

# IMPACT OF DIETARY INULIN SUPPLEMENTATION ON LIPID PROFILE AND MEAT QUALITY OF BROILERS REARED UNDER HEAT STRESS CONDITIONS

I. Varzaru\*, A. Untea, R. Turcu, T. Panaite

National Research-Development Institute for Animal Biology and Nutrition (IBNA),  
Balotesti, Romania

## Abstract

Heat stress conditions affects nutrient utilization, induces oxidative stress, causing detrimental effects on meat quality of broilers. This study was conducted to evaluate the effect of dietary inulin supplementation (1 %) to broilers reared under heat stress, on breast meat lipid profile and lipid degradation products after 7 days of storage. A total of 60 Ross 308 broiler chickens, 14 days old, were randomly divided into 2 treatments (C and E) with 6 replicates per treatment and 5 chicks per pen. The concentrations of PUFA significantly ( $p < 0.05$ ) increased in E group, compared to C. The ratios PUFA/SFA, recommended by FAO to assess the nutritional value of fat, were significantly higher in E compared to C group (1.26 vs 1.15). A higher content of  $\alpha$ -linolenic acid was observed in E group, being associated with the reduced markers of lipid oxidation in E compared to C group, after 7 days of storage. TBARS values, secondary oxidation products, decreased ( $p < 0.05$ ) with almost 22 % in E compared to C group. The results showed a significantly positive effect of inulin supplementation on broiler meat quality, with benefits in delaying oxidation and enhancing the nutritional value of meat.

**Key words:** prebiotic, PUFA, lipid peroxidation, broilers meat

## INTRODUCTION

Poultry farming, particularly broiler production, plays a pivotal role in meeting the increasing global demand for animal protein [1]. However, one of the major challenges faced by poultry farmers is the adverse impact of heat stress on broilers, which can severely compromise their health and productivity. Broilers are highly susceptible to the negative effects of elevated temperatures, making the management of heat stress a critical aspect of poultry farming [2]. Elevated temperatures trigger a series of physiological responses in broilers, including increased respiration rates, reduced feed intake, and behavioral changes [3]. These responses, in turn, contribute to oxidative stress through various mechanisms. Heat stress in broilers is

closely associated with oxidative stress, which is a condition characterized by an imbalance between the production of harmful reactive oxygen species (ROS) and the bird's ability to neutralize them [4]. Oxidative stress can lead to cellular damage, inflammation, and a range of health issues, affecting growth rates, feed efficiency, and meat quality in broilers [5, 6]. As a result, there has been a growing interest in implementing strategies aimed at mitigating the impact of heat stress in broilers.

Prebiotics, which are non-digestible dietary components, have been proposed as a nutritional strategy to improve the resilience of broilers against heat stress [7]. Research studies indicate that prebiotics have several beneficial effects in broilers nutrition, including the improvement of gut

\*Corresponding author: iulia.maros@ibna.ro

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health [8], the modulation of immune function [9], the enhancement of stress resistance [10], and the alteration of the composition of gut microbiota in broilers facing heat stress. Common prebiotics used in broiler nutrition include inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), and mannan oligosaccharides (MOS).

Inulin, a non-digestible carbohydrate, primarily consists of fructans, which are polymers of fructose molecules. As a prebiotic, inulin serves as a substrate for beneficial gut bacteria, such as *Lactobacillus* and *Bifidobacterium*, that ferment it. This fermentation process produces short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate, which reduced the pH of the intestine and thus stimulate the beneficial bacteria proliferation [11], and also decrease bile acid solubility, indirectly enhancing the absorption of minerals [12].

Inulin supplementation to broilers diets has been reported to have a positive effect on gut microbiota, growth performance and nutrient utilization, immune system, mineral metabolism [13] and increase their lipid and cholesterol metabolism [14]. Incorporating inulin into broiler diets has demonstrated a positive impact on blood serum lipids by reducing the levels of triacylglycerides, and also enhances the ability of saturated oils to improve the ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) in intramuscular fat of broilers [15]. This implies that inulin can contribute to a healthier fatty acid profile in broiler meat, which is beneficial from a nutritional and health perspective.

This study was conducted to evaluate the effect of dietary inulin supplementation to broilers reared under heat stress, on breast meat lipid profile and lipid degradation products after 7 days of storage.

## MATERIAL AND METHOD

All the experimental procedures including broiler raising and sample

collection were carried out under the approval of the Ethics Committee of the National Research and Development Institute of Animal Biology and Nutrition, Romania, and in compliance with the Romanian Law 43/2014 for handling and protection of animals used for experimental purposes and Directive 2010/63/EU concerning the protection of animals used for scientific purposes.

## Experimental Design

A total of 60 Ross 308 broiler chickens were obtained from a local commercial hatchery. Birds were fed with a starter diet from day 1 to 14, and after that were randomly divided into 2 experimental dietary groups (C and E) with 6 replicates per treatment and 5 chicks per pen. Both grower and finisher diets were formulated to meet the nutrient requirements of broilers, and were based on corn-soybean meal and corn gluten as main ingredients. In the experimental diet (E), 1% inulin extract was included as presented in Table 1 (published data, [16]). The inulin source used in the current study was a commercial product (Frutafit® IQ, Sensus, Netherlands) obtained from chicory (*C. intybus* L.) roots containing 90% inulin and a polymerization degree of 2–60. Inulin was supplied in powder form and was included in the diet by replacing the same amount of corn powder.

The birds were reared in an experimental hall with digestibility pens, where the temperature was maintained at 32°C during the entire feeding period (1 to 42 days). At 42 days old, 6 birds from each were slaughtered according to the veterinary procedures and breast meat samples were collected.

## Fatty Acids Determination

The fatty acid content of breast meat samples was determined using a gas chromatograph Perkin Elmer Clarus 500 (Massachusetts, United States) according to the method described by [17]. The fatty acids from samples were first transformed

into methyl esters and afterwards were separated on the capillary chromatographic column with a high polar stationary phase TRACE TR-Fame, (Thermo Electron, Massachusetts, United States), with dimensions of 60 m × 0.25 mm × 0.25 μm film. The fatty acids detection was performed using a flame ionization detector (FID) and their identification and quantification was made by reference to the standard chromatograms. The average amount of each fatty acid was used to calculate the sum of the total saturated (SFA), total monounsaturated (MUFA) and total polyunsaturated (PUFA) fatty acids.

Lipid indices of atherogenicity and thrombogenicity and the ratio of hypo and hypercholesterolemia (h/H) in breast meat were determined based on the data obtained from the fatty acid composition of breast samples, and their importance to human food was evaluated. The indices of atherogenicity and thrombogenicity were calculated according to [18] using the following equations:

$$AI = (C\ 12:0 + 4 \times C\ 14:0 + C\ 16:0) / (\Sigma MUFA + \Sigma n6 + \Sigma n3) \quad (1)$$

$$TI = (C\ 14:0 + C\ 16:0 + C\ 18:0) / (0.5 \times \Sigma MUFA + 0.5 \times \Sigma n6 + 3 \times \Sigma n3 + \Sigma n3 / \Sigma n6) \quad (2)$$

$$h/H = (C18:1n - 9 + C18:2n - 6 + C20:4n - 6 + C18:3n - 3 + C20:5n - 3 + C22:5n - 3 + C22:6n - 3) / (C14:0 + C16:0) \quad (3)$$

### Lipid oxidation assessment

Samples of breast meat were stored for 7 days in the refrigerator at a constant temperature of 4°C before evaluating the lipid oxidation parameters. To conduct the evaluation, the samples were minced and subjected to cryogenic treatment using liquid nitrogen at a temperature of -180°C, after which they were grounded. The oxidative stability of the meat was determined by assessing the primary lipid degradation parameters, peroxide index,

dienes and conjugated trienes, respectively, by secondary parameter, represented by the values of thiobarbituric acid-reactive substances (TBARS), involving spectrophotometric methods previously described by [19] and a UV VIS spectrophotometer (Jasco V-530, Japan Servo Co. Ltd., Japan).

Table 1 Diets formulation and chemical composition

Item	Grower		Finisher	
	C	E	C	E
<i>Ingredients, %</i>				
Corn	44.5	43.5	47.23	46.23
Wheat	10.00	10.00	10.00	10.00
Soybean meal	35.45	35.45	32.16	35.45
L-lysine, %	0.32	0.32	0.12	0.32
Di-methionine	0.28	0.28	0.27	0.28
L-threonine	0.04	0.04	0.03	0.04
Calcium carbonate	1.26	1.26	1.17	1.26
Monocalcium phosphate	1.59	1.59	1.48	1.59
Salt	0.37	0.37	0.37	0.37
Vegetable oil	5.12	5.12	6.10	5.12
Choline	0.07	0.07	0.07	0.07
Premix*	1.00	1.00	1.00	1.00
Total	100	100	100	100
<i>Chemical analysis</i>				
ME, kcal/kg	3129		3218	
Crude protein, %	21.50		20.00	
Ether extract, %	6.01		6.49	
Crude fiber, %	3.57		3.36	

**Note:** \*1 kg premix contains: 1,100,000 IU/kg vit A; 200,000 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 Mn; 8000 mg/kg Fe; 500 mg/kg Cu; 6000 mg/kg Zn; 37 mg/kg Co; 18 mg/kg Se ME – metabolizable energy; CP – crude protein; EE – ether extract; CF – crude fiber

### Statistical Analyses

The analytical data were subjected to statistical analysis through a two-way ANOVA, followed by Tukey's test, using XLSTAT software (Addinsoft, France). A

probability level below 5% was considered significant.

## RESULTS

The fatty acid composition of breast meat was influenced by dietary inulin (Table 2). The ANOVA analysis results showed ( $p < 0.05$ ) significant differences in the content of the analyzed fatty acids in

breast meat between birds fed with the control diet and those fed with the inulin supplemented diet. The concentrations of SFA and MUFA were lower ( $p < 0.05$ ), whereas those of PUFA and the ratios PUFA:SFA and  $\Omega 6/\Omega 3$  were higher in birds fed with the inulin supplemented diet compared to the control diet.

Table 2 Fatty acids composition of the breast meat (%)

Fatty acids	C	E	SEM	p-value
SFA	29.68 <sup>b</sup>	28.79 <sup>a</sup>	0.23	0.043
MUFA	36.03 <sup>b</sup>	34.76 <sup>a</sup>	0.30	0.018
PUFA	34.01 <sup>a</sup>	36.26 <sup>b</sup>	0.51	0.011
$\Omega 3$	2.59 <sup>b</sup>	2.19 <sup>a</sup>	0.10	0.030
$\Omega 6$	31.42 <sup>a</sup>	34.08 <sup>b</sup>	0.58	0.006
$\Omega 6/\Omega 3$	12.26 <sup>a</sup>	15.59 <sup>b</sup>	0.72	0.004
PUFA/SFA	1.15 <sup>a</sup>	1.26 <sup>b</sup>	0.03	0.014

**Note:** Values with different superscripts in the same row are significantly different ( $P < 0.05$ ). SFA - Saturated fatty acids, MUFA - Monounsaturated fatty acids, PUFA - polyunsaturated fatty acids

Results regarding the concentration of  $\alpha$ -linolenic acid showed a significantly higher level ( $p < 0.05$ ) in the experimental group, compared to control group (Figure 1), which can be associated with the higher content of PUFA registered in the same group.

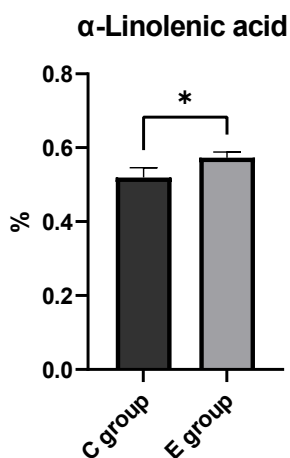


Fig. 1  $\alpha$ -Linolenic acid in breast meat of broiler chicken reared under heat stress conditions

The assessment of lipid quality indices (Table 3) showed no significant differences

( $p > 0.05$ ) between the groups for the thrombogenicity index, but a significant reduction ( $p < 0.05$ ) of the atherogenicity and a significant ( $p < 0.05$ ) increased of the h/H ratio was observed in the group with prebiotic supplement, compared to the control.

Table 3 Lipid quality indices of the breast meat (%)

Indices	C	E	SEM	p-value
TI	0.71 <sup>a</sup>	0.70 <sup>a</sup>	0.01	0.452
AI	0.33 <sup>b</sup>	0.31 <sup>a</sup>	0.01	0.012
h/H	3.04 <sup>a</sup>	3.33 <sup>b</sup>	0.06	0.002

**Note:** Values with different superscripts in the same row are significantly different ( $P < 0.05$ ). AI—atherogenicity index; TI—thrombogenicity index; h/H—hypocholesterolemic /hypercholesterolemic ratio.

Results regarding the oxidative stability of the breast meat stored in refrigerated conditions are presented in Figure 2. Over the storage period of 7 days, the production of conjugated dienes and conjugated trienes was not reduced by the inclusion of inulin in the diets of broilers reared under heat stress conditions, but the peroxide value significantly decreased ( $p < 0.05$ ) in the experimental group, compared to control group.

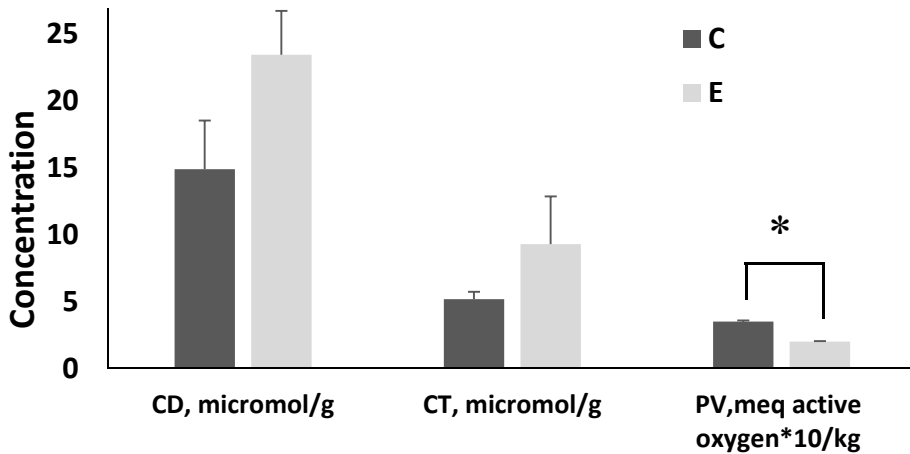


Fig. 2 Effect of dietary inulin on primary oxidation markers in the breast meat of broilers reared under heat stress conditions (\*: significantly different,  $P < 0.05$ ; CD: conjugated dienes; CT: conjugated trienes; PV: peroxide value)

The assessment of secondary parameter of lipid oxidation is presented in Figure 3. The inclusion of prebiotic in the broilers reared under heat stress conditions improved the oxidative stability of breast meat, as expressed by the TBARS value.

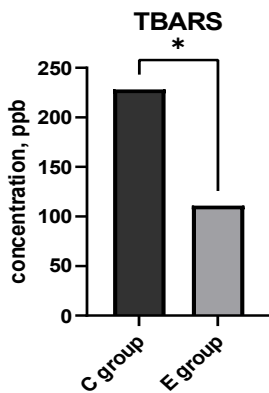


Fig. 3 Effect of dietary inulin on secondary oxidation marker in the breast meat of broilers reared under heat stress conditions (\* - significantly different,  $P < 0.05$ )

A significant ( $p < 0.05$ ) decrease of the TBARS has been observed in the experimental group (almost 22 % reduction), compared to the control.

## DISCUSSIONS

Studies have shown that incorporating herbal additives into chicken feed can positively impact the composition of fatty acids in the meat. The most desirable effects of diet supplementation involve an increase in polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA), along with a reduction in saturated fatty acids (SFA) in the breast muscles. This modified fatty acid profile contributes to the enhancement of poultry meat quality, leading to increased consumer demand [20]. Nevertheless, a potential issue arises from the fact that polyunsaturated fatty acids are more susceptible to oxidation compared to other lipid types, and this susceptibility can adversely affect the sensory qualities of the meat [21]. Herbal additives are rich in antioxidants that can counteract against the oxidation of unsaturated fatty acids and cholesterol found in animal-derived products, while also enhancing the consumer's overall antioxidant status.

In this study, dietary supplementation with 1 % inulin induced changes in the fatty acid profile of breast meat from broilers reared under heat stress conditions. The available literature concerning the influence of the dietary inulin on the tissue fatty acid

composition provides mixed and inconclusive results. In a study conducted by Velasco et al. in 2010 [15], where 3 concentrations of inulin (0, 5, and 10 g/kg of diet) were used in broilers diets, in breast meat the concentration of C18:2n-6 and PUFA and the ratios PUFA:SFA and UFA:SFA increased linearly ( $P < 0.05$ ) with inulin level, whereas SFA concentration had a quadratic response to inulin level. In a research investigation carried out by [22], the inclusion of a mixture of inulin and horse chestnuts had no effect on the fatty acid composition, including the ratio of PUFAs n-6/n-3 in the longissimus dorsi muscle of pigs. According to findings reported by [23], the inclusion of inulin in rabbits' diets resulted in an increase in the levels of beneficial fatty acids, such as CLA and n3-PUFA. This dietary change also led to a more favorable health-promoting index, along with a reduction in the atherogenic and thrombogenic indices in the meat. Similarly, [24] noted that the fatty acid composition and the lipid indices of the backfat and meat of fatteners fed with a diet supplemented with a dried inulin-rich dandelion, indicated a decreased risk of developing atherosclerotic disorders.

Results of this study showed in the inulin supplemented group a decreasing tendency of the thrombogenicity index, while a significant reduction ( $p < 0.05$ ) of the atherogenicity index was registered in the same group. The atherogenicity index (AI) and thrombogenicity index (TI) serve as key parameters for evaluating the nutritional quality and the potential health impact of animal fats on consumers [18]. These indices are not directly related to the quality of meat, such as its taste, tenderness, or overall palatability. However, the composition of the meat, especially the type of fats it contains, can indirectly influence these indices, which are one of the most reliable, and widely used indices to assess the potential effects of fatty acids on cardiovascular disease [25]. The AI measures the potential of a food to promote

atherosclerosis, a condition characterized by the buildup of fatty deposits in the arteries. while the TI assesses the potential of a food to promote blood clot formation, being influenced by the types of fatty acids present in the food [26]. In this study, the decreasing values of the atherogenicity index and thrombogenicity index can be associated with the decreased concentrations of the SFA in the inulin supplemented group. The h/H ratio provides insights into how fatty acids impact cholesterol metabolism, and meat with favorable health attributes should exhibit elevated values [27]. In the present study, the h/H ratio significantly increased ( $p < 0.05$ ) in breast meat under the influence of the dietary inulin.

These results are considered positive, given the decrease in the thrombogenicity and atherogenicity indices, as well as the increase in linolenic acid (n-3), in the context of producing healthy and functional foods for human consumption. [28] highlighted the health benefits of long-chain n-3 fatty acids for both animals and humans, including the reduction of heart disease risk and a decrease in circulating cholesterol levels. As [29] and [15] have highlighted, incorporating inulin into the broilers diet results in a reduction in body fat accumulation, lowers serum cholesterol levels, and decreases abdominal fat weight of chickens [30]. This aligns with the observed gene regulation by [31], being attributed to the enhanced mitochondrial function associated with various fatty acid metabolism processes. Moreover, an important enhancement in the digestibility of the amino acids and an increase in the absorption of fatty acids were detected in broilers that were fed an inulin enriched diet [32].

Meat consumption has been associated with an imbalanced intake of fatty acids among consumers, primarily because certain types of meat naturally exhibit a low PUFA to SFA ratio, often around 0.1 [33]. Thus, a PUFA to SFA ratio of 0.4 is often



recommended [34]. In the current study the PUFA/SFA ratio was higher than 0.4, being observed a value of 1.15 for control group, and a significantly ( $p < 0.05$ ) higher ratio (1.26) in the group supplemented with inulin. In the same line, [35] noted a significantly higher PUFA/SFA ratio in meat from broilers fed a plant-based diet, confirming higher polyunsaturated fatty acids level, which are known to be more susceptible to oxidation.

Heat stress can accelerate metabolic rates, generating substantial quantities of free radicals, causing lipid peroxidation, impairs proteins and DNA, and ultimately induces oxidative stress, resulting in a decline in meat quality [36]. Moreover, due to its high concentration of polyunsaturated fatty acids (PUFA), poultry meat is more prone to oxidative reactions, particularly lipid oxidation, compared to beef or pork [37]. Lipid oxidation is a complex process consisting of several phases, including initiation, propagation, and termination phase. This process results in the formation of both primary and secondary by-products, which have consequences for both meat quality and human health [21]. Conjugated dienes, conjugated trienes and peroxide values are primary indicators of lipid oxidation. They are formed when fatty acids are exposed to oxidative stress and can serve as a measure of the initial stages of lipid oxidation. In this study, among the primary oxidation products only the peroxide value significantly decreased in the inulin supplemented group. A more pronounced effect on delaying the oxidation processes in meat was observed by [38], when symbiotic supplements were used in broilers diets reared in normal thermal conditions. Results of this study showed that inulin supplementation significantly decreased ( $p < 0.05$ ) TBARS values in chicken breast meat, indicating a potential role in decreasing lipid oxidation. Lower TBARS values are generally associated with improved meat quality, reduced off-flavors, and enhanced shelf life. This fact

can be attributed to the antioxidant capacity of inulin, along with the influence of the gut microflora that ferments inulin in the gastrointestinal tract [39]. A previous study [16] reported that antioxidant capacity of broilers meat can be improved under inulin influence. [40] recommend also the inclusion of 1% inulin in the broilers diet for its capacity to improve the antioxidant status.

## CONCLUSIONS

Inulin supplementation in broiler diets appears to be a promising strategy for delaying the onset of lipid oxidation in broilers subjected to heat stress conditions. Moreover, it can lead to a reduction in saturated fatty acids, an increase in monounsaturated and polyunsaturated fatty acids, and an improvement in the omega-6 to omega-3 fatty acid ratio and in the lipid quality indices related to human health. These changes may contribute to the production of poultry meat with improved nutritional profiles, aligning with consumer preferences for healthier foods.

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