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Gut Microbiota and Intestinal Morphology of Growing Quails as Affected by Dietary Addition of Tryptophan or/and Canthaxanthin

Khaled H. El-Kholy; Mohamed E. Sokariaa* and Ibrahim T. El-Ratel
Animal, Poultry and Fish Production Department, Faculty of Agriculture, Damietta University 34517, Egypt.

Corresponding author*: Email:emohamedessam10@gmail.com

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Abstract:

The aim of this study was to determine the influence of dietary addition with tryptophan (Trp) or/and Canthaxanthin (CX) on the meat and cecal microbiota and intestinal morphology of growing Japanese quails. Two hundred day-old unsexed Japanese quails *(Coturnix coturnix japonica*) with an average of initial live body weight (BW) of 33.50± 1.20 g were equally allocated randomly into four groups (five replicates per group of 10 chicks in each). The first group was fed basal diet without dietary addition from 1 to 5 wks (control, T1), while Trp and CX at a rate of 0.01 and 0.005%, respectively were mixed with the basal feed of the 2nd (T2) and 3rd (T3). While the 4th group (T4) of chicks received mixture of trp+CX at the same studied level. The results showed that dietary addition of Trp or CX or mixture of Trp+CX significantly (P<0.05) decreased populations of total aerobic mesophilic and coliform bacteria counts in the meat, while lactic acid bacteria was significantly (P<0.05) increased in meat as a result of dietary Trp and/or CX in compared to control group. Also, *E. coli* and salmonella of the ceca were significantly reduced in the combination treatment compared to the control. The jejunum villi of growing Japanese quails from all experimental groups were significantly higher and (P< 0.001), while their jejunum villi crypts were significantly deeper (P<0.001) in comparison with these parameters in quails belongs of the control group. The villus height to crypt depth ratio of growing Japanese quails, were significantly increased (P<0.001) in experimental groups of growing Japanese quails in compared to control group. In conclusion, these findings suggest that dietary addition of Trp and/or CX has a beneficial effect on growing Japanese quail's intestinal morphology and meat and cecal microbiota.

INTRODUCTION

The nutritional challenges arising from elevated dietary concentrations of polyunsaturated fatty acids, mycotoxins, imbalances in vitamins and minerals, as well as the impacts of climate change, represent significant pressures encountered in contemporary poultry industry **(El-Kholy et al., 2022 & 2024; Attia et al., 2024).** Additionally, many poultry species, particularly quails, have undergone genetic enhancements over the years to achieve faster growth rates. This rapid growth is linked to increased cell proliferation, which in turn elevates the levels of reactive oxygen species (ROS), resulting in oxidative stress **(Surai, 2015; Mund et al., 2020).** The extreme reactivity and instability of ROS pose significant biological risks due to their harmful impact on cellular membranes, as well as on DNA and RNA. Consequently, these ROS can induce stress within the body, disrupting various metabolic and immunological processes **(Halliwell & Gutteridge, 1999).** Certain essential amino acids, notably tryptophan (Trp), have been identified as playing a significant role in mitigating oxidative stress **(Mund**

et al., 2020). The Trp is converted into 5-hydroxytryptophan (5-HT), which plays a crucial role in maintaining membrane fluidity in chickens. In contrast, oxidative stress adversely affects membrane fluidity. Additionally, 5-HT contributes positively to both enzymatic and non-enzymatic antioxidant capacities **(Dong et al., 2012).** Stressful experiences, conversely, can disrupt the equilibrium of intestinal microbiota, resulting in an overgrowth of harmful pathogens and a reduction in the levels of beneficial bacteria, including Lactobacilli and Bifidobacteria **(Zhang et al., 2016; Bello et al., 2018).** The increase in Bifidobacteria and Lactobacilli populations within the gastrointestinal tract may enhance non-anxious behavior in mice via the vagus nerve, potentially mediated by neuroactive compounds derived from these bacteria **(Saulnier et al., 2013).** Pathogenic microbiota colonization presents a significant threat to the health of poultry and can adversely impact food safety. Species within the Lactobacilli and Bifidobacteria genera play a crucial role in suppressing the growth and proliferation of various pathogenic bacteria. They achieve this by producing lactic and acetic acids as their primary metabolic byproducts, along with smaller quantities of succinic, formic acids, and ethanol **(Alakomi et al., 2000).** Besides that, the underdevelopment of the digestive tract during the initial weeks following hatching poses a significant constraint on the growth of productive poultry. This limitation arises from the continuous enhancement of enzyme secretion capacity and absorption surface area, which are facilitated by the longitudinal growth of the small intestine and the elevation of villus height **(Castro et al., 2020).** Studies has been conducted aimed at enhancing the functional capacity of the poultry intestine through the utilization of nutrients that promote the development of the mucosal lining **(Dai et al., 2011; Sakamoto et al., 2006; Rezaei et al., 2018),** so that there is better utilization of the diet. Tryptophan serves as a fundamental structural element of proteins and acts as a significant precursor for the hormone’s serotonin and melatonin. These hormones are crucial for the regulation of various physiological processes, including tissue synthesis and immune function in broiler chickens **(Bai et al., 2017).** Also, Trp plays a role in the biosynthesis of niacin in poultry **(Richard et al., 2009**). Serotonin is an essential neurotransmitter that enhances the ability to adapt to environmental changes and reduces oxidative stress **(Martin et al., 2000).** It is a significant mucosal signaling molecule synthesized by enterochromaffin cells located in the gastrointestinal tract and is associated with various pathophysiological processes **(Coates et al., 2004).** Moreover, Trp, recognized as the precursor of serotonin, is crucial in regulating various physiological functions, including motility of the duodenum and ileum, as well as secretion and sensitivity **(Cirillo et al., 2011),** and intestinal permeability **(Nylander and Pihl, 2006; Yamada et al., 2003)** in the gastrointestinal tract. Red-orange carotenoid that belongs to the xanthophyll group is called canthaxanthin, its naturally present in bacteria, algae and some fungi **(Venugopalan et al., 2013).** Canthaxanthin have possesses high antioxidant activity in vitro model **(Soffers et al., 1999),** in animal experiments in vivo **(Zhang et al., 2011; Rosa et al., 2012),** due to its ability to scavenge singlet molecular oxygen and extinguish other free radicals **(Bohm et al., 2012).** Canthaxanthin is necessary carotenoid that might efficiently deposited in egg yolk and further **(Surai and Speake, 1998).** It's one of the most powerful lipid-soluble antioxidants in nature, and it has specified as a potent free radical scavenger **(Rengel et al., 2000).** The Canthaxanthin (CX) is a carotenoid which can reduce lipid peroxidation and enhance serum total antioxidant capacity **(Rocha et al., 2013).** Other beneficial functions, considered to be responsible for the health-promoting properties of carotenoids, are the facilitation of cell differentiation **(Zhang et al., 1992; Rock et al., 1995),** the regulation of cell proliferation **(Krinsky, 1992; Bertram and Borthiewicz, 1995)** and the enhancement of the immune function **(Bendich, 1991; Hughes, 1999; Moller et al., 2000).** The extensive information is available on Trp requirements of broilers but not for meat-type growing Japanese quails, and the recommendation of NRC (1994) for Trp in quails has not been evaluated with fast growing strain Japanese quails **(Nasar et al., 2016).** Regarding to CX, **Weber et al. (2013)** investigated the tolerance of poultry (broiler chicks, laying hens and egg breeders) to a ten-times overdose of CX. They added that as compared to the control treatment, neither the performance data, nor the health examination or the final post-mortem analysis of the experimental birds evidenced any detrimental or adverse effects of CX when added to the feed at the recommended dose or at the overdose level. Unlike for broiler and layer chickens, there is little information concerning has been done investigating Try or CX or their combined dietary addition on quails. The small intestine is an important organ responsible for the digestion and absorption of nutrients from the diet. Any changes in its function affect the function of other organs and systems in the organism **(Toman et al., 2015).** There are only a few studies that have previously evaluated the effect of Trp and/or CX on the intestinal morphology of quails. This study aimed to determine the impact of tryptophan or canthanthine or their combination in meat and gut health and small intestinal morphology of quail's chicks during the growing phase.

2.Materials and Methods

2.1. Ethical approval

This study has been conducted at the Animal, Poultry and Fish Production Department of the Faculty of Agriculture at Damietta University and a commercial Poultry farm in Mansoura City, Dakahlia Governorate, Egypt. The "Directive 2010/63/EU of the European Parliament and of the council, of 22 September 2010, on the protection of animals used for scientific reasons" was followed in all procedures and experimental protocols.

2.2. Experimental birds

Two hundred one-day-old, unsexed Japanese quails (Coturnix coturnix) chicks, averaging 33.50 ± 1.20 g in weight, were sourced from a local hatchery and randomly assigned to four groups, each consisting of five replicates (10 birds per replicate). The first group was served as the control without any dietary studied additives (control, T1). The second (T2) and third (T3) groups received feed supplemented with Trp and CX at concentrations of 0.01% and 0.005%, respectively. The fourth group (T4) was given a combination of Trp and CX at the suggested levels of T2 and T3. The basal diet was formulated in accordance with the guidelines established by the **NRC (1994)** for the nutritional needs of growing quail (refer to Table 1). All chicks were maintained under the same managerial protocol throughout the duration of the experiment. They were housed in conventional cages measuring 90 × 40 × 40 cm, equipped with a nipple waterer and feeder, and provided with fresh water and feed available for *ad libitum* consumption during the experimental period. During the rearing phase, the temperature was initially set at 37 ºC and was gradually decreased by 3 ºC per week until the fifth week of age, with relative humidity maintained between 56% and 66%. The lighting schedule consisted of 16 hours of light followed by 8 hours of darkness, with consistent housing conditions upheld for all chicks.

2.3. Biosecurity considerations

The study has been conducted under strict sanitary and hygienic conditions. All bio-security precautions were meticulously followed.

Table 1: Ingredient and nutrient composition of basal diet.

Ingredients	**%**
Mazie	56.00
Soybean meal (44% CP)	32.00
Plant concentrate meal (50 % CP)[1]	9.00
Ground lime stone	1.30
Dicalcium phosphate	0.50
Vegetable oil	0.50
DL-methionine	0.10
Salt (NaCl)	0.30
Vitamins and minerals mixture[2]	0.30
Total	100
Calculated analysis[3]	
Metabolizable energy (Kcal/Kg)	2919
Crude protein (CP, %)	24.00
Crude fiber (CF, %)	3.50
Calcium (Ca, %)	0.80
Available phosphorus (Ava. P, %)	0.50

[1]Plant concentrate contains (%): CP 50, CF 1.3, Ca4.72, Av P 3.1, lysine 6, methionine 2 and ME 2650 kcal/kg.

[2]Premix provided per kg of diet: vitamin A, 12.000 IU; vitamin D3, 2.400 IU; vitamin E, 30 mg; vitamin K3, 4 mg; vitamin B1, 3 mg; vitamin B2, 7 mg; vitamin B6, 5 mg; vitamin B12, 15 µg; niacin, 25 mg,Fe, 80 mg; folic acid, 1 mg; pantothenic acid, 10 mg; biotin, 45 mg; choline, 125,000 mg; Cu, 5 mg; Mn, 80 mg; Zn, 60 mg; Se, 150 µg. 3-According NRC, 1994.

2.4. Microbiological analysis:

For microbial population in the meat and caecum content, at 35 day, five quails from each group were randomly selected and slaughtered. According to **Baumgart et al. (1993)**, the meat sample (25 g) was homogenized in 225 ml of sterilized Ringer's solution. Then sterilized solutions were prepared. The pour plate method was used for microbiological analysis. Plate Count Agar (PCA, Merck) was used for the enumeration of total aerobic mesophilic microorganisms (TAM). Plates were evaluated after being incubated at 30 ± 1°C for 72 + 1 hours. Rogosa Acetate Agar (RAA, Merck) was used for the enumeration of lactic acid bacteria. These plates were incubated anaerobically for 5 days at 30 ± 1°C. All colonies produced following the incubation were evaluated. Violet Red Bile Agar (VRBA, Merck) was used for the enumeration of coliform bacteria. The plates were incubated anaerobically at 30 ± 1°C for 24 hours. Red colonies larger than 1 mm were counted after incubation. Bacteria counts were expressed as logarithmic colony-forming units per gram of sample (log CFU/g). According to **Xia et al. (2004) and Mahgoub et al. (2019),** in the caecum, the fresh samples (1 g/quail) of caecal digesta were exposed to a stream of CO_2 in bottles and transferred immediately to the microbiology lab. Violet Red Bile Agar (Biolife) was used for counting coliform after incubation for 24 h at 37°C. Escherichia coli was counted on Eosin Methylene Blue agar plates (Merck) after incubation for 24 h at 37°C. Salmonella was counted on xylose lysine deoxycholate agar plates (Merck) after incubation for 24 h at 37°C.

2.5. Intestinal histomorphology:

At the end of growth trail period (35 days), 5 quails from each group were euthanized using humane methods. Tissue samples were collected from the jejunum and fixed in a 4% formaldehyde solution, dehydrated in xylene, embedded in paraffin, and sectioned into 4 µm slides for subsequent hematoxylin–eosin staining. The resulting sections were examined using a light microscope. To assess villus morphology, villus length, villus width, crypt depth, and their ratio were measured using an automatic image analyzer.

2.6. Statistical analysis

All of the statistical analyses were performed using the **SAS (2001)**. All data were assessed with a one-way ANOVA using the post-hoc Tukey's test. The significance was established at P<0.05.

3. Results

3.1. Microbial population

The results of microbial population in the meat and intestinal content are presented in Table 2. The results indicated that dietary addition of Trp or CX or the mixture of Trp+CX significantly (P<0.05) decreased total counts of aerobic mesophilic and coliform bacteria in meat, while lactic acid bacteria was significantly (P<0.05) increased in meat as a result of dietary Trp and/or CX in compared to control group. Regrading to bacterial count of cecum, dietary addition of CX or Trp+CX shows significantly (P<0.05) decreased populations of *E. coli*, and *Salmonella*.

Table 2: Bacterial count (log CFU/g) of meat and cecum of Japanese quails at 35 days of age as affected by dietary
addition of tryptophan (Trp) or/and canthaxanthin (CX).

Variables	**Control (T1)**	**Treatments***			**SEM**	**P-value**
		Trp (T2)	**CX (T3)**	**Trp + CX (T4)**		
Total aerobic mesophilic and antibacterial activity in the meat (log CFU/g)						
Total aerobic mesophilic	6.57^{a}	4.40^{b}	4.46^{b}	4.35^{b}	0.465	0.0315
Coliform bacteria	3.05^{a}	2.44b	2.30^{b}	2.24^{b}	0.518	0.0519
Lactic acid bacteria	1.98^{b}	1.95 ^{b}	2.20 ^{b}	2.95^{a}	0.552	0.0435
Antibacterial activity in the caecum (log CFU/g)						
Lactobacilli	8.58	8.40	8.33	8.2	0.526	0.9687
E coli	6.04^{a}	5.60^{a}	5.53^{ab}	5.39^{b}	0.778	0.0413
Salmonella	5.54^{a}	5.00^{a}	4.07^{b}	3.84^{c}	0.488	0.0183

Means in the row with different superscript letters are significantly different at P < 0.05
*Trp= Tryptophan (0.01%); CX= Canthaxanthin (0.005%); Trp+CX= 0.01% Tryptophan mixture with 0.005% Canthaxanthin.

3.2. Small intestinal morphology

Table 3 shows the effects of Trp and/or CX addition on villus height (VH) and crypt depth (CD), and villus: crypt ratio (VH/CD) in jejunum of growing Japanese quails at d 35. Morphometric analysis of the jejunum villi of growing Japanese quails revealed differences between the control and experimental groups of quails at the tissue structure level as affected by dietary addition of Trp and/or CX, as shown Table 3. Also, the dietary addition of Trp or CX mixture of Trp+CX significantly (P<0.05) increas VH and ratio of VH:CD in compared to contr group. Another experimental dietary addition did n markedly affect (P>0.05) CD of jejunum at d 35.

Table 3: Intestinal histomorphology of Japanese quails at 35 days as affected by dietary addition of tryptophan (Trp) or/and canthaxanthin (CX).

Variables	Control (T1)	Treatments*			SEM	P-value
		Trp (T2)	CX (T3)	Trp + CX (T4)		
Villus height (VH, µm)	36.40^{b}	36.55^{b}	38.14^{a}	39.31^{a}	4.46	0.0008
Crypt depth (CD, µm)	54.86	49.21	52.80	48.54	4.47	0.7216
VH : CD	0.67^{b}	0.74^{ab}	0.72^{ab}	0.81^{a}	0.068	0.0372

Means in the row with different superscript letters are significantly different at $P < 0.05$.
*Trp= Tryptophan (0.01%); CX= Canthaxanthin (0.005%); Trp+CX= 0.01% Tryptophan mixture with 0.005% Canthaxanthin.

Discussion

Nowadays, researchers are increasingly focused on the gastrointestinal tract health of poultry, recognizing its significance for enhancing their overall health and performance. The interaction between Trp or CX and the intestinal microbiota is a topic that still lacks associated information and clear evidence. In current study, dietary addition of CX or mixture of Trp plus CX significantly (P<0.05) decreased populations of *E. coli*, and *Salmonella* in intestine in compared to other experimental groups. Also, it is an interesting to observe that dietary addition of Trp or CX or mixture of Trp+CX significantly (P<0.05) decreased populations of TAM and Coliform bacteria in meat and significantly (P<0.05) increased lactic acid bacteria as beneficial bacteria. In current study, CX promotes gut microbiota homeostasis by increasing the abundance of beneficial bacteria by significantly (P<0.05) increased of lactic acid bacteria, while decreasing harmful bacterial growth (such as *E. coli* and *Salmonella*). Hence CX alone or mixture with Trp significantly impacts the gut microbiota by reducing local and systemic oxidative stress and inflammation. The most interesting part of the current results is that dietary Trp level has prebiotic-like role in chicken **(Alhassan et al., 2018**). Quantification of selected species of bacteria belonging to pathogenic or non-pathogenic group showed that non-pathogenic bacteria greatly increased along with decrease in pathogenic ones (*E. coli* and *salmonella*). This is a strong prebiotic-like effect as such reported with usage of known prebiotics **(Guo et al., 2004; Abudabos et al., 2015).** To the best of our knowledge, this is the first study reporting such effect for dietary CX alone or with mixture with Trp. The exact reason behind the observation is not clear and further multidisciplinary studies needed to shed light on the issue. It is noteworthy to mention that, reducing pathogenic bacteria such as *E. coli* and salmonella not only improve quail's health and well-being, it may also benefit safety of food industry, as these bacteria are among the major causes of food-borne diseases **(Alhassan et al., 2018).** It is acknowledged that gut microbiota plays an important role in regulating intestine inflammation **(Amoroso et al., 2020; Li et al., 2020).** One of the most important factors known to affect meat quality is the microbial load of meat, as its increase impairs the meat quality, and poses a risk to human health **(Ozbilgin et al., 2021).** Although meat's microbial load is affected by the slaughter and storage conditions, the dietary addition of Trp has a vital positive impact **(Bello et al., 2018).** The role of the dietary addition of Trp and/or CX in reducing TAM of quail meat could be explained by their ability to mitigate the pathogenic intestinal bacteria (mesophilic aerobic, coliform, and *Escherichia coli*) and activate the beneficial ones (lactic acid bacteria), resulting in a decrease in meat contamination with intestinal content during slaughter, thus reducing the meat's microbial load **(Siregar et al., 2024).** Furthermore, the antimicrobial substances found in the structure of meat are highly important for its storage without spoilage **(Gumus and Gelen, 2023).** The used dietary supplements reduced the intestinal Enterobacteriaceae counts in quails. The Trp plus CX group (T4) induced insignificantly lower Enterobacteriaceae count (*E. coli* and *salmonella*) than the Trp group (T2) or CX group (T3). In this respect**, Bello et al. (2018) and Damaziak et al. (2018)** showed lower coliforms and *Salmonella* count in meat and eggs of birds treated with Trp and Cx, respectively in compared to birds fed basal diet without addition. It was reported that Trp and CX have strong antioxidant effect against both gram-positive and gram-negative bacteria. Moreover, as mentioned by **Khattak and**

Helmbrecht. (2019) who showed that after Trp supplementation, the microbial shift in the caecum toward beneficial bacteria. In addition to this, the results obtained by **Dai et al. (2022)** suggested that xanthophylls such as CX has a higher impact on the modification of intestinal microbiota composition than carotenes. This demonstrates that carotenoids are structurally distinct and can affect differently the composition of the intestinal microbiota **Dai et al. (2022)**. Carotenoids have been associated with various health benefits, mainly due to their anti-inflammatory and antioxidant properties that provide protection against lipid peroxidation and damage caused by ROS **(Eroglu et al., 2022).** Therefore, the results obtained from these studies indicate that carotenoids and the intestinal microbiota have a structure–activity relationship and the latter can be a potential target for carotenoids' utilization **(Dai et al., 2022).** However, a comprehensive understanding of the direct interaction between carotenoids and the intestinal microbiota and their relationship is still lacking **(Yin et al., 2019)**. So, dietary addition of CX or mixture of Trp plus CX have provided sufficient evidence to be safe and natural alternatives of antibiotic growth promoters in broiler chicken diets, to prevent microorganisms' contamination of human food and to prevent many diseases. Dietary tryptophan is an essential amino acid, which can be metabolized by gut microbiota into indole derivatives **(Sun et al., 2020; Liu et al., 2022).** The indole pathway has attracted widespread attention as it can maintain intestinal health and improve inflammation **(Sun et al., 2022).** In the study of **Ma et al. (2024),** showed that dietary tryptophan significantly increased the concentration of indole-3-acetic acid (IAA) and indole-3-lactic acid (ILA). In addition, indole derivatives can regulate intestinal inflammation by altering the immune related signaling pathways **(Keszthelyi et al., 2009)**. Many bacteria may use the indolic compounds to thrive over other bacteria in multispecies communities. Indole producing bacteria reported to inhibit the growth and survival of none-indole-producing bacteria such as Enterobacteria, especially within the genera Salmonella and Shigella **(Smith and Macfarlane, 1997). Nowak and Libudzisz. (2006),** also reported the same growth inhibitory effects for indolyl acetic acid added to Lactobacilli culture. It is noteworthy to mention that, reducing pathogenic bacteria such as E. coli and salmonella not only improve quail's health and well-being, it may also benefit safety of food industry, as these bacteria are among the major causes of food-borne diseases. In addition, **Agus et al. (2018)** demonstrated that Trp is the versatile AA and precursor of serotonin, niacin, and different metabolic compounds obtained from kynurenic pathway affecting metabolism and even gut microbiota. Also, during TRP metabolism, several endogenous antioxidants may produce such as melatonin, 5-hydroxytryptophan and 3-hydroxykynurenine **(Huether et al., 1992).** Provision of TRP therefore increases antioxidative activity in body **(Liu et al., 2015**), controlling formation of ROS. High levels of ROS are known to damage cell membrane integrity and membrane-bound receptors and enzymes. Thus, we hypothesize that provision of dietary Trp and/or CX may reduce the adverse effects of stress and bacterial load of the major foodborne pathogens and therefore improve poultry welfare and meat quality. These findings suggested that carotenoids (such as CX) could help to speed up the formation of Short-chain fatty acids (SCFAs). SCFAs, particularly acetic acid, propionic acid, and butyric acid, are essential bacterial metabolites in modulating intestinal immunity, lowering intestinal inflammation, and improving intestinal functioning **(Zhang et al., 2020).** Another study reported that, while modulating the variety of cecal microbiota, carotenoids reduced Ochratoxin A (OTA)-induced cecum damage and inflammation in mice **(Chen et al., 2021).** They observed that OTA decreased gut flora diversity and increased the number of *Firmicutes* and *Lactobacillaceae*. The number of intestinal pathogens is inversely proportional to the abundance of Firmicutes **(Mulder et al., 2009).** Changes in lactobacillus proportions induce the alteration in the abundance of Firmicutes, indicating that lactobacillus is a primary detoxifying bacteria in the OTA group **(Walter, 2008).** Moreover, carotenoids treatment improved gut flora and increased gut microbial diversity (especially *Lactobacillaceae* and *Lachnospiraceae*), leading to cecum barrier recovery from OTA-induced injury **(Hegde et al., 2022).** Based on the above results, we also hypothesized that CX alone or mixture with Trp supplementation could affect intestinal health by altering the composition of gut microbiota. As predicted, we found that dietary addition of CX alone or mixture with Trp changed gut microbiota composition. The VH and CD are important indices of the functional capacity of enterocytes, and the VH: CD ratio affects the nutrient digestibility and absorption capacity of the intestinal mucosa **(Chang et al., 2015; Wu et al., 2018).** Current morphometric results of the jejunum villi in quails as affected by dietary addition of Trp and/or CX revealed that it was significantly higher, while its CD was insignificantly deeper in the CX alone or mixture with Trp (T3 & T4) groups in compared to the control group (T1). These results are consistent with the results of the study by **Sayrafi et al. (2011),** who showed that broiler fed a diet supplemented with a prebiotic had significantly higher and wider intestinal villi of the jejunum in compared to control group. **Liu et al. (2022)** suggested that Trp enhanced intestinal health by a modulated intestinal microbiota composition, improved the short chain fatty acids synthesis, reduced inflammation, increased antioxidant capacity, and improved intestinal barrier function. Also, in Japanese quails, glutamine supplementation resulted in expressive increase in villus height in the jejunum of birds at 21 and 42 days of age **(Salmanzadeh et al., 2013).** In addition, Murakami et al. (2012) showed that dietary Arginine (Arg) supplementation in broiler chickens' diet improved intestinal mucosa development. Xaio et al. (2016) indicated that the supplementation of Arg in rat exert protective effects against oxidative stress in rat's intestine and also increased VH/CD. Although in the present trial the dietary addition of Trp couldn't induce any marked effect on morphometric related CD. It is probably due to the optimum environmental condition in the present study. As reported by **Landy and kavyani. (2014)** the environmental stressors such as high or low temperature can disrupt the normal balance of the intestinal microbial ecology and morphology **(Landy and kavyani, 2014);** thus, may be our chicks didn't respond to Trp addition as a result of optimum environmental condition. **Awad et al. (2008)** stated that the increase of villi height in the chicken intestine is parallel to the increase of digestive and absorption functions as well as a smooth expression of the nutrient transport system throughout the body. Increasing crypt depth causes absorption of more nutrients into the bloodstream, causing the increasing of growth and efficiency of feed used to be better. **Sun et al. (2005)** stated that the development of chicken intestinal villi is related to intestinal function and growth of the chicken. The ability to digest and absorb nutrients is influenced by the surface area of the intestinal epithelium, the number of folds, and the number of villi and microvilli that expand the absorption field **(Awad et al., 2008).** As a result, the development of the digestive tract, including the intestine is also better. The size of the intestine is larger and longer (number and height of the villi) as a place for better absorption of nutrients into all body tissues. **Fitasari (2012)** stated that one of the parameters that can be used to measure the quality of growth is the intestinal morphological structure. The intestinal villi are a place for absorption of nutrients, the wider the villi the more nutrients are absorbed which ultimately has an impact on the growth of the body's organs. These components participate in controlling the proliferation of pathogenic bacteria and the consequent avoidance of possible damage to the intestinal mucosa, which also leads to the reduction of morphometric measures of the intestinal villi **(Sayrafi et al., 2011).**

Conclusion:

In conclusion, the present study showed that the addition of Tryptophan and/or Canthaxanthin to feed mixtures has a significant protective effect on the gut tissue of quail, which is reflected through promotes gut microbiota homeostasis and better morphometric measures of the jejunum villi and jejunum villi crypts of quail from all the experimental groups in compared to quails from the control group. Following the results of this study, the dietary addition of 0.005% of Canthaxanthin alone or mixture with 0.01% Tryptophan showed the strongest positive effect on quail guts. The promising and encouraging results of this study emphasize the importance of the further evaluation of the administration level of investigated supplements in order to maximize their positive effects on the gut tissue of chickens and, consequently, the overall health of growing Japanese quails.

AUTHOR CONTRIBUTIONS:

Conceptualization, K.E., M.S. and I.T.; Methodology, K.E., M.S. and I.T.; Investigation, M.S. and I.T.; Formal analysis, I.T.; Data curation, K.E. and I.T.; Writing—original draft preparation, K.E. and I.T.; Writing—review & editing, K.E. and I.T.; Visualization, K.E., M.S. and I.T.; Supervision, K.E.

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الملخص العربى

ميكروبات الجهاز الهضمي وموفولوجيا الأمعاء للسمان النامي نتيجة التغذية بالتربتوفان مع/أو الكانازانثين

خالد حسان الخولي – محمد عصام سكرية – إبراهيم طلعت الرطل

قسم الإنتاج الحيواني والداجني والسمكي – كلية الزراعة – جامعة دمياط

كان الهدف من هذه الدراسة هو تحديد تأثير الإضافة الغذائية مع التربتوفان (Trp) مع/أو الكانثاكسانثين (CX) على الكائنات الحية الدقيقة للجهاز الهضمي ومورفولوجيا الأمعاء لطيور السمان اليابانية النامية. تم توزيع مائتي طائر السمان الياباني غير المجنس بعمر يوم واحد (Coturnix coturnix japonica) بمتوسط وزن حي أولي يبلغ 33.50 ± 1.20 جم بشكل عشوائي إلى أربع مجموعات (خمس مكررات لكل مجموعة مكونة من 10 كتاكيت سمان في كل منها). غذيت المجموعة الأولى على علف أساسي بدون إضافة غذائية من 1 إلى 5 أسابيع (الكنترول أو الكنترول ، T1)، بينما تم خلط Trp وCX بمعدل 0.01 و0.005% على التوالي مع علف المجموعة الثانية (T2) والثالثة (T3). بينما تلقت المجموعة الرابعة (T4) خليط Trp+CX. أشارت النتائج إلى أن إضافة Trp أو CX أو خليط Trp+CX إلى الغذاء أدى إلى انخفاض معنوي (P<0.05) في تعداد البكتيريا الهوائية المتوسطة والقولونية في اللحوم، في حين زادت بكتيريا حمض اللاكتيك بشكل معنوي (P<0.05) في اللحوم بشكل معنوي (P<0.05). نتيجة للـ Trp و/أو CX الغذائي مقارنة بالمجموعة الكنترول. كانت خملات الصائم في طيور السمان الياباني النامية من جميع المجموعات التجريبية أعلى معنويا (P < 0.001)، في حين كانت خملات الصائم أعمق بكثير (P < 0.001) مقارنة بطيور السمان التي تنتمي إلى المجموعة الكنترول. ارتفعت نسبة ارتفاع الخملات إلى العمق لطيور السمان الياباني النامية بشكل معنوي (P < 0.001) في المجموعات التجريبية لطيور السمان اليابانية النامية مقارنة بالمجموعة الكنترول. في الختام، تشير هذه النتائج إلى أن إضافة Trp و/أو CX إلى علائق السمان النامي له تأثير مفيد على نمو أمعاء طائر السمان الياباني ونوعية الكائنات الحية الدقيقة.