



DOI: 10.22363/2312-797X-2024-19-4-707-719  
UDC 619:636.8:615  
EDN DETGUT


Research article / Научная статья

## A study of blood parameters in rabbits with otitis caused by *Malassezia pachydermatis* and the effect of Farnesol on recovery rates when added to the treatment regimen

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**Abstract.** *Malassezia* otitis is very common disease among animals. In previous years, successful, published work was carried out on modeling *Malassezia* otitis in rabbits using a *Malassezia pachydermatis* strain taken from dogs. We reproduced this experiment — we induced a clinical picture of the disease in order to try different treatment regimens on this model. The study involved 35 rabbits. The animals were divided into 7 groups of 5 rabbits each. Each group received one of the following drugs: Surolan; Otifri; Otoxolan; Surolan + Farnesol 200 µM/ml; Otifri + Farnesol 200 µM/ml; Otoxolan + Farnesol 200 µM/ml; control without treatment. All drugs were applied to the entire affected surface of the ear. The treatment was carried out once a day, the duration of treatment was 30 days. It was found that the use of drugs in combination with Farnesol in animals of the experimental group reduced the clinical signs of the disease; elimination of fungi in smears occurred more quickly; clinical parameters of rabbit blood improved.

**Keywords:** *Malassezia pachydermatis*, experimental otitis, clinical signs, blood test, rabbits

**Authors' contribution:** Sachivkina N.P. — conceptualization and methodology; Olabode I.R. — validation and investigation; Smolentsev S.Y. — data processing. All authors have read and agreed to the published version of the manuscript.

**Conflict of interests.** The authors declare no conflict of interest.

**Article history:** received 7 December 2024; accepted 23 December 2024.

**For citation:** Olabode IR, Sachivkina NP, Smolentsev SY. A study of blood parameters in rabbits with otitis caused by *Malassezia pachydermatis* and the effect of Farnesol on recovery rates when added to the treatment regimen. *RUDN Journal of Agronomy and Animal Industries*. 2024;19(4):707–719. doi: 10.22363/2312-797X-2024-19-4-707-719

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## Introduction

*Malassezia* is a commensal fungus that constitutes the normal skin microbiota. However, under certain conditions and in certain individuals, it can transform into pathogenic yeasts with multiple associated dermatological disorders and various clinical manifestations [1, 2]. Little is known about the virulence properties and infection mechanisms of *Malassezia* spp., and the implementation of infection models may allow for the evaluation of the interaction of these yeasts with hosts, the virulence of different species or strains of a specific species, and antifungal activity [3]. There are different types of suitable models in which virulence and infection can be studied, but it is critical to realize that the results obtained in each model provide partial answers, as it was mentioned before. Therefore, it is important to study virulence properties in different *in vitro* and *in vivo* models and the results obtained can provide complementary answers [4, 5].

Contrary to *in vitro* models, the *in vivo* models mimic the complexity of the host response better [6, 7]. These are rather diverse and can vary from mammalian models to insect models. Mammalian models are phylogenetically the closest to human beings and, generally, are regarded as more accurately reproducing the host-microbe interaction, known as fidelity [8]. Additionally, many of these models are well characterized, allowing for genetic modifications to reach a desirable condition. The drawbacks of these models are the high cost of feeding and maintenance, the limited number of individuals and the need for trained personnel to handle the animals [9]. All mammalian models are limited by ethical considerations — the use of mammalian infection models must be justified and subject to institutional and national regulation. This limits the use of mammalian models to address certain questions, such as large-scale studies of strain-specific differences in virulence or screening of antifungal compounds. These drawbacks can be solved by the implementation of alternative animal models, like invertebrates.

Experimental models may prove valuable in the further elucidation of both yeast virulence and host immune factors that are important in disease processes in various species. A successful model of *Malassezia* otitis in rabbits has been developed using a *Malassezia pachydermatis* strain derived from dogs [10]. Before this, models of vaginal candidiasis in mice were developed at the Department of Microbiology of RUDN University in 2009–2010 [11]. Female mice in estrus were maintained by subcutaneous injections of the hormonal drug Mesalin (Intervet, USA). When creating dysbiosis in laboratory animals, the antibiotics doxycycline and benzylpenicillin were used.

**The purpose of the study** was to determine the effect of Farnesol (Far) on the treatment of *Malassezia* otitis in rabbits and prove the enhancing effect of it on antimicrobial agents.

## Materials and methods

The research involved 35 healthy adult rabbits breed "Soviet chinchilla", weight 5.5 kg, males were used in experiment. Animals were divided into 7 groups of 5 animals. Each group received one of the following drugs:

1) Surolan, the active ingredients of which are: miconazole, polymyxin B, prednisolone;

2) Otifri lotion for cleaning ears with calendula which contains components such as: water, propylene glycol, emulsifier (Cremophor EL), calendula;

3) Otoxolan contains as active ingredients: marbofloxacin, clotrimazole, dexamethasone; and as auxiliary components propyl gallate, medium chain triglycerides, sorbitan oleate, anhydrous colloidal silicon oxide;

4) Surolan + Far 200  $\mu\text{M}/\text{mL}$  in equal proportion;

5) Otifri + Far 200  $\mu\text{M}/\text{mL}$  in equal proportion;

6) Otoxolan + Far 200  $\mu\text{M}/\text{mL}$  in equal proportion;

7) control.

All drugs were sprayed onto the entire affected surface of the ear (Fig. 1). The treatment was done once every day, rabbits were fixed, the duration of treatment was 30 days.



**Figure 1.** Spraying medications into the ear

Source: compiled by I.R. Olabode, N.P. Sachivkina, S.Y. Smolentsev.

Every five days for a month, smears of ear contents and blood samples were taken from animals in the control and 6 experimental groups and clinical signs of the disease were recorded. Blood tests were performed using the Mindray 2800Vet hematological analyzer (Mindray, China) [12].

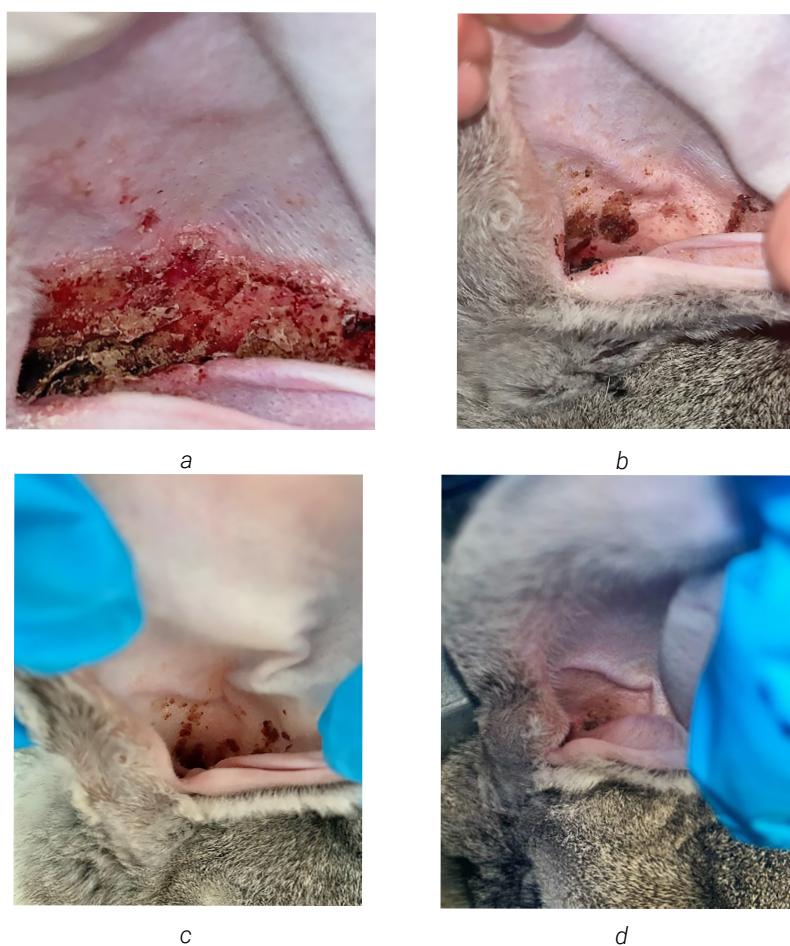
In whole blood, the number of erythrocytes and leukocytes, hemoglobin, as well as the content of leukocytes were determined. In the study, a quantitative counter of shaped elements of animal blood was used, the percentage of different types of leukocytes was calculated in stained blood smears by a unified method [13, 14].

The results obtained were compared in the experimental and control groups with an assessment of the reliability of the differences. The parameters given in the tables

had the following designations:  $M$  is the average,  $m$  is the error of the average,  $n$  is the volume of the analyzed subgroup,  $p$  is the achieved level of significance. In all cases, the critical value of the significance level ( $p$ ) was assumed to be 0.05.

## Results and discussion

The use of medicinal drugs + Farnesol in the animals of the experimental group reduced the signs of hyperemia, swelling, itching, the amount of exudation on the 5–7th days of treatment, and complete clinical recovery of the animals occurred on the 20th day. When using only drugs in animals (Surolan; Otifri; Otoxolan), on average, an improvement in the clinical condition occurred on 25 days, and final recovery followed after a full course of treatment — 30 days, and then when using Otifri once a day, redness of the ears persisted. Animals in the control group maintained clear clinical signs of the disease throughout the experiment. Their condition worsened and did not recover on their own, which proves the excellent effectiveness of the MO model we developed in rabbits.



**Figure 2.** Recovery clinical signs on rabbits' model: *a* – 5 days treatment with Surolan; *b* – 10 days; *c* – 15 days; *d* – 20 days

Source: compiled by I.R. Olabode, N.P. Sachivkina, S.Y. Smolentsev.

Analyzing the results obtained, we can say that both treatment regimens (with and without the addition of Far) turned out to be effective, but the regimen used in the experimental group Far + Surolan/Otifri/Otoxolan gave faster results due to the wide spectrum of action of the drug Farnesol in relation to microorganisms that most often cause otitis media and its anti-inflammatory effect [2].

It is worth noting that in the experimental group there was not a single case of the presence of MP in the smear and on the nutrient medium after combination therapy with the drug + Farnesol after 20 days of therapy. And in the control group of 5 animals, all of them had fungi observed during microscopy of smears of ear exudate and were cultured on the nutrient medium in high concentrations for all 30 days of the experiment. It is important to note that when Farnesol was added to the drug, microbiological clearance from M occurred 5–10 days earlier. The best result in this series of experiments was with the combination of Otoxolan + Far, since clearing of the ears from BY was recorded on the 10th day of therapy [15, 16].

Clinical blood testing is one of the most important diagnostic methods, reflecting the reaction of the hematopoietic organs to the influence of various physiological and pathological factors; it also allows to monitor therapy effectiveness. Clinical blood parameters of rabbits with MO before treatment were characterized by low erythrocyte count values —  $5.20 \pm 0.34 \cdot 10^6/\mu\text{L}$ , which cannot be called anemia, but is a borderline value (Table 1–6).

Also, in sick animals, a decrease in hemoglobin was observed at the beginning —  $9.18 \pm 1.07 \text{ g/dL}$ . After treatment, amount of hemoglobin in blood of experimental rabbits increased to values of 10 g/dL and higher, and a significant difference was present between the experimental groups, which indicates the positive effect of Farnesol in the treatment of Malassezia otitis.

We began to observe statistically significant differences between the experimental groups by the 15–20th day of treatment. For example, number of platelets ( $103/\mu\text{L}$ ) in blood of sick animals in control was significantly higher, but after therapy number of platelets became lower and this decrease occurred earlier in the groups with combination of the drug and Far.

Table 1

**Hematological parameters in rabbits’ model on day 5 of treatment**

Parameters		Surolan	Otifri	Otoxolan	Surolan + Far	Otifri + Far	Otoxolan + Far	Control
Total white blood cells, $103/\mu\text{L}$	5 days	$5.78 \pm 0.52$	$5.64 \pm 0.60$	$5.70 \pm 0.61$	$5.66 \pm 0.60$	$5.74 \pm 0.65$	$5.60 \pm 0.68$	$6.34 \pm 0.41$
Lymphocytes, $103/\mu\text{L}$		$2.70 \pm 0.41$	$2.55 \pm 0.40$	$2.62 \pm 0.48$	$2.71 \pm 0.40$	$2.70 \pm 0.39$	$2.62 \pm 0.41$	$2.71 \pm 0.40$
Monocytes, $103/\mu\text{L}$		$0.54 \pm 0.12$	$0.58 \pm 0.15$	$0.52 \pm 0.19$	$0.57 \pm 0.16$	$0.55 \pm 0.14$	$0.55 \pm 0.12$	$0.60 \pm 0.13$
Granulocytes, $103/\mu\text{L}$ , of them		$3.24 \pm 0.30$	$3.19 \pm 0.39$	$3.18 \pm 0.37$	$3.27 \pm 0.38$	$3.29 \pm 0.43$	$3.06 \pm 0.35$	$3.28 \pm 0.45$
Neutrophils, $103/\mu\text{L}$		$2.18 \pm 0.31$	$2.24 \pm 0.45$	$2.21 \pm 0.40$	$2.10 \pm 0.39$	$2.17 \pm 0.32$	$2.13 \pm 0.37$	$2.18 \pm 0.32$
Red blood cells, $106/\mu\text{L}$		$5.51 \pm 0.50$	$5.68 \pm 0.36$	$5.70 \pm 0.40$	$5.62 \pm 0.37$	$5.22 \pm 0.35$	$5.38 \pm 0.38$	$5.20 \pm 0.34$

Ending tabl. 1

Parameters		Surolan	Otifri	Otoxolan	Surolan + Far	Otifri + Far	Otoxolan + Far	Control
Hemoglobin, g/dL	5 days	8.93 ± 0.91	8.62 ± 0.95	8.73 ± 0.94	8.56 ± 1.02	8.78 ± 0.95	8.63 ± 0.90	9.18 ± 1.07
Hematocrit, %		34.70 ± 3.46	32.71 ± 2.86	34.75 ± 3.27	36.01 ± 3.86	35.61 ± 3.06	33.70 ± 2.90	30.98 ± 2.88
Mean corpuscular volume, fL		67.95 ± 2.83	66.39 ± 2.90	69.25 ± 3.13	68.24 ± 2.93	65.25 ± 2.68	68.30 ± 2.43	68.17 ± 2.86
Mean corpuscular hemoglobin, pg		20.01 ± 0.95	19.71 ± 0.90	19.49 ± 0.96	19.81 ± 1.05	19.92 ± 0.94	19.84 ± 0.97	17.95 ± 1.07
Mean corpuscular hemoglobin concentration, g/dL		31.891 ± 1.86	31.69 ± 1.56	32.83 ± 1.98	30.92 ± 1.48	32.84 ± 1.76	33.01 ± 2.16	30.50 ± 2.08
Platelets, 103/μL		218.20 ± 24.05	220.49 ± 23.91	221.83 ± 21.85	220.35 ± 25.48	221.75 ± 22.05	220.24 ± 23.07	232.94 ± 18.94

Note. There are no statistically significant differences in this table between experience and control ( $p < 0.05$ ).

Source: compiled by I.R. Olabode, N.P. Sachivkina, S.Y. Smolentsev.

On day 5 of our experiment, no statistically significant differences were observed between the 5 experimental groups and one control group. Conclusion: the duration of therapy is very short for visible results.

Table 2

### Hematological parameters in rabbits' model on day 10 of treatment

Parameters		Surolan	Otifri	Otoxolan	Surolan + Far	Otifri + Far	Otoxolan + Far	Control
Total white blood cells, 103/μL	10 days	5.18 ± 0.53	5.41 ± 0.64	5.84 ± 0.58	5.17 ± 0.52	5.23 ± 0.54	5.15 ± 0.66	6.33 ± 0.48
Lymphocytes, 103/μL		2.53 ± 0.41	2.85 ± 0.40	2.62 ± 0.48	2.71 ± 0.40	2.70 ± 0.39	2.62 ± 0.41	2.61 ± 0.44
Monocytes, 103/μL		0.54 ± 0.12	0.58 ± 0.15	0.50 ± 0.19	0.57 ± 0.16	0.58 ± 0.14	0.55 ± 0.12	0.54 ± 0.11
Granulocytes, 103/μL, of them		3.24 ± 0.30	3.19 ± 0.39	3.18 ± 0.37	3.27 ± 0.38	3.29 ± 0.43	3.06 ± 0.35	3.43 ± 0.55
Neutrophils, 103/μL		2.18 ± 0.31	2.24 ± 0.45	2.21 ± 0.40	2.10 ± 0.39	2.17 ± 0.36	2.13 ± 0.37	2.28 ± 0.35
Red blood cells, 106/μL		5.84 ± 0.43	5.61 ± 0.48	5.47 ± 0.43	5.64 ± 0.41	5.61 ± 0.43	5.61 ± 0.43	5.62 ± 0.38
Hemoglobin, g/dL		9.05 ± 1.00	9.25 ± 1.30	9.24 ± 1.20	9.21 ± 1.34	9.26 ± 1.10	9.27 ± 1.28	9.05 ± 1.17
Hematocrit, %		33.24 ± 3.75	35.36 ± 4.20	32.24 ± 4.02	35.08 ± 4.04	36.04 ± 4.08	35.14 ± 3.95	33.20 ± 4.73
Mean corpuscular volume, fL		67.84 ± 3.12	67.80 ± 2.56	67.04 ± 3.02	66.36 ± 3.08	65.24 ± 3.00	67.09 ± 2.93	67.92 ± 2.86
Mean corpuscular hemoglobin, pg		19.08 ± 0.92	18.86 ± 0.90	19.21 ± 0.74	19.28 ± 0.93	19.06 ± 0.72	19.15 ± 0.84	18.50 ± 1.05
Mean corpuscular hemoglobin concentration, g/dL	30.60 ± 2.02	30.60 ± 2.02	31.83 ± 1.98	30.42 ± 1.48	32.84 ± 1.76	32.83 ± 1.98	30.40 ± 2.18	
Platelets, 103/μL	216.90 ± 18.72	211.94 ± 20.79	215.23 ± 19.85	220.35 ± 25.48	226.75 ± 22.05	217.83 ± 21.85	235.74 ± 20.22	

Note. There are no statistically significant differences in this table between experience and control ( $p < 0.05$ ).

Source: compiled by I.R. Olabode, N.P. Sachivkina, S.Y. Smolentsev.

On day 10 of our experiment, also no statistically significant differences were observed between the 5 experimental groups and one control group. Conclusion: the duration of therapy is very short for visible results according to Hematological parameters in rabbits’ model.

Table 3

**Hematological parameters in rabbits’ model on day 15 of treatment**

Parameters	Surolan	Otifri	Otoxolan	Surolan + Far	Otifri + Far	Otoxolan+Far	Control
Total white blood cells, 10 <sup>3</sup> /μL	5.01 ± 0.37	5.23 ± 0.58	5.83 ± 0.45	5.10 ± 0.65	5.41 ± 0.71	5.32 ± 0.47	6.33 ± 0.56
Lymphocytes, 10 <sup>3</sup> /μL	3.06 ± 0.38	2.96 ± 0.40	2.76 ± 0.45	2.58 ± 0.68	3.17 ± 1.28	2.66 ± 0.58	2.61 ± 0.44
Monocytes, 10 <sup>3</sup> /μL	0.29 ± 0.13	0.40 ± 0.17	0.41 ± 0.14	0.36 ± 0.18	0.42 ± 0.15	0.40 ± 0.17	0.50 ± 0.11
Granulocytes, 10 <sup>3</sup> /μL, of them	3.08 ± 0.28	3.19 ± 0.39	3.16 ± 0.37	3.04 ± 0.31	2.88 ± 0.24	3.43 ± 0.58	3.43 ± 0.55
Neutrophils, 10 <sup>3</sup> /μL	2.00 ± 0.33	2.18 ± 0.41	1.99 ± 0.46	2.20 ± 0.58	2.14 ± 0.50	2.11 ± 0.42	2.28 ± 0.35
Red blood cells, 10 <sup>6</sup> /μL	5.29 ± 0.41	5.61 ± 0.48	5.47 ± 0.43	5.59 ± 0.51	5.59 ± 0.51	5.59 ± 0.51	5.62 ± 0.38
Hemoglobin, g/dL	9.43 ± 1.21	9.25 ± 1.30	9.24 ± 1.20	9.40 ± 1.27	9.48 ± 1.01	9.53 ± 1.21	9.05 ± 1.17
Hematocrit, %	35.90 ± 4.80	32.02 ± 3.17	33.94 ± 3.42	34.65 ± 3.81	34.90 ± 3.77	33.52 ± 3.83	33.20 ± 4.73
Mean corpuscular volume, fL	64.01 ± 2.37	64.31 ± 2.65	68.21 ± 2.28	60.53 ± 2.67	69.01 ± 3.08	65.91 ± 2.64	67.92 ± 2.96
Mean corpuscular hemoglobin, pg	19.00 ± 1.04	21.45 ± 0.86	19.74 ± 0.69	19.80 ± 1.02	20.32 ± 0.94	20.80 ± 0.73	18.60 ± 0.98
Mean corpuscular hemoglobin concentration, g/dL	33.53 ± 1.20	32.61 ± 1.37	32.84 ± 1.42	32.41 ± 1.24	33.54 ± 1.26	32.51 ± 2.07	30.40 ± 2.18
Platelets, 10 <sup>3</sup> /μL	232.60 ± 20.34	225.60 ± 17.04	219.60 ± 21.05	218.60 ± 19.36	210.60 ± 16.04	200.32 ± 14.04*	240.34 ± 20.24

Note. The data are represented as mean ± SD. \* – statistically significant difference between experience and control (*p* < 0.05).

Source: compiled by I.R. Olabode, N.P. Sachivkina, S.Y. Smolentsev.

On the 15th day of our experiment, statistically significant differences between 5 experimental groups and one control group are visible in the level of platelets in rabbits that received daily therapy Otoxolan + Far.

Table 4

**Hematological parameters in rabbits’ model on day 20 of treatment**

Parameters	Surolan	Otifri	Otoxolan	Surolan + Far	Otifri + Far	Otoxolan + Far	Control
Total white blood cells, 10 <sup>3</sup> /μL	5.08 ± 0.63	5.23 ± 0.51	5.11 ± 0.62	5.64 ± 0.55	5.09 ± 0.58	5.34 ± 0.43	6.13 ± 0.48
Lymphocytes, 10 <sup>3</sup> /μL	2.46 ± 0.38	2.41 ± 0.40	2.26 ± 0.45	2.38 ± 0.68	3.10 ± 1.23	2.26 ± 0.58	2.61 ± 0.44
Monocytes, 10 <sup>3</sup> /μL	0.29 ± 0.13	0.40 ± 0.17	0.41 ± 0.14	0.36 ± 0.18	0.42 ± 0.15	0.40 ± 0.17	0.50 ± 0.11

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Parameters	Surolan	Otifri	Otoxolan	Surolan + Far	Otifri + Far	Otoxolan + Far	Control
Granulocytes, 103/ $\mu$ L, of them	2.87 $\pm$ 0.38	2.76 $\pm$ 0.39	3.10 $\pm$ 0.50	3.02 $\pm$ 0.41	2.85 $\pm$ 0.39	2.70 $\pm$ 0.34	3.43 $\pm$ 0.45
Neutrophils, 103/ $\mu$ L	2.00 $\pm$ 0.33	2.18 $\pm$ 0.41	1.99 $\pm$ 0.46	2.20 $\pm$ 0.58	2.14 $\pm$ 0.50	2.11 $\pm$ 0.42	2.28 $\pm$ 0.35
Red blood cells, 106/ $\mu$ L	5.28 $\pm$ 0.42	5.14 $\pm$ 0.40	4.97 $\pm$ 0.57	5.24 $\pm$ 0.45	5.35 $\pm$ 0.39	5.40 $\pm$ 0.42	5.62 $\pm$ 0.38
Hemoglobin, g/dL	9.62 $\pm$ 0.81	9.22 $\pm$ 0.94	9.43 $\pm$ 1.05	8.82 $\pm$ 0.90	9.52 $\pm$ 0.81	9.41 $\pm$ 0.94	9.05 $\pm$ 1.17
Hematocrit, %	35.38 $\pm$ 3.64	36.83 $\pm$ 4.08	36.19 $\pm$ 3.06	35.25 $\pm$ 4.03	32.83 $\pm$ 3.41	35.36 $\pm$ 4.15	32.20 $\pm$ 2.93
Mean corpuscular volume, fL	64.57 $\pm$ 1.82	65.53 $\pm$ 1.94	65.69 $\pm$ 2.02	65.97 $\pm$ 2.13	66.05 $\pm$ 1.90	65.74 $\pm$ 1.95	67.92 $\pm$ 2.96
Mean corpuscular hemoglobin, pg	19.90 $\pm$ 1.24	19.20 $\pm$ 1.13	18.56 $\pm$ 1.25	19.70 $\pm$ 1.20	18.84 $\pm$ 1.15	19.30 $\pm$ 1.02	17.94 $\pm$ 0.88
Mean corpuscular hemoglobin concentration, g/dL	32.94 $\pm$ 1.54	35.90 $\pm$ 1.73	36.90 $\pm$ 2.02	37.65 $\pm$ 1.54*	36.82 $\pm$ 1.63*	37.24 $\pm$ 1.54*	30.40 $\pm$ 2.18
Platelets, 103/ $\mu$ L	225.78 $\pm$ 20.43	211.50 $\pm$ 21.43	209.26 $\pm$ 17.93	201.53 $\pm$ 20.42*	205.56 $\pm$ 18.79*	207.28 $\pm$ 17.43*	234.74 $\pm$ 20.22

Note. The data are represented as mean  $\pm$  SD. \* – statistically significant difference between experience and control ( $p < 0.05$ ).

Source: compiled by I.R. Olabode, N.P. Sachivkina, S.Y. Smolentsev.

On the 20th day of our experiment, statistically significant differences between 5 experimental groups and one control group are visible in the level of platelets and mean corpuscular hemoglobin concentration in rabbits that received daily therapy Otoxolan + Far; Surolan + Far; Otifri + Far.

Table 5

### Hematological parameters in rabbits' model on day 25 of treatment

Parameters	Surolan	Otifri	Otoxolan	Surolan + Far	Otifri + Far	Otoxolan + Far	Control
Total white blood cells, 103/ $\mu$ L	4.96 $\pm$ 0.76	5.13 $\pm$ 0.52	5.24 $\pm$ 0.95	4.97 $\pm$ 0.90	4.82 $\pm$ 0.41*	5.03 $\pm$ 0.51*	6.23 $\pm$ 0.38
Lymphocytes, 103/ $\mu$ L	3.21 $\pm$ 0.44	3.14 $\pm$ 0.35	3.15 $\pm$ 0.38	3.06 $\pm$ 0.29	3.18 $\pm$ 0.30	3.01 $\pm$ 0.32	2.65 $\pm$ 0.44
Monocytes, 103/ $\mu$ L	0.29 $\pm$ 0.13	0.40 $\pm$ 0.17	0.41 $\pm$ 0.14	0.36 $\pm$ 0.18	0.42 $\pm$ 0.15	0.40 $\pm$ 0.17	0.50 $\pm$ 0.11
Granulocytes, 103/ $\mu$ L, of them	2.87 $\pm$ 0.38	2.76 $\pm$ 0.39	2.80 $\pm$ 0.40	3.02 $\pm$ 0.51	2.85 $\pm$ 0.49	2.70 $\pm$ 0.34	3.43 $\pm$ 0.55
Neutrophils, 103/ $\mu$ L	2.08 $\pm$ 0.53	2.19 $\pm$ 0.61	2.26 $\pm$ 0.48	1.93 $\pm$ 0.66	2.24 $\pm$ 0.52	2.15 $\pm$ 0.60	2.18 $\pm$ 0.35
Red blood cells, 106/ $\mu$ L	5.28 $\pm$ 0.42	5.14 $\pm$ 0.40	4.97 $\pm$ 0.57	5.24 $\pm$ 0.45	5.25 $\pm$ 0.39	5.40 $\pm$ 0.42	5.62 $\pm$ 0.38



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Hemoglobin, g/dL	9.61 ± 1.03	9.73 ± 1.12	10.08 ± 0.85	10.22 ± 0.95	10.02 ± 1.10	9.43 ± 1.04	9.05 ± 1.17
Hematocrit, %	34.39 ± 2.24	35.18 ± 2.57	33.75 ± 3.12	34.82 ± 3.00	35.19 ± 1.73	35.16 ± 3.10	32.10 ± 2.73
Mean corpuscular volume, fL	64.92 ± 2.56	65.38 ± 2.48	65.36 ± 3.04	65.49 ± 2.93	65.12 ± 3.03	63.34 ± 2.50	67.82 ± 2.56
Mean corpuscular hemoglobin, pg	19.90 ± 1.24	18.20 ± 1.13	18.56 ± 1.25	19.40 ± 1.20	18.84 ± 1.15	19.30 ± 1.02	18.60 ± 0.98
Mean corpuscular hemoglobin concentration, g/dL	36.64 ± 2.26	35.60 ± 2.34	38.45 ± 1.79*	39.61 ± 2.14*	37.94 ± 2.83*	38.62 ± 2.15*	30.40 ± 2.18
Platelets, 103/μL	204.73 ± 16.19	201.26 ± 23.38	197.54 ± 20.70*	201.50 ± 21.74*	200.53 ± 15.67*	199.50 ± 18.72*	233.94 ± 22.48

Note. The data are represented as mean ± SD. \* – statistically significant difference between experience and control ( $p < 0.05$ ).

On the 25th day of our experiment, statistically significant differences between 5 experimental groups and one control group are visible in the level of platelets & mean corpuscular hemoglobin concentration & total white blood cells in rabbits that received daily therapy Otoxolan + Far; Surolan + Far; Otifri + Far & just Otoxolan.

Table 6

**Hematological parameters in rabbits’ model on day 30 of treatment**

Parameters	Surolan	Otifri	Otoxolan	Surolan + +Far	Otifri + Far	Otoxolan + +Far	control
Total white blood cells, 103/μL	4.90 ± 0.76	4.72 ± 0.87	4.97 ± 0.63	4.38 ± 0.74*	4.21 ± 0.82*	4.41 ± 0.55*	6.93 ± 1.28
Lymphocytes, 103/μL	3.14 ± 1.20	3.75 ± 0.69	3.38 ± 0.70	3.24 ± 0.75	3.18 ± 0.84	3.22 ± 0.68	2.71 ± 0.48
Monocytes, 103/μL	0.29 ± 0.13	0.40 ± 0.17	0.41 ± 0.14	0.32 ± 0.18	0.42 ± 0.15	0.40 ± 0.17	0.50 ± 0.11
Granulocytes, 103/μL, of them	2.87 ± 0.38	2.76 ± 0.39	2.83 ± 0.42	3.02 ± 0.51	2.84 ± 0.50	2.70 ± 0.34	3.43 ± 0.55
Neutrophils, 103/μL	2.18 ± 0.53	2.19 ± 0.61	2.16 ± 0.58	1.93 ± 0.60	2.24 ± 0.52	2.15 ± 0.60	2.28 ± 0.35
Red blood cells, 106/μL	5.52 ± 0.41	5.27 ± 0.40	5.20 ± 0.45	5.47 ± 0.50	5.28 ± 0.48	5.12 ± 0.36	5.60 ± 0.41
Hemoglobin, g/dL	9.66 ± 0.47	9.84 ± 0.61	10.35 ± 0.53	10.06 ± 0.49	10.11 ± 0.79	10.60 ± 0.44	9.01 ± 1.10
Hematocrit, %	41.03 ± 1.80*	39.25 ± 1.96*	40.01 ± 1.88*	41.10 ± 1.90*	40.56 ± 1.54*	39.03 ± 1.90*	32.20 ± 3.73
Mean corpuscular volume, fL	62.92 ± 2.56	61.38 ± 2.48	65.36 ± 3.01	65.49 ± 2.73	64.12 ± 3.00	62.34 ± 2.50	67.92 ± 2.96
Mean corpuscular hemoglobin, pg	21.90 ± 1.24	19.20 ± 1.13	20.56 ± 2.05	19.80 ± 1.64	19.87 ± 1.35	19.90 ± 1.52	18.60 ± 0.98

Ending tabl. 6

Parameters		Surolan	Otifri	Otoxolan	Surolan + +Far	Otifri + Far	Otoxolan + +Far	control
Mean corpuscular hemoglobin concentration, g/dL	30 days	40.64 ± 2.26*	41.60 ± 2.34*	40.25 ± 1.79*	41.61 ± 2.14*	39.94 ± 2.03*	40.62 ± 2.05*	30.40 ± 2.18
Platelets, 103/μL		195.73 ± 11.88*	198.06 ± 13.38*	200.51 ± 15.70*	196.40 ± 19.72*	200.53 ± 15.76*	197.55 ± 18.02*	234.74 ± 23.82

Note. The data are represented as mean ± SD. \* – statistically significant difference between experience and control ( $p < 0.05$ ).

Source: compiled by I.R. Olabode, N.P. Sachivkina, S.Y. Smolentsev.

On the 30th day of our experiment, statistically significant differences between 5 experimental groups and one control group are visible in the level of platelets & mean corpuscular hemoglobin concentration & total white blood cells & hematocrit in rabbits that received daily therapy in all 6 groups. In fact, the blood parameters of rabbits in the experimental groups returned by day 30 to the level of hematological indicators before the experimental infection. This return of indicators was observed especially quickly in the group with the addition of Far.

## Conclusion

The rabbit model of otitis ear with *Malassezia pachydermatis* taken from dogs was successful and clinical signs of the disease were observed: loss of appetite or body weight, erythema, itching, the release of abundant ear secretions (cerumen) of a yellow-brown color, often with an unpleasant odor. It was found that the use of drugs in combination with Farnesol in animals of the experimental group reduced the signs of hyperemia, edema, itching, the amount of exudate already on the 5th–7th day of treatment, and complete clinical recovery of animals occurred, on average, on the 20th day of the experiment. When using commercial drugs (Surolan; Otifri; Otoxolan) in monotherapy in animals, on average, an improvement in the clinical condition occurred on the 25th day, and final recovery occurred only after a full course of treatment — 30 days. However, when using Otifri, the clinical manifestation of MO persisted throughout the experiment. Animals of the control group also retained clinical signs of the disease throughout the experiment. Their condition worsened in the dynamics of the study, which proves the effectiveness of the MO model in rabbits developed by us. Analyzing the obtained results, it can be said that all treatment regimens with the addition of Farnesol were effective due to its broad spectrum of action against microorganisms that most often cause otitis, as well as the anti-inflammatory effect. It is worth noting that in the experimental groups there was not a single case of the presence of MR in smears and on nutrient media after combined therapy with Surolan / Otifri / Otoxolan in combination with Farnesol, already on the 20th day of therapy. At the same time, in the control group, all animals were observed DPG during microscopy of smears of ear exudate and there was growth on special nutrient media in high concentrations throughout the experiment. It is important to note that when

adding Farnesol to any drug, microbiological purification from MR occurred, on average, 5–10 days earlier. At the same time, the best result in this series of experiments was with a combination of Otoxolan + Farnesol, since complete sanitation of the ears from MR was recorded in animals of this group already on the 10th day of therapy. Clinical blood parameters of rabbits with MO before treatment were characterized by low values of the red blood cell count —  $5.20 \pm 0.34$   $10^6/\mu\text{l}$  and hemoglobin —  $9.18 \pm 1.07$  g/dl. It was clearly shown that on the 5th-10th days of the experiment, statistically significant differences between the experimental groups and the control group were not observed. On the 15th day of our experiment, statistically significant differences between the experimental and control groups were visible in the platelet level in rabbits receiving daily therapy with Otoxolan + Farnesol. On the 20th, differences were visible in the platelet count and the average concentration of corpuscular hemoglobin in rabbits receiving daily therapy with combinations of Otoxolan + Far; Surolan + Far; Otifri + Far. On the 25th day, differences were recorded in the platelet count, mean concentration of corpuscular hemoglobin and total number of leukocytes in rabbits receiving daily therapy with Otoxolan + Far; Surolan + Far; Otifri + Far and Otoxolan in monotherapy. On the 30th day of our experiment, statistically significant differences were recorded in the platelet level, which was 16.62...14.57% lower in the experimental groups receiving treatment, when compared with the control group. A significant difference was also noticeable when measuring the total number of leukocytes. Moreover, their greatest decrease (by 36.36...39.25%) was observed in the groups receiving Farnesol. The number of erythrocytes in all experimental groups varied in values close to the control, however, significant changes were observed in the hematocrit values, which increased by 27.42% in the group receiving Surolan therapy, by 21.89% — Otifri, by 24.25% — Otoxolan, by 27.64% — Surolan + Far, by 25.96% — Otifri + Far, by 21.21% — Otoxolan + Farnesol, when compared with the control group. This calls for further investigation to clarify the role of *Malassezia* in dermatological disorders and the potential of new treatment approaches.

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## Изучение показателей крови у кроликов с отитом, вызванным *Malassezia pachydermatis*, и влияние фарнезола на показатели выздоровления при добавлении в схему лечения

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**Аннотация.** В связи с широким распространением малассезиозного отита у животных предложена экспериментальная модель данной патологии для отработки эффективной фармакотерапии и разработки протокола лечения. В исследовании задействовано 35 кроликов, животных разделили

на 7 групп по 5 кроликов в каждой (порода «Советская шиншилла», самцы). Каждая группа получала один из следующих препаратов: Суrolан; Отифри; Отоксолан; Суrolан + Фарнезол 200 мкМ/мл; Отифри + Фарнезол 200 мкМ/мл; Отоксолан + Фарнезол 200 мкМ/мл; контроль. Все препараты наносили на всю пораженную поверхность ушной раковины. Обработку проводили один раз в день, продолжительность лечения составила 30 суток. Установлено, что применение лекарственных препаратов в комплексе с Фарнезолом у животных опытной группы уменьшало клинические признаки заболевания; быстрее наступала элиминация грибов в мазках; клинические показатели крови кроликов улучшались.

**Ключевые слова:** *Malassezia pachydermatis*, экспериментальный отит, клинические признаки, анализ крови, кролики

**Вклад авторов:** концепция, методология — Н.П. Сачивкина; валидация и проведение исследования — И.Р. Олабодэ; работа с данными — С.Ю. Смоленцев. Все авторы прочитали окончательную версию рукописи и согласны с ней.

**Заявление о конфликте интересов.** Авторы заявляют об отсутствии конфликта интересов.

**История статьи:** поступила в редакцию 7 декабря 2024 г., принята к публикации 21 декабря 2024 г.

**Для цитирования:** *Olabode I.R., Sachivkina N.P., Smolentsev S.Y. A study of blood parameters in rabbits with otitis caused by Malassezia pachydermatis and the effect of Farnesol on recovery rates when added to the treatment regimen // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. 2024. Т. 19. № 4. 707—719. doi: 10.22363/2312-797X-2024-19-4-707-719*

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