1.6 times, reducing the recurrence level by 1.8 times with their predominant course in mild form and prolonging the remission period by 1.6 times.

ADVANTEGES IN RT-PCR METHOD APPLICATION TO ASSESS INTESTINAL BARRIER FUNCTION IN BROILER CHICKENS

Shatalov S.[1], PhD student,
Nezdvetsky V.[1], D.Sc., Professor,
Baydas G.[2], PhD, Professor,
Baselga R.[3], PhD. Vet.,
Benito A.[3], PhD. Vet.,
Arnal J. L.[3], MSc., Vet-Biochemist
shatalov.s.a@dsau.dp.ua
[1] Department of Animal Physiology, Biochemistry, and Laboratory Diagnostics
Dniprovsk State Agrarian Economic University, Dnipro, Ukraine
[2]Altinbas University, Istanbul, Turkey
[3]EXOPOL SL Polígono Rio, San Mateo de Gállego Zaragoza, España

Relevance. Current technology of industrial poultry farming requires the study the intestinal barrier function as a crucial task for an animal health. In last decades, the requirements of intensive poultry farming have posed several challenges related to the health of broiler chickens. One such problem is the disruption of the intestinal barrier function, leading to compromised immunity and the spread of infectious diseases among birds. Additionally, this disruption has a negative impact on feed efficiency, resulting in financial losses. Investigating this issue will enable the evaluation of the effectiveness of various products and additives used to enhance the birds' immunity and productivity. Morphological parameters and the composition of the intestinal microbiota are key indicators of intestinal health. However, considering the latest scientific advancements, molecular markers of intercellular adhesion in the intestine, as well as markers of cytokine production and programmed cell death, are increasingly being used to assess the intestinal barrier function. Therefore, the utilization of molecular biological methods, such as RT-PCR, for evaluating the intestinal barrier function in broiler chickens is currently more relevant and practical.

The aim of our study was to characterize the intestinal barrier function in broiler chickens via measuring the expression levels of genes encoding molecular markers using RT-PCR.

Materials and Methods. Immunoblotting and real-time reverse transcription polymerase chain reaction (RT-PCR) are two methods that can be used to assess the intestinal barrier function in broiler chickens. Immunoblotting is a method that utilizes antibodies to detect the presence of specific proteins in a sample. This method is useful for evaluating the expression of proteins associated with the intestinal barrier function, such as microvilli and tight junctions. Immunoblotting is a valuable technique for determining the presence of specific proteins in a sample and assessing the expression of proteins associated with the intestinal barrier function. However, it has a few limitations. Firstly, immunoblotting requires the use of specific antibodies, which can limit the number of proteins that can be used if it is unknown which proteins are specifically associated with the intestinal barrier function. Additionally, the specificity of immunoblotting relies on the quality of antibodies, so the formation of specific antibody-antigen complexes can be problematic and lead to false results. Secondly, immunoblotting typically requires a large amount of protein material, which can limit its application in situations where sample volumes are limited. Thirdly, immunoblotting can be sensitive to protein degradation during sample

processing and storage, which can impact result accuracy. Therefore, while immunoblotting is a useful method, its use requires careful antibody selection, investigation of optimal sample processing and storage conditions, and consideration of limitations associated with limited available protein samples.

Real-time reverse transcription polymerase chain reaction (RT-PCR) is a molecular technique used to detect the expression levels of specific genes. It involves reverse transcription of RNA into complementary DNA (cDNA) followed by PCR amplification and quantification of the target genes. RT-PCR allows for the sensitive and specific detection and quantification of RNA molecules, including those related to the intestinal barrier function. It provides quantitative data and can be used to analyze multiple genes simultaneously. However, RT-PCR requires careful primer design and optimization, and appropriate controls should be included to ensure accurate and reliable results.

In summary, immunoblotting and real-time RT-PCR are two methods that can be applicated to assess the intestinal barrier function in broiler chickens. Immunoblotting detects specific proteins, while RT-PCR measures gene expression levels. Both methods have their advantages and limitations, and their applying should be based on the specific features of the research targets and available resources.

Reverse transcription-polymerase chain reaction (RT-PCR) is a method used to measure gene expression in cells. This method is useful for assessing the expression of genes encoding proteins associated with intestinal barrier function, such as tight junction proteins between epithelial cells and proteins that form the mucus layer. RT-PCR has been employed to evaluate the expression of genes involved in the barrier function of the intestine in broiler chickens, and it offers several advantages. Firstly, the method allows for quick and efficient determination of gene expression levels in tissue samples. Secondly, RT-PCR is highly sensitive and specific, meaning it can detect even small changes in gene expression and distinguish them from other genes expressed in the same tissue. Thus, RT-PCR can help accurately determine the levels of expression of molecular markers of intestinal barrier function in broiler chickens. Thirdly, as RT-PCR is a quantitative method, it can measure not only the presence of a gene in a sample but also its quantity. Therefore, RT-PCR is a convenient tool to determine gene expression levels and compare this index among different samples.

Taking into account the overall assessment of the data from these two aforementioned molecular diagnostic methods, it has been decided to further investigate the use of RT-PCR, which is a fast, sensitive, specific, and quantitative method. Consequently, it is an effective means of evaluating gene expression and analyzing molecular markers of intestinal barrier function in broiler chickens.

In order to select target genes for more representative molecular markers (cytokines) in assessing the state of intestinal barrier function, literature sources on this matter were analyzed. The most representative genes identified are occludins and E-cadherins. Occludins consist of four transmembrane domains and contribute to the interaction with molecules of other cells. On the other hand, E-cadherin is an intermediate filament-like protein that provides binding to the cytoskeleton. Interferons alpha and gamma are a group of cytokines that play an important role in regulating the immune response of the body to infection and inflammation. Studies have shown that gamma-interferon has a protective effect on intestinal barrier function by increasing the expression of occludin and E-cadherin in intestinal cells. Meanwhile, interferon-alpha may exert anti-inflammatory effects in the intestine by reducing the permeability of tight junctions and maintaining stable intestinal barrier function. Therefore, occludins, E-cadherin, and interferons are important molecular markers for studying the intestinal barrier function and its connection to immune responses.

Results. For further research, primers were designed for the conserved regions of molecular marker transcripts using the NCBI gene bank (National Center for Biotechnology Information, USA) and the BLAST tool. Specifically, primers were designed for occludin (reference sequence in NCBI NM_205128), E-cadherin (reference sequence in NCBI NM_001039258.3), interferon-

gamma (reference sequence in NCBI NM_205149.2), and chicken beta-actin, which will be used as a reference gene to compare the expression intensity of the selected genes.

To compare the results of immunoblotting with real-time PCR, organ samples (small and large intestine) were collected from broiler chickens of five age groups from four ROSS 308 crosses: 14, 20, 27, 34, and 43 days, with five samples from each group. Further investigations will be conducted using the primers described above.

Conclusion. The application of the RT-PCR method for evaluating the intestinal barrier function in broiler chickens is an effective approach that provides precise and clear information in respect with the intestine health characteristics of the commercial animals.

POLYMORPHISM IN EXON 4 OF THE *PRKAG3* GENE AND PRODUCTION TRAITS OF BEEF CATTLE

Śpiewak M.[1] mgr inż.,
Kowalewska I.[1] dr hab., prof. ZUT,
Czerniawska-Piątkowska E.[2] dr hab. inż., prof. ZUT,
Hanuszewicz P. mgr inż.
e-mail: magdalena.malepsza@zut.edu.pl
[1]West Pomeranian University of Technology, Faculty of Biotechnology and Animal Breeding, Department of Genetics, Piastów Avenue 45, 79-311 Szczecin, Poland
[2]West Pomeranian University of Technology, Faculty of Biotechnology and Animal Breeding, Department of Ruminant Science, Klemensa Janickiego Street 32, 71-270 Szczecin, Poland

Introduction. The bovine *PRKAG3* gene encodes the AMPK gamma3 subunit, one isoform of the regulatory gamma subunit of the AMP-activated protein kinase (AMPK). The AMPK plays a major role in the regulation of energy metabolism. The gamma3 subunit is involved in the regulation of AMPK activity in skeletal muscle and strongly influences glycogen metabolism. Glycogen content in muscle is correlated to meat quality in livestock because it influences postmortem maturation process and ultimate pH (Roux et al., 2006)

AMPK is present in ovarian and testicular cells of various species. This kinase controls steroidogenesis in the gonads and and survival of somatic gonadal cells and in the maturation of oocytes or spermatozoa. In recent years, mounting evidence shows that AMPK is involved in the regulation of reproductive function through multiple mechanisms (Bertoldo et al. 2015; Yang et al., 2020).

Aim. The aim of the study was to estimate of the frequency of alleles and genotypes in relation to the polymorphism in the *PRKAG3* gene (*rs43316209*) in a herd of Simmental cattle and determination of possible relationships between the marked genotypes of the examined polymorphic site of the *PRKAG3* gene and selected production traits of beef cattle.

Material and methods. The study was carried out in a herd of 198 *Simmental* cows grazed in the area of *Greater Poland Voivodeship* in Poland. All animals were kept in similar environmental conditions and fed with standardized feeding doses.

In order to identify and determine the attendance of genotypes and alleles of selected *PRKAG3* gene polymorphisms, the first step was to isolate the genetic material from peripheral blood collected from the external jugular vein of the studied specimens. DNA isolation was performed using a commercial reagent kit for DNA isolation MasterPure™ DNA Purification Kit for Blood Version II (Lucigen, Wisconsin, USA), according to the isolation protocol attached to the kit.