IMPACT ON THE GROWTH PARAMETERS AND MICROBIAL POPULATIONS OF PROBIOTICS AND PREBIOTICS ON RABBITS RAISED IN THE HOUSEHOLD SYSTEM

Andrei Radu SZAKACS³, Maria-Catalina MATEI LATIU¹, Laura Cristina STEFANUT¹, Victoria BUZA¹, Sorana MATEI¹, George NADAS¹, Dan PALCU¹, Adrian MACRI¹

e-mail (first author): andrei.szakacs@usamvcluj.ro

Abstract

The investigations and laboratory determinations were performed within the disciplines of Nutrition and Microbiology of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. The breeding of rabbits and the collection of samples took place in the own household from Sărata locality, Bistrita-Năsăud county. The aim of this study was to demonstrate the impact of the use of probiotics and prebiotics in feeding rabbits raised in the household system. For this experiment, 12 rabbits, common breed, 55 days old, raised in the household system were used. The basic feed ration was a commercial concentrated, ground feed in which the probiotic (Enteroferm® 35G), 0.15 g/kg feed, containing cultures of Enterococcus faecium, the prebiotic (Inulin FOS, ZENYTH), were incorporated, 25 g / kg fodder, powder obtained from chicory root (Cichorium intybus), which contains polysaccharides with a prebiotic role (inulin and fructooligosaccharide - FOS) and a synbiotic (mixture of the two), in the same doses. There were 4 groups of 3 individuals as follows: group number 1, control, group number 2, probiotic, group number 3, prebiotic and group number 4, synbiotic. In addition to the concentrated feed, good quality natural hay and water were added, both the feed and the water were administered ad libitum. In order to achieve the proposed goal, the amount of feed consumed by each group and the weight of each rabbit were determined weekly for 8 weeks. Based on these data, the final average weight of each group, the total feed consumption of each group, the weekly weight increase for each group, the average weekly and final feed conversion rate were calculated. Samples were also collected from the rectum, before and after treatment with probiotics and prebiotics, to determine their impact on the composition of the bacterial flora at this level.

The determinations performed yielded clearly superior results in favor of the experimental groups compared to the control group, highlighting group 2, consisting of rabbits treated with probiotics.

Key words: rabbits, probiotics, prebiotics, synbiotics

INTRODUCTION

Nowadays, rabbits are considered to be an important source of animal protein used in human consumption. They represent a unique segment in which they can be seen as livestock that is easy to manage. Also, the rabbits are highly prolific animals, with a short interval between generations. However, the costs implicated in the growth of the rabbits are very high (Adeyemo et al., 2013).

In the past, different additives were added to the daily food intake, in order to increase the food conversion rate and to obtain a good growth performance. From those additives, antibiotics were used in a high amount. However, in the growing context of antibioresistance, in January 2006, the European Commission forbiden the usage of antibiotics as growth promoters for animals, according to the Reglementation (EC) 1831/2003 (Reg EC 1821/2003). In this context, different alternatives were used with the same expected results for the growth performances, health status, or production performances, like antibiotics. In this category are included probiotics (Falcao-e-Cunha et al., 2007), prebiotics and sinbiotics. The term "probiotic" is defined as a mixture of live microorganisms that are able to provide a health benefit to the host when they are administered in adequate amounts (FAO/ WHO, 2002; Schmitz et Suchodolski, 2016). Prebiotics are composed of selective fermentative ingredients that are able to produce changes at the level of gastrointestinal microbiota (Gibson et al., 2010). The combination between the probiotics and prebiotics represents the synbiotics (Schmitz et Suchodolski, 2016). At present, probiotics are used on a large scale as growth promoters in animal husbandry. The advantage of those formulas is that the presence of residues in animal products used in human consumption is avoided, compared with antibiotics (Sherif, 2018). Moreover, it was demonstrated that probiotics are able to provide a

³ University of Agricultural Sciences and Veterinary Medicine Cluj

health benefit to the rabbits, together with enhancing their growth performance (Bhatt et al., 2017). One of the main mechanisms of action of probiotics is represented by their capacity to improve the microbial intestinal balance of the host. Due to this ability, the microbiota present in the caecum of the rabbits is able to break down the cellulose, pectin, ammonia, urea, and the proteins from the small intestine (Sherif, 2018), improving the digestion and, at the same time, the growth performances.

The majority of the studies that researched the influence of different additives on the growth performances of the rabbits were performed on breeds designed for meat production, in an intensive breeding program, as specified for the industrial systems. The advantages of those systems are represented by the ability to have strict control over the feeding conditions and over the microclimate. However, in our country, the intensive breeding programs that use rabbits from meat breeds are neglected. Those systems are replaced by small household breeding systems. Unfortunately, the data regarding the influence of different additives (as probiotics, prebiotics, or synbiotics) on the rabbits' growth performances in this type of system are lacking. The aim of the present study was to evaluate the impact of probiotics, prebiotics, and synbiotics on the growth performances of the rabbits from household breeding systems.

MATERIAL AND METHOD

Study design

The present study was realized during 8 weeks with 10 day of adaptation. A total number of 12 rabbits, males, and females, aged between 55-60 days, clinically healthy were enrolled in the study. In the first 10 days of the study, the rabbits benefited from a period of accommodation. On day 11. rectal samples were collected (2samples/group), in order to characterize the bacterial microflora resident at this level before the supplements administration. Also, the rabbits were weighed, and the initial mean weight was determined for each group. During the 8 weeks of the study, the rabbits were weighed once a week, and the mean weight for each group was calculated. At the same time, food consumption was monitored. At the end of the study, rectal samples were once again collected in order to compare the results with the initial ones. The weekly body weight gain for each group and the food conversion rate for each group were calculated. The study was approved by the Bioetic Commission of USAMV Cluj-Napoca with no. 254 /2021.

Biological material

Twelve rabbits, males, and females, mixed breed (Californian and English butterfly variety) were included in the study. The animals were randomly divided into 4 groups (3 animals / group). The animals were obtained immediately after weaning, at the age of 45-50 days, from a local breeder. Before the acquisition, a complete clinical examination was performed in order to select only the clinical healthy individuals.

Probiotic, prebiotic and synbiotic administration

The combined feed was represented by a commercial formula produced by S.C. PISANO GROUP S.R.L., presented as pellets, which were processed further by grinding, in order to allow the mixing between the food and the powder additives. The preparation of the mix between the food and the probiotic/prebiotic/synbiotic was realized manually, using a kitchen scale, a large capacity enameled vessel, and a hull for homogenization. After the homogenization, the ratio for each group was stored in plastic recipients, with sealing systems.

The used probiotic was composed of a single bacterial strain - *Enterococcus faecium* (Enteroferm® 35G). The product was administered in a quantity of 0,15 g for each kilogram of food (Kalma et al., 2016). The prebiotic was represented by chicory root (*Cichorium intybus*) (Inulin FOS by ZENYTH) and was administered in a quantity of 25 g for each kilogram of food (Juskiewicz, 2008). The symbiotic was represented by the combination between the probiotic and the prebiotic, in the same concentrations, and the administration followed the same protocol as for the other two groups

RESULTS AND DISCUSSIONS

Analysis of growth and development parameters

Mean body weight of the rabbits at the beginning of the study ranged from 1170 to 1257 g, at the end of the study reaching values between 2497 and 2858 g. Upon completion of the experiment, significantly greater body weights were recorded in the experimental groups, compared to control group. As can be seen in Table 1, the group 2, treated with probiotics, recorded the highest body weight gain (1643.26 g), followed by group number 4 (1601.65 g), treated with synbiotics, then group 3, treated with prebiotic and finally control group number 1.

In this context, we specify that the total body weight gain of group 2 was achieved with a total consumption of 23027 g of feed (table 2). In the case of group 4, the total body weight gain was achieved with a lower total feed consumption (22688 g) compared with second group. Analysis of the feed conversion rate evolution (table 3) shows fluctuations depending on the week, but the final average values indicate the best value for group 2 treated with probiotics (4.67 g of feed consumed / g body weight gain), followed by group 4 treated with symbiotics (4.72 g of feed consumed / g body weight gain).

Dynamics of microbial populations

Following the inoculation of samples collected before the start of treatment with and symbiotics, probiotics, prebiotics the appearance of bacterial colonies was polymorphic. Developed colonies were large, medium and small, of type S or R, white or yellow, morphological characters found in each of the 4 groups. Following the bacterioscopic examination, Gram + cocci were identified in 80% of the analysed microscopic preparations, being arranged in piles (in the form of clusters) and with an alveolar appearance in the microscopic field. These characters are specific to germs of the genus Staphylococcus. In 15% of the microscopic preparations were identified Gram + bacilli, unsporulated, morphological characters that can most likely include these germs in the genus Bacillus. In the remaining 5% of the preparations were identified coccobacilli, Gram-, lactose negative, represented by Escherichia coli.

Following the seeding of the samples collected at the end of the study, a different aspect of the colonies was observed. Medium and small, white and white to gray S and R type colonies developed. Colonies with yellow pigmentation were present, in small numbers, in groups 1 (control) and 3 (treated with prebiotics). The population of germs of the genus Staphylococcus was considerably reduced, they were identified in 15% of the preparations, in the largest proportion in the control group (group 1) and in smaller numbers in groups 2 (treated with probiotic) and 3 (treated with prebiotics). The control group presented a bacterial flora composed of 50% germs of the genus Streptococcus, cocci, Gram +, grouped in long chains, 30% bacteria of the genus Staphylococcus and 20% Escherichia coli. The rabbits in group 2, which were given the probiotic, showed a bacterial flora composed of 70% bacteria of the genus Streptococcus. From the rabbits in group 3, to which the prebiotic was administered, a bacterial flora composed of 80% Escherichia coli and 20% germs of the genus Staphylococcus was identified. From group number 4, treated with symbiotics, a bacterial flora composed of 90% *Escherichia coli* and 10% germs of the genus *Streptococcus* was identified.

In the context of what was observed in our study, Yamani et al. (1992) showed that the administration of probiotics to rabbits has beneficial effects on body weight and body weight gain. The same aspects are reported in 2004 by Amber et al., where after using *Lactobacilus acidophilus* supplements, the average daily gain of tested animals was greater with 9.6% compared to controls. At the same time, it has been observed that the administration of prebiotics and symbiotics significantly increases the body weight gain and the conversion rate of food (Ewuola et al., 2011).

The correlative analysis of the obtained data shows that the administration of probiotics, prebiotics and symbiotics modified the structure of the microbial population at the level of the rectum, with beneficial effects on the parameters of growth and development.

CONCLUSIONS

The results obtained indicate a favorable impact of probiotics, prebiotics and synbiotic on the growth indicators and on the microbial population from intestine of rabbits raised in the household system.

REFERENCES

- Adeyemo A.A. O.A.Adeyemi. D.A. Ekunseitan. O.S Taiwo. 2013 - Effect of concentrate to forage ratio on the performance and Haematological parameters of growing rabbits. G.J B.A.H.S.. Vol.2 (2) 2013:114-118
- Amber, K. H., Yakout, H. M., & Hamed Rawya, S. 2004 - Effect of feeding diets containing yucca extract or probiotic on growth, digestibility, nitrogen balance and caecal microbial activity of growing New Zealand White rabbits. Proceedings of 8th World Rabbit Congress, Puebla, Mexico (pp. 737-741).
- Bhatt. R. S., Agrawal. A. R., & Sahoo. A. 2017 Effect of probiotic supplementation on growth performance. nutrient utilization and carcass characteristics of growing Chinchilla rabbits. Journal of Applied Animal Research. 45(1). 304-309.
- Ewuola, E. O., Amadi, C. U., & Imam, T. K. 2011 -Performance evaluation and nutrient digestibility of rabbits fed dietary prebiotics, probiotics and symbiotics. International Journal of Applied Agricultural and Apicultural Research, 7(1&2), 107-117.
- Falcao-e-Cunha. L.. Castro-Solla. L.. Maertens. L.. Marounek. M.. Pinheiro. V.. Freire. J.. & Mourao. J. L. 2007 - Alternatives to antibiotic growth promoters in rabbit feeding: A review. World Rabbit Science. 15. 127-140.
- **FAO/WHO. 2002 -** Working group for drafting guidelines for the evaluation of probiotics in food

- Gibson G.R.. Scott K.P.. Rastall R.A.. Tuohy K.M.. Hotchkiss A.. Dubert-Ferrandon A., 2010 -Dietary prebiotics: current status and new definition. Food Scince and Technology Bulletin. Vol 7. 1–19;
- Juśkiewicz J., L. Ašmanskaitė, Z. Zduńczyk, P. Matusevičius, M. Wróblewska, A. Žilinskienė, 2008 - Metabolic response of the gastrointestinal tract and serum parameters of rabbits to diets containing chicory flour rich in inulin. Journal of Animal Physiology and Animal Nutrition 92, p 113– 120:
- Kalma R. P., Patel V. K., Joshi A., Umatiya R.V., Parmar K. N., Damor S.V., Chauhan H.D., Srivastava A. K., Sharma H. A., 2016 - Probiotic supplementation in rabbit: a review. International Journal of Agriculture Sciences, Vol. 8, Issue 53, p. 2811-2815
- Schmitz S. J., Suchodolski, 2016 Understanding the canine intestinal microbiota and its modification bt

pro-. pre- and synbiotics- what is the evidence?. Veterinary Medicine and Scienc. vol 2. 71- 94;

- Sherif Sara Khalil. 2018. Effect of Dietary Additives on Growth Performance. Carcass Traits and Some Blood Constituents of Rabbits. Journal of Agricultural Science; Vol. 10. No. 1
- Yamani, K. A., Ibrahim, H., Rashwan, A. A., El-Gendy, K. M. 1992 - Effects of a pelleted diet supplemented with probiotic (Lacto-Sacc) and water supplemented with a combination of Probiotic and acidifier (Acid-Pak 4-Way) on digestibility, growth, carcass and physiological aspects of weanling New Zealand White rabbits. Journal of Applied Rabbit Research, 15, 1087-1100.
- https://eur-lex.europa.eu/legal-
- content/RO/TXT/PDF/?uri=CELEX:32003R1831&f rom=RO
- ***ftp://ftpfaoorg/es/esn/food/wgreport2pdf 2002

Table 1

Mean weekly weight gain (WG) and total weight gain (TWG) of the 4 experimental groups (grams)

Week 1	Week 2	Week3	Week 4	Week 5	Week 6	Week 7	Week 8	TWG
136.89	142.95	161.64	159.44	158.34	162.91	186.92	217.68	1326.78
176.64	187.60	218.99	208.95	238.06	211.97	188.39	212.67	1643.26
131.36	156.10	198.60	191.57	201.81	200.86	200.98	196.86	1478.12
159.33	167.08	184.55	187.54	202.47	205.00	240.17	255.51	1601.65
	136.89 176.64 131.36	136.89 142.95 176.64 187.60 131.36 156.10	136.89 142.95 161.64 176.64 187.60 218.99 131.36 156.10 198.60	136.89142.95161.64159.44176.64187.60218.99208.95131.36156.10198.60191.57	136.89142.95161.64159.44158.34176.64187.60218.99208.95238.06131.36156.10198.60191.57201.81	136.89142.95161.64159.44158.34162.91176.64187.60218.99208.95238.06211.97131.36156.10198.60191.57201.81200.86	136.89142.95161.64159.44158.34162.91186.92176.64187.60218.99208.95238.06211.97188.39131.36156.10198.60191.57201.81200.86200.98	136.89142.95161.64159.44158.34162.91186.92217.68176.64187.60218.99208.95238.06211.97188.39212.67131.36156.10198.60191.57201.81200.86200.98196.86

Table 2

Weekly and total feed consumption of each group (grams / group)

	Week 1	Week 2	Week3	Week 4	Week 5	Week 6	Week 7	Week 8	Total
Group 1	1825	2385	2781	3145	3261	3384	3578	3892	24251
Group 2	1909	2251	2887	2924	3177	3304	3167	3408	23027
Group 3	1814	2545	2906	3370	3629	3789	4002	4317	26372
Group 4	1810	2138	2428	2638	3044	3242	3521	3867	22688

Table 3

Feed conversion rate (FCR) in the experimantal groups for 8 weeks and total period (TFR)

	Week 1	Week 2	Week3	Week 4	Week 5	Week 6	Week 7	Week 8	TFR
Group 1	4.43	5.66	5.81	6.50	6.84	6.94	6.39	5.99	6.09
Group 2	3.60	4.03	4.42	4.65	4.43	5.18	5.60	5.33	4.67
Group 3	4.66	4.79	4.82	5.02	5.23	5.51	5.27	5.77	5.95
Group 4	3.78	4.27	4.39	4.69	5.01	5.28	4.93	5.09	4.72

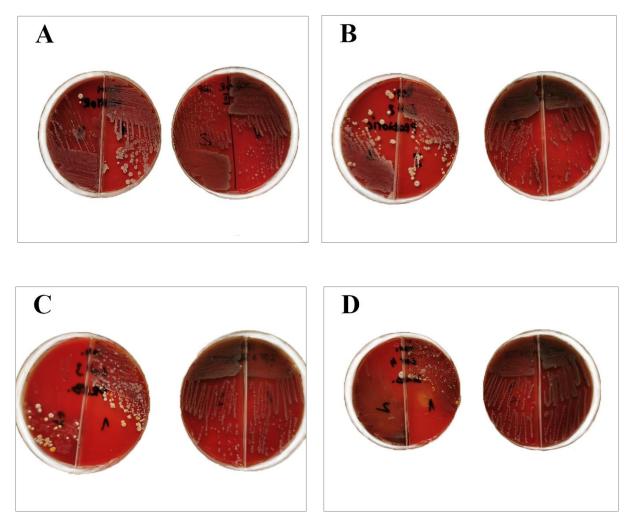


Figure 1 The morphological aspect, ante-therapeutic (left) and post-therapeutic (right), of the bacterial colonies: A. Lot 1 (control); B. Lot 2 (probiotic); C. Lot 3 (prebiotic); D. Lot 4 (synbiotic).