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SOURCES AND METHODS FOR OBTAINING PRIMARY CULTURES OR CELL LINES USED IN ANIMAL NUTRITION RESEARCH – SCREENING STUDIES PRIOR TO IN VIVO STUDIES

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Abstract

In recent decades, research on the bioavailability, absorption and transport of bioactive compounds, including drugs and nutrients, in cell models has developed rapidly. In vitro models, such as primary cultures and cell lines, play a key role as screening tools for evaluating feed additives, bioactive ingredients and substances affecting gastrointestinal health and function. They enable analysis of absorption mechanisms, cytotoxicity, inflammatory responses and intestinal barrier integrity without the need to involve animal models. In addition, preliminary selection of compounds under in vitro conditions makes it possible to reduce the number of animals used in in vivo studies. The aim of this paper is to present selected, most commonly used commercial cell lines applied in animal nutrition research and to describe selected methods for isolating primary enterocyte cultures.

Keywords: enterocytes, Caco-2 cells, cell lines, primary cultures, in vitro cultures

Introduction

(KusResearch on the bioavailability, absorption and transport of bioactive compounds, drugs and nutrients in cell models is developing rapidly (Rein et al., 2013). This approach can reduce the number of animals used in experiments and enable faster identification of the most promising compounds for further biological and pharmacological analyses (Arora et al., 2011). Both commercial cell lines (Kus et al., 2023) and cell lines isolated and characterized by the laboratory conducting the study (Loret et al., 2009; Romanek et al., 2018) are used in analyses of feed additives and/or drugs. Commercial cell lines are standardized biological models available for purchase and provide reproducibility and comparability of results between research centres (Capes-Davis et al., 2019). A major limitation, however, is the availability of suitable commercial cell lines, especially when less commonly studied animal species are investigated. Most

commercially available cell lines originate from humans or from a few commonly studied animal models, such as mice, rats or cattle. For less common species, including poultry, farmed fish and wild animal species, the range of commercial cell lines is much more limited or may be unavailable altogether (https://www.cytion.com/; https://www.dsmz.de/). This limits the possibility of conducting studies under conditions that reflect the actual responses of a given species. Primary cultures and/or cell lines isolated and selected in-house may better reflect species-specific biological conditions relevant to a particular study. This enables a more precise assessment of the efficacy and safety of feed additives or drugs (Kaur and Dufour, 2012; Park et al., 2024). Unfortunately, isolating primary cell lines or primary cultures is associated with a number of important technical, organizational and economic limitations and difficulties (Romanek et al., 2018). The process requires considerable time, labour and financial resources. Successful isolation depends heavily on access to appropriate laboratory facilities, including sterile rooms, specialized cell-culture equipment and high-quality biological reagents. Highly qualified and experienced research personnel are also essential, because technical errors may lead to culture contamination, loss of cell viability or changes in biological properties. Additional challenges include the limited lifespan and high susceptibility of primary cells to culture-related stress, meaning that they can be maintained in culture only for a short period without losing their phenotype and function. Following isolation, the cells also require detailed characterization to confirm their identity and stability, which entails additional and often time-consuming and costly molecular and biochemical analyses. The choice of cell line depends on the aim of the study, the availability of biological material, and the validation and standardization requirements of the assays to which the cells will be subjected (Geraghty et al., 2014).

Commercially available cell lines used in studies of feed additives and/or drugs

Caco-2 cells

The Caco-2 cell line, derived from human colorectal adenocarcinoma, is a model system used to assess intestinal absorption and the effects of bioactive substances on intestinal epithelial cells (Kamiloglu et al., 2015). After reaching confluence, Caco-2 cells spontaneously differentiate toward an enterocyte-like phenotype, forming tight monolayers with functional properties similar to those of the human intestinal epithelium (Isayama et al., 2023). Owing to these characteristics, the Caco-2 line is used for screening active substances present in both drugs and feed additives, allowing assessment of their absorption, cytotoxicity, bioavailability and effects on intestinal barrier integrity (Sasaki et al., 2022). This model is particularly valuable as an alternative to *in vivo* studies in accordance with the 3R principles (Replacement, Reduction, Refinement), enabling preliminary selection and dose optimization before proceeding to animal experiments (Garcia et al., 2018).

For feed additives, Caco-2 cells can be used to assess, among other things, their effects on intestinal barrier function, the expression of nutrient transporters, and potential immunomodulatory and cytotoxic effects (Sambuy et al., 2005). This is particularly important when searching for new, safe and effective bioactive substances, such as probiotics, prebiotics and phytobiotics, that may support the health and productivity of livestock (Kus et al., 2023). In drug studies, the Caco-2 model allows estimation of potential bioavailability and identification of transporter interactions, which is crucial for evaluating the safety and efficacy of pharmaceutical products (Hubatsch et al., 2007).

The use of Caco-2 cells in *in vitro* studies is practical and economical, while also being scientifically justified as an initial stage in assessing the effects of active substances in both veterinary and human medicine.

Other cell lines used in studies of feed additives and/or drugs

One of the most commonly used is the IPEC-J2 line, derived from porcine enterocytes obtained from the jejunum of piglets. These cells serve as a model of the porcine intestinal barrier and are used to assess the effects of feed additives on intestinal health, absorption processes, cytotoxicity and inflammatory responses. Owing to their physiological properties, IPEC-J2 cells are frequently used in veterinary and nutrition research (Wang et al., 2021). Another example is the CLAB line, derived from chicken liver cells. It is used mainly in studies of feed-additive metabolism and to assess antioxidant effects and potential toxicity (Steube et al., 2012). The MAC-T line, derived from bovine mammary epithelial cells, is used to evaluate the effects of feed additives on milk secretion and mammary-gland health (Ogunnaike et al., 2021). In contrast, the HT-29 cell line is derived from human colorectal cancer; unlike Caco-2 cells, HT-29 cells can differentiate toward goblet-cell-like phenotypes, allowing investigation of mucus secretion and interactions between dietary components and the intestinal mucus layer. This line is widely used in studies of intestinal immunity and the effects of bioactive substances, including prebiotics and probiotics (Pradhan et al., 2023). HIEC (Human Intestinal Epithelial Cells), derived from human intestinal epithelium, are also used in studies of intestinal development and digestibility. They provide a model that more closely reflects physiological intestinal function than tumour-derived cell lines and allow more accurate reproduction of digestion and nutrient-absorption processes (Lopez-Escalera and Wellejus, 2022).

A variety of cell lines are used in cytotoxicity analyses of a given additive and/or drug, each suitable for a particular range of applications. One of the oldest and best-characterized is the HeLa line, derived from human cervical cancer. These cells are widely used in anticancer-drug screening and general toxicity studies (Park, 2017). The HepG2 line, derived from human hepatocellular carcinoma, is valued for its ability to synthesize detoxification enzymes such as cytochrome P450 and is therefore used in studies of metabolic toxicity and hepatotoxicity (Kitano et al., 2022). L929 mouse fibroblasts are frequently used in cytotoxicity tests conducted according to ISO standards and play an important role in evaluating the biocompatibility of medical materials (Kim et al., 2022). The Vero line, derived from African green monkey kidney, is widely used in studies of viruses, vaccines and the toxicity of biological products (Todd and Tripp, 2020). MDCK (Madin-Darby Canine Kidney) cells, isolated from canine kidney, are used as a model for membrane transport and renal toxicity studies (Ye et al., 2021).

Each of the above cell lines has specific biological and metabolic properties that determine its suitability for a particular type of study. The simultaneous use of multiple cell lines enables a comprehensive assessment of toxicity and identification of specific mechanisms of action of the tested substances.

Primary cultures used in studies of feed additives and/or drugs

As mentioned above, the availability of commercial cell lines from some species is limited. Laboratories therefore isolate primary cultures directly from tissues for the needs of a particular study and its analyses. Enterocyte cultures are used in animal nutrition research. Enterocytes are specialized epithelial cells of the small intestine that play a key role in the absorption of nutrients from the gastrointestinal tract (Tümer et al., 2013). Their surface is covered with microvilli, which substantially increase the absorptive surface area and enable efficient uptake of vitamins, minerals and other nutrients (Kiela and Ghishan, 2016). Enterocytes are also involved in the immune barrier, protecting the body against pathogens present in the intestinal contents. Their proper function is essential for maintaining metabolic homeostasis and normal gastrointestinal function (Ambrogi and Vezina, 2024).

Cell isolation methods

Enterocytes, i.e. the cells lining the intestine, can be isolated in several different ways depending on the aim of the study and the methods available (Table 1).

Table 1. Examples of methods for isolating enterocytes from small intestine tissue

Method of cell isolation	Description / application
Isolation using dissociation solution (Vermeire et al., 2021)	Separation of cells by loosening intercellular junctions using dissociation solutions
Isolation using enzymatic digestion (Bader et al., 2000)	Degradation of the extracellular matrix using enzymes (e.g. trypsin, collagenase, hyaluronidase)
Method using differential centrifugation (Rath et al., 2018)	Separation of cells based on differences in mass and density through centrifugation-based fractionation
Isolation method using commercial reagents (e.g. Gentle Cell Dissociation Reagent, StemCell Technologies) (Stem Cell, 2023)	Gentle separation of cells using a commercial dissociation reagent
Isolation using explant cultures (Chougule et al., 2012)	Migration of cells from tissue fragments placed in culture
Isolation using a homogenizer (Dópido et al., 2004)	Mechanical disruption of tissues or cell aggregates
Isolation using scrapings (Tsvilikhovs'kyĭ, 1997)	

Cell isolation begins by excising 10-cm fragments of jejunum. The fragments are immediately immersed in cold sterile PBS to prevent tissue degradation. They are then thoroughly rinsed in PBS supplemented with 100 U/ml penicillin and 100 µg/ml streptomycin to remove intestinal contents and bacterial contamination (Rath et al., 2018; Vermeire et al., 2021).

Isolation using dissociation solution

The next step is to cut intestinal fragments approximately 5 cm long. The tissue is incubated in 35 ml of cold dissociation buffer (I) containing:

- 30 mM ethylenediaminetetraacetic acid (EDTA),
- 1.5 mM dithiothreitol (DTT),
- 6 µM Rho kinase inhibitor (Y-27632),

The samples are incubated on ice for 30 minutes and shaken vigorously for 10–15 seconds every 5 minutes to facilitate crypt detachment.

The samples are then transferred to warm (37°C) dissociation buffer (II) containing:

- 30 mM EDTA,
- 6 µM Y-27632

The mixture is incubated for 10 minutes on an orbital shaker to further separate the crypts. The tissue is then transferred to cold sterile PBS and incubated for 5 minutes, increasing the shaking frequency to every 1–2 minutes. The contents of the tube are subsequently passed through a 70-µm cell strainer into a new 15-ml conical tube. The isolated crypts are centrifuged at 200 × g for 5 minutes and then resuspended in 1 ml of cold PBS. Crypts isolated in this way can be seeded in culture medium (Vermeire et al., 2021).

Isolation using enzymatic digestion

Before digestion, the intestine is cut into small 1–2-mm pieces and thoroughly washed in ice-cold PBS without magnesium or calcium ions. The next step is tissue digestion for 30 minutes at 37°C in a mixture containing high-glucose DMEM culture medium, 1% fetal bovine serum (FBS), 200 U/ml collagenase, 100 U/ml hyaluronidase and 1× antibiotic-antimycotic solution (AAS). After digestion, 5 ml FBS is added to stop the reaction, and the sample is centrifuged at 100 × g for 3 minutes to separate cells from tissue debris. The resulting pellet is resuspended in PBS containing AAS and then filtered through a 100-µm cell strainer. The recovered cell aggregates can be suspended in culture medium, yielding purified enterocyte fractions ready for further studies (Bader et al., 2000).

Method using differential centrifugation

The collected intestine is cut into 1–2-mm pieces, which are then washed with ice-cold PBS. The tissue is centrifuged at 300 × g for 10 minutes. The resulting pellet is suspended in 0.1% hyaluronidase dissolved in DMEM and digested for 30–40 minutes, followed by centrifugation to remove the supernatant. The tissue is then digested in 0.025% trypsin-EDTA for 30 minutes. The treated cells are centrifuged on Histopaque medium (1.119) for 30 minutes at 400 × g. The cell layer at the gradient interface is collected and washed three times in DMEM by centrifugation. Finally, the cell pellet and cell clusters are seeded into PureCol™-coated T25 culture flasks, enabling purified enterocyte fractions to be obtained for further studies (Rath et al., 2018).

Isolation method using a commercial reagent: Gentle Cell Dissociation Reagent (STEMCELL Technologies)

Using sterile scissors, the tissue should be minced as finely as possible. Ten millilitres of Gentle Cell Dissociation Reagent (STEMCELL Technologies) is added to the tube containing the tissue fragments, and the sample is incubated on ice for 30 minutes. During incubation, the tube contents should be mixed every 5 minutes. After incubation, the sample is centrifuged at 290 × g for 5 minutes and the supernatant is removed. One millilitre of ice-cold DMEM supplemented with 1% BSA is added to the pellet, and the tube contents are vigorously pipetted approximately 20 times using a 1-ml pipette to detach intestinal crypts from the tissue. The suspension is filtered through a 70-µm cell strainer. The resulting suspension contains isolated intestinal crypts that can be used to establish intestinal organoid cultures with IntestiCult™ Organoid Growth Medium (STEMCELL Technologies). The method is based on protocols provided on the manufacturer's website (Stem Cell, 2023).

Isolation using explant cultures

Culture of pre-digested intestinal fragments under a coverslip involves preparing tissue fragments that have first undergone mild enzymatic digestion to partially loosen intercellular junctions. The prepared intestinal pieces are placed in a suitable culture medium and then covered with a sterile coverslip, which creates a uniform thin layer of material and improves cell adhesion to the substrate (Chougule et al., 2012). As the cells proliferate, they migrate beyond the coverslip and form colonies.

Isolation using a homogenizer

Mechanical isolation of enterocytes from the small-intestinal epithelium involves disrupting the tissue structure under controlled conditions. First, an intestinal fragment is thoroughly cleaned, intestinal contents are removed and the intestine is rinsed with an antibiotic-containing solution. The tissue can then be mechanically disrupted, for example by homogenization in an isotonic buffer, which breaks up the tissue and releases enterocytes from the epithelium. This process separates enterocytes from other mucosal structures and prepares them for further biochemical or molecular analyses (Dópido et al., 2004). Depending on the requirements, the procedure may be supplemented with differential centrifugation to facilitate separation of enterocytes from tissue fragments and other cells.

Isolation using scrapings

Enterocytes can also be mechanically isolated by gently scraping the intestinal epithelium with a scalpel. For this purpose, a prepared small-intestinal fragment is cut longitudinally and opened with the mucosal surface facing upward. The epithelial cells are then gently scraped off with a sharp scalpel while avoiding damage to deeper tissue layers. The collected material is suspended in an appropriate medium, allowing enterocytes to be separated and maintained in cell

suspension (Tsvilikhovs'kyĭ, 1997). The suspension can subsequently undergo further processing, such as filtration or centrifugation, depending on its intended use.

The techniques described above make it possible to separate cells from tissue, purify them and prepare them for culture or further analyses. Each method aims to obtain the purest and most functional enterocyte population possible for scientific research.

Studies of nutritional factors conducted at the National Research Institute of Animal Production using cell cultures

The National Research Institute of Animal Production conducts research on the potential effects of selected plant-derived additives on the health and gastrointestinal function of livestock. The substances analysed include garlic (*Allium sativum*), hops (*Humulus lupulus*), purple coneflower (*Echinacea purpurea*), oak bark (*Quercus robur*), turmeric (*Curcuma longa*), oregano (*Origanum vulgare*) and ginger (*Zingiber officinale*). These natural additives, known for their antibacterial, antioxidant and immunomodulatory properties, are evaluated for their effects on small-intestinal barrier integrity, enterocyte viability, and the response of porcine and chicken intestinal epithelial cells to induced oxidative stress. These studies are carried out both in primary models using cells obtained directly from animals and in established cell lines, including the human Caco-2 line and the porcine IPEC-J2 line. Cell cultures make it possible to conduct preliminary high-throughput assessment of selected plant extracts or their mixtures in terms of intestinal barrier function, cytotoxicity and responsiveness to reactive oxygen species.

The aim of the Institute's research is to identify the most promising individual additives or mixtures that exhibit a beneficial activity profile without adversely affecting intestinal epithelial cells. Selected substances can then be used in subsequent *in vivo* studies in experimental animals. This two-stage strategy, combining *in vitro* and *in vivo* studies, not only reduces the number of animals used in experiments in accordance with the 3R principles, but also increases the efficiency of developing new feed additives that support gastrointestinal health and the overall condition of livestock.

Conclusions

Cell cultures, including both independently isolated primary cultures and commercial cell lines, are an important component of modern animal nutrition research. They enable screening studies that provide an early assessment of the biological properties and safety of feed additives before their use in *in vivo* models. Commercial cell lines offer reproducibility and availability, making them a convenient tool for *in vitro* research. Primary cultures, although more technically demanding, provide data that more closely reflect physiological conditions in the organism. The overall assessment of tested nutritional factors can be supplemented by other *in vitro* assays, for example evaluation of antimicrobial activity against pathogenic microorganisms, effects on probiotic bacteria, or antioxidant activity, thereby allowing a more comprehensive assessment of the potential effects of the tested factors under real-world conditions.

References

Ambrogi M., Vezina C.M. (2024). Roles of airway and intestinal epithelia in responding to pathogens and maintaining tissue homeostasis. Front. Cell. Infect. Microbiol., 14: 1346087.

Arora T., Mehta A.K., Joshi V., Mehta K.D., Rathor N., Mediratta P.K., Sharma K.K. (2011). Substitute of animals in drug research: An approach towards fulfillment of 4R's. Indian J. Pharm. Sci., 73(1): 1–6.

Bader A., Hansen T., Kirchner G., Allmeling C., Haverich A., Borlak J.T. (2000). Primary porcine enterocyte and hepatocyte cultures to study drug oxidation reactions. Br. J. Pharmacol., 129(2): 331–342.

Capes-Davis A., Bairoch A., Barrett T., Burnett E.C., Dirks W.G., Hall E.M., Healy L., Kniss D.A., Korch C., Liu Y., Neve R.M., Nims R.W., Parodi B., Schweppe R.E., Storts D.R., Tian F. (2019). Cell lines as biological models: Practical steps for more reliable research. Chem. Res. Toxicol., 32(9): 1733–1736.

Chougule P., Herlenius G., Hernandez N.M., Patil P.B., Xu B., Sumitran-Holgersson S. (2012). Isolation and characterization of human primary enterocytes from small intestine using a novel method. Scand. J. Gastroenterol., 47(11): 1334–1343.

Dópido R., Rodríguez C., Gómez T., Acosta N.G., Díaz M. (2004). Isolation and characterization of enterocytes along the intestinal tract of the gilthead seabream (*Sparus aurata* L.). Comp. Biochem. Physiol. A Mol. Integr. Physiol., 139(1): 21–31.

Garcia M.T.J., de Vasconcellos F.L.L., Raffier C.P., Roberts M.S., Grice J.E., Benson H.A.E., Leite-Silva V.R. (2018). Alternative methods to animal studies for the evaluation of topical/transdermal drug delivery systems. Curr. Top. Med. Chem., 18(4): 287–299.

Geraghty R.J., Capes-Davis A., Davis J.M., Downward J., Freshney R.I., Knezevic I., Lovell-Badge R., Masters J.R., Meredith J., Stacey G.N., Thraves P., Vias M., Cancer Research UK. (2014). Guidelines for the use of cell lines in biomedical research. Br. J. Cancer, 111(6): 1021–1046.

Hubatsch I., Ragnarsson E.G., Artursson P. (2007). Determination of drug permeability and prediction of drug absorption in Caco-2 monolayers. Nat. Protoc., 2(9): 2111–2119.

Isayama K., Rini D.M., Yamamoto Y., Suzuki T. (2023). Propionate regulates tight junction barrier by increasing endothelial-cell selective adhesion molecule in human intestinal Caco-2 cells. Exp. Cell Res., 425(2): 113528.

Kamiloglu S., Capanoglu E., Grootaert C., Van Camp J. (2015). Anthocyanin absorption and metabolism by human intestinal Caco-2 cells – a review. Int. J. Mol. Sci., 16(9): 21555–21574.

Kaur G., Dufour J.M. (2012). Cell lines: Valuable tools or useless artifacts. Spermatogenesis, 2(1): 1–5.

Kiela P.R., Ghishan F.K. (2016). Physiology of intestinal absorption and secretion. Best Pract. Res. Clin. Gastroenterol., 30(2): 145–159.

Kim J.S., Lim H., Seo J.Y., Kang K.R., Yu S.K., Kim C.S., Kim D.K., Kim H.J., Seo Y.S., Lee G.J., You J.S., Oh J.S. (2022). GPR183 regulates 7α,25-dihydroxycholesterol-induced oxiapoptophagy in L929 mouse fibroblast cell. Molecules, 27(15): 4798.

Kitano H., Kawabe Y., Kamihira M. (2022). HepG2-based designer cells with heat-inducible enhanced liver functions. Cells, 11(7): 1194.

Kus M., Ibragimow I., Piotrowska-Kempisty H. (2023). Caco-2 cell line standardization with pharmaceutical requirements and *in vitro* model suitability for permeability assays. Pharmaceutics, 15(11): 2523.

Lopez-Escalera S., Wellejus A. (2022). Evaluation of Caco-2 and human intestinal epithelial cells as *in vitro* models of colonic and small intestinal integrity. Biochem. Biophys. Rep., 31: 101314.

Loret S., Rusu D., El Moualij B., Taminiau B., Heinen E., Dandrifosse G., Mainil J. (2009). Preliminary characterization of jejunocyte and colonocyte cell lines isolated by enzymatic digestion from adult and young cattle. Res. Vet. Sci., 87(1): 123–132.

Ogunnaike M., Wang H., Zempleni J. (2021). Bovine mammary alveolar MAC-T cells afford a tool for studies of bovine milk exosomes in drug delivery. Int. J. Pharm., 610: 121263.

Park G., Rim Y.A., Sohn Y., Nam Y., Ju J.H. (2024). Replacing animal testing with stem cell-organoids: Advantages and limitations. Stem Cell Rev. Rep., 20(6): 1375–1386.

Park W.H. (2017). Gallic acid induces HeLa cell death via increasing GSH depletion rather than ROS levels. Oncol. Rep., 37(2): 1277–1283.

Pradhan D., Gulati G., Avadhani R., H.M.R., Soumya K., Kumari A., Gupta A., Dwivedi D., Kaushik J.K., Grover S. (2023). Postbiotic lipoteichoic acid of probiotic *Lactobacillus* origin ameliorates inflammation in HT-29 cells and colitis mice. Int. J. Biol. Macromol., 236: 123962.

Rath N.C., Liyanage R., Gupta A., Packialakshmi B., Lay J.O. Jr. (2018). A method to culture chicken enterocytes and their characterization. Poultry Sci., 97(11): 4040–4047.

Rein M.J., Renouf M., Cruz-Hernandez C., Actis-Goretta L., Thakkar S.K., Da Silva Pinto M. (2013). Bioavailability of bioactive food compounds: a challenging journey to bioefficacy. Br. J. Clin. Pharmacol., 75: 588–602.

Romanek J., Opiela J., Lipiński D., Smorąg Z. (2018). Effect of high hydrostatic pressure applied before cryopreservation on the survival rate and quality of porcine mesenchymal stem cells after thawing. Anim. Biotechnol., 29(4): 283–292.

Sambuy Y., De Angelis I., Ranaldi G., Scarino M.L., Stammati A., Zucco F. (2005). The Caco-2 cell line as a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics. Cell Biol. Toxicol., 21(1): 1–26.

Sasaki Y., Tatsuoka H., Tsuda M., Sumi T., Eguchi Y., So K., Higuchi Y., Takayama K., Torisawa Y., Yamashita F. (2022). Intestinal permeability of drugs in Caco-2 cells cultured in microfluidic devices. Biol. Pharm. Bull., 45(9): 1246–1253.

Stem Cell (2023). https://www.stemcell.com/products/intesticult-organoid-differentiation-medium-human.html#section-protocols-and-documentation

Steube K.G., Koelz A.L., Uphoff C.C., Drexler H.G., Kluess J., Steinberg P. (2012). The necessity of identity assessment of animal intestinal cell lines: A case report. Cytotechnology, 64(4): 373–378.

Todd K.V., Tripp R.A. (2020). Vero cells as a mammalian cell substrate for human norovirus. Viruses, 12(4): 439.

Tsvilikhovs'kyĭ M.I. (1997). Otrymannia fraktsiĭ plazmatychnoï membrany z enterotsytiv tonkoï kyshky tvaryn riznoho viku [The isolation of plasma membrane fractions from the enterocytes of the small intestine in animals of different ages]. Fiziol. Zh., 43(3–4): 101–105. PMID: 9303792

Tümer E., Bröer A., Balkrishna S., Jülich T., Bröer S. (2013). Enterocyte-specific regulation of the apical nutrient transporter SLC6A19 (B0AT1) by transcriptional and epigenetic networks. J. Biol. Chem., 288(47): 33813–33823.

Vermeire B., Gonzalez L.M., Jansens R.J.J., Cox E., Devriendt B. (2021). Porcine small intestinal organoids as a model to explore ETEC–host interactions in the gut. Vet. Res., 52: 94.

Wang D., Zhou L., Zhou H., Hou G. (2021). Chemical composition and anti-inflammatory activity of n-butanol extract of *Piper sarmentosum* Roxb. in the intestinal porcine epithelial cells (IPEC-J2). J. Ethnopharmacol., 269: 113723.

Ye X., Wang J., Qiao Z., Yang D., Wang J., Abudureyimu A., Yang K., Feng Y., Ma Z., Liu Z. (2021). Quantitative proteomic analysis of MDCK cell adhesion. Mol. Omics, 17(1): 121–129.

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SOURCES AND TECHNIQUES FOR OBTAINING PRIMARY CULTURES AND CELL LINES IN ANIMAL NUTRITION RESEARCH – SCREENING TESTS BEFORE *IN VIVO* STUDIES

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SUMMARY

In recent decades, there has been rapid progress in research on the bioavailability, absorption, and transport of bioactive compounds, including drugs, and nutrients in cell models. *In vitro* models, including primary cultures and cell lines, serve as important screening tools for evaluating the effects of feed additives, bioactive ingredients, and substances that affect gastrointestinal health and function. They allow analysis of absorption mechanisms, cytotoxicity, inflammatory responses, and intestinal barrier integrity without relying on animal models. Additionally, selecting compounds *in vitro* helps reduce the number of animals needed for *in vivo* studies. This paper aims to present the most commonly used commercial cell lines in animal nutrition research and to describe selected methods for isolating primary enterocyte cultures.

Keywords: enterocytes, Caco-2 cells, primary cultures, cell lines, *in vitro* culture