Journal of
Food and Nutrition

JScholar
Fostering Scholarly Communication

Research Open Access

Are They What They Eat? The Influence of Feed on Helix Aspersa's Microbiota

Yuste A*, Arosemena EL, Nogué E, Calvo MA

Research Group in Applied and Environmental Microbiology, School of Veterinary Medicine. Autonomous University of Barcelona. Cerdanyola del Vallés, Barcelona, Spain

***Corresponding author:** Aida Yuste Parareda, Research Group in Applied and Environmental Microbiology, Faculty of Veterinary Science. Autonomous University of Barcelona. Cerdanyola del Vallés, Barcelona, Spain. Tel: 608113096, Email: aidayuste@gmail.com

Received Date: January 19, 2019 **Accepted Date:** February 27, 2020 **Published Date:** March 03, 2020

Citation: Yuste A (2020) Are They What They Eat? The Influence of Feed on Helix Aspersa's Microbiota. J Food Nutr 6: 1-5.

Summary

Helix aspersa is the terrestrial and edible snail. For that reason, it is essential that the health status of these animals is the most suitable. One of the most important variables to achieve this is feed hygiene. This study is an analysis of the hygiene of two different types of feed over time and the evolution it causes in the microbiota of the animals through the analysis of the females.

The three feeds have the same blackberry flour base. One without supplements, another one will be enriched with probiotic through the addition of the *Lactobacillus plantarum* CA-7 lyophilized strain, and the last one was added the same probiotic plus thymol and oxalic acid. But one of them will be enriched with a probiotic (*Lactobacillus plantarum* CA-7) and in the other, apart from the probiotic, oxalic acid and thymol will also be added.

The results of the evolution of the microbiota over time vary because in the short term the feed that presents a better recount of acid-lactic bacteria is the only enriched with probiotics, but in the longterm, there is a change of tendency and the one that causes this increase is the one that contains probiotic, oxalic acid and thymol.

All in all, it must be considered that the feed presents deficient hygiene due to the high presence of growth in the fungi plate. Therefore, the results of this study could vary with a feed with good hygiene.

Keywords: Snails; probiotics; feed.

Introduction

Helix aspersa is a Mollusca gastropod terrestrial of mainly Mediterranean origin that belongs to the Helicidae family. Range from 20 to 40 mm in height from 24 to 45 mm in amplitude [1].

The heliciculture consists in the breeding in captivity of edible terrestrial snails.1It is a very important agricultural sector in our country since it is estimated that 16 million kilos of snails are consumed per year, being the second country of the world consumer of these animals [2]. It is a food that contains great nutritious power and is very rich in proteins and minerals such as calcium and magnesium [2,3].

Heliciculture may be intensively or extensively exploited. It is important that the breeding environment is fully controlled with a temperature between 18-20º C, a humidity of 75- 90% and a photo period of 12 hours of light and 12 hours of darkness; but it is also important to maintain strict control of feed hygiene [3].

At the time of feeding, it must be borne in mind that they are phytophagous animals, that is to say, they are based on vegetarian products [4] But in the case of farms, they base the feeding of snails on a feed of flour of different types of cereals and fodder, of very fine texture[3] Nowadays, functional food is also taken into account which provides benefits to the physiological functions of the organism such as the cases of freeze-dried probiotics or plant extracts, apart from the nutritional value [5]. These probiotics can displace pathogenic microorganisms through different mechanisms of action [6]. *H. aspersa* is not exempt from various pathologies associated with microorganisms, the main one being caused by *Pseudomonas aeruginosa* that can provoke high mortality; but it can also be affected by other etiological agents such as *Escherichia, Klebsiella, Listeria, Clostridium,* among others [3,7] In the case of suffering a pathology, it is seen an increase of the bacteria that belongs to the Enterobacteriaceae family while it is seen a decrease of the acid-lactic bacteria which are the ones that constitute the most the intestinal microbiota of these animals [3,7].

It can also be affected by parasites such as Riccardoella limacum (Schrank, 1776) giving rise to one of the most serious snail diseases because it acts as an ectoparasite and feeds on the blood of the respiratory tissue of its host producing a reduction in growth and activity taxes causing the death of the animal [3]. Moreover, the transmission of this mite is very easy between animals and also between cargoes and the ground [8].

In one study it was observed that oxalic acid and thymol composts added to feed are ideal for combating acariosi [9].

The objective is to know what composition of the food causes an improvement in the health status of these snails through microbiological analysis of the faeces in order to assign it to the breeding and fattening granges of this animal. It will also make control throughout the time of the different feeds.

Material and Methods

Microbiological evaluation of three feeds of different composition distributed by a factory specialized in the production of feed in the province of Barcelona is analyzed.

The three feeds have the same base that is maize flour, corn, barley, and soya. One without supplements, another one will be enriched with probiotic through the addition of the *Lactobacillus plantarum* CA-7 lyophilized strain, and the last one was added the same probiotic plus thymol and oxalic acid.

This microbiological evaluation is performed with different culture media in a Petri dish to determine the presence or absence of different microorganisms. These culture media are:

1. Soy Trypticase Agar (TSA)
2. ManRogosa Sharpe agar (MRS)
3. Baird Parker agar (BP)
4. MacConkey agar(MK)
5. Sabouraud dextrose agar with chloramphenicol(S)
6. XLT4 agar

Figure. 1: Group of snails with a type of feed. Original Photo.

Weigh 6 grams of feed and make a 1/10 dilution with Lactose Broth and make serial dilutions upto-3. Sowon all plates dilution -1 and -3 except in XLT4 agar as the container where the Lactose broth has been added is incubated in the 37ºC stove for 24 hours and then sowed by immersion in Rappaport broth and incubated at 42ºC for 24 hours and then inoculated in the Petri dish with XLT4 agar. For each dilution, three plates are sown and thus the average is obtained to be statistically more representative. 18 adult snails are obtained from a production farm in the province of Barcelona. These 18 loads are distributed in three groups of 6 members each. Each group is given a different feed.

The analysis of the microbiota through the faeces is performed with different culture measures in Petridish to determine the presence or absence of different microorganisms. These culture media are:

1. Soy Trypticase Agar (TSA)
2. ManRogosa Sharpe Agar (MRS)
3. MacConkey Agar (MK)
4. Sabouraud dextrose agar with chloramphenicol(S)
5. XLT4 Agar

The stool is collected with a sterilized spatula and weighed all the faeces from the same group together to make a 1/10 dilution with Lactose broth. Serious dilutions are made until -8 and -3, -5, -7 and -8 are sown to all plates except XLT4 which follows the previous pattern. For each dilution, three plates are sown and thus the average is obtained to be statistically more representative.

TSA, MRS, BP, and MK plaques are incubated at 37ºC while S plaques are incubated at 28ºC. Both microbiological evaluations are performed once a week. In order to eliminate from the feed, the possible contaminants it is carried out treatment with UV light during a couple of hours in order to eliminate the undesirable microorganisms. As the probiotic is also eliminated, 10^9 CFU/kg is added.

Results

The results are expressed in the form of graphs comparing the CFU/mL of the different plates of the three feeds or of the feces obtained. The three feeds are represented like Feed (only the base), P feed (base with *L. plantarum*) and PTAO feed (base with *L. plantarum plusoxalic* acid and thymol).

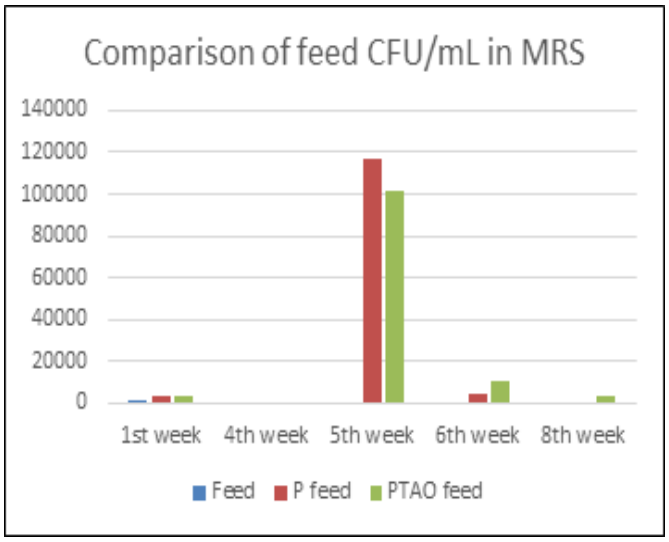

Figure. 2: Comparison of CFU/mL of MRS in 6 grams of three feeds over time.

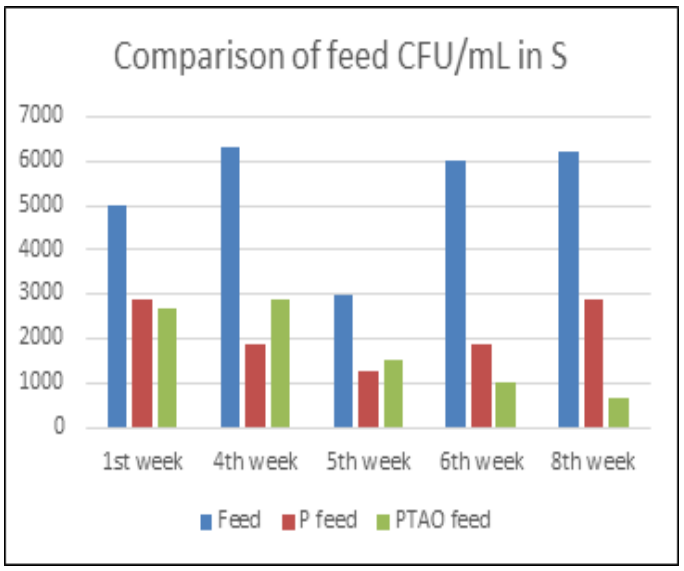

Figure. 3: Comparison of CFU/mL of S in 6 grams of three feeds over time.

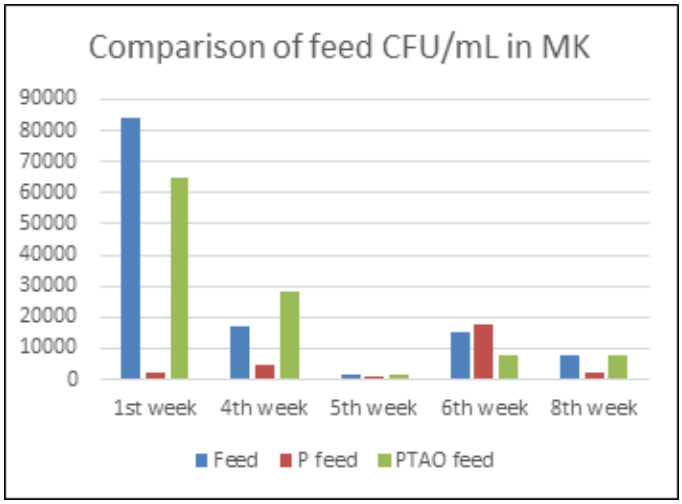

Figure. 4: Comparison of CFU/mL of MK in 6 grams of three feeds over time

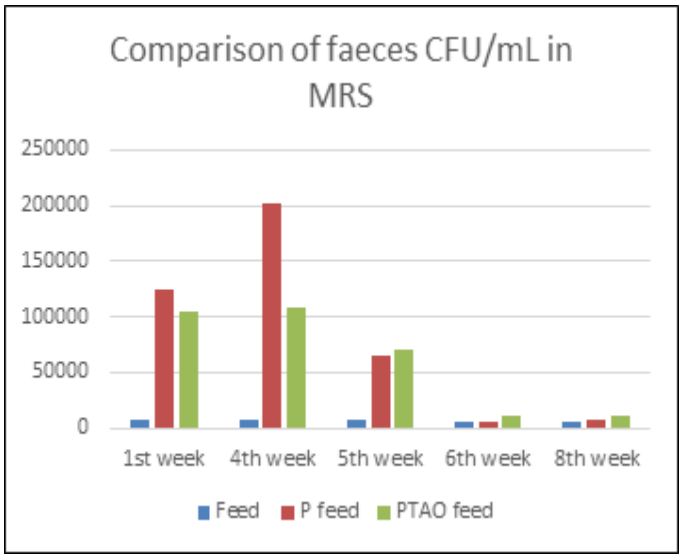

Figure. 5: Comparison of CFU/mL of MRS in faeces over time.

Discussion

At the beginning of the analysis, there were already very few colony-forming units on the MRS plates in three feeds and a few weeks later it was completely lost. Due to the null presence of acid-lactic bacteria in the five weeks, it was decided to make a treatment with UV light and the subsequent addition of previously lyophilized probiotics with a concentration of 10^9 CFU/kg in the feed with probiotic, but the preservation is deficient since after three weeks were lost again (see Figure 2).

This preservation can be affected by the high presence of fungi and for that reason, the acid-lactic bacteria were lost so quickly. It is seen a reduction of the colony-forming units in the fifth week when the treatment is carried out with UV light for two hours, but even though the reduction is not very marked, it is necessary to search for an antifungal compound (see Figure 3).

In the case of the feed formed by probiotic, oxalic acid and thymol at the beginning presented a high number of bacteria that belongs to the Enterobacteriaceae family in comparison to the other feed, but due to the treatment with UV light it is seen a reduction of these in both cases and, although they later increase, they remain in lower concentrations than in the beginning (see Figure 4).

The BP plate counts were negative during all weeks, that is to say, there was no contamination by Staphylococcus.

There was a problem when it came to ingest the fodder because the one that was composed of probiotic, oxalic acid and thymol, cost more, and even weeks that did not consume it. Therefore, the difference between this food and the other is in the presence of oxalic acid and thymol that can suppose a change in the taste or in the smell, is to say, in the organoleptic characteristics.

At first, the feed that caused an increase in the bacterial acid-lactate count was the feed enriched with probiotics and this factis maintained for a few weeks but, around week 6, there is a change in this trend inceitis an increase in the bacterial acid-lactic in the faeces of the animals that ingest the feed enriched with probiotic, oxalic acid and thymol see (Figure 5).

Conclusions

1. Feed hygiene is not adequate due to the high presence of fungi and Enterobacteriaceae.
2. The treatment with UV light is effective when finished, especially in the case of enterobacteria because later, although they increase, they remain at a low level.
3. The addition of probiotics is very effective at the moment, but then it is lost quickly, is to say, there is no preservation. Therefore, a study is necessary to obtain a new way of preserving the probiotic in the feed for longer periods of time.
4. It would be necessary to find a component that acts against fungi without being harmful to the health status of the animals because the treatment with UV light is not effective.
5. In the short term, feed enriched with probiotic has better results than feed enriched with probiotic, thymol and oxalic acid; but in the long term this trend is reversed and the one with better results is formed by probiotic, oxalic acid and thymol.
6. The feed with probiotic, oxalic acid and thymol has cost more to be ingested by the animals due to their organoleptic characteristics.
7. The population of fungi predisposes to a faster loss of acid-lactic bacteria.

References

1) Olivares CA (2005) (Mollusca: Gastropoda: Stylommatophora) in the IV region of Chile. Biociencias 13: 177-192.

2) El País (2018) Los caracoles step on the accelerator on the farm.

3) Vera RE (2016) Microbiology of the snail Helix aspersa Müller. Biotechnological applications for their health improvement with impact on their commercialization. Ph.D. Dissertation, UniversitatAutònoma de Barcelona, Bellaterra.

4) Gorain Gipuzkoa. Caracoles y Babosas.

5) Alvídrez-Morales, González-Martínez BE, Jímenez-Salas Z (2002) Trends in food production: Functional foods. RESPYN, 3.3.

6) Rodríguez JM (2015) Probiotics: from the consumer laboratory. Nutrición Hospitalaria 31: 33-47.

7) Kiebre-Toe MB, Borges E, Maurin F, Richard Y, Kodjo A (2003) EtudedelaflorebactérienneaérobieàGram négatif de l'escargotd'élevage (Helix aspersa). Revue de MédecineVétérinaire, 154:605-610.

8) Schüpbach HU, Baur B (2008) Experimental evidence for a new transmission route in a parasitic mite and its mucus-dependent orientation towards the host snail. Parasitology 135: 1679-1684.

9) Partnership J, Izquierdo J (2018) Anàlisi i tractament de l'acariosi caused by Riccardoellalimacum (Schrank, 1776) en grangesd'engreixd'Helixaspresa. Senior thesis, UniversitatPolitècnica de Catalunya, Barcelona.

greixd'Helixaspresa. Senior thesis, UniversitatPolitècnica de Catalunya, Barcelona.