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Immunofluorescence localization of cannabinoid receptor type 2 in the ileum of juvenile and adult chickens (*Gallus domesticus*) and its association with gga-mir-200a-3p and oxidative stress

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Abstract

While Cannabinoid Receptor Type 2 (CB2R) is known to influence mammalian gastrointestinal function, its distribution in avian guts and associations with gga-miR-200a-3p and oxidative stress remain understudied. This study aimed to immunohistochemically map the cellular distribution of CB2R in the ileum of healthy chickens and to explore its correlation with gga-miR-200a-3p expression. Ileal samples were collected from 32 male and female chickens. The tissues were analyzed to evaluate the localization and expression of CB2R and gga-miR-200a-3p. The findings revealed an age-related difference in the distribution of CB2R throughout the ileum. In the juvenile epithelial cells, components of the lamina propria, enteric neuronal plexus, ileal crypts, smooth muscle cells of the *tunica muscularis*, and serosal cells exhibited predominantly intense immunoreactivity for CB2R, in contrast to the older age group. While significant differences were observed between juveniles and adults, with higher distribution levels in juveniles, no significant differences were noted between males and females regarding CB2R distribution. Furthermore, in both age and gender groups, the CB2R levels correlated negatively with gga-miR-200a-3p and malondialdehyde, and positively with total antioxidant capacity levels. This study indicates that CB2R is strategically positioned to influence intestinal motility and immune functions, and is associated with gga-miR-200a-3p expression and markers of oxidative stress.

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Introduction

The gastrointestinal tract plays a central role in nutrient absorption, making it a critical organ in the animal body (1,2). Its function is regulated by complex neural, hormonal, and signaling networks, among which the endocannabinoid system (ECS) has emerged as a key modulator. The ECS, through its cannabinoid receptors, influences appetite, gut motility, secretion, and inflammatory processes (3,4). The main components of the ECS are cannabinoid receptors, endogenous cannabinoids, and the metabolic enzymes (5-7). Among these, cannabinoid receptor types 1 (CB1) and 2 (CB2) are the principal cannabinoid receptors. While CB1 receptors are predominantly expressed in the nervous system, CB2 receptors are mainly found in immune cells, such as macrophages (7,8). The distribution and functional role of CB2 have been well-documented in the intestines of numerous mammalian species, including dogs (9), horses (10), cats (11), bulls (12), and pigs (13). However, despite the economic and biological importance of chickens, the precise localization and distribution of CB2 in the avian gut remains largely unexplored. Furthermore, the mechanisms regulating CB2 expression in the gut are not fully understood. MicroRNAs (miRNAs) are key post-transcriptional regulators of gene expression and have been implicated in various gut pathologies, including inflammatory bowel disease and Crohn's disease (14-16). Notably, (Gallus gallus: gga)-mir-200a-3p is upregulated in

chicken models of necrotic enteritis, suggesting a potential role in gut inflammation. A possible link between miR-200 family members and inflammatory pathways relevant to ECS function has been suggested but not yet investigated in this context (17). Significantly, miR-200 has been implicated in regulating *Eimeria tenella* infection in chickens through various circRNA-miRNA-mRNA networks (18). The immunomodulatory role of gga-miR-200a-3p via the MAPK signaling pathway in necrotic enteritis-afflicted chickens has also been investigated (19). Furthermore, microRNA profiling revealed that an abundant mir-200a-3p promotes chicken skeletal muscle satellite cell development by targeting different pathways including transforming growth factor 2 (20).

Given the knowledge gap regarding CB2 in avian species and the potential regulatory role of gga-mir-200a-3p, this study first aimed to characterize the immunofluorescence localization pattern of CB2 in the ileum of juvenile and adult chickens. Secondly, it sought to analyze the association between CB2 distribution and the expression levels of gga-mir-200a-3p. Finally, the study aimed to investigate the potential relationship between CB2 localization and markers of oxidative stress.

Materials and methods

Ethics approve

The Animal Ethics Committee of Ilam University approved all chickens and experimental protocols in this study (Permit Number: IR.ILAM.REC.1403.046).

Tissue sampling

Ileum organ samples were obtained from 32 male and female healthy chicks (Ross 308) at two distinct age groups: juvenile (3-week-old; n=8 female/8 male) and adult (32-week-old; n=8 female/8 male). The birds were sourced from a chicken farm commercial hatchery (Zarbal hatchery, Mazandaran, Iran). The selected birds had no prior history of gastrointestinal diseases and exhibited no anatomical alterations in the gastrointestinal wall. The chickens were considered healthy based on a summary clinical visit before euthanasia. Animals, eight per group, were euthanized by pentobarbital sodium solution (50 mg/kg body weight) delivered by wing vein injection. After euthanasia, the abdominal cavity was dissected, and tissue samples were collected from the midsection of the ileum within 20 minutes post-mortem. Anatomically, no vascular abnormalities or serosa thickening were noted. The dissected segments of the ileum were opened along the mesenteric border and cleaned by being washed with phosphate-buffered saline. One portion of the intestinal tissue sample was placed in 4% paraformaldehyde fixative for paraffin embedding before IHC experiments. In contrast, the other portion was stored in -80 °C for subsequent analysis of gga-miR-200a-3p and oxidative stress markers.

Immunofluorescence staining

Fixed ileal samples were routinely dehydrated in ascending graded levels of ethyl alcohol, cleared in xylene, and embedded in paraffin wax. Sections of five microns in thickness were made by a rotatory microtome. The sections were deparaffinized through xylene and hydrated by descending graded levels of ethyl alcohol. For antigen retrieval, the samples underwent boiling in 0.01 mol/L sodium citrate (pH = 6) to expose antigenic components, and then cooled. After that, to quench peroxidase activity, the slides were incubated in 3% hydrogen peroxide for 10 min at room temperature, and then washed in distilled water and phosphate-buffered saline (PBS) (P4417, Sigma-Aldrich) again for 20 min (21,22). Next, samples were washed in PBS and blocked with 10% goat serum albumin (Sigma-Aldrich, G9023) at 37°C for 45 min to prevent nonspecific binding. Subsequently, sections were incubated overnight at 4°C with rabbit polyclonal anti-cannabinoid receptor type 2 (CB2) antibody (1:100; Abcam, UK), which is validated for reactivity with chicken tissues by the manufacturer via Western blot and immunohistochemistry. After four washes with PBS, sections were incubated at 37°C in the dark with fluorescently labeled secondary antibody (Goat anti-rabbit IgG conjugated to Alexa Fluor® 488; 1:150; Abcam, UK) for 90 min. After 3 washes in PBS, DAPI (D9542, Sigma-Aldrich) was added to the tissues for staining in the dark for 15 min. The localization of CB2R was observed and photographed under a fluorescent microscope (Olympus, Japan) (21,22). Immunopositivity for CB2 was evaluated quantitatively by three blinded observers focusing on cellular localization patterns. To document distribution differences, the percent immunopositive area was measured using Image J (v1.53) and computerized densitometry as follows: % area positivity = (positive pixels / total tissue pixels) × 100 (23). Furthermore, by employing a blend of the unique morphological traits of the various cells examined at high magnification, along with their clearly defined spatial organization within the different layers of intestinal tissue, the distinction between the various cell types in the ileum was achieved.

Real-time polymerase chain reaction

Total RNA was extracted from approximately 30 mg of homogenized ileal tissue using TRIzol™ reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. The concentration and purity (A260/A280 ratio ~2.0) of the isolated RNA were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, USA). Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using the Easy cDNA Synthesis Kit (Parstous, Mashhad, Iran) with oligo(dT) and random hexamer primers according to the manufacturer's instructions. The reaction was performed in a 20 µL volume under the following conditions: 25°C for 10 min, 42°C for 60 min, and 70°C for 10 min to terminate the

reaction. The RT-PCR experiment was performed with the kit (SYBR Master with high Rox; 2X; addbio, Korea). PCR amplification was performed on a Real-time PCR thermocycler (ABI StepOne, USA). The condition of reaction was as: Initial denaturation; 95 °C for 5 min, Amplification; 95 °C for 15 s, 60 °C for 15-20 s, 72 °C for 15-30 s, Melting curve; 95 °C for 15 s, 65 °C for 1 min, and Cooling; 30 °C for 20 s. Threshold cycle (Ct) values were normalized to those of U6 snRNA as an internal reference. The primer sequences for gga-miR-200a-3p and U6 snRNA were designed and provided by Synaclone Biotech (Synaclone, Tehran, Iran). The sequences are listed in Table 1. The expression levels of gga-miR-200a-3p were calculated using the comparative 2^ (-ΔΔCt) method. All RT-PCR reactions were performed using three technical replicates. Two separate reactions without cDNA or with RNA were performed in parallel as controls (19, 24-26).

Table 1: Primer sequences

Gene name	Sequence
STEM LOOP	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAC
STEM LOOP-r	AACTGGTGTCGTGGAGTC
gga-miR-200a-3p	TAACACTGTCTGGTAAG
U6 Forward primer	CGCTTCGGCAGCACATATAC
U6 Reverse primer	CCAGTGCAGGGTCCGAGGTATT

Oxidative stress

Ileal segments were longitudinally opened and thoroughly rinsed with ice-cold phosphate-buffered saline (PBS, 0.01 M, pH 7.4) to remove luminal contents. The mucosal layer was then carefully scraped off using a sterile glass microscope slide on an ice-cold plate. The collected mucosa was immediately weighed and stored at -80 °C in an ultra-low temperature freezer (Eppendorf, Germany) until analysis. For homogenization, approximately 100 mg of mucosal tissue was homogenized in 1 mL of ice-cold PBS using a mechanical homogenizer (IKA T10 basic Ultra-Turrax, Germany). The homogenate was centrifuged at 12,000 × g for 15 minutes at 4°C using an Eppendorf 5430 R centrifuge. The resulting supernatant was aliquoted and used for the subsequent assays. The protein concentration in the supernatant was determined using the Bradford protein assay method to normalize the oxidative stress markers (27). Briefly, 5 µL of supernatant or standard was mixed with 250 µL of Bradford reagent (Bio-Rad, USA) in a 96-well microplate. After a 10-minute incubation at room temperature, the absorbance was measured at 595 nm using a microplate reader (BioTek Synergy H1, USA).

The total antioxidant capacity (TAC) was assessed utilizing an Antioxidant Assay Kit (Sigma-Aldrich). Approximately 30 µg of the protein extract was incorporated into the reaction mixture, which included myoglobin and 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS). The introduction of hydrogen peroxide generates a ferryl myoglobin radical that oxidizes ABTS into a chromogen, which is then detected spectrophotometrically at a wavelength of 415 nm. A Trolox™ curve was utilized as a standard for determining the concentration of antioxidants, and the results were expressed as nmol Trolox™ per mg of protein (28).

Malondialdehyde (MDA) content was quantified using a commercial thiobarbituric acid reactive substances (TBARS) assay kit (A003-1-1, Nanjing Jiancheng Bioengineering Institute, China). The method is based on the reaction of MDA with thiobarbituric acid (TBA) under high temperature and acidic conditions to form a pink-colored MDA-TBA adduct. The assay was performed following the kit protocol. The absorbance of the resulting supernatant was measured at 532 nm (29).

Statistical analysis

The statistical analyses were performed using IBM SPSS® version 24 (IBM Corp, New York, NY) and GraphPad Prism 9 (New York, USA) software. Results are presented as mean ± standard error of the mean (SEM). The normality of data was approved by Shapiro-Wilk or Kolmogorov-Smirnov tests. A one-way analysis of variance followed by the Bonferroni post-hoc test was employed to assess the differences in CB2R tissue expression within the ileum across different groups. Additionally, a Pearson correlation test was utilized to explore the relationship between the ileal tissue levels of CB2R and the expression of gga-miR-200a-3p, as well as the parameters of oxidative stress among the various categories. A p-value of less than 0.05 was considered to indicate significant differences between the groups.

Results

Distribution of CB2R immunoreactive cells in the chicken ileum

Immunofluorescence analysis revealed a widespread distribution of CB2R immunoreactivity within the ileum. The levels of CB2R localization were notably higher in young chickens compared to adults ($P \leq 0.0001$). In the mucosal layer of the juvenile group, cells exhibiting CB2R immunoreactivity demonstrated intense positive staining, in contrast to the older age group ($P \leq 0.0001$). The epithelial

cells, ileal crypts, and components of the lamina propria displayed moderate to intense immunoreactivity. Notably, goblet cells were found to be CB2R negative in both age groups. Within the submucosal tela, the morphology of CB2R-immunoreactive cells appeared irregular. At the same time, the enteric neuron plexus exhibited predominantly bright immunoreactivities for CB2R, particularly in the juvenile group.

Furthermore, CB2R immunoreactivity was also detected in the smooth muscle cells of the *tunica muscularis* and serosal cells of juvenile poultry, in comparison to the older group ($P \leq 0.0001$). In the myenteric plexus of the outer longitudinal muscle layer from the juvenile group, CB2R-immunoreactive cells showed strong positive staining relative to adult animals ($P \leq 0.0001$). However, no significant differences were observed between males and females concerning CB2R immunoreactive expression (Figures 1-4).

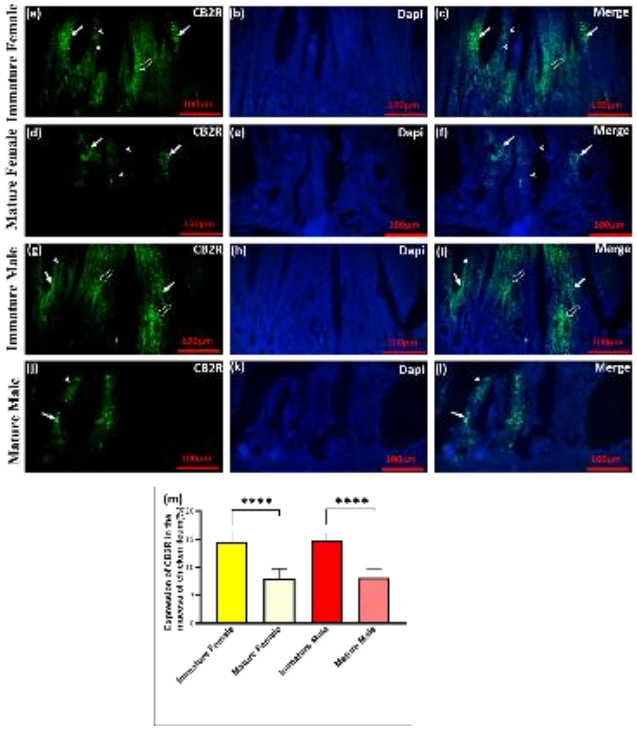

Figure 1: Immunofluorescence images showing CB2R-positive components in the chicken ileum. CB2R-immunoreactivity of cells located in the mucosal layer (white arrows) as well as in the lamina propria immunocytes (open arrows) of immature female (a-c), mature female (d-f), immature male (g-i), and mature male (j-l). The arrowheads indicate CB2R-negative goblet cells. (m): Percent immunopositive area of CB2 receptors in different cell components of the mucosal layer of the chicken ileum. **** $P \leq 0.0001$ indicates significant differences between the designated groups. Scale bar = 100 µm.

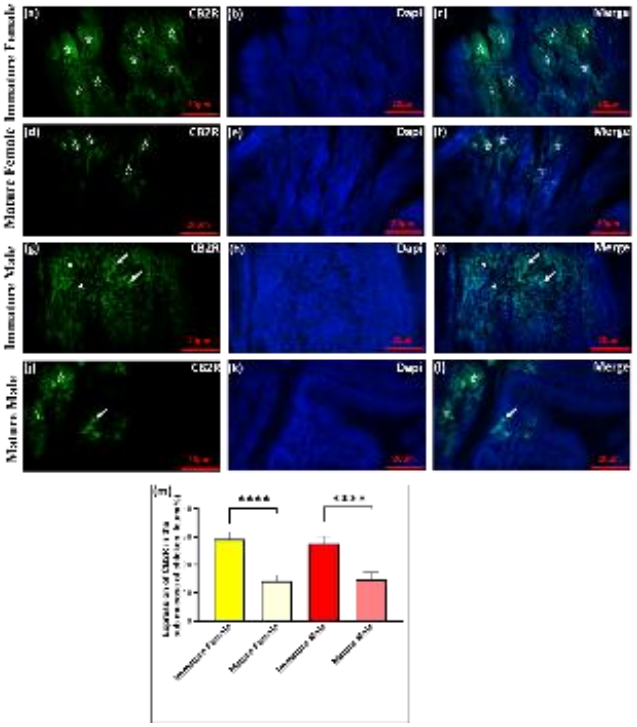

Figure 2: Immunofluorescence images showing CB2R-positive components in the chicken ileum. CB2R-immunoreactivity of cells located in the submucosal plexus neurons (stars) as well as in the submucosal cells (white arrows) of immature female (a-c), mature female (d-f), immature male (g-i), and mature male (j-l). The arrowheads indicate CB2R-negative blood capillaries located in the submucosa layer. (m): Percent immunopositive area of CB2 receptors in different cell components of the submucosal layer of chicken ileum. **** $P \leq 0.0001$ indicates significant differences between the designated groups. Scale bar = 20 µm.

Changes in gga-miR-200a-3p levels

In juvenile chickens, the ileal distribution of gga-miR-200a-3p was markedly reduced compared to the older cohort ($P \leq 0.01$ for females and $P \leq 0.0001$ for males, respectively). There were no significant differences in gga-miR-200a-3p levels between male and female subjects (Figure 5).

Changes in oxidative stress parameters

In juvenile chickens, the ileal distribution of MDA was significantly lower ($P \leq 0.001$ for females and $P \leq 0.0001$ for males, respectively). At the same time, TAC was considerably higher than in the older group ($P \leq 0.0001$). Similar to gga-miR-200a-3p, MDA and TAC levels did not show significant differences between genders (Figure 6).

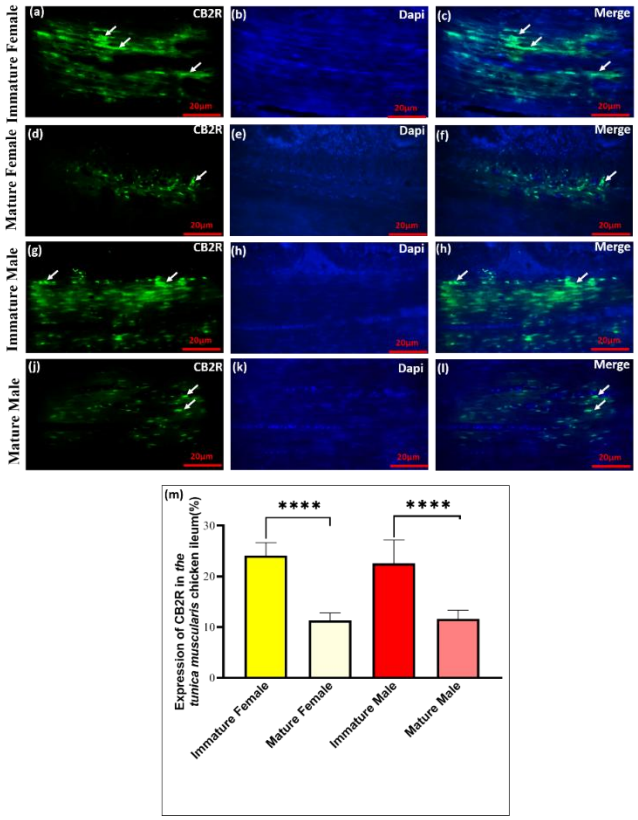

Figure 3: Immunofluorescence images showing CB2R-positive components in the chicken ileum. CB2R-immunoreactivity of cells located in the components of the *tunica muscularis* (white arrows) of immature female (a-c), mature female (d-f), immature male (g-i), and mature male (j-l). (m): Percent immunopositive area of CB2 receptors in different cell components of the *tunica muscularis* layer of chicken ileum. **** $P \leq 0.0001$ indicates significant differences between the designated groups. Scale bar = 20 µm.

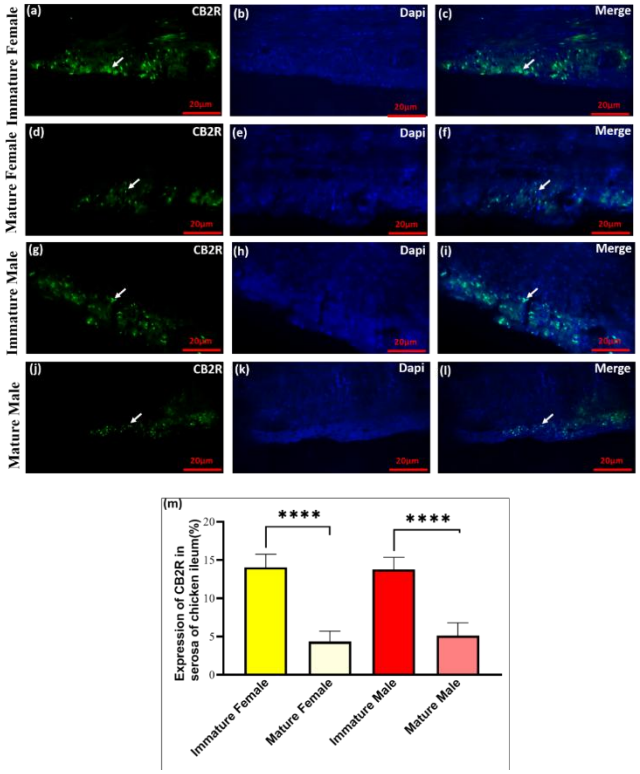

Figure 4: Immunofluorescence images showing CB2R-positive components in the chicken ileum. CB2R-immunoreactivity of cells located in the components of the serosa tela (white arrows) of immature female (a-c), mature female (d-f), immature male (g-i), and mature male (j-l). (m): Percent immunopositive area of CB2 receptors in different cell components of serosa tela of chicken ileum. **** $P \leq 0.0001$ indicates significant differences between the designated groups. Scale bar = 20 µm.

Association between CB2R, gga-miR-200a-3p, and oxidative stress parameters

Our analysis using Pearson correlation reveals a notable negative correlation between the tissue levels of CB2R and the expression of gga-miR-200a-3p ($P \leq 0.001$ for immature females, mature females, and immature males; $P \leq 0.05$ for mature males). Additionally, a significant negative correlation was found with MDA ($P \leq 0.001$ for immature females and immature males; $P \leq 0.01$ for mature females and mature males). Conversely, a significant positive correlation was identified with total antioxidant capacity levels across both sexes ($P \leq 0.001$ for immature males, $P \leq 0.01$ for immature females and mature males; $P \leq 0.05$ for mature females) (Figures 7-9).

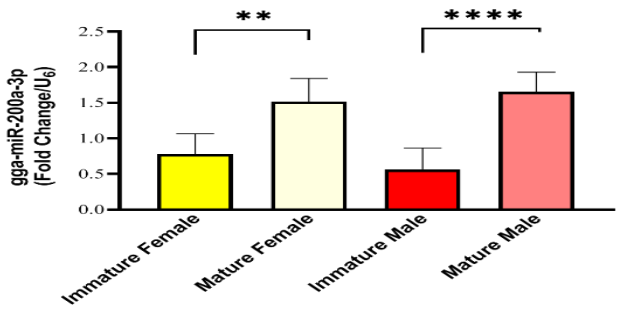

Figure 5: Developmental distribution of gga-miR-200a-3p in the chicken ileum: Age-dependent regulation and sex-independent expression in chickens. Age-associated decline in ileal gga-mir-200a-3p expression in chickens reveals developmental regulation without sexual dimorphism. Data are presented as mean ± SEM, n = 8. **$P \leq 0.01$ and ****$P \leq 0.0001$ indicate significant differences between the designated groups.

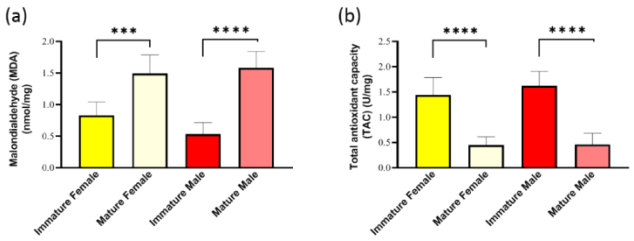

Figure 6: Age-dependent shifts in ileal oxidative stress markers: reduced lipid peroxidation (MDA) and enhanced antioxidant capacity (TAC) in juvenile chickens independent of sex. Data are presented as mean ± SEM, n = 8. ***P ≤ 0.001 and ****P ≤ 0.0001 indicate significant differences between the designated groups.

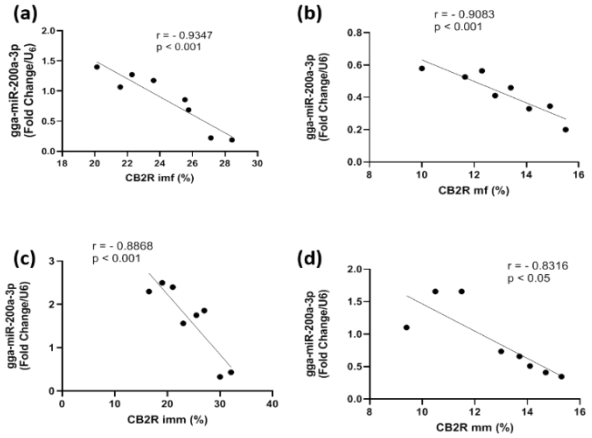

Figure 7: Inverse CB2R coordinates with gga-mir-200a-3p in the avian ileum (sex-independent). Imm: immature male; mm: mature male; imf: immature female; mf: mature female.

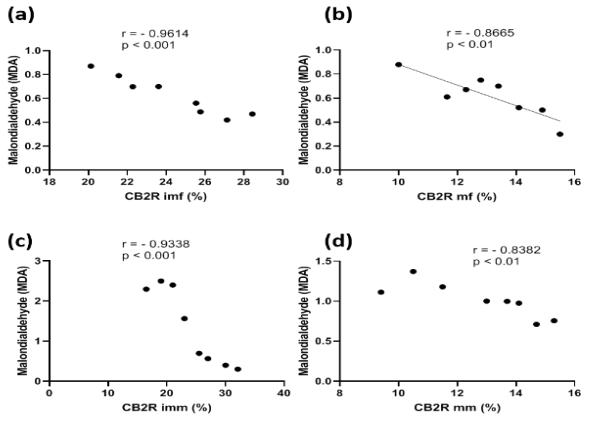

Figure 8: Inverse CB2R coordinates associated with MDA in the avian ileum (independent of sex). Imm: immature male; mm: mature male; imf: immature female; mf: mature female.

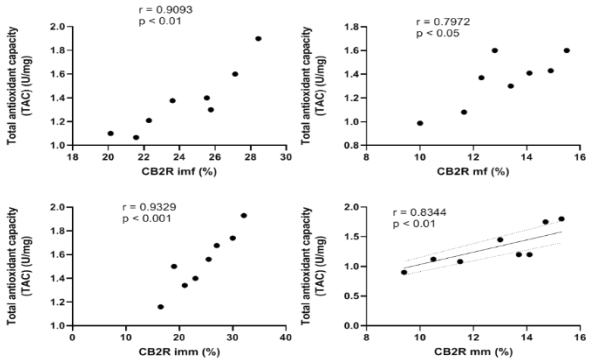

Figure 9: The positive correlation of CB2R is associated with TAC in the avian ileum, regardless of sex. Imm: immature male; mm: mature male; imf: immature female; mf: mature female.

Discussion

The ECS plays a crucial role in regulating various gastrointestinal functions and its distributions reveals segment-specific in various animals including horse, cat, and Holstein bull intestines, highlighting species-specific roles in gut function and suggest potential therapeutic targets for improving animal gut health and productivity (10-12). In this study, authors investigated the immunohistochemical distribution of CB2R receptors and its association with gga-miR-200a-3p expression as well as oxidative stress parameters in the small intestine of chickens, with the aim of contributing to the general knowledge about its potential roles in poultry gastrointestinal homeostasis. The present study provides the first comprehensive evidence of CB2 localization in the ileum of juvenile and adult chickens and its potential regulatory link with gga-miR-200a-3p, as well as MDA and TAC. The current immunofluorescence data revealed distinct CB2 distribution patterns across various ileal stratums, predominantly in epithelial cells, components of the lamina propria, enteric neuronal plexus, ileal crypts, smooth muscle cells of the *tunica muscularis*, and serosal cells). This aligns with mammalian studies where CB2 modulates gut motility and inflammation (10-12), but extends the paradigm to avian species, highlighting evolutionary conservation of the endocannabinoid system in gut physiology. In addition, the CB2R receptors observed in the structural ingredients of the chicken ileum confirmed the results obtained in another species (9-12), and could reflect the possible role of the receptor in the regulation of intestinal integrity including nociception, appetite regulation, and immune response (30).

The observed age-dependent variation in CB2 receptor immuno-positivity, with higher expression in juveniles compared to adults, strongly suggests a developmental role for the endocannabinoid system in the avian gut. This pattern may be linked to the heightened state of immune surveillance

and tissue remodeling required in the juvenile intestine, which is still maturing and establishing its microbiome (1,4). The higher CB2 presence could modulate inflammatory responses and promote tolerance during this critical period. This finding aligns with several human and mammalian studies indicating that CB2R activation exerts a protective role in models of inflammatory bowel disease by reducing the production of pro-inflammatory cytokines and dampening immune cell activation (31,32). The present results extend this concept ontogenetically, implying that this protective mechanism may be particularly vital during early post-natal development. Furthermore, the differential therapeutic application of cannabinoids based on age, with cannabis -rich products favored for pediatric disorders and delta-9-tetrahydrocannabinol-rich ones for adult conditions (33), supports the notion of an evolving endocannabinoid system.

Given that, CB2 activation in mammals suppresses pro-inflammatory cytokines (34); its increased distribution in juvenile chickens might reflect a developed immunomodulatory feedback mechanism, potentially critical for maintaining gut barrier integrity in immature birds. This finding gains further relevance in poultry farming, where gut health directly affects productivity.

In both age groups, the CB2R localization was approved in almost all compartments of the chicken ileum, suggesting a possible role of this receptor in promoting ileal homeostasis (35,36). Interestingly, other studies have pointed out that the CB2R distribution in the epithelial cells of the intestine might have an analgesic effect in visceral pain (37). The presence of CB2R in the cells located in the lamina propria is well known (10). The immunomodulatory effect of cannabinoids in different brain cells and their role in the pathophysiology of psychiatric and neurodegenerative disorders is probably mediated by the CB2R (38). The studies using CB2R agonists have shown encouraging results in mitigating gut inflammation in rodent models of IBD (39,40). There are studies supporting the fact that CB1R and CB2R suppress T cell function and macrophage populations by altering IL-6 and TGF-β (41). CB1R and CB2R play critical, non-redundant roles in host defense against Salmonella, with CB1R deficiency exacerbating bacterial dissemination and CB2R knockout causing gut dysbiosis and immune dysregulation. These receptors distinctly modulate immune cell responses, cytokine profiles, and microbiome stability during infection (41).

Notably, the identification of gga-miR-200a-3p as a putative regulator of *Cannabinoid Receptor 2* (*CNR2*; the CB2 gene) introduces a novel layer of endocannabinoid system regulation in chickens. This is supported by the present Pearson correlation test, which reveals a significant negative correlation between CB2 protein levels and gga-miR-200a-3p expression. This inverse relationship suggests that this microRNA may be a key post-transcriptional repressor responsible for the observed downregulation of CB2 in adult chickens. The miR-200 family implicated in modulating inflammation and epithelial-mesenchymal transition by inhibiting epithelial-mesenchymal transition in mammalian models (42). Given that CB2 receptor activation is also known to exert potent anti-inflammatory effects (39-41), a compelling hypothesis emerges the age-dependent increase in gga-miR-200a-3p may simultaneously fine-tune immune responses and contribute to the decline in CB2 expression, representing a coordinated mechanism for immune adaptation in the mature gut. However, the mechanistic interplay between gga-miR-200a-3p and CB2 warrants further investigation, like through luciferase assays or knockout models to confirm direct targeting and functional consequences. A prior investigation illustrated how microarrays influence the development of skeletal cells in broiler chickens (20). Similarly, a study reveals that the endocannabinoid anandamide exerts anti-inflammatory effects in vascular smooth muscle cells by binding nuclear receptors NR4A1/2, suppressing inflammatory genes like CCL2 via NCoR1 recruitment. These findings identify NR4A-targeting AEA analogs as potential novel anti-inflammatory therapeutics for atherosclerosis (43).

In this work, the Pearson correlation test shows a significant negative correlation between the tissue levels of CB2R and MDA, but a significant positive correlation with total antioxidant capacity levels, in both genders. In accordance with our results, recently He *et al.* (44) demonstrated that CB2 receptor activation via agonist GW842166X alleviates psoriasis-like lesions in mice by reducing inflammation and oxidative stress. The therapeutic effects involve suppression of inflammatory cytokines and modulation of the Keap1/Nrf2 signaling pathway. These findings position CB2R activation as a promising novel strategy for psoriasis treatment (44). Previous study demonstrates that CB2 receptor agonist effectively protects against diabetic cardiomyopathy by reducing oxidative stress, fibrosis, and inflammasome activation. This finding is consistent with the broader protective role of CB2 receptors across various organ systems. In fact, they have concluded that CB2 receptor agonists can act as a promising therapeutic candidate for diabetic complications through their multi-targeted CB2 receptor-mediated mechanisms (45).

This study has many limitations. While we established CB2's spatial distribution, its functional role in avian gut motility/immunity remains untested. Electrophysiological studies or CB2 agonist/antagonist trials could clarify this. In addition, the miR-200a/CB2 axis should be explored in disease contexts like *Salmonella* infection, given CB2's immunoprotective roles in mammals. Furthermore, single-cell RNA-seq could resolve CB2's cell-specific expression and miRNA interactions.

Conclusion

This study provides the first evidence for the distribution of CB2R in the ileum of chickens, a finding that bridges a

critical gap in our understanding of the avian endocannabinoid system. It has been examined localization of CB2R across multiple intestinal layers. Furthermore, a novel link between CB2R expression, gga-mir-200a-3p, and oxidative stress, suggesting a complex regulatory mechanism. Future functional studies are warranted to validate the therapeutic potential of CB2R agonists and to elucidate the precise mechanisms of the miR-200a/CB2R axis.

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Conflict of interest

There is no conflict of interest.

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الفحص المتاعي المشع وتمركز مستقبلات القنب نوع ٢ في اللفائفي للصغار والبالغين من دجاج (*Gallus domesticus*) وارتباطه مع gga-mir-200a-3p والإجهاد التأكسدي

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الخلاصة

إن مستقبلات القنب من النوع ٢ معروفة بالتأثير على وظيفة الجهاز الهضمي للثدييات، وإن توزيعها في أحشاء الطيور وارتباطاتها مع gga-miR-200a-3p والإجهاد التأكسدي لا يزال غير مدروس. تهدف هذه الدراسة إلى رسم خريطة كيميائية مناعية للتوزيع الخلوي مستقبلات القنب من النوع ٢ في اللفائفي للدجاج السليم واستكشاف ارتباطه مع التعبير gga-miR-200a-3p. تم جمع عينات اللفائفي من ٣٢ دجاجة من الذكور والإناث. تم تحليل الأنسجة لتقييم تمركز والتعبير عن مستقبلات القنب من النوع ٢ و gga-miR-200a-3p. كشفت النتائج عن اختلاف مرتبط بالعمر في توزيع مستقبلات القنب من النوع ٢ في جميع أنحاء اللفائفي. في الخلايا الظهارية اليافعة، أظهرت مكونات الصفيحة المخصوصة، والضفيرة العصبية المعوية، والخبايا اللفائفية، وخلايا العضلات الملساء في الغلالة العضلية، والخلايا المصلية نشاطا مناعيا مكثفا في الغالب لـمستقبلات القنب من النوع ٢، على عكس الفئة العمرية الأكبر سنا. وفي حين لوحظت فروق ذات دلالة إحصائية بين الصغار والبالغين، مع ارتفاع مستويات التوزيع في الصغار، لم تلاحظ فروق ذات دلالة إحصائية بين الذكور والإناث فيما يتعلق بتوزيع مستقبلات القنب من النوع ٢. وعلاوة على ذلك، في كل من الفئات العمرية والجنسية، ترتبط مستويات مستقبلات القنب من النوع ٢ سلبا مع gga-miR-200a-3p و مالوندیالدهید، وإيجابية مع مجموع مستويات القدرة المضادة للأكسدة. تشير هذه الدراسة إلى أن مستقبلات القنب من النوع ٢ في موقع استراتيجي للتأثير على حركية الأمعاء ووظائف المناعة، ويرتبط بتعبير gga-miR-200a-3p وعلامات الإجهاد التأكسدي.