ABSTRACT

This doctoral thesis, entitled "RESEARCH ON THE EFFECT OF NATURAL ANTIOXIDANTS ON THE DIGESTIVE TUBE IN INTENSIVELY RAISED PIGS", was elaborated according to the methodology for writing a doctoral thesis, comprising 2 parts: the first part summarizes the current state of the literature in the field of the thesis, followed by the second part, where the results obtained from our own research on the topic are reported.

The first part of the thesis consists of 3 chapters summarizing information on the morphophysiology of the oral cavity, salivary glands, stomach and intestinal tract (small and large) of the Pietrain pig; the intestinal immune system with emphasis on the lymphoid tissue associated with the intestinal mucosa and the use of grape polyphenols as antioxidants in pigs with the description of their chemical structure, bioavailability and bioeffects at the intestinal level.

The second part of the thesis comprises 6 chapters which: state the aim and objectives of the thesis; and also report the results of research on the histological and physiological peculiarities of the small intestine, strictly in Pietrain pigs and the response induced by black grape tescovine added to the diet on intestinal health and productive indices in pigs.

All of the above is presented in 51 original figures and 10 tables, and is consulted by 496 bibliographical references.

Chapter 4 is entitled "**Aim and Objectives of the Thesis**", highlighting the main objectives for the realization of the thesis:

- Histological evaluation of the small intestine in Pietrain pigs, with emphasis on the analysis of the height of the intestinal villi, the depth of the Lieberkühn crypts, the morphology of the columnar epithelium with striated plateau, the number of goblet cells, the cell populations infiltrating the lamina propria, the thickness of the mucosal musculature and the lymphoid tissue present in the jejunum and ileum;
- To follow the intestinal mucosal response after challenge with grape tescovine polyphenols in pig feed over a 3-month experimental period. In this study, variations in the height of intestinal villi, length of Lieberkühn's glands, villous height/crypt depth ratio and the dimensions of the lymphopods making up the Peyer's plaques were calculated;
- Inumohistochemical technique to highlight the reduction of some pro-inflammatory markers and to promote those responsible for the induction of the antioxidant effect at intestinal level, following the inactivation of black grape tescovine in the basic diet of pigs for 3 months;
- Calculation of indices reflecting production efficiency in intensively reared pigs fed black grape tescovin.

Chapter 5 is entitled "**The organizational framework in which the research was carried out**". This chapter describes the herd of pigs used in the thesis experiments, giving details of the breed, age and rearing conditions applied on the farm. The diet with black grape tescovine is also described in terms of total phenolic and flavonoid content and antioxidant activity.

Chapter 6 is entitled "**Investigations on morphostructural and functional particularities of the small intestine in Pietrain pigs**" with the main aim of highlighting the physiological and histological differences in the small intestine of Pietrain pigs.

The pigs (n=10) were included in the experiment at 180 days of age and an average weight of 85.09 ±5.11 kg. The feed received was a complete pelleted fattening pelleted feed given ad libitum for 90 days. At the end of the experimental period, the pigs were slaughtered in a slaughterhouse and the small intestine was removed. The research then continued to the laboratory where histologic preparations were obtained.

The mucosa of the duodenum had an average thickness of 960µm, consisting of intestinal villi (250µm), glandular layer in the lamina propria (420µm) and muscularis mucosae (25µm). The surface epithelium is columnar with a striated plateau and consists of numerous enterocytes and goblet cells. Enterocytes vary in size depending on their position: in Lieberkühn's glands (where they originate) they are cubic in shape, gradually growing to a prismatic shape at the base of the villi (17µm high) and at the apex of the villi (30µm high). The height of the microvilli is lower in enterocytes at the base of the villi (0.5µm) and higher (1.5µm) for those at the apex of the villi. Bruke's muscle is found at the central axis of each villi and originates from the muscle fibers of the muscularis mucosae. The thickness of this muscle can reach up to 50µm. The thickness of the submucosa can reach up to 500µm, here the mixed Brünner's glands are found, which are made up of both serous and mucosal secretory units. The muscularis consists of the inter layer with the muscle fibers arranged circularly (200µm) and the outer layer with the fibers oriented longitudinally (125µm).Between these layers of muscle is located the Auebach's vegetative myenteric nervous plexus. The last tunic of the duodenum is the serosa with a thickness of up to 80µm.

In jejunum, the highest values were obtained for total mucosal height (1006µm), villous height (246µm) and the longest length of Lieberkühn glands (604µm). Higher villi means a larger absorptive surface area, hence better digestion and absorption in the jenun. Also more goblet cells were observed in the jejunum than in the duodenum.

The most important structures observed in the distal submucosa of the jejunum, but also in the ileum are Peyer's plaques. These lymphoid formations are large in both jejunum (985µm) and ileum (760µm) and imprint the submucosa with a thickness twice that found in the duodenum.

In the ileum the smallest values were recorded for the total thickness of the mucosa (454µm), for the height of the villi (125µm) and the length of the intestinal glands (347µm) and the highest value for the thickness of the muscularis mucosae (80µm).This explains the fact that the main function of the

ileum is not in digestion and absorption, but in immune defense through the lymphoid tissue associated with the mucosa, which is highly developed.

The muscle belonging to both the jejunum and the ileum thickens as it approaches the large intestine. Although the thickness increases for both layers of muscle in the muscularis (circular and longitudinal), they remain proportionally equal.

The enteric nervous system takes part in both modulating the gut immune response and digestion. Nerve fibers, following impulses from adrenergic and cholinergic neurons, release neuropeptides with modulatory functions.

Pietrain pigs have a high productive performance due to the correlations formed between the morphoarchitecture of the intestinal tract, the neuro-endocrine messages and the resistant lymphoid tissue in the small intestine.

Chapter 7 is entitled "**Histologic analysis on the reactivity of the intestinal mucosa following the introduction of grape tescovine in Pietrain pigs**". The main aim was to observe what effects the inclusion of black grape tescovine in pigs' feed has on the histologic appearance of the digestive tract. The experiment was carried out in an intensive system with pigs (n=50) of the same age (180 days) and initial weight (85.09 ± 5.11 kg). In order to find the optimal dose of tescovine for the diet of pigs that can modify the morphology of the intestinal mucosa and submucosa, it was administered in different gram doses for 3 months. The total group of animals was also divided into smaller experimental groups according to the dose of tescovine, resulting in the following experimental protocol: LM (10 pigs on basal diet), LE1 (10 pigs on 1g/kg tescovine), LE2 (10 pigs on 5g/kg tescovine), LE3 (10 pigs on 10g/kg tescovine) and LE4 (10 pigs on 15g/kg tescovine).

The effects of tescovine throughout the experiment on the small intestine were represented by significant changes in the mucosal structure as well as in the lymphoid tissue asocytosed in the intestinal mucosa. At the dose of 10g/kg, in the duodenum, the highest values were observed for the height of the intestinal villi, the depth of the crypts, as well as the ratio between them. The lowest values for intestinal morphoarchitecture were reported in LE1, with values approximately equal to those of LM.

Morphometric indices measured in the jejunum and ileum had the highest values at LE3 (10g/kg testis) and the lowest at LE1 (1g/kg testis). Lymph nodes measured in the jejunum and ileum were largest at 10g/kg tescovine. This dose also promoted the best morphostructure in the cecum of pigs. LE3 had the longest intestinal glands, the most numerous goblet cells and the most numerous lymph nodes in the cecum of the pigs. The conclusion of this chapter was that the recommended dose to promote the morphoarchitecture and associated lymphoid tissue of the intestinal mucosa occurred at the dose of 10g/kg black grape tescocholine as an addition to the pigs' feed.

Chapter 8 is entitled "**Immunohistochemical expression of pro-inflammatory (IL-1β, TNF-α and MCH-II) and redox (Nrf2 and p65) markers in the intestine of pigs fed black grape tescovin**". The aim of this chapter was to follow the evolution of inflammation and antioxidant potency of black grape tescovin when added to the pigs' diet, by immunohistochemical technique.

The pigs (n=50) at the beginning of the experiment were of the same age and weights. They were divided into 4 experimental groups and a control group (10 pigs per group) and fed for 3 months as follows: LM (basal diet), LE1 (basal diet +1g/kg tescotin), LE2 (basal diet + 5g/kg tescotin), LE3 (basal diet +10g/kg tescotin) and LE4 (basal diet + 15g/kg tescotin).

The effect of black grape tescovine polyphenols down-regulated the expression of pro-inflammatory markers in the digestive tract (small intestine and cecum). The Il-1β experiment was most potent in LM in all digestive tract segments analyzed. Among the experimental groups, the most intense staining (+++) was in the duodenum in LE1 and LE2, in the jejunum in LE1, LE2 and LE4; and in the cecum LE1. The weakest expression of Il-1β (+) was in the ileum in LE3 and LE4, and in the cecum in LE3. TNF-α expression was most intense (+++) in LM in all areas studied. The weakest staining (+) was in the duodenum in LE3 and LE4, in jejunum, ileum and cecum in LE3. For the MCH-II marker, the strongest (+++) expression was in the duodenum in LM, and in jejunum, ileum and cecum in LM and LE1. The weakest (+) expression was observed in the duodenum in LE3 and LE4, and in the jejunum and cecum in LE3.

In order to analyze the redox ratio at the intestinal level, the expression results of two markers were followed in parallel: Nrf2 and p65. The weakest expression (+) of Nrf2 was in the duodenum, jejunum and ileum in LE1, whereas the strongest expression (+++) of p65 was in the jejunum and cecum in LE1. The strongest staining (+++) of Nrf2 was in duodenum, jejunum, ileum and cecum in LE3, while the weakest staining (+) of p65 was in duodenum and cecum in LE3 and LE4, and in jejunum in LE3. The weakest expression of pro-inflammatory markers but also the best redox ratio in the intestine of pigs fed black grape tescovin was obtained at a dose of 10g/kg.

Chapter 9 is entitled ”**Investigations on the evolution of growth performance parameters of pigs fed a diet supplemented with black grape tescovin”** with the main aim to calculate growth parameters (ADFI, ADG, TCF and initial weight) and to establish the performance of pigs fed a black grape tescovin admixture.

A total of 50 pigs of the same initial weight and age were divided 10 pigs each into 4 experimental groups and a control group. For 3 months, the pigs were fed with tescovin adducts at the following doses: LM (basal diet), LE1 (tescovin adduct 1g/kg), LE2 (tescovin adduct 5g/kg), LE3 (tescovin adduct 10g/kg) and LE4 (tescovin adduct 15g/kg).

The highest values of final weight, ADFI and ADG, were obtained by feeding 10g/kg black grape tescovin (LE3). Pigs that were fed 1g/kg black grape tescovin were the weakest at last weighing, and had the lowest ADG and ADFI values. The best feed conversion rates were at LE3.

General conclusions end the thesis.