The Inulin Functionality as a Prebiotic Used in Heat Stress Conditions in Broilers' Diet on Growth Performance, Intestinal Microbiota, Blood Parameters and Intestinal Histomorphometry

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Abstract
A possible alternative source to antibiotics could be the utilization of specific probiotics and prebiotics, such as inulin. In this study, we conducted a 42-day experimental study on 75 one-day-old ROSS 308 broiler chickens, divided into 3 groups (CON_{NTC}, CON_{HTS}, INL_{HST}), 25 per group, housed in an experimental hall with controlled environmental condition, according to the guide ROSS 308, on permanent litter rearing system.
During the starter phase (days 1–10) all chickens received a conventional basal diet under normal temperature conditions. In grower and finisher phases, the broilers were allocated as follows: CON_{NTC} (standard diet under normal temperature); CON_{HST} (standard diet under heat stress conditions 35°C) and INL_{HST} (1% inulin diet supplementation under heat stress conditions 35°C). There were significant differences in body weight between groups, the CON HST group registered a significant decrease (p=0,045) compared to CON_{NTC} group and INL_{HST} group registered a increase compared to CON_{HST}. Also, the weight of the gizzard was significantly (p=0,001) reduced in the CON_{HST} group. A significant increase in *Lactobacillus sp*. population (p=0,038) and a significant decrease in *Enterobacteriaceae sp.* and *Staphylococcus sp.* (p=0,004; p=0,005) were observed in INL_{HST} group, compared to CON_{NTC} and CON_{HST}. Concerning the haematological and biochemical profile, a significant increase in heterophils was observed in the CON_{HST} group (p<0,001) compared to CON_{NTS} and INL_{HST} groups; serum protein (p=0,013) and total protein (p=0,013) levels increased CON_{HTS} si INL_{HTS} compared to CON_{NTS}. The intestinal histomorphometry evaluation of villi width recorded a significant increase (p=0,003) in the INL_{HST} group compared to CON_{HST} group. In conclusion, 1% inulin administered in the diet of broilers are significantly increased beneficial bacterial populations in the intestinal microflora, without influencing growth performance, blood parameters and histomorphometry of villi and crypts in the broiler's duodenum.

Keywords: antibiotics, alternative source, inulin, nutrition, poultry, prebiotics.

1. Introduction

In the scientific effort to reduce the use of antibiotics in animal feed by replacing them, possible alternative sources have been studied such as bioactive compounds, bacteriophages, bacteriocins, as well as the use of specific probiotics and prebiotics, for example, inulin. A prebiotic is a selectively fermented ingredient that allows specific changes in the composition or activity of the gastrointestinal microbiota and is not digested by digestive enzymes, being a nutrient for probiotics (beneficial bacterial

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species) such as *Lactobacillus* and *Bifidobacterium*. The growth of endogenous microbial populations, such as *Bifidobacteria* and *Lactobacilli*, is specifically stimulated, being beneficial for animal health, by improving the microbial balance of the host. Probiotics and prebiotics are administered together in a symbiotic form [1]. Possible effects of inulin on mineral metabolism have also been investigated, with the interaction between inulin and *L. acidophilus*, which promotes Ca absorption by its metabolites, being investigated [2]. Another study evaluated the effects of adding exogenous enzymes (xylases and beta-glucanases) which have been shown to reduce intestinal viscosity and improve digestibility and nutrient performance [3]. Prebiotics, probiotics, acidifiers, bacteriocins and some phytobiotics are promoters of homeostasis of the gastrointestinal tract ecosystem. Inulin-type fructans could modulate the immune response by activating the expression of genes and cytokines involved in immune-mediated processes. Inulin generally decreases the expression of proinflammatory cytokines, for example, lipopolysaccharide-induced tumor necrosis factor (LITAF), interferon γ (IFN-γ), interleukin-6 (IL-6), as well as inducible nitric oxide synthase, which, in turn, modify the intestinal barrier and immune function [4]. The main sources from which commercial inulin is extracted are chicory, dahlia and Jerusalem artichoke. The technology of inulin production has 2 stages, respectively, in the first stage the extraction and purification of the raw syrup is carried out, which in the second stage is refined to produce the commercial product [5]. The effects of inulin in the body are diverse, for example, inulin is a regulator of lipid parameters, has antioxidant action, increases zinc absorption, reduces the level of triglycerides and cholesterol in serum, improves the immune response, facilitates the growth of the *Bifidobacterium* population, as well as increases the absorption of calcium and magnesium in animals in the growth phase [6]. Studies have shown that inulin can be used as a fat substitute in low-fat meat products and incorporated as a functional ingredient, as fat is a high-calorie nutrient that plays an important role in creating a feeling of satiety. The ability of inulin to replace fat is due to its particle gel properties. Poultry meat is particularly susceptible to lipid peroxidation, due to its high levels of unsaturated fatty acids, and consequently its shelf life is reduced [7]. There is some evidence that inulin can increase mineral utilization in rats [8] and pigs [9]; however, the effects of inulin on Ca and P absorption are variable [10]. Studies in animal models have shown that inulin-type fructans stimulate the absorption of minerals, calcium and magnesium, with a beneficial effect on bone health [11]. In poultry farming, antibiotics have been widely used to prevent various infectious pathologies, including enteric diseases, as well as to maintain health and improve growth performance. Thus, this continuous and abusive use of antibiotics in poultry feed has led to the emergence of antibiotic-resistant microbial strains, with the transfer of antibiotic resistance to humans [12]. Indigestible carbohydrates in the diet are perceived to improve health through the gut microbiota-dependent generation of products such as short-chain fatty acids (SCFA). In addition, SCFA are also precursors for the synthesis of lipids and cholesterol, which may have undesirable effects on lipid metabolism [13].

In the current context of multidrug resistance to antibiotics, this study aimed to determine the effects of supplementing the diet of broiler chickens with inulin on production, hematology and biochemistry parameters, meat quality, intestinal microbial population, and intestinal villi. Thus, inulin can be considered as a possible alternative source to antibiotics.

2. Materials and methods

2.1 Experimental design

The experiment was conducted over a period of 42 days in which 75-day-old ROSS 308 hybrid chickens were included, divided into 3 groups, with 25 chickens/groups. The chickens were housed in experimental hall with controlled environmental conditions (average temperature/total growth period 27.07±2.75^{0}C; humidity 64.80±9.57%; ventilation/chick 0.50±0.24%; CO2 level 686.39±104.38 ppm) on permanent litter rearing system. The feed consumption, body wight and health status were recorded daily throughout the experiment. The chickens were not subjected to drug treatment and vaccinated at the hatchery, against Marek's disease and infectious bronchitis. In the first phase (starter) for a period of 10 days, all 3 batches received the same conventional diet and were subjected to the same thermal regime. The

following 2 phases (growth and finishing) compared to the CON_{NTC} group, respectively, the CON_{HST} group received the same conventional diet, but the environmental temperature was increased (35^0 C) and the experimental INL_{HST} group received a diet supplemented with 1% inulin and was subjected to increased thermal stress (35^0 C) (*Table 1*). The raw material, inulin, was purchased commercially in powder form. The experiment was conducted according to a protocol approved by the IBNA Ethics Committee established by decision no. 118/02.12.2019 and operating under the Board of Directors and the Scientific Council of IBNA, in accordance with Romanian and European Union legislation.

Table 1. Nutritional diet structure and chemical analysis

Specification	Starter 0 -10 d	Grow 11 - 24 d		Finishing 25 - 42 d	
	Basal diet	CON	INL	CON	INL
Ingredients, %					
Maize	34.87	44.5	43.5	47.23	46.23
Wheat	20.00	10	10	10	10
Soybean meal	37.98	35.45	35.45	32.16	32.16
L-lysine HCl	0.25	0.32	0.32	0.12	0.12
DL- methionine	0.35	0.28	0.28	0.27	0.27
L- threonine	0.11	0.04	0.04	0.03	0.03
Calcium carbonate	1.28	1.26	1.26	1.17	1.17
Monocalcium phosphate	1.24	1.59	1.59	1.48	1.48
Salt	0.40	0.37	0.37	0.37	0.37
Vegetable oil	3.47	5.12	5.12	6.1	6.1
Choline	0.05	0.07	0.07	0.07	0.07
Inulin	-	-	1	-	1
Premix	1	1	1	1	1
TOTAL	100	100	100	100	100
Theoretical calculation, %					
ME broiler, kcal/kg	3,000.00	3,100.00	3,100.00	3,326.66	3,327.91
Crude protein	23.00	21.50	21.50	20.00	20.00
- CP dig.	20.24	18.90	18.26	17.58	16.93
Crude fat	5.51	7.32	8.25	8.35	9.35
Raw cellulose	3.49	3.30	4.18	3.18	4.06
Calcium	0.96	0.87	0.87	0.81	0.81
Available phosphorus	0.48	0.44	0.44	0.41	0.41
Lysine dig.	1.44	1.29	1.29	1.06	1.06
Methionine dig.	0.65	0.59	0.59	0.56	0.56
Met.+cist. dig	1.02	0.87	0.87	0.83	0.83
Threonine dig.	0.88	0.77	0.77	0.71	0.71
Tryptophan dig.	0.24	0.19	0.18	0.17	0.17
Arginine dig.	1.27	1.23	1.19	1.15	1.11
E.M.P./P.B, ratio	130.43	150.56	150.55	166.33	166.40

Where: CON – control diet; INL – conventional diet supplemented with 1% inulin;
*1kg premix IBNA (A1) contains: = 1100000 IU/kg vit. A; 200000 IU/kg vit. D3; 2700 IU/kg vit. E; 300 mg/kg Vit. K; 200 mg/kg Vit. B1; 400 mg/kg Vit. B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 300 mg/kg Vit. B6; 4 mg/kg Vit. B7; 100 mg/kg Vit. B9; 1.8 mg/kg Vit. B12; 2000 mg/kg Vit. C; 8000 mg/kg manganese; 8000 mg/kg iron; 500 mg/kg copper; 6000 mg/kg zinc; 37 mg/kg cobalt; 152 mg/kg iodine; 18 mg/kg selenium; 6000 mg/kg antioxidant

2.2 Samples collection and analysis

At the end of the experiment (42 days) the chickens were sacrificed by cervical dislocation, followed by exsanguination and evisceration for biological samples (blood, intestinal contents, intestinal segments, organs, muscles). To obtain data on growth performance, individual body weight, mortality rate, average daily consumption and average daily gain were monitored. For microbiology determinations, intestinal content samples (caecum) were collected and stored at -20 ^{0}C for determination of intestinal microflora *(Escherichia coli, Staphilococcuss spp. Salmonella spp., Lactobacillus spp.)* The Scan 300

Colony Counter (Interscience, Paris, France) was used for determination of colony-forming units. The results were expressed as logarithm based on 10 CFU/g cecal content. Before slaughter, for blood parameters, blood was collected aseptically from the brachial vein in vacutainers with anticoagulant (EDTA) for hematological samples, respectively in vacutainers with coagulation activator, and for biochemical samples a SPOTCHEM EZ SP-4430 Arkray analyzer (Kyoto, Japan) and for hematology VH30, Genrui Biotech Inc. (Hong Kong, China) analyzer was used. Tissue samples were taken by sectioning, in containers with 10% buffered formaldehyde solution, for fixation. The samples, fixed and modeled, are passed through the processing steps, paraffin inclusion, microtome sectioning and deparaffinization. The slides are stained by the *Hematoxylin-Eosinophil* (H.E) bichrome method. The histological preparations were examined with an Olympus CX43 microscope (Tokyo, Japan) equipped with a microphotography system, with x4, x10, x20 objectives.

2.3 Statistical analysis

A completely randomized design was applied to analyze the results regarding productive parameters, biochemical parameters, gut microbial population and small intestinal histomorphometry following dietary inulin supplementation. The experimental results were tested by analysis of variance (ANOVA) using Minitab for Windows (SAS, version 17, SAS Institute Inc., Cary, NC, USA). Significance between individual means was identified using the Tukey's multiple range test. Mean differences were considered significant at $p<0.05$.

3. Results and discussion

The results regarding the effects of including 1% inulin in the diet on growth parameters are presented in *Table 2*. It can be observed, inulin inclusion did not influence the growth performance of the chicks compared to the CON_{HST} and CON_{NTC} groups, respectively. However, under heat stress conditions, the CON_{HST} group recorded a significant decrease ($p=0.045$) in average body weight (BW) compared to the CON_{NTC} group, without significant differences compared to the INL_{HST}.

No significantly differences were registered about the production efficiency regarding the: protein efficiency ratio (PER), European production efficiency factor (EPEF), efficiency of energy utilization (EEU) and performance index (PI).

Table 2. Influence of dietary supplementation with inulin on production performance and economic efficiency indices (average values/group)

Parameter	Diets			SEM	p-Value
	CON $_{NTC}$	CON $_{HST}$	INL $_{HST}$		
BW 0 d, g	39.20	39.64	39.89	0.635	0.788
BW 42 d, g	2715.50 ^{a}	2405.33 ^{b}	2619.09 ^{ab}	80.20	0.045
FCR, kg feed/kg gain	1.39	1.56	1.54	0.049	0.084
PER	3.39	3.02	3.10	0.098	0.059
PI	196.8	158.0	178.0	11.0	0.091
EU	4.59	5.09	5.03	0.160	0.121
EPEF	368.50	376.10	323.80	26.10	0.091

Where: CON $_{NTC}$ – Control group, conventional diet and normal thermal regim; CON $_{HST}$ – experimental group, conventional diet and heat stress conditions; INL $_{HST}$ – experimental groups, diet supplemented with 1% inulin and heat stress conditions; SEM - standard error of mean; $^{a-b}$ mean value within a row having different superscripts are significantly different at p 0.05

Abbreviations: PER, Efficiency of Protein in the diet; PI, Performance Index; EU, Energy Efficiency; EPEF, The European Efficiency Factor;

Similar results were obtained by [13] who included 7 g inulin and 7 g oligofructose in the diet of broiler chickens, concluded that performance parameters were not affected. However, [1] observed that supplementing the diet with 1% inulin increased weight gain, but without significant differences in the feed conversion rate. Following the inclusion of 0.7% inulin and 0.014% beta-glucan in the diet, [15] did not observe significant differences in feed intake, weight gain, feed conversion rate, and carcass yield. In another study on the impact of supplementing the diet of broiler chickens with 25 mg/kg inulin on meat quality with *Clostridium*

perfringens, [12] did not observe significant differences in growth performance. Similar research was also conducted by [16] who concluded that following supplementation with 2% inulin and 10% wheat bran, the groups with diets supplemented with wheat bran induced weight gain and the highest crude fat digestibility, while the diets with wheat bran and inulin showed a lower feed conversion rate.

In *Table 3* are presented the effects of heat stress conditions and 1% inulin dietary inclusion on carcass and organ development. There was no significant difference ($p \geq 0.05$) in carcass weight and organ development for any of the groups under different thermal conditions, except for gizzard weight, which in the groups subjected to high thermal stress (CON_{HST} and INL_{HST}) gizzard weight was significantly (p=0.001) lower compared to the CON_{NTC} group.

Table 3. Influence of dietary inulin supplementation on carcass and organ development (average values/group)

Parameter	Diets			SEM	p-Value
	CON $_{NTC}$	CON $_{HST}$	INL $_{HST}$		
Live weight at slaughter (g)	2327.50	2307.50	2338.80	64.40	0.942
Carcass (%)	81.49	84.17	83.73	2.28	0.682
Breast (%)	22.32	24.96	20.56	1,21	0.080
Thigh (%)	18.27	19.42	20.90	0.78	0.109
Liver (%)	1.72	1.88	1.78	0.18	0.794
Gizzard (%)	1.46 ^{a}	0.81 ^{b}	0.83 ^{b}	0.09	0.001
Proventriculus (%)	0.40	0.39	0.34	0.04	0.544
Heart (%)	0.40	0.41	0.38	0.03	0.667
Spleen(%)	0.08	0.09	0.08	0.01	0.059
Gall bladder (%)	0.11	0.14	0.13	0.02	0.396

Where: CON $_{NTC}$ – Control group, conventional diet and normal thermal regim; CON $_{HST}$ – experimental group, conventional diet and heat stress conditions; INL $_{HST}$ – experimental groups, diet supplemented with 1% inulin and heat stress conditions; SEM – standard error of mean; $^{a-b}$ – mean value within a row having different superscripts are significantly different at p 0.05

Other researchers [23] after determining the weight of the thigh and breast, they concluded, at the end of the experiment (day 42), that the group with 1% inulin had the lower relative weight of the legs ($p < 0.05$). After day 14, the groups that received a diet supplemented with 1, 2 or 4% inulin had lower relative weights ($p<0.05$) of the breast muscles than those that received the control diet. Bucław [24] in a overview about the use of inulin in poultry show that, Ortiz et al. (2009) and Kucukersan et al. (2011) applied various inulin concentrations (5, 10, 15 or 20 g/kg of feed), but failed to demonstrate its effect on carcass yields in broiler chickens. Świątkiewicz et al. (2011) observed that the weight of carcass, breast, abdominal fat and liver did not change as a result of inulin supplementation. Yusrizal and Chen (2003) noted that supplemental inulin gave a significant increase in carcass yields in male broiler chickens. Park and Park (2011) stated that carcass weight and meat yield from the carcass were higher in groups fed inulin in diets.

Table 4. Influence of dietary supplementation with inulin on the microbial population in the intestine (average values/group)

Parameter (CFU/g)	Diets			SEM	p-Value
	CON $_{NTC}$	CON $_{HST}$	INL $_{HST}$		
Lactobacillus sp.	7.69^{b}	7.69^{b}	8.95^{a}	0.588	0.038
Enterobacteriaceae sp.	5.47^{a}	4.60 ^{a}	4.00^{b}	0.229	0.004
Staphylococcus sp.	6.67 ^{a}	5.00 ^{a}	3.75^{b}	0.413	0.005

Where: CON $_{NTC}$ – Control group, conventional diet and normal thermal regim; CON $_{HST}$ – experimental group, conventional diet and heat stress conditions; INL $_{HST}$ – experimental groups, diet supplemented with 1% inulin and heat stress conditions; SEM – standard error of mean; $^{a-b}$ – mean value within a row having different superscripts is significantly different at p 0.05, CFU/g – colony forming units

In *Table 4*, are presented the effects of including 1% inulin in broiler diet in heat stress conditions compared to normal thermal condition and conventional diet. Concerning the *Lactobacillus sp*. population the results show a significantly (p=0.038) increased in INL_{HST} group compared to

CON_{NTC} and CON_{HST} groups, while for *Enterobacteriaceae sp.* and *Staphylococcus sp*. population significantly (p=0.004; p=0.005, respectively) decreased in CON_{HST} and INL_{HST} groups compared to CON_{NTC} group.

Similar results to another study on the same topic conducted by [2] regarding the effects of including *Lactobacillus acidophilus* and 2% inulin in the diet decreased the population of *Enterobacteriaceae* and increased the number of *Lactobacilli* and *Bifidobacteria*, and the gastrointestinal pH was not affected. Including 10g/kg, respectively 20 g/kg inulin, [3] observed that the number of *Bifidobacteria* and *Lactobacilli* in the ileal and cecal contents increased significantly. Microbiological analyses, conducted by [1] demonstrated that supplementation with 0.5%, respectively 1% inulin increased the number of *Bifidobacteria* and reduced the number of *Escherichia coli* in the cecal contents, without a significant effect on the pH and the number of microorganisms in the ileal contents. Awad et al. [18] concluded that in chickens subjected to heat stress there were no interactions between the bacterial populations of *Lactobacilli, E. coli* or *Clostridium spp*. in the cecum and temperature, temperature had no impact (p>0.05) on the cecal microbial populations.

Results regarding the effects of heat stress conditions and inulin dietary inclusion on hematological profile are presents in *Table 5*. No significant differences (p≥0.05) were recorded regarding the determination of hematology parameters, with the exception of lymphocytes showed a significant decrease (p<0.0001) in chickens subjected to high heat stress, respectively, the CON_{HST} group, compared to CON_{NTC}. On the other hand, heterophils recorded a significant increase (p<0.0001) in the CON_{HST} group, compared to the CON_{NTC} and INL_{HST} groups.

Table 5. Influence of dietary supplementation with inulin on the hematological profile (average values/groups)

Parameter (%)	Diets			SEM	p-Value
	CON $_{NTC}$	CON $_{HST}$	INL $_{HST}$		
Leukocyte (WBC), %	13.80	12.50	14.05	0.558	0.164
Lymphocytes, %	60.38 ^{a}	33.50 ^{b}	57.63 ^{a}	2.930	<0.0001
Monocytes, %	5.88	5.75	4.13	1.380	0.622
Eosinophils, %	2.38	2.13	3.12	0.898	0.724
Heterophile, %	31.38 ^{b}	58.63 ^{a}	35.13 ^{b}	2.220	<0.0001
Lymphocytes, K/µL	8.32 ^{a}	4.18 ^{b}	8.08 ^{a}	0.452	<0.0001
Monocytes, K/µL	0.80	0.72	0.57	0.173	0.648
Eosinophils, K/µL	0.33	0.26	0.43	0.126	0.650
Heterophile, K/µL	4.33 ^{b}	7.32 ^{a}	4.96 ^{b}	0.409	<0.001

Where: CON $_{NTC}$ – Control group, conventional diet and normal thermal regim; CON $_{HST}$ – experimental group, conventional diet and heat stress conditions; INL $_{HST}$ – experimental groups, diet supplemented with 1% inulin and heat stress conditions; SEM - standard error of mean; $^{a-b}$ mean value within a row having different superscripts are significantly different at p 0.05

Similar research conducted by [21] on a diet supplemented with fumaric acid under heat stress conditions observed that birds fed 0.5% fatty acid had significantly (p<0.05) higher erythrocyte and hemoglobin concentrations compared to birds in the heat stressed group. There were no significant variations, such as those related to mean corpuscular volume (MCV), leukocyte (MCH), lymphocyte (WBC), granulocytes and monocytes, among all experimental groups. On the other hand, [23] showed that supplementation with synbiotics (inulin extracted from gembili tuber and *Lactobacillus plantarum*) did not affect erythrocytes, leukocytes, hemoglobin (Hb) and cellular cell value (PCV). This study was in contrast to the study by Beski et al. (2015), who reported that supplementation with probiotics and synbiotics significantly increased erythrocytes, Hb and PCV in broilers. Borges et al. (2004) and Hrabcakova et al. (2014) stated that hematological parameters were remarkably influenced by species, age, sex, nutrition, physiological state, stress and environmental temperature.

The influence of dietary supplementation with 1% inulin in heat stress conditions on biochemical profile are presented in *Table 6*. There were no significant differences (p≥0.05) in the determination of biochemical parameters, except for serum proteins and total proteins, which recorded increases (p=0.013) in both experimental

groups, CON_{HST} and INL_{HST}, compared to the CON_{NTC} group. Also, an increased level (p=0.020) of total cholesterol was observed in both experimental groups, compared to the CON_{NTC} group.

Table 6. Influence of dietary supplementation with inulin on the biochemical profile (average values/groups)

Parameter	Diets			SEM	p-Value
	CON $_{NTC}$	CON $_{HST}$	INL $_{HST}$		
Creatinine, mg/dL	0.06	0.06	0.08	0.009	0.134
Serum protein, g/dL	2.30 ^{b}	2.75 ^{a}	2.73 ^{a}	0.093	0.013
Cholesterol, mg/dL	94.50 ^{b}	131.75 ^{a}	133.00 ^{a}	8.73	0.020
Gamma glutamyl transferase, UI/L	18.25	22.00	17.25	1.78	0.193
Serum glucose, mg/dL	205.8	192.3	218.1	11.5	0.329
Triglycerides, mg/dL	32.3	41.0	62.0	10.9	0.194
Urea, mg/dL	0.66	1.25	1.75	0.332	0.122
Urea nitrogen (BUN), mg/dL	0.23	0.49	0.70	0.131	0.085
Globulin, g/dL	1.25	1.43	1.38	0.047	0.057
Total protein, g/dL	2.30 ^{b}	2.75 ^{a}	2.73 ^{a}	0.094	0.013
Albumin, g/dL	1.08 ^{b}	1.31 ^{a}	1.33 ^{a}	0.064	0.043

Where: CON $_{NTC}$ – Control group, conventional diet and normal thermal regim; CON $_{HST}$ – experimental group, conventional diet and heat stress conditions; INL $_{HST}$ – experimental groups, diet supplemented with 1% inulin and heat stress conditions; SEM - standard error of mean; $^{a-b}$ mean value within a row having different superscripts are significantly different at $p \leq 0.05$

Similar research conducted by [15] on the influence of inulin on blood parameters, by including 0.7% inulin and 0.014% beta-glucan in the diet, determined a decrease in serum cholesterol and total triglyceride levels. In a study conducted by [4] on the effects of inulin supplementation on intestinal barrier function in chickens with salmonella infection, they showed that inulin supplementation decreased INF-gamma levels and significantly increased IgA and IgG levels. Specialized studies [14], [17] indicate that even relatively short periods of inulin consumption in mice with an intact gut microbiome have negative effects on metabolism and liver function. On serum biochemistry, [20], [19] showed that, compared with the control group, the heat stress group showed a significant increase in glucose, TG, TC, and LDL-C, but the concentrations of TP, albumin, globulins, and HDL-C in serum did not show significant changes. Similar results obtained by [22] show that groups that received 2 or 4% inulin in the diet had higher HDL-C concentrations ($p < 0.05$) after day 42 than those that received 1% inulin, those that received 4% inulin or 400 ppm flavomycin had lower TG concentrations ($p < 0.05$) than birds that received the control diet on day 21.

In *Table 7* are presented the influence of 1% inulin dietary supplementation in heat stress conditions on intestinal histomorphometry. There were no significant differences ($p \geq 0.05$) in determining the histomorphometry of villi and crypts in the duodenum, except for the experimental groups (CON $_{HST}$ and INL $_{HST}$), where the width of the villi was significantly lower (p=0.003) compared to the CON_{NTC} group, probably negatively influenced by the heat stress conditions that limited the intestinal absorption of the nutrients. The similar results were obtained for depth crypts which are significantly decrease ($p<0.0001$) for both experimental groups compared to CON_{NTC} group.

Table 7. Influence of dietary inulin supplementation on intestinal morphometry (average values/groups)

Parameter (μm)	Diets			SEM	*p*-Value
	CON $_{NTC}$	CON $_{HST}$	INL $_{HST}$		
Intestinal villi length (LV)	1820.1	1621.1	1667.0	85.8	0.302
Intestinal villi width (WV)	201.5 ^{a}	155.3 ^{b}	162.0 ^{b}	8.99	0.003
Intestinal crypts depth (CD)	616.4 ^{a}	308.3 ^{b}	306.3 ^{b}	33.4	<0.0001

Where: CON $_{NTC}$ – Control group, conventional diet and normal thermal regime; CON $_{HST}$ – experimental group, conventional diet and heat stress conditions; INL $_{HST}$ – experimental groups, diet supplemented with 1% inulin and heat stress conditions; SEM - standard error of mean; $^{a-b}$ mean value within a row having different superscripts is significantly different at $p \leq 0.05$; LV=length villi; WV=width villi; CD=depth crypt.

The following microscopic images (fig. 1, 2, 3, 4, 5, 6) show the measurements of the length and width of villi and depth of crypts in the duodenum of broiler.

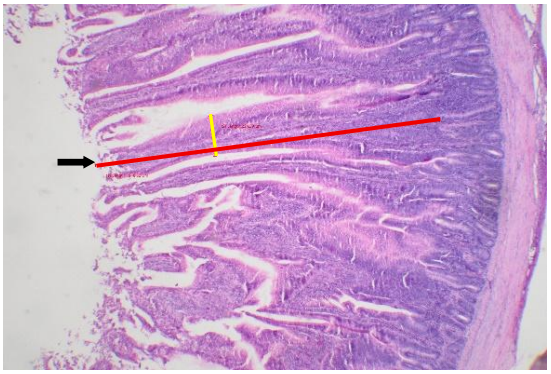

Figure 1 – CON NTC group – Duodenum – villi (HE stain, ob. 10x), black arrow - detail

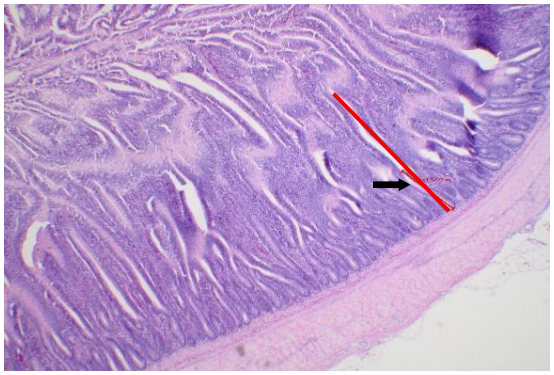

Figure 2 – CON NTC group – Duodenum – crypt (HE stain, ob. 10x, black arrow - detail

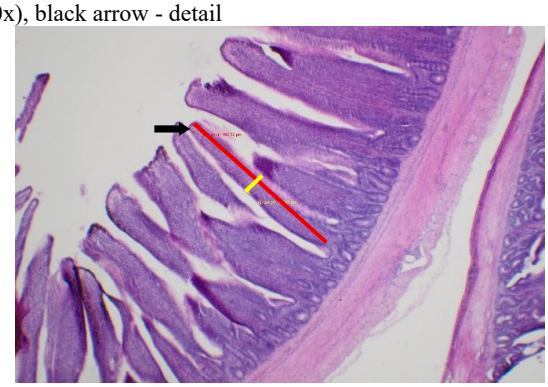

Figure 3 – CON HST group – Duodenum – villi (HE stain, ob. 10x), black arrow - detail

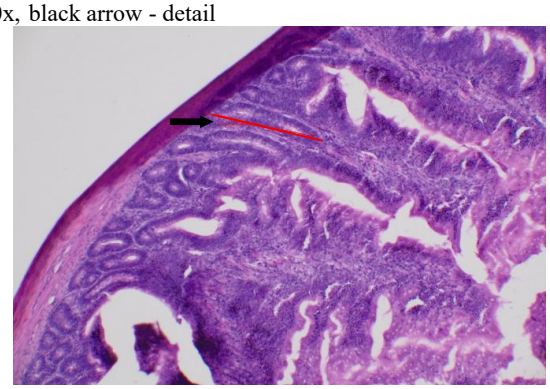

Figure 4 – CON HST group – Duodenum – crypt (HE stain, ob.10x), black arrow - detail

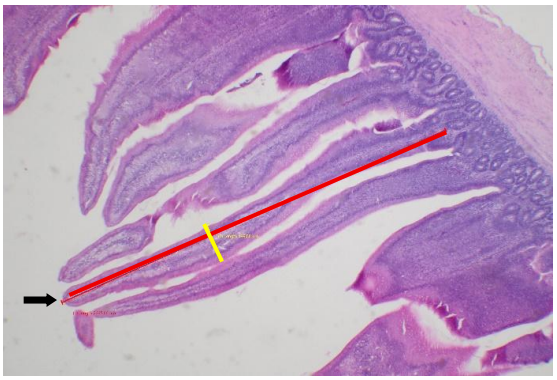

Figure 5 – INL HST group – Duodenum – villi (HE stain, ob. 10x), black arrow - detail

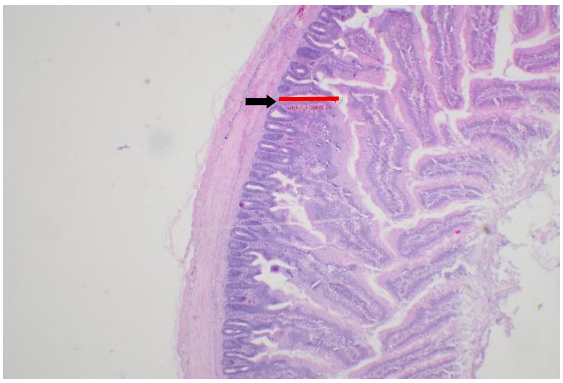

Figure 6 – INL HST group– Duodenum – crypt (HE stain, ob. 10x), black arrow - detail

In a similar study in which the diet of chickens was supplemented with 0.5% and 1% inulin, respectively, [1] showed that the inclusion of inulin had no effect on the length of villi, crypt depth or length/depth ratio in the duodenum and jejunum at day 42. But, in the ileum, it was found that the length of villi increased significantly. Similarly, [16] by including 2% inulin and 10% wheat bran in the diet of broiler chickens, it was observed that the length of villi increased in the jejunum and ileum, but without differences in crypt depth, and the villus/crypt ratio was higher in the jejunum. Elrayeh et al. [15] by supplementing the diet of broiler chickens with 0.7% inulin and 0.014% beta-glucan concluded that the total length of the intestine was significantly greater. In an experiment with *Salmonella spp*. infection, [4] show that supplementing the diet with inulin attenuated the atrophy and detachment of the mucosa of the villi

in the duodenum, jejunum and ileum, induced by *Salmonella spp*. infection. In contrast, [3] show that the inclusion of inulin had no effect on the length of the villi and the depth of the crypts, a significant increase in the villus/crypt ratio was observed in the experimental group with 10 g/kg inulin. Also, [20] in an experiment on the direct effect of high heat stress on intestinal integrity, exposure to heat stress conditions for 24 hours significantly decreased both the ratio of villus height to crypt depth and the number of proliferating cells (PCNA) in the duodenum and increased plasma endotoxin concentration, also intestinal integrity and function were not affected by 12 hours of heat stress. On the other hand, chickens exposed to heat stress for 72 hours showed significantly damaged intestinal morphology in the duodenum, as well as increased plasma endotoxin concentration. These findings suggest that the changes in intestinal morphology and permeability observed in chickens heat-stressed for 24-72 hours are due to heat stress conditions and not to reduced food intake.

4. Conclusions

Summarizing the experimental results we obtained, it can be concluded that 1% inulin administered in the diet of broilers are significantly increased beneficial bacterial populations in the intestinal microflora, without influencing growth performance, blood parameters and histomorphometry of villi and crypts in the broiler's duodenum. The present study will serve as a base for future studies in which the rate of inulin inclusion in broilers 'diet can be increased up to 4%.

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