ABSTRACT

Key words: sources of unconventional feeds, vegetal by-products, medicinal plants, enriched eggs, amino acids, liposoluble micronutrients.

The PhD thesis entitled „**Research regarding the increasing of egg nutritional value**" has been developed by PhD student Eng. Maroş (Vărzaru) Iulia under the scientific coordination of Mr. Prof. Univ. Dr. Van Ilie, within the framework of the Doctoral School Plant and Animal Resources Management and Engineering, from the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

The experimental research performed during the PhD studies were conducted in the Laboratory of Chemistry and Nutrition Physiology, National Research Development Institute for Animal Biology and Nutrition (IBNA Balotesti).

The purpose of the thesis was to investigate the effect of using unconventional feed sources, low-cost and rich in PUFA, on amino acids digestibility from compound feeds and their recovery in eggs, as well as the possibility of enriching eggs in liposoluble micronutrients, by using medicinal plants / vegetal by-products from food supplements industry for humans.

The PhD thesis is structured into 8 chapters, including 106 tables, 49 figures, pictures and graphics, and a list of 219 references from books and scientific articles.

PART I BIBLIOGRAPHIC STUDY, has five chapters, as follows:

CHAPTER I EGG AND LAYING HEN NUTRITION highlights the nutritional value of eggs, as well as the importance of laying hen diet in increasing the egg nutritional value.

CHAPTER II PHYSIOLOGICAL AND NUTRITIONAL ROLE OF AMINO ACIDS describes the metabolic pathways of amino acids absorption, requirements and recommandations of amino acids for laying hen nutrition, raw feed materials, natural and synthetic, as sources of amino acids, and analytical methods for amino acids determination.

CHAPTER III PHYSIOLOGICAL AND NUTRITIONAL ROLE OF VITAMINS AND CAROTENOIDS presents the importance of liposoluble micronutrients in laying hen nutrition, requirements and recommandations as well as liposoluble micronutrients sources. Also contained in this chapter are issues related to the physiology of absorption, metabolism and excretion of liposoluble vitamins and carotenoids, and chemical methods used for determination of these micronutrients.

CHAPTER IV INDUSTRY DEVELOPMENT FOR ACHIEVING EGGS WITH ENRICHED NUTRITIONAL QUALITIES describes the functional food concept, types of functional

components / functional food, and the evolution of functional food market. There are also presented different ways of obtaining eggs enriched in vitamins, and a study regarding methods of amino acids digestibility assessment from feeds and their profile in eggs.

CHAPTER V STUDIES ON THE USE OF NEW UNCONVENTIONAL FEEDS IN LAYING HENS NUTRITION presents researches regarding the use of unconventional feeds in laying hen nutrition, recommended for their remarkable nutritional value.

PART II OWN RESEARCH contains three chapters which present the materials and methods used in order to achieve the objectives, experimental research performed in order to investigate the effects of selected unconventional feeds on increasing the egg nutritional value, obtained results, general conclusions and recommandations regarding the use of tested unconventional feeds in laying hen nutrition.

CHAPTER VI RESEARCH METHODOLOGY describes the materials and methods used to accomplish goals. Also it presents the developments and validations of two analytical methods for amino acids, and lutein and zeaxanthin determination from samples of animal and vegetal origin, by using high performance liquid chromatography (HPLC).

CHAPTER VII RESULTS AND DISCUSSIONS contains the six experiments conducted on laying hens, as follows:

- **Experiment 1** has investigated the influence of feeds rich in PUFA (linseeds and camelina seeds), as well as an antioxidant source (fenugreek seeds), on amino acids digestibility from compound feeds in which were included. The control group received a conventional diet based on corn - soybean meal. The experimental diets were enriched in PUFA by using 5% linseed and 2 % camelina seeds (E1); 2 % linseed, 3 % camelina seed and 1 % fenugreek seed (E2). The digestibility coefficients of the amino acids were significantly ($P \leq 0.05$) lower for E2 group, when compared with the control. The concentrations of sulfur amino acids (cystine and methionine) from albumen decreased significantly ($P \leq 0.05$) in experimental groups (E1 and E2) compared with the control group (C).
- **Experiment 2** was aimed to study the effects of camelina and linseed meal included in compound feeds for laying hens, on amino acids digestibility. The control group received a conventional diet based on corn - soybean meal. In the experimental diets, 5% linseed meal and 2 % camelina meal (E1); 3% linseed meal, 3 % camelina meal and 1 % fenugreek seed (E2) were included. Essential amino acids for laying hen nutrition registered significant decreases of the digestibility coefficients from E1 and E2 compound feeds, decreases which were also observed at the albumen level.

- **Experiment 3** studied the effect of copper addition on amino acid digestibility from compound feeds enriched in PUFA, by inclusion of by-products resulted from the oil industry (camelina and linseed meal). The basic structure of the compound feeds was the same for all 4 groups used in experiment (conventional compound feed in which were included 5 % linseed meal and 2 % camelina meal). In the E2 and E3 diets, 100 mg/kg Cu and 150 mg/kg Cu respectively were included. Results showed digestibility coefficients significantly ($P \leq 0.05$) lower for almost all the studied amino acids, in case of E1, E2 and E3 groups, compared with C group. Between the experimental groups no significant differences were recorded.
- **Experiment 4** aimed to solve the problem identified in experiment 2 and unsolved in experiment 3 by copper addition. The experiment investigated the effect of addition in compound feeds of cellulolytic enzyme with or without copper, on amino acids digestibility. Experimental diets (E1 and E2) differed from the control diet by the inclusion of 5 % linseed meal and 2 % camelina meal. In E2 and E3 diets, 0.0125 % cellulolytic enzyme was included. Besides the enzyme used also in E2 group, in E3 diet 150 ppm Cu were added. The digestibility coefficients of essential amino acids increased in groups E2 and E3 compared to C, with the most pronounced increases being observed for E3 which was distinguished from E2 by copper supplement. Consequently, the highest concentrations of essential amino acids from albumen were noted in E3 group, being significantly ($P \leq 0.05$) different from E1 and E2 groups.
- **Experiment 5** was conducted in order to evaluate the effects of two medicial plants included in the compound feeds for laying hens, on lutein and zeaxantin, vitamin A and vitamin E concentrations from eggs. Twelve plants, known in traditional medicine for their pharmacological effects in different diseases, including inflammatory eye disorders, were chemically characterized in order to assess their nutritional value. Pot marigold and St John's wort were selected for the feeding trial. The inclusion in laying hens diets of 5 % pot marigold (E1) and 5 % St John's wort (E2) caused a significant ($P \leq 0.05$) increase of lutein and zeaxanthin concentrations with 9.8 % (E1) and 14.9 % (E2), of vitamin E concentrations with 20.3 % (E1) and 53.4 % (E2), and of vitamin A concentrations with 56.1 % (E1) and 155.1 % (E2), compared to C, after 21 days.

Experiment 6 aimed the recovery of several mixtures based in vegetal by-products, resulted from the industry of food supplements for humans, in laying hens nutrition, in order to increase the xantophylls concentrations in egg. Four vegetal mixtures containing plants rich in xantophylls were analyzed, and two of them were included in the compound feeds for laying hens (E1 – 2 % vegetal mixture no. 2, E2 – 1 % vegetal mixture no. 4, E3 – 2 % vegetal mixture no. 4). After 14 days of experiment, lutein and zeaxanthin concentrations increased significantly ($P \leq 0.05$) in E1, E2, E3 compared to C. Data regarding the lutein and zeaxanthin content in

vegetal mixtures, compound feeds and egg yolks were strongly correlated and highlight the efficient utilization of the vegetal mixtures for obtaining eggs enriched in lutein and zeaxanthin.

CHAPTER VIII GENERAL CONCLUSIONS AND RECOMMENDATIONS

- Amino acids digestibility assessment from feeds showed that enriched compound feeds in PUFA, due to the inclusion of linseed and camelina seed with / without fenugreek seeds, caused a decrease of amino acids digestibility coefficients, which were generally lower than C group, and significantly ($P \leq 0.05$) lower for essential amino acids, compared to C.
- Reduction of amino acids digestibility from feeds caused by the inclusion of 5 % linseed meal and 2 % camelina meal in the compound feed, negatively influenced the egg quality by diminishing the concentrations of essential amino acids.
- Copper addition (100 ppm / 150 ppm) in compound feeds enriched in PUFA by 5 % linseed meal and 2 % camelina meal, did not solve the negative effects of the oily byproducts on amino acids digestibility from compound feeds.
- The inclusion of 5 % linseed meal, 2 % camelina meal, 0.0125 % enzyme and 150 ppm Cu in the compound feed, led to higher amino acids digestibility coefficients (significantly ($P \leq 0.05$) higher for essential amino acids), than conventional compound feed, enriched compound feed by 5 % linseed meal, 2 % camelina meal, and also compared to the compound feed wich included 5 % linseed meal, 2 % camelina meal 0.0125 % enzyme.
- Using in laying hens diet of pot marigold and St John's wort / vegetal by-products from the industry of food supplements for humans led to an increase of liposoluble micronutrients concentrations in egg yolk.
- Comparative evaluation of the two experiments which aimed to increase liposoluble micronutrients content in egg, revealed a higher bioavailability of xantophylls from vegetal by-products showing upper concentrations of lutein and zeaxanthin in egg yolk.
- Beside the bioavailability advantage, using vegetal by-products brings economic benefits and can be considered low-cost source of bioavailable micronutrients with positive impact on feed cost.

Nutrition is the way the egg can be nutritional enriched in a naturally manner.

This PhD thesis provide a high degree of novelty, due to the topics addressed and the solutions proposed: a study of amino acids digestibility from compound feeds enriched in PUFA by the inclusion of unconventional feed sources, and also ways to increase amino acids digestibility from these feeds; the use of medicinal plants / recovery of vegetal by-products from

the industry of food supplements for humans in order to obtain eggs enriched in liposoluble micronutrients.

The impact of research performed is supported by the fact that the results have applicability, usability and economic efficiency (by-products being considered *low-cost* sources) in the field of laying hens nutrition by designing viable solutions with impact in increasing the nutritional value of eggs.

The obtained results of this doctoral research were disseminated in scientific meetings and articles published in scientific journals, thus increasing the visibility of Romanian research.