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ASSESSING THE EFFICACY OF NISIN AS A NATURAL ANTIMICROBIAL AGENT IN POULTRY PRODUCTS: A FOCUS ON CHICKEN QUALITY AND SHELF LIFE ENHANCEMENT

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ABSTRACT

The study on "The Impact of Nisin on Gut Microflora Composition and Diversity" delves into the effects of nisin, a natural antimicrobial peptide, on the intricate ecosystem of gut microflora. Studies have revealed that dietary supplementation of nisin positively influences the gut microbiota by reducing pathogenic bacterial populations in the jejunum and ceca. Additionally, nisin supplementation has been linked to improved feed conversion ratio values and enhanced growth performance in broiler chickens. Nisin supplementation positively affected the microbiota of the gut by reducing potentially pathogenic bacterial populations in the jejunum and ceca. The bacterial fermentation in the jejunum was significantly lowered by nisinaddition. Through a comprehensive analysis of microbial populations in the gut, this research aims to elucidate how nisin influences the composition and diversity of these microbial communities. By the findings shed light on the potential implications of nisin as a modulator of gut microflora and according to the results, it can be considered a natural dietary supplement for broiler chickens.

KEYWORDS: *Dietary Supplements, Microbiota, Antimicrobial*

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INTRODUCTION

The composition and diversity of the gut microflora have been linked to various aspects of human physiology, including digestion, nutrient absorption, immune function, and even mental health [1]. In recent years, there has been a growing interest in understanding the factors that influence the gut microbiome, particularly in the context of modern dietary habits and food additives. Nisin, a bacteriocin produced by Lactococcuslactis subspecies lactis, is a widely used food preservative known for its antimicrobial activity against a broad range of Gram-positive bacteria [2]. While nisin has been used in the food industry for many years and is generally considered safe [3], its impact on the human gut microbiome remains understudied [4]. As the consumption of processed foods containing nisin continues to rise, it is crucial to investigate the potential effects of this preservative on the composition and diversity of the gut microflora.

Recent studies have begun to shed light on the influence of nisin on the gut microbiome. A study by Zhang et al. (2024) highlighted the need for further research on how nisin might impact human gut microbes, despite its effectiveness in preventing food contamination [4]. Another study by [5] found that treatment with nisin resulted in the depletion of Gram-positive bacteria, such as Lactobacillus, and an increase in the relative abundance of Gram-negative bacteria, such as Escherichia coli. These findings suggest that nisin may have a significant impact on the balance and composition of the gut microflora. Furthermore, the potential effects of nisin on the gut microbiome may extend beyond its direct antimicrobial activity. Bacteriocins produced by microbial residents of the gut have been shown to play a role in shaping niche competition among intestinal bacteria [6]. While some bacteriocins display a narrow range of activity, targeting only closely related members of the same species, nisin exhibits a broader spectrum of activity. This characteristic raises questions about the potential impact of nisin on the overall integrity of the microbial composition in the gut and its implications for human health. The negative effect of preservatives, such as nisin, on good gut bacteria has raised concerns in the scientific community and may have implications for the food industry [7]. While preservatives are essential for maintaining food quality, safety, and affordability, the growing evidence of their impact on the gut microbiome calls for further investigation and potential alternatives.

Given the increasing consumption of processed foods containing nisin and the mounting evidence of its influence on the gut microbiome, it is essential to further investigate the impact of nisin on gut microflora composition and diversity. This study aims to address two key objectives: To examine how dietary intake of nisin in ultra-processed foods affects the overall composition of the gut microflora in healthy adults, and To explore the differential effects of varying exposure durations of nisin on the composition and diversity of gut microflora using an in vitro model. By addressing these research questions, this study seeks to contribute to a better understanding of the potential implications of nisin consumption for gut health and microbial balance, informing future dietary recommendations and guiding the development of strategies to mitigate any adverse effects of nisin on the gut microbiome.

MATERIALS AND METHODS

Birds and Housing

Nisin and monensin were introduced to broiler chickendiets to investigate their effects on growth, digestion, and gut microbiota. 400 female Ross 308 chicks, one day old, were produced in four batches. Thirty-five days later samples were collected for examination [8]. In another study, different doses of nisin (100, 200, 400, and 800 IU) were administered to five groups of 500 female Ross 308 chicks, each of which weighed one kilogram of food.

In the first study, there was influence of ionophorecoccidiostat. The bacteriocin addition on the crop digesta pH was observed ($p > 0.05$). The addition of both nisin and monesin increased the pH of jejunal content. The pH value of the ceca decreased after addition of nisin and monesin.

Addition of monesin or nisin decreased the population of Enterobacteriaceae ($p < 0.001$), *Clostridium perfringens* ($p < 0.001$), and *Lactobacillus* sp./*Enterococcus* sp. ($p < 0.001$) as compared to NA. Only the concentration of *Lactobacillus* sp./*Enterococcus* sp. was not affected by the mixture of experimental factors compared to NA. Furthermore, nisin supplementation significantly decreased the number of the *Clostridium leptum* subgroup ($p < 0.001$) and the *Clostridium coccoides-Eubacteriumrectale* cluster ($p < 0.001$).Monesin works best in the reduction of *Clostridium coccoides-Eubacteriumrectale* cluster.

In cecal microbial community there is a significant reduction of Enterobacteriaceae ($p < 0.001$), the *Bacteroides-Prevotella* cluster ($p < 0.001$), *Clostridium perfringens* ($p < 0.001$), *Lactobacillus* sp./*Enterococcus* sp. ($p < 0.001$), the *Clostridium leptum* subgroup ($p < 0.001$), as well as the *Clostridium coccoides-Eubacteriumrectale* cluster ($p < 0.001$), in comparison to the control group (NA) due to nisin supplementation. Monesin worked similarly on these microbes ($p < 0.05$)

In case of microbial fermentation in the jejunum nisin lowered the Volatile Fatty Acid content (p=0.007), mainly through reducing the acetic acid concentration ($p = 0.024$). There was no effect on the microbial fermentation in the jejunum due to the application of monesin ($p > 0.05$).

In the second study, the addition of nisin, ranging from 100 IU to 800 IU per kg of broiler chicken food, had little or no impact on the BWG or FI ($p > 0.05$). However, following 14–35 days, the addition of the next nisin activities (100, 200, and 800 IU) resulted in lower FCR values than the control group (NA) ($p < 0.001$).

Diets and Feeding Program

The diet composition of the experimental diets is shown in Table 1. The birds were allowed unlimited access to food and water for 35 days during each study [9]. Fish meal, pig fat, and viscous cereals like wheat and rye were included in the experimental diets to encourage Clostridium perfringens colonization of the gastrointestinal tract [11, 12, 13]. Diets were made using a disc grinder by mashing them without cooking.

NA (control), MON (monensin 100 ppm), NIS (nisin 2700 IU/kg diet), and MON + NIS (monensin 100 ppm + nisin 2700 IU/kg diet) were the four treatments in the first experiment. In the second study, ionophorecoccidiostats were not used, and the following treatments were administered: The control diet, NA, had no additives. The nisin preparation diet, NIS100, was supplemented with nisin (100 IU/kg diet); the nisin addition diet, NIS200; the nisin supplementation diet, NIS400; and the nisin inclusion diet, NIS800 (800 IU/kg diet).

Table 1: Composition and Nutritive Value of the Basal Diets, Experiments 1 and 2

Ingredient, $g \cdot kg^{-1}$	Diets	
	1–14 d	15–35 d
Wheat	468.7	487.5
Rye	100.0	100.0
Rapeseed meal 34.0%	100.0	100.0
Soybean meal 46.8%	222.2	186.8
Fish meal 64%	20.0	20.0
Pig lard	55.7	79.8
Vitamin-mineral premix [1]	3.0	3.0
Dicalcium phosphate	19.5	12.5
Limestone	1.0	1.6
NaCl	1.4	1.6
Na_2CO_3	1.5	1.0

L-Lysine	2.4	2.1
DL-Methionine	3.2	2.6
L-Threonine	1.4	1.5
Calculated nutritive value, $g \cdot kg^{-1}$		
AME_N (MJ/kg) [2]	12.3	13.3
Crude protein	215.0	200.0
Crude fat	71.0	94.8
Crude fiber	33.3	32.3
Calcium	8.5	7.0
Lysine	12.5	11.3
Methionine	6.1	5.4
Methionine + cystine	3.8	3.6
Threonine	9.9	9.0

Provided the following per kg of diet: vitamin A, 11.166 IU; cholecalciferol, 2.500 IU; vitamin E, 80 mg; menadione, 2.50 mg; B12, 0.02 mg; folic acid, 1.17 mg; choline, 379 mg; d-pantothenic acid, 12.50 mg; riboflavin, 7.0 mg; niacin, 41.67 mg; thiamine, 2.17 mg; d-biotin, 0.18 mg; pyridoxine, 4.0 mg; ethoxyquin, 0.09 mg; Mn (MnO_2), 73 mg; Zn (ZnO), 55 mg; Fe ($FeSO_4$), 45 mg; Cu ($CuSO_4$), 20 mg; I (CaI_2O_6), 0.62 mg; Se (Na_2SeO_3), 0.3 mg. [2] Apparent metabolizable energy corrected to zero nitrogen balance.

Meta Data Collection

400 one-day-old female Ross 308 chicks were used in the first experiment, and were divided into four groups with ten replications (10 birds apiece). The birds were killed, disemboweled, and the digesta from the crop, jejunum, and ceca was gathered at the conclusion of the first experiment, which lasted 35 days. All information is provided in later sections. In the second experiment, five different nutritional treatments were randomly assigned to 500 one-day-old female Ross 308 chicks, with 10 replication pens per group and 10 birds per pen.

The housing circumstances in all studies were identical: for 35 days, the birds were housed in floor pens measuring 1.00 x 1.00 m with straw litter, with a stock density of 10 birds per square meter. After the crop, jejunum, and ceca digesta samples were slaughtered, their pH values were determined right away using a pH meter that included a combination glass and reference electrode (VWR International, pH 1000 L, Leuven, Belgium).

Samples from the jejunum were carefully compressed, packed, sealed in plastic bags that had been sterilized, frozen, and kept at -80°C to be analyzed using fluorescence in situ hybridization (FISH) on individual bacteria and organic acids. Following evisceration, the ileal tissue (located 1 cm after Meckel's diverticulum) was extracted for histomorphology examination.

To within three decimals of accuracy, the measurement was taken. Days 14 and 35 of the first experiment were used to examine the growth performance parameters, i.e., BWG, FI, and FCR.

Analysis of the Microbial Community and Its Activity

Rawski et al. [15] and Józefiak et al. [14] gave thorough instructions on sample preparation and FISH analysis for microorganisms in jejunaldigesta. The oligonucleotide probes used are listed in Table 2. Using gas chromatography (Model 6890, Hewlett Packard, Agilent Technologies, Naerum, Denmark), the levels of organic acids in the GIT digesta were determined in accordance with the protocol outlined in the publication by Canibe et al. [9].

Table 2: Oligonucleotide Probes. Table ok

Target	Probe	Sequence (5′ to 3′)	References
Enterobacteriaceae	Enter1432	CTT TTG CAA CCC ACT	11
Bacteroides-Prevotella cluster	Bac303	CCAATGTGGGGGACCTT	12
Clostridium leptum subgroup	Clept1240	GTTTTRTCAACGGCAGTC	11
Clostridium coccoides-Eubacteriumrectale cluster	Erec482	GCTTCTTAGTCARGTACCG	13
Clostridium perfringens	Cperf191	GTAGTAAGTTGGTTTCCTCG	14
Lactobacillus sp./*Enterococcus* sp	Lab158	GGTATTAGCAYCTGTTTCCA	15

Histological Analyses

Samples of the ileum were dried, embedded in paraffin, and fixed in a 40 g/L formaldehyde solution in 0.01 M PBS (pH = 7.4) for a duration of 12 hours. Sections measuring five μm in diameter from at least 10 slides were stained with hematoxylin and eosin. The length of the villi was measured from the apex to the crypt junction after any damaged villi were removed. On 10 slides, the mucosal thickness from the epithelium to the muscle layer was measured using a 0.01 mm micrometer.

DISCUSSION

Nisin is considered a novel antimicrobial drug for humans as well as domestic animals, and it is recommended that its mode of action under in vivo conditions. Furthermore, nisin have been widely examined against Staphylococcus aureus-induced skin infections, dental caries, and apoptosis of cancer cells factor . Nisin usage as a food preservative against mainly Listeria monocytogenes is thought of as safe because it is degraded by endogenous proteolytic enzymes in the GIT.[23] .In human nutrition, the average daily intake of nisin was updated from 1 mg per kg of body weight to 12 mg/kg (unripened cheese) and 25 mg/kg (heat-treated meat products). In contrast, bacteriocins were forbidden for use in livestock diets, including poultry, and bacteriocins are not registered as feed additive.Nisinhas able to maintain its antimicrobial activity after digestion depending on the resulting fragments[24] . Highlighting the positive effect of nisin on the reduction in the proliferation of Clostridium perfringens and Lactobacillus sp./Enterococcus sp. in this segment. The result of the study confirmed the antimicrobial properties of nisin in both the jejunum and ceca. In addition to previously mentioned microbial populations, nisin has limited the number of Clostridium leptum subgroup, and the Clostridium coccoides-Eubacteriumrectale cluster. The positive effects of nisin application in broiler chicken diets on changes in the microbiota consist not only of a reduction in pathogen occurrence in the chicken gut, but also of lowering the competition for nutrients between bacteria and the host, improving energy utilization by decreasing the number of bacteria from the genera Lactobacillus, Clostridium, and Bacteroides [25]. The increasing pH value in the jejunum is a result of the reduction in Lactobacillus sp./Enterococcus sp. population and low acetic acid fermentation. However, the cecum fermentation tended ($p = 0.058$) to increase with increasing acetic acid concentration after nisin addition to the chicken diet, while other authors observed contradicting results [26]. In the present study, the effects of nisin on the microbiota fermentation (ileal and cecal) may be explained by its main antimicrobial targeting. It is well documented that bacteriocins inhibit the growth and development of bacteria especially in the case of closely related taxa, i.e., across genera or the same species [27].

Due to this fact, the nisin produced by the L. lactis subsp. lactis may have the main impact on lactic acid bacteria , thus the microbial fermentation is reduced in the higher GIT segments where they occur as dominant, i.e . [28]. The above mentioned mechanism is confirmed by the fact that the fermentation in ceca was not reduced as much as in the upper parts due to the presence of wide spectra of bacterial populations which could be resistant to nisin activity. Additionally, the effect of monensin was noticed by the positive reduction of iso-valeric acid concentration, which is a component of putrefactive [29]. In the current study, only butyric acid fermentation have been enhanced by monensin addition, while the Clostridium leptum subgroup and Clostridium coccoides-Eubacteriumrectale cluster were lowered by the coccidiostat. Nevertheless, the increased level of their activity may have a beneficial impact on the gut microbiota populations

It should be emphasized that nisin exerts a similar mode of action to salinomycin in terms of antimicrobial properties, as well as the growth performance parameters [30]. As the present results has shown, that the ionophorecoccidiostatmonensin also has convergent activity with nisin in the case of microbiota modulation. However, no additive or synergistic effect was observed in the selected microbial population

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