DIETARY PREBIOTIC SUPPLEMENT IN BROILERS NUTRITION AND THEIR EFFECTS ON PERFORMANCES, BLOOD PARAMETERS AND INTESTINAL HEALTH

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Abstract

A-28 days feeding trial was conducted on broiler chickens (grower and finisher stage) with the aim to evaluate the effects of dietary inulin presence on performance and intestinal microflora. The animals were housed in an experimental hall, under heat stress conditions, with controlled humidity, ventilation and light regime. The experimental diet differed from control diet by inclusion of 1% inulin. Weekly was monitored broiler body weight and daily was registered the feed intake and leftovers. At the age of 42 days, 6 broilers per group were slaughtered and blood samples were collected for hematological and biochemical determinations. Samples of caecal content were collected upon slaughtering, for bacteriological examination (Enterobacteriaceae, Lactobacillus sp., Staphylococcus sp.). The presence of inulin in the diets of broilers didn't influence the productive parameters. The hematological profile presented increased values of lymphocyte and heterophile concentrations in experimental group and also heterophile to lymphocytes ratio. The content of Enterobacteriaceae and Lactobacillus sp. in the cecum was positively influenced by the prebiotic dietary supplement that contributed to maintaining the balance of the intestinal microflora.

Key words: *prebiotics, inulin, broiler chicken, performances, health status*

INTRODUCTION

The poultry industry plays a crucial role in meeting the ever-growing global demand for high-quality protein. To maintain and enhance poultry production, it is essential to optimize the nutrition and health of broilers. One avenue of interest in recent years has been the use of dietary prebiotic supplements to improve broiler nutrition, enhance productive parameters, modulate blood parameters, and promote intestinal health [1].

Heat stress in broiler chickens farming is a common challenge to poultry farmers because can lead to reduced feed intake, impaired growth, and increased mortality rates, negatively impacting their overall welfare and productivity [2].

Prebiotics offer a promising approach to alleviating heat stress in animals, by promoting the growth and activity of beneficial gut bacteria, they can enhance gut health and function, which, in turn, supports the resilience of animals to heat stress [3]. This modulation of the gut can help improve nutrient absorption and reduce the physiological strain caused by high temperatures, ultimately mitigating the adverse effects of heat stress on the animals' well-being and performance [4].

Among some common prebiotics like fructooligosaccharides, galacto-

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The manuscript was received: 17.10.2023
Accepted for publication: 03.11.2023

oligosaccharides, and xylooligosaccharides, inulin is a natural, non-digestible carbohydrate which belongs to a class of prebiotics that selectively promote the growth and activity of beneficial gut microorganisms, primarily *Bifidobacteria* and *Lactobacilli* [5]. Inulin is characterized by its ability to ferment in the colon, producing short-chain fatty acids, which contribute to a healthier gut environment [6].

Inclusion of inulin prebiotic supplements in broiler diets has been linked to enhanced growth performance. Broilers fed with inulin-supplemented diets often exhibit increased body weight gain and improved feed conversion ratios [7]. Inulin can improve feed efficiency by promoting the breakdown of complex carbohydrates in the gut, making them more readily available for absorption. This not only reduces feed wastage but also contributes to better productivity and increase the antioxidant defense system [8].

The gut and the immune system are intricately connected, and inulin can help modulate the immune response in broilers [9]. By promoting the growth of beneficial gut bacteria, inulin contributes to a balanced immune system, enhancing broilers' ability to combat infections and diseases [10]. This can result in improved blood parameters, including a reduction in inflammatory markers. High serum lipid levels can be detrimental to broiler health. Studies have shown that inulin supplementation can lead to a reduction in serum lipid levels, promoting better cardiovascular health and reducing the risk of conditions such as fatty liver syndrome in broilers [11].

By supporting the growth of beneficial gut bacteria, prebiotics help create a favorable gut environment for nutrient absorption and utilization [12]. Inulin supplementation can enhance gut barrier function by promoting the growth of beneficial bacteria, which contribute to a healthier gut lining.

This study was conducted to test the effects of inulin on broiler chickens' production performances, blood parameters and intestinal health, raised in heat stress conditions.

MATERIAL AND METHOD

Ethical Consideration

The experiment conducted in the present study adhered to Directive 2010/63/EU, which concerns the welfare of animals utilized for scientific research. All the procedures outlined in this study received approval from the Ethical Commission of the National Research and Development Institute for Biology and Animal Nutrition.

Experimental Design and Diets

In this study, a total of 60 Ross 308 unsexed broiler chickens were utilized to test the effect of 1% inulin supplement. The broilers used for the study were purchased from a commercial hatchery. During the starter phase (1-14 day) all chickens were fed the same basal diet which contained equivalent amount of metabolizable energy (2975 kcal/kg) and crude protein (21.50%). After the starter phase, when the birds reached 14 days, were individually weighted and distributed into two groups having 30 chickens/group each. During the trial (15-42 days), an inulin nutritional supplement was assessed. The control diet (C) was formulated using a combination of corn-soybean meal and corn gluten as the primary ingredients, while the experimental diet (E) was the same basal diet supplemented with 1% inulin (Frutafit® IQ) extracted from chicory root. The inulin was added to the control diet by substituting 1% of corn. This dietary setup was employed throughout both the grower and finisher phases, as outlined in Table 1.

The research was conducted within a controlled experimental facility equipped with digestibility pens and carefully managed microclimate conditions, including humidity, ventilation, and lighting, in accordance with the broiler breeding management guidelines [13]. The temperature was set to be constantly maintained at 32°C throughout the entire feeding period, spanning from day 1 to day 42. Each treatment was replicated six times, with each replicate pen accommodating five chicks. The broiler chickens were provided with *ad libitum* access to ground feed and unrestricted access to water during the entire experimental period.

Table 1 Ingredients and chemical composition of broilers basal diets, during grower and finishing phases

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

Premix: 11.000 IU/kg vit A; 2.000 IU/kg vit D3; 27 IU/kg vit E; 3 mg/kg vit K; 2 mg/kg vit B1; 4 mg/kg vit B2; 14.85 mg/kg pantothenic acid; 27 mg/kg nicotinic acid; 3 mg/kg vit B6; 0.04 mg/kg vit B7; 1 mg/kg vit B9; 0.018 mg/kg vit B12; 20 mg/kg vit C; 80 mg/kg Mn; 80 mg/kg Fe; 5 mg/kg Cu; 0.60 mg/kg Zn; 0.37 mg/kg Co; 1.52 mg/kg I; 0.18 mg/kg Se.

Production Performances Measurement

Throughout the experimental period, bodyweight (g/broiler), average daily weight gain (g/broiler/day), average daily feed intake (g/day/broiler), feed conversion ratio (kg feed/kg weight), and viability (%) were monitored.

Bodyweight was measured at the beginning (14 days) and end (42 days) of the trial, and with this data average daily gain was calculated. Feed intake was calculated based on the feed given and the refusals. To calculate these data, the cage was considered the experimental unit. Throughout the trial period, no medical treatment was applied to the chickens. Based on the production performances data, the European Production Efficiency Factor (EPEF) and the European Broiler Index (EBI) were calculated with appropriate formula [14].

Blood Sampling and Analyses

At the end of the trial, six broilers form each group were randomly selected and sampled for blood. For the biochemical analysis, venous blood samples were carefully collected into 9 mL plain plastic tubes with lithium heparin and without anticoagulants designed for plasma separation (Vacutest, Arzergrande, Italy). After centrifugation, serum was isolated.

For hematological assessments blood samples were collected in vacutainer tubes that included ethylene diamine tetra-acetic acid heparin anticoagulant.

Cecal Sampling and Microbiological Analyses

After blood sampling, same broiler chickens were slaughtered following the Romanian Law 43/2014 for the handling and protection of animals used for experimental purposes according to a prior approved experimental protocol. After the birds were humanely slaughtered by severing the jugular vein and carotid artery, allowing for a 2-minute exsanguination period, the intestinal tract was carefully removed from the abdominal cavity. Subsequently, cecal samples were collected and placed into sterile plastic tubes and sent to laboratory for analyses.

One gram of cecal content was homogenized with 7 mL Brain Heart Infusion broth (Oxoid LTD, England CM1135) supplemented with 2 mL sterile glycerol and the enumeration of colony forming units (CFU) of *Lactobacillus* spp., *Staphylococcus* spp., *Enterobacteriaceae*, and *Salmonella* spp. were performed [15].

Statistical Analyses

The graphs and statistical interpretation of the results obtained were made in GraphPad Prism software, version 13.2 (GraphPad Software, La Jolla, CA, USA). The effect of inulin was carried out using one-way analysis of variance (ANOVA), and the values were determined to be significant at $P<0.05$ between groups.

RESULTS

Effect of Dietary Inulin as Prebiotic on Production Performances and Efficiency of Broiler Chickens

Under heat stress conditions, the final body weight (Figure 1a) and average weight gain (Figure 1b) of broiler chickens fed with dietary prebiotic supplement showed significant ($P<0.05$) difference compared with control group. Experimental broiler chickens showed higher weight under heat stress conditions, as a response to dietary inulin supplement.

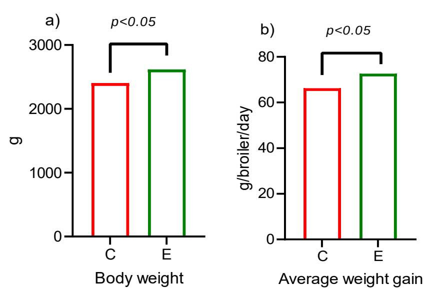

Fig. 1 Effect of dietary inulin supplement on final body weight (a) and average weight gain (b) of broiler chickens after 42 experimental days, under heat stress conditions (32°C)

In terms of feed consumption, the broilers fed the inulin supplemented diet showed slightly higher average feed intake (Figure 2a), but the difference was not statistically significant ($P>0.05$). On the contrast, the feed conversion ratio, was significantly lower ($P<0.05$) in broilers from experimental group (1.56 kg feed/kg weight) as a response to dietary inulin (Figure 2b), compared to control group (1.66 kg feed/kg weight).

The production efficiency factors, were significantly higher ($P<0.05$) in the inulin supplemented group when compared with the control group, as presented in Figure 3.

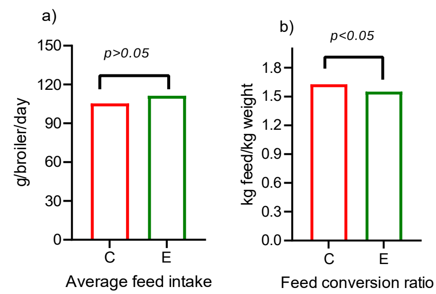

Fig. 2 Effect of dietary inulin supplement on average feed intake (a) and feed conversion ratio (b) of broiler chickens after 42 experimental days, under heat stress conditions (32°C)

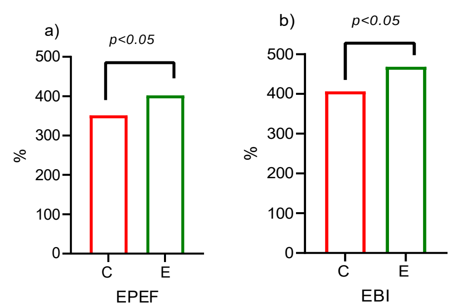

Fig. 3 Effect of dietary inulin supplement on European production Efficiency Factor (a - EPEF) and European Broiler Index (b - EBI) of broiler chickens after 42 experimental days, under heat stress conditions (32°C)

Effect of Dietary Inulin as Prebiotic on Biochemical and Hematological Blood Parameters of Broiler Chickens

The effect of inulin supplement in broilers raised under heat stress conditions showed no significant effect on health status, given by the biochemical serum parameters (Table 2). Although the tendency for triglycerides, glucose and urea nitrogen were observed. However, from the hematological profile, the lymphocyte and heterophile parameters as well as their ratio were significant ($P<0.05$) among the two groups.

Table 2 Effect of dietary inulin supplement on biochemical serum and hematological blood parameters, of broiler chickens after 42 experimental days under heat stress conditions (32°C)

Item	C	E	SEM	P
Biochemical parameters				
Cholesterol, mg/dL	132	133	3.201	0.156
Triglycerides, mg/dL	41.0	62.3	8.580	0.246
Glucose, mg/dL	192	218	8.721	0.189
Total protein, g/L	2.75	2.72	0.072	0.140
Albumin, g/dL	1.31	1.32	0.052	0.522
Globulin, g/dL	1.43	1.38	0.039	0.186
Creatinine, mg/dL	0.06	0.08	0.007	0.295
Urea, mg/dL	1.25	1.75	0.280	0.155
Urea nitrogen, mg/dL	0.70	0.49	0.111	0.155
Gama- glutamyl transferase, UI/L	22.0	17.3	1.383	0.212
Hematological parameters				
Lymphocyte (%)	33.50^{b}	57.63^{a}	3.084	0.001
Heterophile (%)	58.63^{a}	35.13^{b}	2.925	0.001
H: L	1.83^{a}	0.62^{b}	0.133	0.001

a and b superscripts in the same row differ significantly (p < 0.05).C – control diet; E – experimental diet with 1% inulin supplement; SEM – standard error of the mean; P – significance.

Effect of Dietary Inulin as Prebiotic on Intestinal Health of Broiler Chickens

The findings from this study demonstrate a significant impact of dietary inulin supplementation on the microbial composition within broilers exposed to heat-stress conditions. The results of this research indicate a significant increase (P<0.05) in beneficial bacteria, specifically *Lactobacillus sp*., when compared to the control group, as presented in Figure 4a.

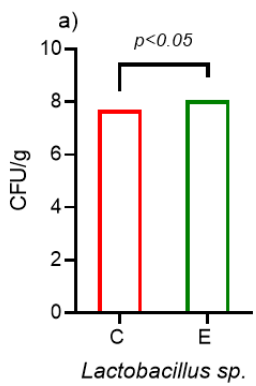

Fig. 4a Effect of dietary inulin supplement on *Lactobacillus sp*. of broiler chicken after 42 experimental days under heat stress conditions (32°C)

Significantly higher (P<0.05) *Enterobacteriaceae* counts were also registered in the experimental group compared with the control group (Figure 4b).

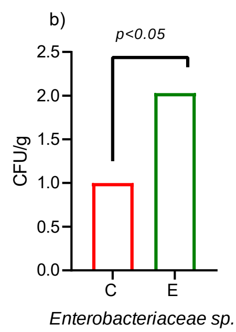

Fig. 4b Effect of dietary inulin supplement on *Enterobacteriaceae* counts of broiler chicken after 42 experimental days under heat stress conditions (32°C)

Moreover, a significant (P<0.05) reduction in the total counts of harmful bacteria like *Staphylococcus sp.* was observed in response to the dietary inulin supplementation (Figure 4c).

Salmonella sp. was absent in all cases.

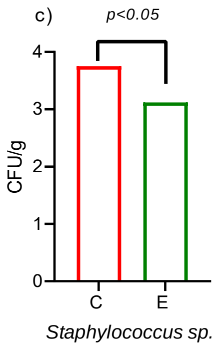

Fig. 4c Effect of dietary inulin supplement on *Staphylococcus sp*. of broiler chicken after 42 experimental days under heat stress conditions (32 °C)

DISCUSSIONS

Effect of Dietary Inulin as Prebiotic on Production Performances and Efficiency of Broiler Chickens

The effect of incorporating dietary inulin in the diets of broiler chickens facing heat stress conditions suggest that the inclusion of 1% dietary inulin has a substantial positive effect on growth and production parameters. Specifically, it was observed that dietary inulin led to a significant increase ($P<0.05$) in body weight (2619 g vs. 2405 g) and average weight gain (72.61 g/day vs. 66.22 g/day), while also contributing to improvements in feed conversion ratio and production efficiency factors. These findings align with literature data that underscores the potential benefits of inulin supplementation in broilers, especially when they are exposed to environmental stressors like heat [16]. The improved body weight and weight gain are consistent with previous research highlighting inulin's role in enhancing nutrient utilization and absorption, ultimately leading to better growth performance [17]. This effect suggests a positive correlation between inulin and improved growth parameters. Moreover, the positive impact on feed conversion ratio and production efficiency is also in line with previous studies where 0.5% or 1% inulin improved feed consumption and efficiency [18]. Although the mechanism responsible for the growth-promoting action in broilers is still unclear, an increase in nutrient digestibility is likely a main contributing factor in the growth-promoting effect of inulin. This prebiotic is known to support gut health by promoting the growth of beneficial bacteria, such as *Lactobacilli*, which can lead to more efficient nutrient utilization [17]. This can result in reduced feed wastage and better utilization of the feed, leading to improved feed conversion and overall production efficiency. Further, higher production performances and 100% viability rate in the experimental group, led to significantly higher efficiency factors compared with control group, as we observed previously [14]. Under heat stress conditions inulin might exhibit anti-inflammatory and antioxidant properties [19] which maintained the viability rate higher.

Although these findings underscore the potential of inulin as a valuable nutritional strategy to mitigate the adverse impacts of heat stress on poultry production, further research, and investigations into potential interactions with other dietary components, is necessary to comprehensively understand the full spectrum of benefits and optimize its application in the poultry industry. While the current findings are encouraging, it's essential to acknowledge that variations may exist in the outcomes of inulin supplementation depending on factors like dosage, duration, and environmental conditions.

Effect of Dietary Inulin as Prebiotic on Biochemical and Hematological Blood Parameters of Broiler Chickens

In this study no significant effect on the biochemical blood profile was observed. However, there was a noteworthy tendency in some parameters, such as triglycerides, glucose, and urea nitrogen. Additionally, the levels of lymphocytes and heterophiles, two types of white blood cells, showed significant differences (P<0.05) between the two groups.

This results, particularly the tendency in triglycerides, glucose and urea nitrogen aligns with some prior research that suggests inulin may play a role in modulating lipid metabolism and reducing urea nitrogen levels [20]. Inulin's impact on glucose and triglycerides is likely associated with its role in improving gut health and the subsequent enhancement of nutrient absorption [17]. In the study of [20] inulin supplement at 0.2%, 0.4% and 0.6% had significant effect on protein and lipid metabolic plasma parameters, especially cholesterol, glucose, and uric acid, without other significant changes in the biochemical profile. Inulin is a prebiotic, which means it provides a favorable environment for the growth of beneficial gut bacteria. These beneficial bacteria can have a positive influence on lipid metabolism as they can ferment dietary fibers, like inulin, into short-chain fatty acids, which can reduce cholesterol and triglycerides synthesis in the liver [6]. The tendency in urea nitrogen levels with inulin supplementation could be associated with the fiber's role in reducing protein fermentation and nitrogen excretion [21]. Inulin, as a non-digestible carbohydrate, is known to slow down the fermentation of dietary proteins in the gut. This could lead to decreased urea production and thus, lower urea nitrogen levels in the blood. Further, the significant differences in lymphocytes and heterophiles, key immune cells, are intriguing findings. These immune cells play a crucial role in defending the body against pathogens and responding to stressors, including heat stress. The significant variation in their levels suggests that dietary inulin may have some immunomodulatory effects [22]. Inulin's effect on these immune cells is linked to its influence on the gut microbiota. A balanced gut microbiota can positively affect the immune system. Prebiotics have the ability to promote the growth of beneficial bacteria, which can help modulate the immune response [12]. An increase in lymphocytes may suggest a bolstered immune response, while changes in heterophiles could be indicative of the stress response to factors like heat stress [23].

However, it's important to consider that the impact of dietary components, like inulin, on blood parameters can be multifaceted and context dependent. The existing literature supports the idea that inulin may influence various metabolic and immunological parameters, but the specific outcomes can vary depending on factors such as dosage, duration of treatment, and the overall health and stress levels of the animals involved.

Effect of Dietary Inulin as Prebiotic on Intestinal Health of Broiler Chickens

The results of this study revealed a significant increase in beneficial bacteria, such as *Lactobacillus sp*. and *Enterobacteriaceae*, alongside a noteworthy reduction in *Staphylococcus sp.* counts in the group that received inulin supplementation, compared to the control group. Lactobacilli, often considered the "good guys" of the gut, are known for their role in maintaining a balanced and harmonious gut microbiome [24]. They aid in digestion, contribute to the synthesis of essential nutrients, and compete with harmful bacteria for resources [25]. The proliferation of *Lactobacillus sp*., as seen in this study, is in line with the widely recognized ability of inulin to stimulate the growth of these beneficial microbes [26]. The significant reduction in *Staphylococcus* counts is an important result. *Staphylococcus* species can include pathogens that are detrimental to both gut health and overall well-being [27]. By diminishing their presence in the broiler chickens' intestines, inulin appears to contribute to a healthier gut environment. Research has consistently highlighted the positive impact of inulin on the gut microbiota, specifically in promoting the growth of beneficial bacteria like Lactobacilli [5,17]. Moreover, the reduction in *Staphylococcus* counts is not only in line with similar findings in other studies but also aligns with the broader understanding of how prebiotics can help establish a gut environment that is less hospitable to harmful bacteria [12, 28]. It was reported that 0.8% inulin supplement alone or combined with postbiotics in broilers significantly inhibit the proliferation of pathogenic bacteria [28]. Considering the antibiotics banning in animals feeding, using prebiotic supplements have an ecological advantage in the gut because they can multiply more efficiently than the antibiotic resistant forms [29]. In this way the broilers will consume less energy for maintenance of the resistance factors rather than for depositing muscle mass. In this case, inulin supplement it's particularly promising given that broiler chickens raised under heat stress conditions are more vulnerable to gastrointestinal issues.

CONCLUSIONS

In conclusion, dietary inulin supplement in broiler chicken raised under heat stress condition has beneficial impact compared to a control diet. By maintaining a healthy gut and proliferation of beneficial bacteria, such as Lactobacillus, inulin was very efficient in improving production performances, and maintained the health status of animals, raised under heat stress conditions.

ACKNOWLEDGMENTS

This research was funded by the Ministry of Research, Innovation and Digitalization, Project PN 20.23-03.01, and the National Research Development Project to Finance Excellence (PFE)-8/2021.

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