S U M M A R Y

Research on the negative effects of heat stress on oxidative processes in monogastrics and their combat by using non-conventional feed additives in feed

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The main objectiv of the thesis is the development, based on research activities, for new nutritional solutions (tested and validated "in vivo") intended for laying hens raised in thermal stress 30°C respectively 35°C, solutions intended to ensure bioproductive performance, animal health and obtaining some products high quality livestock (eggs).

The objectives of the research were:

(1) Establishing and characterizing some phytoadditives and (2) testing the effects of their inclusion in the fodder rations of laying hens subjected to high temperatures with the aim of evaluating bioproductive performance and egg quality.

The secondary objective was:

-comparison of nutritional ratios and phytoadditives used to determine the health status of animals.

This paper has been splited into two parts in accordance with the drafting rules. The first part is made up of the bibliographic study that addresses the general framework of the subject of the thesis and consists of two chapters. The second part consists of the own research part, being structured in five chapters.

Part I

Chapter I is called **GENERAL CONSIDERATIONS REGARDING THE EFFECT OF HIGH THERMAL STRESS ON POULTRY** and represents the importance of the problem addressed in the thesis, the effects of global thermal stress on poultry production and the need to find solutions to mitigate them.

In the first part, the general characteristics of thermal stress and its effects on animals exploited in these conditions are presented. Heat stress is one of the most stressful and costly events for farm animals, with negative effects on health, productivity and product quality. Rising global temperatures and frequent heat waves caused by global warming, as well as the move of livestock production to tropical environments, have increased the risk of heat stress in animals. It causes significant economic losses, including slow growth, reduced fertility, high veterinary costs and animal welfare problems.

Heat stress will continue to be a problem if improving production traits is prioritized over thermotolerance and climate adaptation. Birds are particularly vulnerable due to their lack of sweat glands and complete plumage. Heat stress seriously affects their health, leading to high mortality and morbidity and high economic losses in meat and egg production.

Studies show that heat stress increases mitochondrial reactive oxygen species generation and induces oxidative stress in muscle, causing oxidative tissue damage. That is why researchers and producers have been concerned with this problem for decades, especially in warm regions. There is a need to examine manipulation methods to combat heat stress, including environmental improvement and nutritional manipulation, to reduce negative impacts by understanding responses at the molecular and cellular level.

Chapter II is called **POSSIBLE STRATEGIES FOR IMPROVING AND COMBATING THERMAL STRESS IN THE POULTRY INDUSTRY**

Considering the multitude of negative effects and economic repercussions, the issue of thermal stress has become an increasingly intensively researched and debated topic in the poultry sector. Antibiotics represented a solution in combating the negative effects of thermal stress, but in the context of antibiotic resistance, a ban on the use of antibiotics in feed was imposed. Thus, it was necessary to apply well-developed nutritional strategies to mitigate the negative consequences of heat stress. In this chapter, different alternatives are presented for their testing as supplements in the feed of laying hens, such as phytoadditives, such as plants, yeasts and prebiotics. Numerous studies aimed at testing phytoadditives with antioxidant properties in the feed of laying hens exposed to heat stress, obtaining results beneficial for animals as well as eggs (Lin et al., 2006)

Numerous research studies have shown that supplementing rations with vitamin C and E improved feed intake and body weight of birds raised under heat stress (El-Gawad et., 2008, Chung etal., 2005;). Supplementing the rations of laying hens with phytoadditives such as zinc-enriched yeast, parsley and inulin, can improve the antioxidant activity in the bodies of animals subjected to thermal stress, thus reducing primary oxidative products from secondary products, such as eggs. Studies show that supplementation with vitamins, minerals, plants, probiotics and their mixture can reduce the negative effects of thermal stress on birds. It seems that these phytoadditives can have antioxidant effects and improvement effects on the immune function of birds (Lin et al., 2006).

The inulin used in the feed of laying hens had a multitude of benefits according to the studies carried out. Among these benefits we can list the increase in the body's own antioxidant activity, the increase in the absorption of minerals in the body, but also the improvement of the egg shell (Chen and Chen, 2004)

Another phytoadditive used in the feed of laying hens is yeast, a by-product of the beer industry. From a nutritional point of view, yeast contains free amino acids, proteins, enzymes and minerals such as zinc. The use of yeast in the feed of laying hens led to a decrease in the cholesterol content of the egg, an increase in the frequency of laying and an increase in the size of the eggs (Liu et al., 2002)

Parsley contains high amounts of flavonoids, tocopherol and volatile oils with antioxidant activities (Fejes et al., 2000). Lutein and zeaxanthin are relatively polar carotenoid pigments found in high levels in parsley, spinach, kale, egg yolk, and lutein-enriched foods. The macular carotenoids are lutein and zeaxanthin and these convert to meso-zeaxanthin, which is a non-provitamin A carotenoid. Important representatives of oxygenated carotenoids are lutein, zeaxanthin, β-cryptoxanthin, capsanthin, astaxanthin and fucoxanthin. It is estimated that approximately 90% of the total carotenoids in North American diets consist of lycopene, β-carotene, α-carotene, lutein, β-cryptoxanthin, and zeaxanthin (Rao, 2007).

Part II

Chapter III is entitled **THE PURPOSE OF THE RESEARCHERS.**

The main purpose of the conducted research was to investigate the effects of including phytoadditives in feed recipes administered to laying hens raised under heat stress, observing their influence on bioproductive performances, the quality of the eggs obtained and the well-being of the animals. The experiments took place in the experimental rooms of the Nutrition Physiology Laboratory in the "Gh. Burlacu" which belongs to the National Research - Development Institute for Animal Biology and Nutrition - IBNA Balotești. In order to be able to fulfill the general objectives of the doctoral thesis, the research activities resulted in four experiments, two of which were carried out under conditions of thermal stress (30°C) and the other two were carried out under high thermal stress (35°C). In both thermal regimes, the fodder recipes were similar. The experiments were structured as follows:

Experiment I (CHAPTER V): The experiment took place in thermal stress (30°C), for 3 weeks, the age of the chickens at the beginning of the experimental period was 47 weeks. The present experiment was performed on 60 laying hens, divided into 3 (M, E1, E2) experimental groups. The objective of the research was to investigate the effects of including parsley 2% (E1) and inulin 2% (E2) in the ration of laying hens.

Experiment II (CHAPTER VI): The experiment took place in thermal stress (30+C), for 3 weeks, the age of the chickens at the beginning of the experimental period was 47 weeks. The present experiment was performed on 80 laying hens, divided into 4 (M, E1, E2, E3) experimental groups. The objective of the research was to investigate the effects of including yeast 1% (E1), yeast 1% + parsley 2% (E2), yeast 1% + inulin 2% (E3) in the ration of laying hens.

Experiment III (CHAPTER VII): The experiment took place in thermal stress (35+C), for 3 weeks, the age of the chickens at the beginning of the experimental period was 50 weeks. The present experiment was performed on 128 laying hens, divided into 4 (M, E1, E2, E3) experimental groups. The objective of the research was to investigate the effects of including yeast 1% (E1), parsley 2% (E2), yeast 1% + parsley 2% (E3) in the ration of laying hens.

Experiment IV (CHAPTER VII): The experiment took place in thermal stress (35+C), for 3 weeks, the age of the chickens at the beginning of the experimental period was 50 weeks. The present experiment was performed on 126 laying hens, divided into 3 (M, E1, E2) experimental groups. The objective of the research was to investigate the effects of including inulin 2% (E1) and yeast 1% + 2% inulin (E2) in the ration of laying hens.

Chapter IV is called MATERIALS AND METHODS. In this chapter, the structure of the work plan, the experimental conditions and the monitored parameters are presented. The conditions for taking the samples taken in the analysis are also described. This chapter also presents the methods used for the biochemical determinations related to the experiments.

Chapter V is called **RESEARCH ON THE EFFECT OF THE INCLUSION OF PARSLEY AND INULINE ON THE PRODUCTIVE PERFORMANCE AND THE QUALITY OF THE NEW IN CONDITIONS OF THERMAL STRESS.**

The experiment took place in thermal stress (30°C), for 3 weeks, the age of the chickens at the beginning of the experimental period was 47 weeks. The present experiment was performed on 60 laying hens, divided into 3 (M, E1, E2) experimental groups. The objective of the research was to investigate the effects of including parsley 2% (E1) and inulin 2% (E2) in the ration of laying hens.

After running the experiment under thermal stress conditions, the following results were obtained:

From the point of view of zootechnical performances, the use of parsley and inulin did not influence the average daily consumption, the specific consumption, the laying intensity and the weight of the eggs.

From the point of view of the color of the yolks, it changed positively after the administration of parsley (2%) in the ration of the laying hens.

The use of inulin (2%) and parsley (2%) in the rations of laying hens had a positive impact on the oxidative stability of the egg, through the transfer of polyphenols from the feed to the egg content.

The use of inulin (2%) and parsley (2%) in the feed of laying hens leads to a decrease in the amount of malondialdehyde present in the egg, but also to maintaining a lower value during storage.

Following the use of these phytoadditives, the amount of glucose and triglycerides in the blood of laying hens subjected to thermal stress was reduced.

Chapter VI is called **RESEARCH ON THE EFFECT OF THE INCLUSION OF YEAST AND ITS COMBINATION WITH PARSLEY AND INULINA ON THE PRODUCTIVE PERFORMANCE AND THE QUALITY OF THE NEW IN CONDITIONS OF THERMAL STRESS.**

The experiment took place in thermal stress (30°C), for 3 weeks, the age of the chickens at the beginning of the experimental period was 47 weeks. The present experiment was performed on 80 laying hens, divided into 4 (M, E1, E2, E3) experimental groups. The objective of the research was to investigate the effects of including yeast 1% (E1), yeast 1% + parsley 2% (E2), yeast 1% + inulin 2% (E3) in the ration of laying hens.

Analyzing the results obtained in the second experiment, we obtained the following results:

The use of yeast in the rations of the laying hens, the mixture of yeast and parsley and the mixture of yeast and inulin did not influence the average daily consumption, the feed conversion rate, the laying percentage or the weight of the eggs.

Following the use of the mixture of yeast and parsley (E1) and yeast and inulin (E2), the value of the Haugh unit of fresh eggs was significantly reduced, while the pH value of the white increased significantly.

The inclusion in the ration of the laying hens of the mixture of yeast and egg yolk significantly increased the concentration of Vitamin E and Xanthophylls in the egg yolks.

Chapter VII is called **RESEARCH ON THE EFFECT OF THE INCLUSION OF YEAST AND PARSLEY AND THEIR MIXTURE ON PRODUCTIVE PERFORMANCE, EGG QUALITY AND HEALTH STATUS IN CONDITIONS OF HIGH THERMAL STRESS.**

The experiment took place in thermal stress (35°C), for 3 weeks, the age of the chickens at the beginning of the experimental period was 50 weeks. The present experiment was performed on 128 laying hens, divided into 4 (M, E1, E2, E3) experimental groups. The objective of the research was to investigate the effects of including yeast 1% (E1), parsley 2% (E2), yeast 1% + parsley 2% (E3) in the ration of laying hens.

After running the experiment under conditions of high thermal stress, the following results were obtained:

The use of 2% parsley (E2) and a mixture of 2% parsley and 1% yeast (E3) led to a significant increase in feed consumption in the case of laying hens subjected to high thermal stress (35°C).

The inclusion of 1% yeast in the feed of laying hens subjected to high thermal stress (35°C) led to a significant decrease in the specific consumption of combined feed.

Following the administration of 2% parsley (E2) and 1% yeast and 2% parsley (E3) had a significant positive impact on the weight of eggs from laying hens exposed to high thermal stress (35°C).

Following the storage of hen's eggs, it was found that after 14 days the value of the Haugh unit was the highest in the case of the batch where 2% parsley was administered (E2). After 28 days of storage, the highest value of the Haugh unit was recorded in batch E3 where the mixture of yeast 1% and parsley 2% was included in the combined feed.

Following the administration of parsley in the ration of laying hens exposed to high thermal stress (35°C), a significant increase in the antioxidant capacity detected by the egg yolks, as well as an improvement in their color, was observed.

The addition of yeast, parsley and their mixture led to a reduction in the amount of malondialdehyde present in fresh egg yolks, but also after 14 and 28 days of storage.

Chapter VIII is called **RESEARCH ON THE EFFECT OF THE INCLUSION OF INULINA AND YEAST ON PRODUCTIVE PERFORMANCE AND EGG QUALITY IN CONDITIONS OF HIGH THERMAL STRESS.**

The experiment took place in thermal stress (35°C), for 3 weeks, the age of the chickens at the beginning of the experimental period was 50 weeks. The present experiment was performed on 126 laying hens, divided into 3 (M, E1, E2) experimental groups. The objective of the research was to investigate the effects of including inulin 2% (E1) and yeast 1% + 2% inulin (E2) in the ration of laying hens.

After running the experiment under conditions of high thermal stress, the following results were obtained:

The use of yeast phytoadditives and the mixture of yeast and inulin in the feed of laying hens exposed to high thermal stress (35°C) led to an increase in average daily consumption compared to the control group.

Following the use of yeast and inulin in the combined feed administered to laying hens, the specific consumption decreased, while the use of inulin led to an increase in the specific consumption of the combined feed.

The addition of a mixture of 1% yeast and 2% inulin in the rations of laying hens raised in high thermal stress (35°C) led to an increase in the percentage of eggs laid by 11% compared to the control group.

By using a mixture of yeast and inulin, the color of the yolk improved, this being visible even after 14 and 28 days of storage.

The administration of yeast and inulin respectively inulin in the ration of laying hens led to the reduction of the amount of malondialdehyde in the egg yolks both in the initial phase and after 14 and 28 days of storage respectively.

CONCLUSIONS

Under thermal stress conditions of 30°C, the inclusion of the phytoadditives used in these experiments (yeast, parsley, and inulin, as well as their mixture) did not influence the average daily feed consumption, egg weight, feed conversion rate or egg weight. The use of parsley or parsley together with yeast or inulin positively influenced the color of the yolk due to the presence of lutein and zeaxanthin, this being proven by the presence of these carotenoids in the yolk of the analyzed eggs. The use of yeast phytoadditives and inulin negatively affected the value of the Haugh unit of the egg white. From the point of view of lipid oxidation in the yolk, it was found that the use of parsley and inulin in the feed of laying hens leads to a decrease in the primary oxidative products present in the yolk (malondialdehyde), but also maintaining this parameter at a lower value during storage. From the point of view of the biochemical parameters of the blood, the use of these additives decreases the content of glucose and triglycerides when the birds are subjected to high temperatures.

Under the conditions of using the phytoadditives yeast, inulin, parsley and their mixture at a temperature of 35°C, it was found that the use of yeast, parsley and their mixture increase the daily consumption of feed, which is beneficial because normally feed consumption decreases during exposure to high temperatures, thus depriving the animals of the nutrients they need daily. When inulin was used in the feed of laying hens, the daily consumption was significantly reduced. From the point of view of the state of freshness of the eggs measured by the height of the white (Haugh units), it was found that after 14 days the value of the Haugh unit was the highest in the case of the batch where 2% parsley was administered (E2). After 28 days of storage, the highest value of the Haugh unit was recorded in batch E3 where the mixture of yeast 1% and parsley 2% was included in the combined feed. Following the administration of parsley in the ration of laying hens exposed to high thermal stress (35°C), a significant increase in the antioxidant capacity detected by the egg yolks, as well as an improvement in their color, was observed. The inclusion of yeast, parsley and their mixture led to a reduction in the amount of malondialdehyde present in fresh egg yolks but also after 14 and 28 days of storage respectively. The addition of 1% yeast and 2% inulin in the rations of laying hens raised under high thermal stress (35°C) led to an 11% increase in the egg percentage compared to the control batch. By using a mixture of yeast and inulin, the color of the yolk improved, this being visible even after 14 and 28 days of storage.

The administration of yeast and inulin respectively inulin in the ration of laying hens led to the reduction of the amount of malondialdehyde in the egg yolks both in the initial phase and after 14 and 28 days of storage.