD) for 10^3, 10^4 and 10^5 dilutions, were not associated with the gender variable (Mann-Whitney U test with value of p = 0.421 to 1.000). In first immunisation, for 10^6 dilutions a difference between males and females was found (OD 0.1182±0.0577 in female vs. 0.1975±0.0885 for male, at p=0.035). Moreover, there was a significant difference observed in the final body weight between males and females (3.864±364 vs. 3.507±491 at p=0.037).

Effects of the protocol (antigens) used for pAbs production had the estimated impact, with statistically significant differences on optical densities as well as on technological indicators such as: final body weight (p<0.000), total gain (p<0.004), average daily gain (p<0.001) and feed conversion (p<0.015). Thus, in conclusion, the value of body weight, average daily gain and feed conversion are influenced by the protocol – some antigens had a higher impact and others had a lower impact on those parameters, but in terms of real values, the antibody production did not influence drastically the technological parameters.

Conclusions

- Polyclonal antibody production has an impact on the final body weight, total gain, average daily gain and feed conversion depending on the antigen type (protocol), but the level of impact is tolerable by the animals.
- The rabbits between 4 to 7 months of age can be used for the convenient level of antibody production against allergens extracted from pollen of the plant *Ambrosia artemisiifolia*.
- The gender can influence some optical densities al lower dilution but this impact becomes less important with increasing dilution of serum.

Ethic statement and acknowledgments

Study protocol was designed and followed in strict accordance with the Specific Procedures of Experimental Units under Veterinarian Authority Authorization no. 001 / 29.09.2017 and was supported by the project *INSPIRED - Strategii inovative pentru prevenția, diagnosticul și terapia afecțiunilor respiratorii induse de polenul de ambrozia*, ID: P-37-747, cod MySMIS: 103663, contract no. POC 92/09.09.2016. The research was done in *Horia Cernescu* Research Unit of Banat University of Agricultural Science and Veterinary Medicine, Timisoara.

References

[1]Fishback, E.J., Stronsky, S.M., Green, C.A., Bean, K,D., Froude J.W., *Antibody production in rabbits administered Freund's complete adjuvant and carprofen concurrently* Lab Anim (NY), 2016, 45(2):63-66, doi: 10.1038/laban.937.

[2]Hutu, I., *Manual de bune practici in unitătile experimentale* vol, 1&2, Agroprint, Timişoara, Romania, 2017.

[3]Hutu, I., Mircu, C., Lungu, B., Panaitescu, C., Chen, K-W., Polyclonal antibody production in several rabbit models, Proceedings of the International Scientific Congress "Life sciences, a challenge for the future" (17th-18th October 2019, Iasi, Romania), Editor Prof. Liviu-Dan MIRON, Filodiritto Editore – Proceedings. Pages: 422 - 425

[4]Hutu, I., Mircu, C., Patras, I., *Health monitoring of animals in the experimental units,* Revista Romana de Medicina Veterinara, 2017, 27(4):33-42.
[5]Hutu, I., Patras, I., Gherghel D., Lungu, B., Mircu, C., *Application of Infrared Thermography in Rabbit Orthopaedic Models*, Lucrări științifice – Medicină veterinară, Editura "Ion Ionescu de la Brad" Iași, 61(3):9-14. 2018.
[6]Johnston, B.A., Eisen, H., Fry, D., *An evaluation of several adjuvant emulsion regimens for the production of polyclonal antisera in rabbits,* Lab Anim Sci, 1991, 41(1):15-21.
[7]Lai RPJ, Seaman MS, Tonks P, Wegmann F, Seilly DJ, Frost SDW, et al. (2012) *Mixed Adjuvant Formulations Reveal a New Combination That Elicit Antibody Response Comparable to Freund's Adjuvants*. PLoS ONE 7(4): e35083. https://doi.org/10.1371/journal.pone.0035083
[8]Masoud, I., Shapiro, F., Kent, R., Moses, A., *A longitudinal study of the growth of the New Zealand white rabbit: cumulative and biweekly incremental growth rates for body length, body weight, femoral length, and tibial length.* J. Orthop. Res.1986;4(2):221-231.
[9]Stills, H.F. *Polyclonal Antibody Production. In: The Biology of the Laboratory Rabbit*, 2nd Edn. (eds. P.J. Manning, D.H. Ringler& C.E. Newcomer). Pp. 435-448. San Diego CA: Academic Press, 1994.