

SUMMARY

of the doctoral thesis entitled:

STUDY OF THE INFLUENCE OF SOME BIOTECHNOLOGICAL PRODUCTS ON THE NUTRITION OF MONOGASTRIC SPECIES

PhD-student: **VLAD (GRIGORE) Daniela-Mihaela**

Scientific coordinator: **Professor, PhD BĂBEANU Narcisa Elena**

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This research is part of the PhD thesis "*Study of the influence of some biotechnological products on the nutrition of monogastric species*" within the Doctoral School "Vegetal and Animal Resources Engineering and Management" of USAMV Bucharest.

The valorization of waste and by-products from the food industry is a major challenge, intensively addressed from both sustainability and economic perspectives. Massive industrialization has led to the generation of considerable amounts of waste, and the brewing sector is no exception, producing large volumes of by-products, such as spent brewer's yeast and waste water, which are often insufficiently recovered. With efficient management and processing, these wastes can become a valuable resources, contributing to the development of new nutritional sources with high energy potential, useful not only in the livestock sector but also in other industries. Spent brewer's yeast, in particular, is a rich source of essential proteins, vitamins and trace elements with various applications in animal feed and in improving the quality of food of animal origin, contributing to a more sustainable and efficient food resource.

Microbial biopreparations based on yeasts of the genus *Rhodotorula* sp. are increasingly used due to their ability to produce natural pigments, such as carotenoids, with multiple industrial applications. These microorganisms have been studied for their potential to replace synthetic colorants in the food, cosmetics and pharmaceutical industries, while having a low environmental impact. The pigments produced by *Rhodotorula* sp. have antioxidant and antimicrobial properties, which give them additional benefits, including product stability and safety. Recent research has shown that culture conditions, such as environmental composition and growth parameters, can

significantly influence pigment production, providing opportunities for optimizing this process on an industrial scale.

Thus, by integrating spent brewer's yeast and *Rhodotorula* sp. based biopreparations in animal feed, a double objective can be achieved: valorization of waste resources and increased sustainability of agriculture and food industry. This approach contributes to reducing waste, optimizing industrial processes and developing healthier and more nutritious animal products.

The scientific research activity was carried out during 2018-2022 in several locations: INCDBNA Balotești, ICDCB Balotești, Microbiology Laboratory of the Faculty of Biotechnology, Nutrition Laboratory of the Faculty of Animal Production Engineering and Management.

The present work investigates the potential valorization of by-products and wastes from various industries, focusing on the utilization of microbial biopreparations and by-products from the brewing process. Spent brewer's yeast and waste water are generated in considerable quantities and contain valuable components including high quality proteins, B-complex vitamins, essential fatty acids and minerals (such as calcium, magnesium and phosphorus). These by-products have the potential to be used effectively in feed formulation, contributing to increased nutritional value and supporting zootechnical performance. In addition, microbial biopreparations obtained from yeasts of the genus *Rhodotorula* sp. are of particular interest due to their ability to synthesize carotenoid pigments (e.g. β -carotene, torulene and torularhodin), which possess antioxidant and antimicrobial properties.

These pigments have demonstrated beneficial effects on animal health and the quality of animal products by improving natural pigmentation and nutritional value. The integration of these biopreparations in animal feed can contribute to the development of more sustainable animal husbandry systems and maximize the added value of industrial waste, while contributing to the reduction of environmental impact.

The PhD thesis is structured in six chapters, and has been divided into two main parts. The first two chapters are based on current information from the literature. **The first chapter** of the thesis is dedicated to the economic importance of monogastric species, with respect to in monogastric species nutrition, including current trends addressed. **Chapter II** comprises a comprehensive overview of yeast biopreparations, their production techniques, including aspects of fermentation, extraction and conditioning for animal feed use.

Chapters III-V represent an original contribution by presenting in detail the possibility of obtaining, utilization and valorization of yeast biopreparations.

The aim of the PhD thesis was to obtain new innovative biopreparations and their inclusion in compound feed for monogastric animals, in particular broilers. In order to achieve the proposed aim, several variants of yeast biopreparations were obtained and tested, both brewery-derived subroot and yeast biopreparations of the genus

Rhodotorula sp. The resulting biopreparations were tested to assess their nutritional quality.

The specific objectives targeted were:

- identification of a microbial strain with pigmentation capacity;
- evidence of the effects of food industry waste utilization on microbial growth and carotenoid pigment production capacity under controlled culture conditions;
- separation and physico-chemical evaluation of brewery wastes;
- conditioning them with a view to their use in the livestock sector, more specifically in broiler broiler rations;
- to evaluate the effects of single administration and the interaction of the biopreparations obtained on the specific growth and development parameters of broiler chicks;
- to evaluate the effects of single administration and the interaction of the biopreparations obtained on the health status of broilers;
- to evaluate the effects of single administration and the interaction of the biopreparations obtained on the quality of chicken meat;
- evaluation of consumer perception of chicken meat quality.

Chapter III entitled "**Biopreparations obtained from yeasts belonging to the genus *Rhodotorula* sp.**" presents data on the isolation and morpho-cultural characterization of the yeasts, molecular identification of red yeasts by PCR-ITS RFLP technique, evaluation of the optimal biosynthesis parameters of the yeasts and the profile of pigments obtained. Total phenolic content and antioxidant activity of the obtained biopreparations were tested. The molecular identity of the candidate yeast showed 99.98% similarity to the genus *Rhodotorula mucilaginosa*. For the evaluation of the optimal biosynthesis parameters of *Rhodotorula mucilaginosa* yeast, three nutrient variants were proposed (variant 1-previously developed , variant 2-white potato peel and variant 3-sweet potato peel). Regarding the amount of β -carotene accumulated, $6040.79 \pm 247.95 \mu\text{g/L}$, obtained for variant 2, in 72 h of fermentation, was observed.

Chapter IV entitled "**Biopreparations obtained from protein biomass**", presents data on the physicochemical composition and conditioning techniques of waste from the brewing industry. Samples (brewer's spent yeast and waste water) were taken from the brewing of Blonde (BD), Pale Ale (PA) and Atizanal (BA) beer assortments. The pH, total dry matter (SU%), optical density OD 600 nm, chemical oxygen demand (COD), ammonium ion nitrogen content ($\text{NH}_4\text{-N}$), phosphorus from phosphate ($\text{PO}_4\text{-P}$) determination, total nitrogen Kjeldahl (NKT) and volatile fatty acid (VFA) profile. Brewer's residual yeast was analyzed physicochemically (pH, crude protein, total protein, total lipids, total fiber and total dry matter). Two methods of sample processing were proposed for the determination of the yeast aminoacid profile, namely by enzymatic hydrolysis (alkalase) and by mechanical cell wall destruction. The tested

variants had a high content of essential amino acids such as methionine (220.24-387.43 mg/L), lysine (304.66-415.54 mg/L) and leucine (607.95-802.75 mg/L).

In **Chapter V** entitled "**Studies regarding the biological activity of the biopreparates obtained**", experimental data are presented on:

- influence of the obtained biopreparations on the growth parameters of broilers;
- influence of the biopreparations obtained on the health status of broilers;
- influence of the obtained biopreparations on the quality of chicken meat;
- evaluation of consumer perception of chicken meat quality.

The main objective of this chapter was the inclusion of the two biopreparations obtained in the combined feed formula. Seven batches of broilers feed formulas, specific as for the starting, growing and finishing phases were obtained. Tests were carried out on a group of 320 broilers (Ros 308), during 42 days trial. Individual growth performance (live weight and average daily gain) was evaluated on days 0, 7, 12, 22 and 42. At the end of the experiment, chick health status (hematological and serum biochemical profile) was evaluated. On day 42, five chickens/batch were slaughtered to evaluate productive performance (carcass yield, carcass weight of major parts, carcass organ weight), carcass and chicken meat quality, respectively. There was a significant difference in serum glucose after feeding protein biomass biopreparations compared to all experimental groups. Furthermore, protein biomass biopreparations decreased carcass fat. Biopreparations from yeasts belonging to the genus *Rhodotorula* sp. had a significant effect on the color of chicken meat. In the perception of consumers, meat from chickens fed with biopreparations is more pigmented. In addition, meat from chickens fed with bioprepared protein biomass biopreparations showed significant differences for the parameters: tenderness, juiciness and odor.

The concluding chapter summarizes the data obtained on the production and valorization of yeast biopreparations. Experimental data on the biological activity of the biopreparations was similarly summarized. Multiple combined feed formulations have been developed and tested on the Ross 308 hybrid. In the consumer's perception, the appearance of poultry meat fat showed significant differences, in particular it showed a pleasant, more pronounced than characteristic pleasantness.