Theses of doctoral (PhD) dissertation

Investigation of the immunomodulatory effects of Antimicrobial Peptides on chicken hepatic cell cultures and intestinal explants

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Introduction

Poultry, the largest meat-producing livestock species kept today, are highly exposed to various infectious diseases due to crowded housing conditions, which can be exacerbated by stressors such as heat or transportation. Some of these diseases are of bacterial origin and are proving increasingly difficult to treat since the spread of antimicrobial resistance (AMR). Antibiotics are becoming less and less effective and the use of agents to which pathogens are still susceptible is increasingly restricted by the need to protect human life. The demand for natural and synthetic substitutes for antibiotics is therefore growing.

Living organisms have various ways to defend themselves against harmful effects, for example by secreting biologically active substances. Antimicrobial peptides (AMPs), also known as host defense peptides (HDPs) are an important part of this system, produced by almost all organisms, and are promising candidates in the treatment of bacterial and inflammatory diseases. They are composed of up to 100 amino acids and are considered to have a direct antimicrobial effect as part of the innate immune system and to be able to modulate the immune response. Although their discovery is mainly due to their direct antimicrobial property, it has subsequently been demonstrated that in vertebrates, including humans, they can protect against pathogens by stimulating the body's self-defense and by suppressing harmful inflammatory processes. Their direct antimicrobial effect is poorly observed *in vivo* which is the reason why the term HDP is nowadays often used in literature to refer to their protective function in assisting the immune system. To date, more than 3500 AMPs have been described in the University of Nebraska Medical Center database (https://aps.unmc.edu/).

As part of this dissertation, an AMP of natural (chicken cathelicidin-2, Cath-2) and one of synthetic origin (innate defense regulator-1002, IDR-1002) were investigated. Naturally derived AMPs have a larger history of literature and therefore more information is available about them, but they have several disadvantages compared to synthetic AMPs, such as higher levels of toxicity and species specificity. However, synthetic AMPs have been developed considering aspects such as smaller size and increased potency.

Our main aim was to investigate the effects of two of these AMPs on cell viability and immune response. Two *in vitro* models were employed for this purpose: a primary hepatocyte – non-parenchymal cell co-culture and an intestinal explant model, both of chicken origin. The reason behind choosing these models was that most of the infections affecting chickens originate from the intestines and are often caused by pathogenic bacteria or by an imbalance in the gut bacteriome. Therefore, it is beneficial to investigate the effects these promising compounds have on the immune response of the intestinal wall. Moreover,

the liver is the first organ to interact with molecules entering the bloodstream from the intestines, therefore it has an especially important role in the protection against infections and pro-inflammatory agents from this direction.

Aims of the study

Our research group has already established and characterized a primary co-culture comprised of hepatocytes and non-parenchymal cells (predominantly resident macrophages, the Kupffer cells) and an intestinal explant model, both of which can serve as a potent tool to investigate inflammatory processes related to infections in chickens. In order to do that, first, an inflammatory model had to be established, therefore this was our first goal. As former studies presented that this kind of cell culture model reacts somewhat differently to inflammatory stimuli when compared to cell cultures of mammalian origin, several PAMPs were needed to be tested.

Our next question was whether the AMPs chosen for this study, Cath-2 and IDR-1002 have any decreasing effect on the viability of cells in either the hepatocyte – non-parenchymal cell co-culture or the intestinal explants. As AMPs have been proven to have damaging effects on cell membranes and even found to be toxic to some cell types when applied in higher concentrations, it is certainly an important aspect to determine their usability.

Our main goal, besides investigating their potential toxicity was to define the immunomodulatory effects these peptides may have on our models. As most AMPs, Cath-2 and IDR-1002 are also proven to have broad immunomodulatory effects, and they affected the immune function of different cell types diversly. Confirmed by previous studies that these effects can vary between species and cell types, we believe that it is crucial to have reliable information on the specific characteristics these peptides have on the hepatic and intestinal cells of chickens. Furthermore, the recognition of the interrelations between redox and immunological processes in our models may be of great importance.

Goals of my PhD thesis are summarized as follows:

Ad 1, to develop an inflammatory model on our hepatocyte – non-parenchymal cell co-culture model of chicken origin.

Ad 2, to test the effect Cath-2 and IDR-1002 have on the cell viability of the hepatocyte – non-parenchymal cell co-culture and the intestinal explants.

Ad 3, to investigate the immunomodulatory effects of Cath- 2 on the hepatocyte – non-parenchymal cell co-culture and the intestinal explants.

Ad 4, to investigate the immunomodulatory effects of IDR-1002 on the hepatocyte – non-parenchymal cell co-culture and the intestinal explants.

In order to fulfil the abovementioned criteria, the below described study plan has been followed:

	Model	**Investigated compounds**	**Cell viability parameters**	**Inflammatory parameters**	**Redox parameters**
Preliminary Study	Chicken hepatocyte – non-parenchymal cell co-culture	LPS, LTA, PMA	Metabolic activity and extracellular LDH activity	CXCLi2 and IL-6 concentrations	-
Study I.	Chicken hepatocyte – non-parenchymal cell co-culture	Chicken cathelicidin-2, LTA and PMA	Metabolic activity and extracellular LDH activity	CXCLi2, IFN-γ, IL-10 and M-CSF concentrations	H_2O_2 and MDA levels
Study II.	Chicken hepatocyte – non-parenchymal cell co-culture	IDR-1002, LTA	Metabolic activity and extracellular LDH activity	CXCLi2, IL-6, IL-16, IFN-γ, IL-10, M-CSF and RANTES concentrations	H_2O_2, Nrf2 and PC levels
Study III.	Chicken intestinal explant	Chicken cathelicidin-2, LTA	Metabolic activity and extracellular LDH activity	CXCLi2, IL-6, IL-2, IFN-γ, IL-10 concentrations and IFN-γ/IL-10 ratio	-
Study IV.	Chicken intestinal explant	IDR-1002, LTA	Metabolic activity and extracellular LDH activity	CXCLi2, IL-2, RANTES, IFN-γ, IL-10 concentrations and IFN-γ/IL-10 ratio	-

Materials and methods

Preliminary study: induction of inflammation

Our first goal was to establish an inflammatory model on our primary hepatocyte – non-parenchymal cell co-culture of chicken origin previously characterized by our research group. After mixing the cell suspensions in the ratio of 6:1 (hepatocytes to non-parenchymal cells) – which models a mild hepatic inflammation and therefore creates the conditions for an inflammatory response – the hepatocyte- non-parenchymal cell co-cultures were seeded onto 96-well plates previously coated with collagen type I. The seeding volume was 100 µL/well.

In this study, the effects of lipopolysaccharide (LPS), lipoteichoic acid (LTA) and phorbol myristate acetate (PMA) were investigated on cell viability and inflammatory parameters. In the first part of the study, cell cultures were treated with 0 (control), 10 or 50 µg/mL LPS from *Escherichia coli* (O55:B5), and with 10 or 50 µg/mL LTA from *Staphylococcus aureus.* In the second part of the study, cell culture medium was supplemented with 0 (control), 100 or 1000 ng/mL PMA. Cells were incubated with the treatment solutions for 24 hours.

To determine cell viability, the metabolic activity of the cells was measured on 96-well plates using Cell Counting Kit-9 (CCK-8) essay, and extracellular lactate dehydrogenase (LDH) was measured to assess the membrane integrity. Cytokine concentrations, namely interleukin-6 (IL-6) and chicken chemotactic and angiogenic factor (CXCLi2, also known as chicken interleukin-8 [IL-8]) were measured by chicken specific ELISA kits.

Study I and II: Effects of Cath-2 and IDR-1002 on a primary hepatic cell culture

In both Study I and Study II, the preparation of hepatocyte – non-parenchymal cell co-cultures was carried out following the methodology explained in the Preliminary study. After isolation of the cells, cell cultures were prepared using 24-well and 96-well culture plates pre-coated with collagen type I. On the 24-well plates, the seeding quantity was 400 µL/well, while 100 µL/well on the 96-well plates. In Study I, cells were treated with Cath-2 in 5 nmol/mL and 10 nmol/mL concentrations. *Staphylococcus aureus*-derived LTA was used in 50 µg/ml, and PMA in 1000 ng/mL concentrations. In Study II, IDR-1002 was added in concentrations of 10, 30, and 90 µg/mL alone and in combination with 50 µg/mL LTA from *Staphylococcus aureus.*

Assessments of cell viability were carried out the same as in the Preliminary study. Chicken specific ELISA kits were employed to measure CXCLi2 (Study I), or CXCLi2 and

IL-6 (Study II) concentrations. Luminex xMAP method was used to measure IFN-γ, IL-10, and macrophage colony-stimulating factor (M-CSF) concentrations (Study I), and IL-16, IFN-γ, IL-10, M-CSF and RANTES (Regulated On Activation, And Normal T-cell Expressed and Secreted, Study II). Moreover, in both studies, extracellular H_2O_2 content was measured by the Amplex Red method. In study I, malondialdehyde (MDA) concentrations were detected using a specific colorimetric assay. In study II, protein carbonyl (PC) and nuclear factor erythroid 2-related factor 2 (Nrf2) levels were measured by a chicken specific ELISA method.

Study III and IV: Effects of Cath-2 and IDR-1002 on an intestinal explant culture

The isolation of the intestinal explants was performed using the methodology recently developed by our research team, for which a 3-week-old male Ross-308 broiler chicken was sacrificed. These explant cultures can provide a tool to determine the effects of AMPs on the intestinal wall. After preparation of the explants, cell culture media was supplemented with 5, 10 and 25 nmol/mL chicken cathelicidin-2, 10 µg/mL *Staphylococcus aureus* derived LTA, and the combination of the formers. Explants were incubated with the solutions for 12 hours, thereafter samples were taken from the cell culture medium

Cell viability was measured by the previously explained methods. In both Study III and Study IV, the concentration of CXCLi2 was determined through a chicken-specific sandwich ELISA. Luminex xMAP method was used to determine the concentrations of the following cytokines and chemokines: IL-2, IL-6, IFN-γ and IL-10 (Study III), IL-2, IFN-γ, IL-10 and RANTES (Study IV). In both Study III and Study IV, for a more precise assessment of the inflammatory state of the explants, the IFN-γ to IL-10 ratio was calculated.

Statistics

All statistical analyses were performed in R v. 4.0.3 (R Core Team, 2020). Pairwise comparisons were performed using Wilcoxon signed-rank test, as some of the treatment groups showed non-normal distribution based on Shapiro-Wilk tests. We have considered a difference statistically significant if the p-value was less than 0.05.

Results and discussion

Preliminary study: induction of inflammation

This preliminary study aimed to identify suitable compounds for inducing an inflammatory response in chicken hepatocyte – non-parenchymal cell co-cultures, which is essential for evaluating the effects of potential immunomodulatory agents *in vitro*. Given the limited data on cell cultures of chicken origin, this research fills a crucial gap by establishing a reliable inflammatory model using co-cultures of primary hepatocytes and non-parenchymal cells at a 6:1 ratio reflecting a mild hepatic inflammatory state, allowing investigation of interactions between stress and immune responses.

The study first assessed traditional bacterial endotoxins—LPS from Gram-negative- and LTA from Gram-positive bacteria—as potential inducers of inflammation. While LPS and most treatments did not elicit a proinflammatory cytokine response, LTA at 50 µg/mL moderately increased CXCLi2 levels, indicating some activity. Then, in the next part of the study, the effects of PMA (phorbol 12-myristate 13-acetate) were evaluated.

Results showed that LTA caused membrane damage, evident from elevated extracellular LDH levels, while PMA decreased metabolic activity, signaling cellular stress or toxicity. Importantly, only PMA increased significantly the levels of both IL-6 and CXCLi2 cytokines, while LTA elevated CXCLi2.

Conversely, LPS failed to induce cytokine production, likely due to the deficient TLR4 signaling pathway in chickens, which lacks some mammalian gene orthologs and alternative signaling routes. This deficiency may explain the low or absent IL-6 and CXCLi2 secretion observed, despite previous reports of upregulated mRNA expression in similar models. The discrepancy highlights the importance of post-transcriptional and translational regulation in cytokine production, which may affect the final protein levels in culture media.

In conclusion, PMA and LTA were identified as effective inducers of inflammation in chicken hepatic co-cultures. These findings support their use in future *in vitro* studies on immunomodulatory interventions, offering a reliable model for investigating avian immune responses at the cellular level.

Study I: Effects of Cath-2 on a primary hepatic cell culture

In Study I, the effects of Cath-2 were examined in the same chicken hepatocyte–non-parenchymal cell co-culture model. The study aimed to assess Cath-2's cytotoxicity and its impact on the inflammatory response. AMPs, while promising therapeutic agents, can have cytotoxic effects due to their membrane-disrupting action. Cath-2 was shown to decrease cellular metabolic activity and increase LDH release in a dose-dependent manner, suggesting mild to moderate cytotoxicity, particularly at higher concentrations. Interestingly, these effects were mitigated when Cath-2 was administered together with LTA, likely due to its known binding and neutralization of endotoxins like LTA, which may limit off-target cytotoxic effects.

The immune response was evaluated by measuring cytokine and chemokine levels (CXCLi2, IL-10, IFN-γ, and M-CSF). High-dose Cath-2 increased IFN-γ, indicating pro-inflammatory activity, while the lower dose reduced LTA-induced IFN-γ, suggesting anti-inflammatory potential. Cath-2 also elevated CXCLi2 and IL-10 levels in a dose-dependent manner, supporting its immunomodulatory rather than purely anti-inflammatory function. In contrast, LTA and PMA alone had limited effects on these mediators.

M-CSF, involved in macrophage regulation, was suppressed by Cath-2, especially when combined with LTA or PMA, suggesting the AMP's influence on macrophage function. Additionally, Cath-2 increased hydrogen peroxide (H_2O_2) production dose-dependently, although lipid peroxidation (measured by MDA) remained unchanged, and high-dose Cath-2 even reduced MDA levels, indicating a protective effect on membrane lipids.

Overall, Cath-2 modulated multiple aspects of hepatic immune response—altering cytokine production, oxidative status, and cell viability—in a concentration-dependent manner. While high doses posed some risk of cytotoxicity, lower doses maintained cellular integrity and showed beneficial immunoregulatory effects. These findings underscore Cath-2's potential as a therapeutic immunomodulator, offering an alternative to antibiotics by modulating inflammation without causing significant cellular harm.

Study II: Effects of IDR-1002 on a primary hepatic cell culture

Innate defense regulators (IDRs) are host-defense peptides that modulate immune responses without directly killing pathogens. This study investigated the effects of IDR-1002 on the hepatic immune response in chickens using the previously mentioned co-culture model by measuring cytokine and chemokine levels (IL-6, CXCLi2, IFN-γ, IL-16, IL-10, M-CSF, and RANTES), and assessing cell viability.

IDR-1002, tested at 10, 30, and 90 µg/mL, showed no significant cytotoxicity. However, combined treatment with LTA and 90 µg/mL IDR-1002 modestly increased LDH activity, indicating mild membrane damage. The peptide notably increased RANTES and M-CSF levels, both of which influence macrophage polarization: RANTES promotes pro-

inflammatory M1 macrophages, while M-CSF favors anti-inflammatory M2 differentiation. Interestingly, IDR-1002 reduced both pro-inflammatory (IFN-γ, IL-6, IL-16) and anti-inflammatory (IL-10) cytokines, suggesting it induces an intermediate macrophage phenotype.

RANTES levels correlated positively with IFN-γ, IL-16, and IL-10, while M-CSF negatively correlated with CXCLi2, indicating a complex regulation of immune signaling. Despite previous findings that IDR-1002 can increase pro-inflammatory cytokines, in this study it suppressed LTA-induced IFN-γ, IL-6, and CXCLi2, consistent with other models showing anti-inflammatory effects.

IDR-1002 also reduced LTA-induced H_2O_2 levels, likely through activation of the Nrf2/KEAP1 pathway, which regulates antioxidant responses. Elevated Nrf2 levels were observed following IDR-1002 and LTA treatment. While ROS were reduced, carbonylated protein levels increased with IDR-1002, potentially linked to its antibiofilm activity, though this did not harm cells.

In conclusion, IDR-1002 demonstrated immune-modulating and antioxidant effects in chicken liver cells, reducing pro-inflammatory responses and shifting macrophage activity toward a mixed phenotype. It may hold potential as a therapeutic agent for managing inflammation from bacterial infections, though further studies are needed to clarify its mechanisms.

Study III: Effects of Cath-2 on an intestinal explant culture

This study investigated the immunomodulatory and cytotoxic effects of the antimicrobial peptide Cath-2 on chicken intestinal explant cultures. Unlike its previously observed cytotoxic effects in hepatic cells at high concentrations, Cath-2 showed no detrimental impact on intestinal cell viability at 5 and 10 nmol/mL. Interestingly, 25 nmol/mL significantly increased metabolic activity, likely due to enhanced $NADPH+H^+$ production, which plays a role in inflammatory signaling and fatty acid synthesis during immune responses.

Cath-2 induced the release of pro-inflammatory cytokines (IL-2, CXCLi2, and IL-6) in non-inflamed cells, consistent with its known role in stimulating innate immunity through mechanisms like NLRP-3 inflammasome activation. However, under LTA-induced inflammation, Cath-2 exhibited anti-inflammatory properties. It significantly reduced LTA-elevated levels of IL-2, IL-6, and the IFN-γ/IL-10 ratio - an established marker of inflammatory balance in the gut. Moreover, it restored IL-10 levels, which were decreased by LTA, suggesting a protective regulatory effect.

Although IFN-γ levels remained unchanged by Cath-2, its reduction of the IFN-γ/IL-10 ratio further underscores its anti-inflammatory potential. The peptide's strongest regulatory effects were observed at 25 nmol/mL, which both suppressed pro-inflammatory cytokine release and enhanced anti-inflammatory responses without harming tissue viability.

These findings align with earlier studies showing Cath-2's ability to neutralize inflammatory stimuli like LPS and LTA and suppress macrophage activation. IL-2 and IL-10, key regulators of gut immunity, were particularly responsive to Cath-2, indicating its role in promoting immune tolerance and preventing chronic inflammation.

In conclusion, Cath-2 shows promise as a therapeutic agent for managing intestinal inflammation in poultry. It provides dual benefits: stimulating protective immune responses in non-inflamed conditions and reducing harmful inflammation under pathogenic stress. Given its efficacy and safety in this ex vivo model, Cath-2 may serve as a valuable alternative to antibiotics for treating enteric bacterial infections, though further in vivo studies are warranted.

Study IV: Effects of IDR-1002 on an intestinal explant culture

This study investigated the immunomodulatory and cytotoxic effects of IDR-1002 on chicken intestinal explants. The results confirmed that IDR-1002 is non-cytotoxic at appropriate concentrations, with no adverse impact on metabolic activity. Interestingly, 30 µg/mL of the peptide reduced extracellular LDH activity, possibly due to cytoprotective autophagy, although this hypothesis could not be directly confirmed.

The study found that IDR-1002, particularly at 30 µg/mL, increased the production of the pro-inflammatory markers CXCLi2 and IL-2, which may reflect a protective immune activation. All tested concentrations enhanced IL-2 levels, supporting previous findings that link IL-2 to immune tolerance and the prevention of chronic intestinal inflammation. Furthermore, while LTA was used to induce an inflammatory response—marked by elevated IFN-γ, CXCLi2, IL-2, RANTES, and a higher IFN-γ/IL-10 ratio—IDR-1002 effectively counteracted many of these effects.

Among the tested doses, 30 µg/mL of IDR-1002 was the most effective in reducing LTA-induced elevations in CXCLi2, IL-2, and RANTES, highlighting its strong anti-inflammatory potential. Conversely, the highest dose (90 µg/mL) increased IL-10 production significantly and lowered the IFN-γ/IL-10 ratio, suggesting a shift toward an anti-inflammatory state despite an increase in IFN-γ itself. This finding emphasizes the importance of IL-10 in restoring immune balance in the intestinal mucosa.

In conclusion, IDR-1002 demonstrated a dose-dependent immunomodulatory effect on chicken intestinal explants. While enhancing innate defense responses via IL-2 and CXCLi2 in non-inflamed conditions, it mitigated inflammatory responses under LTA challenge, particularly through the modulation of cytokine levels and the IFN-γ/IL-10 ratio. These results position IDR-1002 as a promising therapeutic candidate for managing intestinal inflammation caused by bacterial infections, with further studies needed to determine optimal dosing.

Conclusion

Our five studies demonstrate the diversified effects of AMPs, while showing their dependence on different biological factors. Therefore, two AMPs might influence the immune response of the same cell type in somewhat different ways, while one AMP might provoke diverse immune responses in different tissues or organs. Consequently, it can be concluded that a more substantial understanding of the cellular effects of AMPs is necessary before they can be introduced into clinical research. Our results can contribute to this goal considerably by providing valuable data on cell viability and immune response provoked by two important peptides, the Cath-2 and IDR-1002 in two *in vitro* model systems.

New scientific results

Ad 1,

A reliable inflammatory model has been successfully established for the hepatocyte – non-parenchymal cell co-culture framework. LTA, and, to a lesser extent, PMA have been proven to effectively induce inflammation and are suitable for the testing of immunomodulatory materials using our model.

Ad 2,

Cath-2 decreased cell viability in the hepatocyte – non-parenchymal cell co-culture model, especially when used in higher than 5 nmol/mL concentrations. The viability of the intestinal explants was not reduced by Cath-2. In contrast, IDR-1002 did not have an effect on cell viability of either the hepatocyte – non-parenchymal cell co-culture model or the intestinal explants.

Ad 3,

Cath-2 had a diversified role in the modulation of the immune response of the hepatocyte – non-parenchymal cell co-culture model and the intestinal explants. It had a pro-inflammatory effect in both models when applied alone, especially at higher concentrations. However, it also demonstrated anti-inflammatory effects as it alleviated inflammation provoked by LTA in both models.

Ad 4,

IDR-1002 had a complex role in modulating the immune response of the hepatocyte – non-parenchymal cell co-culture model and the intestinal explants. It had a remarkable anti-inflammatory effect as it decreased the pro-inflammatory cytokine release induced by LTA in both models. Meanwhile, based on the RANTES, M-CSF and cytokine concentrations, it steered inflammatory processes into an intermediate phase between the anti- and proinflammatory direction when used on the hepatocyte – non-parenchymal cell co-culture model.

Own scientific publications

Publications related to the topic of the present thesis

Full text papers in peer-reviewed journals:

Sebők C, Tráj P, Vörösházi J, Mackei M, Papp M, Gálfi P, Neogrády Z, Mátis G (2021) **Two Sides to Every Question: Attempts to Activate Chicken Innate Immunity in 2D and 3D Hepatic Cell Cultures**. Cells 10:1910. https://doi.org/10.3390/cells10081910

Sebők C, Walmsley S, Tráj P, Mackei M, Vörösházi J, Petrilla J, Kovács L, Kemény Á, Neogrády Z, Mátis G (2022) **Immunomodulatory effects of chicken cathelicidin-2 on a primary hepatic cell co-culture model**. PLOS ONE 17:e0275847. https://doi.org/10.1371/journal.pone.0275847

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Cell Co-Culture Model, 15th International Scientific Conference On Probiotics, Prebiotics, Gut Microbiota And Health, Pozsony, 2022.06.27-30.

Sebők, C; Tráj, P; Mackei, M; Kemény, Á; Neogrády, Zs; Mátis, G: **The immunomodulatory action of the antimicrobial peptide IDR-1002 in a hepatic cell culture model of chicken origin**, Proceedings of the 13th International Veterinary Immunology Symposium, Kruger National Park, South Africa. 17-21 November 2023, page 86, abstract 123., 2023

Oral presentations at Hungarian national conferences

Kulcsár A, Sebők C, Mátis G, Talapka P, Hatala P, Petrilla J, Fébel H, Neogrády Zs (2017) **Az inzulin és a glukagon jelpálya különböző takarmányozási faktorok segítségével történő szabályozása broilercsirkében**, Akadémiai Beszámolók 2017. évi 44. füzet.

Sebők C, Orbán K, Mackei M, Vörösházi J, Neogrády Zs, Mátis G (2018) **A chicken heterophil peptide 1 (CHP-1) gyulladáscsökkentő hatásának vizsgálata csirke eredetű májsejt-tenyészetek**en, Akadémiai Beszámolók 2019. évi 46. füzet.

Sebők C, Vörösházi J, Mackei M, Tráj P, Szentgyörgyi Á, Neogrády Zs és Mátis G (2019) **Bakteriális sejtfalkomponensek hatásainak vizsgálata két-, illetve háromdimenziós csirke eredetű májsejttenyészeteken**, Akadémiai Beszámolók 2020. évi 47. füzet

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Sebők C, Tráj P, Mackei M, Márton R A, Vörösházi J, Kemény Á, Neogrády Zs és Mátis G (2024) **Az IDR-1002 ntimikrobiális peptid gyulladásos folyamatokra kifejtett hatásainak vizsgálata csirke eredetű vékonybél explantokon**, Akadémiai Beszámolók 2023. évi 50. füzet

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