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Phytogenic Compounds for Enhancing Intestinal Barrier Function in Poultry–A Review

Authors
Urszula Latek, Magdalena Chłopecka, Wojciech Karlik, Marta Mendel

Affiliation
Division of Pharmacology and Toxicology, Department of Preclinical Sciences, Institute of Veterinary Medicine, Warsaw University of Life Sciences – SGGW, Warsaw, Poland

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Correspondence
Marta Mendel, DVM, PhD
Division of Pharmacology and Toxicology, Department of Preclinical Sciences, Institute of Veterinary Medicine, Warsaw University of Life Sciences (SGGW)
Ciszewskiego Street 8, 02-786 Warsaw, Poland
Phone: + 48 2 25 93 60 37
marta_mendel@sggw.edu.pl

ABSTRACT

After the European Union ban of antibiotic growth promoters, works on different methods of improving gut health have intensified. The poultry industry is struggling with problems that were previously controlled by antibiotic growth promoters, therefore the search for optimal solutions continues. Simultaneously, there is also increasing social pressure to minimize the use of antibiotics and replace them with alternative feed additives. A variety of available alternatives is considered safe by consumers, among which phytogenics play a significant role. However, there are still some limitations that need to be considered. The most questionable are the issues related to bioavailability, metabolism of plant derivatives in birds, and the difficulty of standardizing commercial products. There is still a need for more evidence-based recommendations for the use of phytogenics in livestock. On the other hand, a positive influence of phytogenic compounds on the health of poultry has been previously described by many researchers and practical application of these compounds has auspicious perspectives in poultry production. Supplementation with phytogenic feed additives has been shown to protect birds from various environmental threats leading to impaired intestinal barrier function. Phytogenic feed additives have the potential to improve the overall structure of intestinal mucosa as well as gut barrier function on a molecular level. Recognition of the phytogenics' effect on the components of the intestinal barrier may enable the selection of the most suitable ones to alleviate negative effects of different agents. This review aims to summarize current knowledge of the influence of various phytogenic constituents on the intestinal barrier and health of poultry.

Introduction

The poultry industry accounts for a huge part of the world's livestock production. According to the Food and Agriculture Organization, global poultry meat production increased from 9 to 122 million tons between 1961 and 2017, reaching about 37% of world meat production in 2017, and continues to grow. Optimizing the production process to be both consumer and environmentally friendly, as well as efficient, is now an enormous challenge for researchers, veterinarians, nutritionists, and breeders.

In recent years, the concept of "gut health" has received a lot of attention as it has been recognized as one of the key elements in determining animal performance. After the European Union ban of AGPs [1], works on different methods of improving gut health intensified. The debate on the use of anti-coccidial drugs has also begun due to the fear of increasing resistance among parasites and environmental residues [2]. In 2016, the Federation of Veterinarians of Europe published a position paper on coccidiostats or anticoccidials, recommending strict veterinary supervision of their use in the European Union [3]. Also noteworthy is that social pressure to use alternative feed additives is increasing, and the rise in awareness among customers results in a higher demand for antibiotic-free or "organic" poultry products as well [4–6].

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ABBREVIATIONS

AGPs	antibiotic growth promoters
AH	*Allium hookeri*
C10	sodium caprate
CD	crypt depth
CLDN	claudin
cOCM	coated cinnamon oil
CUR	curcumin
DON	deoxynivalenol
EO	essential oil
FD-4	fluorescein isothiocyanate-dextran 4000
HS	heat stress
JAMs	junctional adhesion molecules
LPS	lipopolysaccharide
MUC-2	mucin 2
NE	necrotic enteritis
OCLN	occludin
OTA	ochratoxin A
PFAs	phytogenic feed additives
PO	per o. s.
RT	total resistance
TEER	transepithelial electrical resistance
TJs	tight junctions
V : C	villus height to crypt depth
VH	villus height
VS	villus surface area
VW	villus width
ZO	zonula occludens protein

According to EU legislation, feed additives mean substances, micro-organisms, or preparations that are intentionally added to feed or water for the purpose of satisfying the nutritional needs of animals, or to favorably affect animal production, performance, or welfare (particularly by affecting the gastrointestinal biota or digestibility of feeding stuffs), or to favorably affect the characteristics of feed or animal products or the environmental consequences of animal production. Feed additives also have a coccidiostatic or histomonostatic effect [1].

There is a variety of available alternative feed additives (pro-, pre- and synbiotics, phytogenics, and organic acids [7–10]) that are considered safe and are welcomed by consumers. Most of the studies on their efficacy have concentrated mainly on their influence on growth performance [11–45], antimicrobial and antiparasitic activity [46–55], or digestibility [13, 17, 19, 21, 28, 33, 34, 40, 41]. Despite the fact that much research has already been carried out in this field, the search for the most effective feed additives continues.

Although there are many factors that influence gut health, an integral and intact gut barrier is a vital component for its maintenance. The knowledge of mechanisms behind proper functioning of the intestinal barrier is rapidly changing. Enhanced understanding of the issue has determined that a lot of matters have been previously oversimplified [8, 56, 57]. Although numerous studies have investigated the effect of feed additives on the morphology of the digestive tract [12, 15, 17, 18, 23, 24, 27, 29, 31–33, 39, 41, 58, 59], their direct influence on individual elements of the intestinal barrier is still poorly described. An interesting review of the influence of plant bioactive compounds on the intestinal barrier of poultry, also in terms of immunology, was published by Patra [60]. Phytogenics are a promising group of feed additives that has the potential to directly improve gut barrier function in addition to exhibiting other positive effects on gut health [61]. Gut health is a complex issue, the improvement of which requires multitarget action. Plant extracts, thanks to their rich composition and variety of active components, have a chance to act multidirectionally and represent a promising alternative to AGPs [62]. The use of molecular technologies might be helpful for better understanding the mode of action of feed additives and what would justify their implementation into animal feeding. Although feed additives of natural origin are gaining popularity among veterinarians and poultry producers, there is still a need for more evidence-based scientific data to justify their use, prove their effectiveness, and gain general acceptance. This review aims to summarize current knowledge of the influence of various phytogenic components, plant extracts, their mixtures, and isolated ingredients on the intestinal barrier in poultry.

The search strategy for this topic included a screening of the electronic publication libraries PubMed and Google Scholar. The search was narrowed down to years 2000–2020. It was based on key words and combinations of them, such as “intestinal barrier”, “gut health”, “tight junctions”, “permeability”, “leaky gut”, “intestinal”, “gastrointestinal”, “poultry”, “chicken”, “broiler”, “phytogenics”, “essential oil”, “polyphenol”, “plant extracts”, “alternative feed additives”, “phytochemical feed additives”, and “flavonoid”. The available literature has also been studied for specific phytogenic components, such as “carvacrol”, “cinnamaldehyde”, “curcumin”, and “resveratrol”, and references of the selected papers were checked. In addition, several studies with other animal species or cell culture models were cited to present the perspectives for future research in poultry.

Gut Health and the Intestinal Barrier

The digestive system is a complicated, complex machinery. Its proper functioning is necessary for the effective absorption of nutrients and the right rate of animals’ development and growth, and therefore, the economic profit. The integrity of the intestinal barrier provides protection against pathogens and xenobiotics entering the body by the alimentary route and the gut immune system is crucial for overall immunity of an animal.

The intestinal barrier is a complicated structure formed by different components: a layer of mucus, gut microbiota, elements of immunological system, and, most importantly, adjacent intestinal epithelial cells [63]. All of these parts remain in dynamic interaction with each other and with the environment. The integrity and permeability of the intestinal barrier is largely maintained by the unimpaired epithelial cells monolayer and the functional junctions between them. Enterocytes are connected by different kinds of junctions, including desmosomes, adherent junctions, gap junctions, and TJs [61, 63–68]. TJs play a crucial role in the regulation of paracellular permeability and maintenance of barrier function [61, 63, 66, 67, 69–71].

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TJs are located on the basolateral side of the apical end of epithelial cells. They are multiprotein complexes formed by the transmembrane proteins, creating the extracellular and intracellular domains and plaque proteins connecting the transmembrane proteins to the perijunctional actomyosin ring [64, 66, 67, 69]. Over 50 TJ proteins have been identified so far [67, 69]. Transmembrane proteins include CLDNs, OCLN, JAMs, the Coxsackie and adenovirus-associated receptor, and tricellulin [66, 69]. They are linked with the cytoplasmic plaque, formed mainly by the ZO [64, 66, 67, 69].

TJ proteins are dynamic structures that can be modified depending on environmental conditions. For example, ZO proteins can shift cyclically between membrane and cytosolic pools, and can also be redistributed into the intracellular compartment as a response to various stressors [72–75]. OCLN can undergo internalization in cytoplasmic vesicles, which results in changes in permeability of the epithelium [63, 66, 69, 74, 75]. On the contrary, the localization of CLDNs in TJs is relatively stable [74, 75], but their distribution and properties can significantly differ, which is reflected in the variable tightness of the epithelia [66, 69, 76]. It is well known that TJs presence and proper functioning is necessary for maintaining mucosal homeostasis. Apart from connecting the epithelial cells and regulating paracellular permeability, TJ proteins also play an important role in the signaling pathways [69, 75, 76].

Impairment of intestinal barrier function

A number of agents that can jeopardize intestinal health and, as a result, animal health has been described [69, 77–88]. Changes in expression, phosphorylation, and distribution of different TJ proteins have been associated with many gastrointestinal and systemic diseases in humans and animals [66, 69, 89–93]. Significant changes in the structure of the intestinal barrier on the molecular level have been observed in cases of exposure to different agents, such as mycotoxins [79, 81, 85, 94–98], pathogens [69, 84, 86, 99, 100], and HS [78, 101–106].

Pathogens

The role of TJs in the regulation of intestinal barrier function and its disruption by pathogens in chickens was widely described by Awad et al. [69], so it is not discussed further here. In brief, some of enteric pathogens, such as enteropathogenic *Escherichia coli* or *Salmonella*, can disrupt mucosal barrier function in chickens by modifying TJs. Disruption of specific TJ elements can result from degradation by pathogen-derived proteases, changes in the phosphorylation state of the proteins, and altered protein synthesis [107].

Withdrawal of antibiotic growth promoters

The AGPs withdrawal also, undoubtedly, brought some hardships for the poultry industry that are associated with gut health impairment, such as an increase in the feed conversion ratio, the reemergence of previously controlled diseases like NE, wet litter, or leaky gut syndrome, and the occurrence of illnesses caused by commensal microbiota capable of crossing the intestinal barrier due to its reduced integrity [69, 108–111].

In an experimental drug-free program described by Gaucher et al., a number of negative effects had been noted [112]. The observations demonstrated serious challenges that must be addressed while considering drug-free poultry production on a mass scale. This program was associated with a higher prevalence of NE (clinical and subclinical) and increased litter moisture content. Animals reared without any medications also had a significantly lower live weight at slaughter and daily body weight gain, and there was an increase in the feed conversion ratio. Moreover, the poultry industry has to deal with the emerging problems of climate change, such as HS and increasing feed contamination by mycotoxins.

Heat stress

It has been reported that broiler chickens exposed to HS resulted in higher permeability of the intestinal barrier, manifested by increased serum endotoxin, inflammatory cytokines concentration, and translocation of intestinal pathogens (*Salmonella* spp.) [77]. Similarly, in another study, it was noted that broiler heat exposure lead to negative changes in jejunal morphology and increased paracellular permeability and the downregulation in the expression of TJ proteins OCLN and ZO-1 [102].

Mycotoxins

Although chickens are known to be relatively insensitive to toxic effects of DON, it has been proven that even subclinical exposure to this mycotoxin can lead to significant changes on a molecular level [96] and be an NE predisposing factor [113]. In cases of both *in vivo* and *in vitro* exposure to DON in broiler chickens, a decrease in TEER has been noted, which is indicative of increased gut barrier permeability [113, 114]. DON exposure has also been associated with poorer nutrient absorption in chicken intestines [114–116]. *In vivo* exposure to OTA in Pekin ducks resulted in growth impairment, reduced villous length, and downregulation of TJ proteins ZO-1 and OCLD expression [97]. Similarly, exposure to aflatoxin B1 in chickens led to changes on a molecular level, increased gut permeability, reduced amino acid digestibility, and impaired growth performance [85]. All the mentioned factors overlap and are known to play a role in the vicious cycle of disease, which negatively impacts gut health and overall bird performance (▶ **Fig. 1**).

Phytogenic additives for intestinal barrier enhancement

PFAs are plant derivatives that can be incorporated into livestock diets to improve their productivity and performance [117] (▶ **Fig. 2**). This group of compounds includes herbs, spices, EOs, and oleoresins [117]. Positive influences of phytogenic compounds on the health of poultry have been previously observed by many researchers [11, 16, 17, 20–22, 24, 26, 30–40, 42, 49, 52, 53, 55, 59, 60, 86, 97, 118–123] and practical application of these compounds is known to have auspicious perspectives in animal production [11, 17, 62, 124–126]. The growing interest in the use of PFAs is reflected in the latest survey on PFAs conducted in 2020 by Biomin [127]. The survey was completed by almost 700 respondents from 79 countries and revealed that over half of them currently use PFAs as part of their feeding program. What is even more promising is the fact that almost 70% of respondents declared that their PFA use will increase in the next 12 months.

Currently, there are several products on the market that contain either one phytogenic compound or, more often, a mixture

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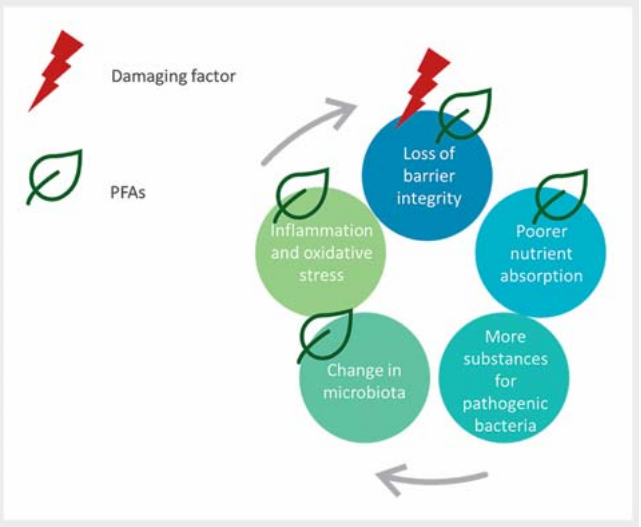


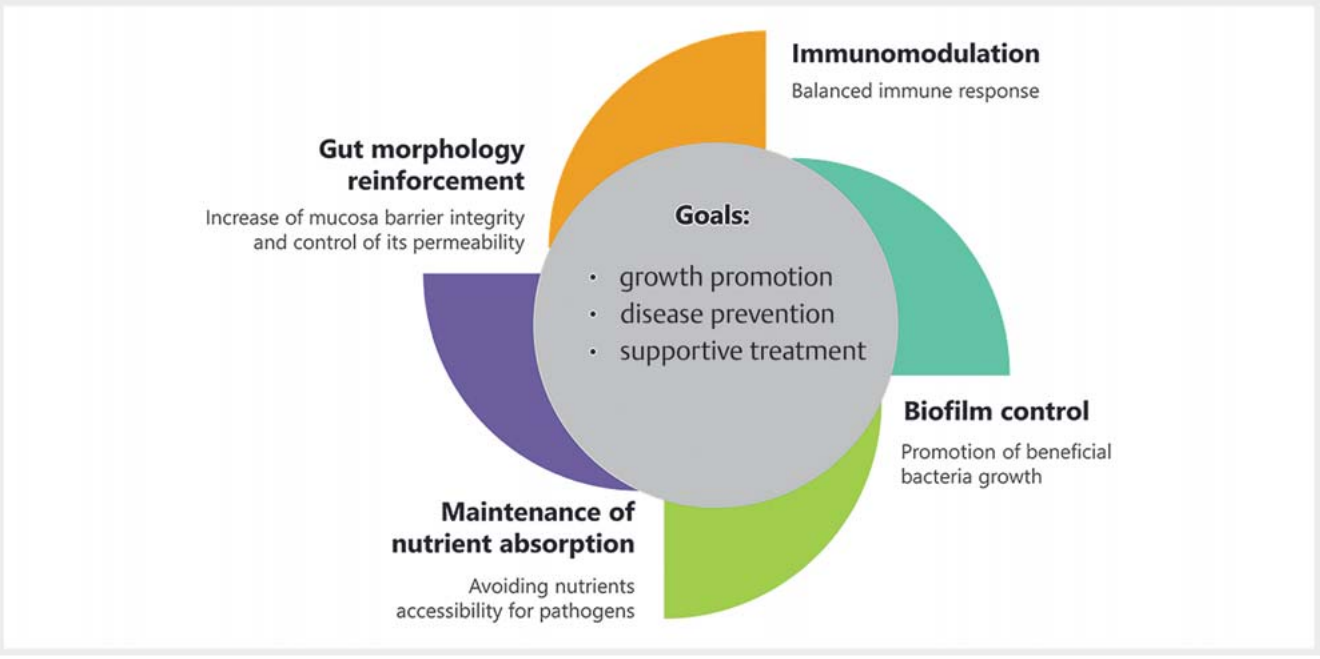
▶ **Fig. 1** PFAs potential to break the vicious cycle of disease.

of phytogenics, sometimes combined with other additives, such as pre-and probiotics or organic acids.

The unique, complex composition of many phytogenics is responsible for several positive properties related to plant-driven products, such as antioxidant, anti-inflammatory, immunomodulative, antimicrobial, and antiparasitic effects, that contribute to better animal health. Benefits of PFAs in poultry also include improved palpability, a stimulating effect on digestive activity, overall improvement of gastrointestinal morphology, and higher meat quality. These aspects of phytogenic implementation in animal nutrition have been reviewed extensively [6, 7, 9, 10, 117, 128–142] and will not be discussed in detail here. The main purpose of this paper was to collect and analyze the effects of phytogenic additives on the epithelial part of the intestinal barrier in poultry.

The vital part of the intestinal barrier is the physical barrier formed mainly by tightly connected enterocytes, goblet cells, and undifferentiated cells [123]. An important indicator of intestinal barrier quality and integrity might be the morphology of the mucosa [33, 143]. The phytogenic supplementation-induced changes in the structure of intestinal mucosa are presented in ▶ **Table 1**. Features most commonly used for evaluation of the physical barrier condition are VH, CD, V:C, and number of goblet cells. The V:C ratio is directly correlated with the balance between VH and CD [143]. The anti-inflammatory effect of PFAs is recognized as one of the mechanisms involved in their positive effects on gut morphology, and regulation of cell growth and apoptosis may also play a role, but the exact mechanisms behind this are not yet known [61]. Another possible protective feature of phytogenics is their antioxidant properties [117].

Intestinal villi are the most important part responsible for nutrients absorption, thus, changes in their length may directly affect a bird's performance. The greater the height of the villi, the greater its surface area, which equates to a larger area for effective absorption [33, 143]. It was observed that supplementation of various PFAs has the ability to increase the villous height in poultry [12, 20, 24, 31, 33, 42, 49, 58, 59, 144]. However, in some cases, although several positive effects of phytogenic supplementation were noted, it did not result in a significant change in growth performance. Moreover, apart from a positive influence on intestinal morphology in some aspects, PFA supplementation was sometimes associated with a decrease in villous height in the supplemented groups [49, 144, 145]. Dietary supplementation of chicken broilers with cOCM was associated with improved intestinal integrity manifested by villus development and modulation of the gene expression of TJ proteins and MUC-2, but no improve-



▶ **Fig. 2** Target sites for PFAs to improve gut health and enhance bird performance.

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▶ **Table 1** PFAs: effects on intestinal morphology.

Feed additive	Dose[a]	Animal model	Challenge	Effect	Reference
Plants and plant extracts					
Berberis vulgaris extract	2 and 4% of total water consumption	mixed- sex broiler chicks (Cobb 500)	none	4%: Significantly increased duodenal VH and VS; 2 and 4%: significantly increased jejunal VH; a tendency to increase ileal VS	[59]
Allium sativum extract	75 and 150 mg/kg	male broiler chicks (Ross)	none	75 mg/kg: Increased jejunal VH	[33]
Lavandula angustifolia powder	0.3, 1, or 1.7%	male broiler chicks (Ross 308)	none	Significantly decreased jejunal CD and increased the V:C ratio	[35]
Mixtures of plant derivatives – complex products					
Blend of three active ingredients (20% curcuminoids, 30% cinnamaldehyde, and 30% glycerol monolaurate)	1 kg/t	male broiler chicks (Cobb)	none	Greater VH and CD in the blend and control groups than in the antimicrobial-treated group; lower V:C ratio in the blend group compared to control group	[181]
Commercial product Anta Phyt (containing hops, licorice, and arabic gum derivatives)	400 mg/kg diet (starter), 300 mg/kg diet (grower), 200/kg diet (grower II and finisher)	male broiler chicks (Ross 308)	none	Significantly greater jejunal wall thickness at day 35; significantly increased jejunal CD and decreased VW at day 42	[180]
Commercial product Digestarom (containing cinnamon 20 g/kg, cumin 20 g/kg, peppermint oil 170 g/kg, garlic oil 150 g/kg, 50 g/kg anise oil g/kg, 40 g/kg fennel oil, and SiO_2 and NaCl)	100, 125, and 150 mg/kg	male broiler chicks (Ross 308)	none	100 mg/kg: increased muscularis thickness and goblet cell number at day 21 and day 42; 125 mg/kg: decreased muscularis thickness and increased goblet cell number at day 21, increased VH and muscularis thickness at day 42; 150 mg/kg: significantly higher VH, muscularis at day 21	[12]
Commercial product Enterosan (containing 21.55 mg carvacrol/g, 18.76 mg thymol/g, and 27.62 mg cinnamaldehyde/g)	100 mg/kg	male broiler chicks (Cobb 500)	none	Increased jejunal VH and decreased CD	[49]
Commercial product Enterosan + CUR	100 mg/kg + 50 mg/kg	male broiler chicks (Cobb 500	none	Significantly higher jejunal V:C ratio	[49]
Commercial product Next Enhance150 (containing 54.13% carvacrol and 45.87% thymol)	100 and 200 mg/kg	male broiler chicks (Ross 308)	none	Improved VH, VS, V:C, and mucosal layer of jejunum at 21 and 42 days of age Increased ileal VH, V:C, mucosal layer and goblet cells number at day 21 and mucosal layer and goblet cells number at 42 days of age	[58]
Commercial product Sangrovit	20–50 g/t	male broiler chicks (Cobb 500)	C. jejuni	Increased VH and V:C ratios in the ileum and jejunum	[32]
Commercial product Tecnaroma Herbal Mix PL	100, 200, 300, 400, and 500 g/t	male broiler chicks (Ross 308)	none	300 g/t: Significantly increased VW and VS	[27]

continued

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▶ **Table 1** *Continued*

Feed additive	Dose[a]	Animal model	Challenge	Effect	Reference
Commercial product Xtract (containing 5% carvacrol, 3% cinnamaldehyde, and 2% *Capsicum* oleoresin)	100 mg/kg	male broiler chicks (Arbor Acres Plus)	cold stress	Intestinal diameter enlargement; increased VH and V:C ratio	[20]
Commercial product Xtract (5 carvacrol, 3% cinnamaldehyde, and 2% *Capsicum* oleoresin)	100 mg/kg	male broiler chicks (Hubbard HI-Ye hybrids)	none	Higher mucus secretion intensity and accumulation inside cells of the gastrointestinal mucosa; decreased jejunal VH and CD in chickens fed maize + XT at day 21	[145]
Herbal medicine complex (5 ppm carvacrol, 3 ppm cinnamaldehyde, 2 ppm capsaicin)	100 mg/kg	male broiler chicks (Ross)	none	Increased jejunal VH	[33]
Specific combination microencapsulated active plant extracts (containing 5.04% carvacrol, 2.9% cinnamaldehyde, 2.18% *Capsicum* oleoresin)	100 g/t	male broiler chickens (Arbor Acres Plus)	none	Increase in VH and V:C ratio in the ileum; decrease in ileal CD	[42]
Single phytoconstituents and essential oils					
Blend of protected organic acids and EOs (containing organic acids fumaric, sorbic, malic, and citric and EOs thymol, vanillin, and eugenol)	300 g/t of feed	male broiler chicks (Cobb × Cobb 500)	*Eimeria* spp. at 1 day and with *C. perfringens* at 11, 12, and 13 days	Lower blood FITC-d concentration and histologic lesions score; increased MUC-2 mRNA expression compared to the challenged control group	[179]
Commercial carvacrol EO (containing 63.5% carvacrol, 3.4% thymol, and 13.1% paracymene)	200, 300, and 400 µL directly PO every day	mixed-sex broiler chicks (Ross 308)	none	Significantly higher goblet cell content in the small intestine epithelium (the highest in the 300 µL treatment group)	[123]
Commercial cOCM (containing 37.5% cinnamaldehyde)	50, 100, 200, and 300 mg/kg	mixed-sex broiler chicks (Cobb 500)	none	50, 100, 200, and 300 mg/kg: decreased jejunal CD; 100, 200, and 300 mg/kg: higher jejunal V:C ratio; 50 and 300 mg/kg: elevated duodenal CD; 300 mg/kg: significantly increased duodenal VH and the jejunal V:C ratio at day 21; 50 mg/kg increased the duodenal V:C ratio at 42 days and reduced jejunal VH	[144]
Commercial EO product (containing 25% thymol and 25% carvacrol)	60, 120, or 240 mg/kg	male broiler chicks (Cobb 500)	*C. perfringens*	Challenged birds: linear alleviation of the gut lesions and improved V:C ratio	[86]
Commercial product Orego-Stim (containing 5% EO of *Origanum vulgare* subsp. *Hirtum* plants and 95% natural feed grade inert carrier)	300 and 500 mg/kg	broiler chicks (Ross 308)	none	3 mg/kg: Significantly increased VH and decreased muscularis thickness in the jejunum and ileum; increased V:C ratio and VS in the jejunum and decreased CD in the jejunum; 5 mg/kg: significantly decreased muscularis thickness and CD in the jejunum and ileum	[31]

continued

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▶ **Table 1** *Continued*

Feed additive	Dose[a]	Animal model	Challenge	Effect	Reference
CUR	50 mg/kg	male broiler chicks (Cobb 500)	none	Decreased jejunal VH and CD	[49]
	400 mg/kg	mixed-sex White Pekin duckling	OTA (2 mg/kg)	Partly restored villus length in the jejunum of OTA challenged birds	[97]
Genistein Hesperidin mixture of genistein and hesperidin (1 : 4)	5 mg/kg 20 mg/kg 5, 10, and 20 mg/kg	broiler chicks (Arbor Acres)	LPS challenge	Increase in gut VH and VW (21 and 42 days) and reduction in CD (in the duodenum and ileum at 21 days and duodenum at 42 days); alleviation of LPS-induced changes; decrease of VH and increase of CD	[24]
Piperine	60, 120, and 180 mg/kg	male broiler chicks (Cobb)	none	60 mg/kg: Increased VS in the duodenum and ileum; 120 and 180 mg/kg: reduced absorption surface of the jejunum; decrease in CD in the duodenum and jejunum	[15]
S. officinalis L. EO (0.1 g/kg diet containing α-thujone 0.04 g/kg, limonene 0.02 g/kg, camphor 0.02 g/kg, and α-humulene 0.01 g/kg)	0.1, 0.25, 0.5, and 1 g/kg	non-sexed laying strain chicks (Isa Brown)	none	Duodenum in Ussing chambers *ex vivo*: 0.1 and 0.25 g/kg: significantly higher TEER	[120]
S. officinalis L. EO (containing camphor 14.9 %, α-thujone 14.8 %, eucalyptol 8.5 %, β-thujone 7.2 %, borneol 3.7 %)	0.01, 0.025, 0.05, and 0.1 % EO + sodium selenite (0.4 ppm)	female laying chickens (Isa Brown)	none	0.05 %: Increased thickness of the mucus layer in the duodenum; decreased the number of goblet cells containing acidic and neutral mucins in the duodenum and jejunum and increased in the ileum	[4]
Thyme EO and oregano EO mixture (encapsulated or non-capsulated)	200 mg/kg	mixed-sex broiler chicks (Ross 308)	none	Encapsulated EOs: significantly higher VH, VW, and CD; significantly lower V : C ratio	[182]
Thymus zigis L. EO (containing 0.1 mg/kg p-cymen and 0.08 mg/kg thymol)	0.5 g/kg	mixed-sex broiler chicks (hybrid Ross 308)	none	Duodenum in Ussing chambers *ex vivo*: significantly higher TEER	[119]

[a] Dose in feed, unless stated otherwise

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ment in the birds' growth performance was observed [144]. This is consistent with the results of PFA supplementation (commercial phytogenic product Digestarom) obtained by Ahsan et al., where there was no difference among the dietary treatments for growth performance and cecal microbe populations at any phase [12]. However, in the supplemented group, increased VH and VW were observed in comparison to birds fed control diets. Similarly, in a study conducted by Khattak et al., inclusion of EO (blend of EOs from basil, caraway, laurel, lemon, oregano, sage, tea, and thyme – Tecnaroma Herbal Mix PL) in poultry feed did not improve growth performance during the starter phase [27]. The authors associated it with relatively low digestive enzyme secretion capacity in young chicks, especially since an improvement in growth efficiency was later noted during the grower and finisher phases.

Crypts of Lieberkühn are the center of enterocyte production, so their depth is equivalent to the intensity of the epithelial cell synthesis process [33]. In order to maintain the integrity of the epithelium, damaged cells require intensive replacement with new ones, which results in rapid cell turnover. The more the epithelium is exposed to various harmful factors, the greater the depth of the crypts. The positive effect of PFAs (a combination of carvacrol, cinnamaldehyde, and *Capsicum* oleoresin, piperine, genistein and hesperidin, oregano EO, lavender powder, a combination of CUR, carvacrol, thymol and cinnamaldehyde, cOCM) on gut morphology includes reduced CD [15, 24, 31, 35, 42, 49, 144, 145], which can be interpreted as limited exposure to various stressors, lesser inflammatory response, and sloughing. The cell turnover is also an energy consuming process and shallow crypts suggest that the bird can spare nutrients for growth [12]. An increase in VH and a decrease in CD leads to an increased V : C ratio, which indicates the presence of mature enterocytes, balanced enterocyte migration and sloughing, and efficient nutrient absorption for growth [86, 143]. Increased villi length without increased CD demonstrates a longer survival of villi, without the need for intensive production of new cells [24]. It is consistent with the results of observations carried out in challenged animals. In the study conducted by Du et al. [86] *Clostriudium perfringens* challenge was associated with remarkably deeper crypts in the ileum and the presence of intestinal lesions. The dietary EO supplementation (commercial product containing 25% thymol and 25% carvacrol) linearly alleviated the intestinal lesions, and 60–240 mg/kg EO increased VH and decreased CD, which resulted in a significantly elevated V : C ratio. Similarly, *Campylobacter jejuni* challenge resulted in a decrease in villous length and an increase in CD, which were successfully alleviated by supplementation of PFAs (commercial product Sangrovit manufactured from extracts of *Macleaya cordata*) [32]. In the study conducted by Kamboh and Zhu, a significant increase in gut villus length and VW (on day 21 and day 42) and a reduction in CD (in the duodenum on day 42 and ileum on day 21) was observed in birds supplemented with dietary genistein and hesperidin regardless of LPS challenge [24]. However, LPS injection itself caused a deterioration in intestinal morphology as manifested by a shortening of the villi and an increase in CD, which was not observed in the supplemented groups.

The epithelial cells are covered by a layer of mucus that is produced and secreted by goblet cells distributed along the villi [4, 146, 147]. The main components of the mucus layer are glycoproteins called mucins, which have polymeric, viscoelastic, and protective properties [146]. The most important tasks of the mucus layer is protection against pathogens and other harmful factors present in the lumen of the intestine [146], and transport between the lumen and the brush border membrane [147]. Apart from creating a physical barrier, mucins contain mannosyl receptors, which competitively bind to the type 1 fimbriae of gram-negative bacteria [148]. Dietary PFA (*Salvia officinalis* EO, Digestarom, a commercial blend of cinnamon, cumin, and the EOs peppermint, garlic, anise, and fennel oil, a mixture of thymol and carvacrol, carvacrol EO, a blend of carvacrol, cinnamaldehyde, and *Capsicum* oleoresin) supplementation was associated with an increase in the number of goblet cells [4, 12, 58, 123, 145] and a higher expression of MUC-2, the major mucin gene in the small intestine [118, 122, 144, 149]. This could indicate the protective properties of PFAs related to villi [145] and a reduction of pathogen adhesion to the epithelium [148]. However, Guo et al. obtained two opposite modulatory effects of cOCM on intestinal MUC-2 expression at two sampling time points – days 21 and 42 [144]. In this study, supplementation of 50 and 300 mg/kg of cOCM increased MUC-2 expression in the jejunum on day 21 but decreased it in the duodenum on day 42. The authors associated this opposite effect with the fact that the results could have been influenced by hygienic conditions or the microbial environment of the intestinal sections. Downregulation of the MUC-2 gene was associated with LPS challenge in chicken broilers, and supplementation with 1% (but not 5%) AH fermented root resulted in significantly higher MUC-2 expression [122]. Contrary, Du et al. did not observe any significant changes in
MUC-2 expression either from *C. perfringens* infection or from EO supplementation (commercial EO product containing 25% thymol and 25% carvacrol) [86].

The use of molecular technologies might broaden the knowledge of phytogenics' mechanism of action, and it may enable the selection of the most suitable ones to alleviate negative effects of different factors. Unfortunately, there are not many papers that describe the direct influence of phytogenic substances on the presence and distribution of TJ proteins in poultry. The key results on this matter are presented in ▶ **Table 2**.

In a study by Liu et al. [123], administering carvacrol EO at various doses to standard-reared birds increased the expression of important TJ proteins ZO-1 and -2, OCLN, and CLDN-1, -3 and -5. The positive modulatory influence of PFAs (cOCM) on TJ protein expression was also observed by Guo, although the effect was strongly dose-, segment-, and age-related [144]. cOCM supplementation commonly increased mRNA expression of CLDN-1, but it did not have a significant effect on ZO-1 mRNA expression. Moreover, cinnamon oil supplementation caused the upregulation of OCLN mRNA in the jejunum and downregulation in the duodenum. Similarly, Paraskeuas and Mountzouris reported that PFA (Digestarom, a commercial blend of cinnamon, cumin, and the EOs peppermint, garlic, anise, and fennel oil) administration significantly affected ileal mucosa gene expression of CLDN-5 and it was higher in broilers fed a diet supplemented with 100 mg PFAs/kg compared with the unsupplemented group [118]. However, the gene expression levels of ZO-1, CLDN-5, and OCLN in cecal mucosa were not affected by PFA inclusion.

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▶ **Table 2** PFAs: effects on TJs.

Feed additive	Dose[a]	Animal model	Challenge	Effect	Reference
Plants and plant extracts					
AH (root or fermented root)	1 or 5%	male broiler chicks (Ross 708)	LPS	LPS challenged groups, all AH treatments: significantly higher OCLN expression level; 5% fermented root: OCLN level same as the control group; 1% fermented root: increased JAM-2 and MUC-2 expression	[122]
AH root	1 or 3%	male broiler chicks (Ross)	NE (*Eimeria maxima* followed by *C. perfringens*)	NE challenged groups with 1 or 3% AH: significantly higher JAM-2, ZO-1, OCLN, and MUC-2 expression level compared to birds fed a basal diet	[149]
Enzymatically treated *A. annua*	1 g/kg	male broiler chicks (Arbor Acres)	HS	Increased ileal OCLN, jejunal ZO-1, and OCLN mRNA expression in the HS group	[103]
Mixtures of plant derivatives – complex products					
Commertial product Digestarom	100 and 150 mg/kg	male broiler chicks (Cobb 500)	none	100 mg/kg: Increased ileal mucosa CLDN-5 and MUC-2 mRNA expression	[183]
Single phytoconstituents and essential oils					
Blend of protected organic acids and EOs (containing organic acids fumaric, sorbic, malic, and citric and EOs thymol, vanillin, and eugenol)	300 g/t	male broiler chicks (Cobb × Cobb 500)	*Eimeria* spp. followed by *C. perfringens*	Increased CLDN-1 and OCLN mRNA expression compared to challenged and non-challenged control groups	[179]
Commercial carvacrol EO (containing 63.5% carvacrol, 3.4% thymol, and 13.1% paracymene)	200, 300, and 400 µL directly PO every day	mixed-sex broiler chicks (Ross 308)	none	Significantly increased OCLN, CLDN-1, CLDN-5, ZO-1, and ZO-2 mRNA expression; 300 or 400 µL: increased CLDN-3 mRNA expression	[123]
Commercial cOCM (containing 37.5% cinnamaldehyde)	50, 100, 200, and 300 mg/kg	mixed-sex broiler chicks (Cobb 500)	none	Dose-, age-, and intestinal segment-dependent changes in mRNA expression of CLDN-1, OCLN, ZO-1, and MUC-2; 300 mg/kg: increased CLDN-1 mRNA expression at both day 21 and day 42	[144]
Commercial EO product (containing 25% thymol and 25% carvacrol)	60, 120, or 240 mg/kg	male broiler chicks (Cobb 500)	*C. perfringens*	Linear dose-dependent tendency to upregulate OCLN mRNA expression	[86]
CUR	400 mg/kg	mixed-sex White Pekin duckling	OTA (2 mg/kg)	Significantly higher OCLN and ZO-1 mRNA and protein expression in CUR + OTA group	[97]

[a] Dose in feed, unless stated otherwise

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The results obtained in challenged birds are extremely valuable as they clearly demonstrate the beneficial potential of PFAs. The LPS challenge affects the molecular structure of TJs and is a good model of inflammation [122]. In a study by Lee et al. [122], different doses of dietary AH root or fermented root were effective in alleviating the negative effects of LPS challenge by increasing the expression of TJs. A similar effect was observed by this research group in the case of AH root treatment and NE challenge [149]. Du et al. [86] also noted the beneficial influence of a mixture of phytogenic additives (containing mainly thymol and carvacrol as active compounds) on intestinal barrier of broilers exposed to *C. perfringens* challenge.

In the study conducted by Song et al. [103], administration of enzymatically treated *Artemisia annua* improved intestinal barrier function in heat-stressed broilers by upregulating the mRNA expression of jejunal and ileal OCLN and jejunal ZO-1. In this way, the treatment mitigated the negative effects of HS. However, no differences were found for jejunal and ileal CLDN-2 and -3 and ileal ZO-1 mRNA expression levels among treatments.

The dietary supplementation of CUR was reported by Ruan at el. to be effective in alleviating the toxic influence of OTA on Pekin ducks intestinal barrier [97]. Feeding the birds an ochratoxin-contaminated diet resulted in the significantly decreased expression of OCLN and ZO-1 proteins and mRNA. However, in ducks fed CUR in addition to OTA, the expression levels of both proteins and mRNA were significantly higher than with the toxic diet alone. The structure of enterocytes and TJs was also examined by transmission electron microscopy (TEM), which showed that enterocytes from ducks exposed to OTA had damaged microvilli and widened intercellular spaces. These adverse effects were alleviated in ducks receiving CUR supplementation in addition to the OTA-contaminated diet.

Perspectives

The collected results indicate that PFAs have the potential to improve the overall structure of intestinal mucosa as well as the gut barrier function on a molecular level. Moreover, supplementation with PFAs has been shown to protect birds from various environmental threats leading to impaired intestinal barrier function. This presents great prospects for including PFAs in the poultry diets. Promising results from phytogenic supplementation have also been obtained in other animal models, for example, berberine has been shown to ameliorate TJ damage in a mouse model of endotoxemia [150]. The inclusion of PFAs in the pig's diet has also been widely discussed [61, 140, 151–153]. Jang et al. proved that flavanol-enriched cocoa powder contributes to gut health improvement by a positive influence on gut microbiota and modulation of markers of localized intestinal immunity [154]. In the study conducted by Gessner et al., the addition of grape seed and grape marc extract in the pig diet showed the potential to suppress the inflammation process in the small intestine and improved the gain : feed ratio in growing pigs [155]. Similarly, Han et al. demonstrated that dietary grape seed proanthocyanidins improved intestinal microbiota and the mucosal barrier of weaned pigs [156]. Obtained results showed that the efficacy of this feed additive was comparable to antibiotics. It has been also demonstrated that oregano EO and thymol promote intestinal integrity in pigs and weaned piglets [157, 158].

The idea of using PFAs in human medicine is also gaining popularity, for example, as a possible treatment of inflammatory bowel disease [159–161]. Interest in the implementation of phytogenics in human treatment protocols has resulted in numerous studies in this field, mainly based on the use of human cell line models. The influence and modulation of TJs by phytogenics have been previously reviewed [67, 159, 162, 163]. Although, the results obtained in cell cultures cannot be directly extrapolated to the *in vivo* situation of poultry, especially when human cell lines are used. However, they can be an inspiration for further research in poultry species. Numerous studies indicated the potential of phytogenic compounds for preventing or mitigating epithelial barrier disruption by various factors, such as inflammatory cytokines [160, 164–167] or mycotoxins [95, 98], which can also be of interest in poultry production. The results of studies on phytogenic compounds in various *in vitro* models are presented in ▶ **Table 3**. However, more research is needed to select the most beneficial phytogenics, as well as their formulation and dosage for application in poultry diets.

Limitations

Despite the promising results of *in vitro* and *in vivo* studies, there are still some issues that need to be addressed. First of all, the bioavailability of phytogenic compounds still remains a controversial topic [168]. Especially when the positive effects of their use are observed *in vitro*, the question arises about their *in vivo* effectiveness. In birds, the metabolism of phytogenics also remains an under-explored problem. There are extensive reviews of the bioavailability of various phytogenics in humans [169–171], some including animals, but mainly rodents [172]. The fate of phytogenic pigments in animal nutrition has been reviewed by Faehnrich et al. [173]. In relation to poultry studies on absorption and metabolism of phytogenics, they mainly concentrate on their content in eggs and tissues intended for consumption [174–178].

Moreover, the metabolism of phytogenics results in the formation of a large number of compounds with various chemical structures, which makes it difficult to assess their individual effects and modes of action [168]. On the other hand, it is clear that dietary PFAs reach the gastrointestinal tract, where they can affect its structural components. CUR, despite its poor bioavailability, has been shown to alleviate the negative effects of OTA exposure [97]. However, for EOs, the use of a delivery method, for example, microencapsulation, may be needed [19, 144].

Another possible complication is the fact that most of the products available on the market are multi-ingredient, which makes it difficult to assess the effects of using individual components and differentiating between them. It is also a serious obstacle in evaluating the published results. Sometimes evaluating and comparing published results can also be problematic as botanical species may be unclear, especially if only the common name is used or only the name of commercial product is stated, without its detailed composition [27, 179, 180]. Moreover, PFAs are usually characterized by variable chemical composition, depending on their ingredients and environmental conditions like

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▶ **Table 3** PFAs: effect on *in vitro* models.

Active compound	Dose	*In vitro* model	Challenge	Effects	References
6-Gingerol	1, 5, 10, 50, and 100 µM	Caco-2 cell line	dextran sodium sulfate exposure	Restoration of the integrity of Caco-2 monolayer with a dose-dependent increase in TEER	[184]
Berberine	50 µM	HT-29/B6 cells	TNF-α 500 U/mL	Significant increase in TEER; prevention of TNF-α-induced TEER decrease and paracellular permeability increase; increase in CLDN-1 protein expression with or without TNF-α challenge; decrease in CLDN-2 protein expression with or without TNF-α challenge	[164]
	100 µM	Caco-2 cell line	simultaneous IFN-γ (10 ng/mL) and TNF-α (10 ng/mL) exposure	Small increase in TEER in control monolayers; significant attenuation of TEER decrease and paracellular permeability increase in IFN-γ- and TNF-α-treated monolayers; Attenuation of IFN-γ and TNF-α caused reorganization of ZO-1, OCLN, and CLDN-1	[166]
	50, 100, and 200 µM	Caco-2 cell line	none	Increase in TEER; 50 and 100 µM: decrease in mannitol flux; 100 µM: significant decrease in CLDN-2 expression, tendency to upregulate CLDN-3, -7, and OCLN expression	[185]
Biochanin A	50 µmol/L	Caco-2 cell line	none	TEER increase; reduced tyrosine phosphorylation of ZO-1 protein	[167]
	50 µmol/L	Caco-2 cell line	TNF-α (100 ng/mL) exposure	Prevention of TNF-α- dependent TEER decrease	[167]
Chrysin	100 µmol/L	Caco-2 cell line	none	TEER decrease; increased FD-4 flux; decrease in OCLN, JAM-1 and CLDN-1, -3, and -4 expression	[159]
Cinnamicaldehyde	12.5 or 25 µmol/L	IPEC-1 cell line	none	Dose-dependent reduction of FD-4 flux; increase in ZO-2 expression; 25 µmol/L: TEER increase, increase in CLDN-4, ZO-1, -2, and -3 expression; promotion of the localization of CLDN-1 and -3 to the plasma membrane	[186]
CUR	10 µmol/L	Caco-2 cell line	none	TEER increase	[167]
Daidzein	100 µmol/L	Caco-2 cell line	none	TEER increase	[159]
Epigallocatechin gallate	218 µM (100 µg/mL)	Caco-2 cell line	indomethacin (250 µM) exposure	Total protection against the induced decrease of TEER; dose-dependent protection against the induced increase of the FD-4 flux	[187]
Ferulate	5 or 15 µM	Caco-2 cell line	tertbutyl hydroperoxide (100 µM) exposure	Attenuation of t-BHP-induced barrier disruption; prevention of t-BHP-induced decrease in ZO-1 and OCLN expression; 15 µM: increase in ZO-1 expression	[188]
Ferulic acid	20, 50, 100, 500, and 1000 µM	T84 cell line	short-term apical C10 exposure	20 µM: Increased ZO-1 and CLDN-4 mRNA expression, decreased OCLN mRNA expression, TEER increase in C10-treated and C10-untreated T84 monolayers; 20–500 µM: dose-dependent TEER increase	[189]

continued

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▶ **Table 3** *Continued*

Active compound	Dose	*In vitro* model	Challenge	Effects	References
Genistein	100 µmol/L	Caco-2 cell line	none	TEER normalization after transient decrease	[159]
	50 µmol/L	Caco-2 cell line	none	TEER increase; reduced tyrosine phosphorylation of ZO-1 protein	[167]
	50 µmol/L	Caco-2 cell line	TNF-α (100 ng/mL) exposure	Prevention of TNF-α-dependent TEER decrease	[167]
Hesperetin	100 µmol/L	Caco-2 cell line	None	TEER increase, slightly lower FD-4 flux; increase in OCLN and CLDN-4 expression	[159]
Hydroxytyrosol	10 µmol/L	Caco-2 cell line	none	TEER increase	[167]
Isoferulic acid	20 µM	T84 cell line	short-term apical C10 exposure	Increased ZO-1 and CLDN-4 mRNA expression; decreased OCLN mRNA expression; TEER increase in C10-treated and C10-untreated T84 monolayers	[189]
Kaempferol	10, 30, and 100 µmol/L	Caco-2 cell line	none	Dose-dependent TEER increase; promotion of the actin cytoskeletal association of ZO-1, ZO-2, OCLN, CLDN-1, -3, and -4	[190]
Luteolin	100 µmol/L	Caco-2 cell line	none	TEER increase after transient decrease; decrease in CLDN-1, -3, and -4 expression and increase in ZO-2 expression	[159]
Morin	100 µmol/L	Caco-2 cell line	none	TEER increase	[159]
Myricetin	10, 30, and 100 µmol/L	Caco-2 cell line	none	dose-dependent reduction of **LY** flux (no difference between 10–30 µmol/L)	[191]
Naringenin	100 µmol/L	Caco-2 cell line	none	TEER increase, slightly lower FD-4 flux; increase in OCLN and CLDN-4 expression	[159]
	10, 30, and 100 µM	Caco-2 cell line	none	At the levels of 30 and 100 µM: dose-dependent TEER increase and decrease in FD-4 flux; 100 µM: increase in the cytoskeletal association of TJ proteins; ZO-2, OCLN, CLDN-1, and -4 increased OCLN phosphorylation; increase in the total expression of CLDN-4	[192]
Oxyresveratrol	25 µM	IPEC-J2 cell line	DON (4 µM) exposure	Increase in TEER and reduction of FD-4 diffusion; significantly reduced DON-induced bacterial translocation; enhanced expression of CLDN-4 in untreated cells and reduced DON-induced decreased expression of CLDN-4	[98]
Polyphenolic extract obtained from red wine	200, 400, and 600 µg/mL	HT-29 cell line	mixture of proinflammatory cytokines (20 ng/mL TNF-α; 10 ng/mL IL-1; 50 ng/mL INF-γ) exposure	600 µg: Decrease in the paracellular permeability of FD-4; increase in OCLN, CLDN-5, and ZO-1 mRNA expression; dose-dependent increase in OCLN, CLDN-5, and ZO-1 expression; protection from cytokine-induced OCLN, CLDN-5, and ZO-1 expression decrease; inhibition of cytokine-induced CLDN-2 mRNA expression increase	[160]
Prunetin	50 µmol/L	Caco-2 cell line	none	TEER increase; reduced tyrosine phosphorylation of ZO-1 protein	[167]
	50 µmol/L	Caco-2 cell line	TNF-α (100 ng/mL) exposure	Prevention of TNF-α- dependent TEER decrease	[167]

continued

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▶ **Table 3** *Continued*

Active compound	Dose	*In vitro* model	Challenge	Effects	References
Quercetin	200 µM	HT-29/B6 cell line	TNF-α (1000 U/mL) exposure	Decrease of CLDN-2 and -3 expression; partial inhibition of TNF-α-dependent decrease of RT	[165]
	50, 100, and 200 µM	rat (male Wistar rats) small and large intestine *in vitro* in Ussing-type chambers	TNF-α 104 U/mL, IFN-γ 100 or 1000 U/mL exposure	Tendency toward CLDN-2 and -4 downregulation; RT increase; 200 µM: partial inhibition of the cytokine-induced RT decrease	[165]
	50, 100, 150, and 200 µmol/L	Caco-2 cell line	none	Dose-dependent TEER increase; 200 µmol/L: increase in CLDN-4 expression; increase of CLDN-4 mRNA expression;	[193]
	100, 200, or 400 µM	LLC-PK_1 cell line		Dose-dependent increases in TEER; 400 µM: reduction in transepithelial mannitol leak; increase in CLDN-5 and -7 concentration, decrease in CLDN-2 concentration	[163]
	33 µM (10 µg/mL)	Caco-2 cell line	indomethacin (250 µM) exposure	Total protection against the induced decrease of TEER; total protection against the induced increase of the FD-4 flux	[187]
	66 µM (20 µg/mL)	Caco-2 cell line	indomethacin (500 µM) or rotenone (40 µM) exposure	Inhibition of induced ZO-1 immunofluorescence decrease; total protection against the ZO-1 and OCLN expression decrease caused by indomethacin	[187]
	10, 30, and 100 µmol/L)	Caco-2 cell line	none	Dose-dependent reduction of **LY** flux and TEER increase; Enhancement of ZO-2, OCLN, CLDN-1, and -4 binding to the actin cytoskeleton, dose-dependent increase in CLDN-4 expression; 100 µmol/L: assembly of CLDN-1 and -4 at the TJ in the confocal images	[191]
	100, 200, and 400 µM	Caco-2 cell line	none	Dose-dependent tendency in TEER increase; 400 µM: increase in CLDN-2, -4, and -5 expression, decreased tricellulin expression	[185]
Resveratrol	50 µM	IPEC-J2 cell line	DON (4 µM) exposure	Partial reduction of DON-induced bacterial translocation; reduction of FD-4 diffusion and TEER drop; Protection from DON-induced disassembly of CLDN-4, complete restoration of the level of CLDN-4 assembly	[95]
	438 µM (100 µg/mL)	Caco-2 cell line	indomethacin (250 µM) exposure	Total protection against the induced decrease of TEER; total protection against the induced increase of the FD-4 flux	[187]
	25 µM	IPEC-J2 cell line	DON (4 µM) exposure	Increase in TEER and reduction of FD-4 diffusion; enhanced expression of CLDN-4 in untreated cells and reduced DON-induced decreased expression of CLDN-4	[98]
Theaflavins (TF-3′-O-gallate)	5, 10, and 20 µM	Caco-2 cell line	none	Reduction in the apparent permeability of fluorescein; at the level of 10 µM: increase in mRNA levels for OCLN, CLDN-1, and ZO-1; enhancement of OCLN, CLDN-1, and ZO-1 protein expression	[194]

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mate, location, harvest stage, or storage conditions [7]. To maintain the constant properties of commercial products, standardization of their active components is needed, which is not always easy [7].

All this causes problems with determining optimal doses for poultry, especially since most of the additives are included in feed or in water, which makes it difficult to control the intake of individual birds. The doses are crucial for obtaining the desired result, because a low dose may not be effective, while a high dose may already be toxic and, inversely, impair barrier function [61]. Other factors influencing the efficacy of PFA application in poultry diets are the differences in bird genetics and overall diet composition [135]. Moreover, the possible interactions between phytogenic and other feed additives is another fact that needs to be considered [117]. The stability of phytogenic compounds during feed processing is also often questionable [62].

Another issue that is worth mentioning is the fact that although there are numerous examples of effective supplementation with phytogenic preparations, there are also a few that do not report any effect of this type of dietary treatment. For example, in a study conducted by Akbarian et al., lemon peel or orange peel extract did not have any effect on ileal histomorphology of birds exposed to HS [23]. In a drug-free experimental program led by Gaucher et al., alternative treatment of a diagnosed clinical NE with commercial EO-based products was ineffective in controlling disease outbreaks under field conditions as efficiently, economically, and quickly as antibiotics [112].

Moreover, while most phytogenics are generally considered as safe feed additives, there is hardly any information available regarding the safety and residual toxicity of these ingredients [62, 178]. Yu et al. tested five phytogenic compounds, among others, berberine, in this regard and concluded that their use in starter, grower, and finisher feeds for broiler chickens is safe [178]. However, more studies of this kind are needed to evaluate the use of various phytogenic compounds as feed additives for poultry.

Conclusions

The presented data on the use of PFAs in poultry indicate their significant influence on intestinal morphology and gut barrier physiology, especially in challenged animals. The development of molecular biology techniques and deepening the knowledge about the functions and regulation of the intestinal barrier offer great opportunities to improve compositions of alternative feed additives. EOs are a particularly promising group of PFAs because they include the major part of active substances in the plant [17]. The studies presented in this review confirm EOs potential to ameliorate mucosal morphology and modulate TJ proteins in both challenged and unchallenged animals. Moreover, many studies have focused on this group, so there is a greater chance that evidence-based data will form the basis of EOs use in animal diets. However, there is still a need for more research into phytogenic ingredients in poultry nutrition. In particular, determining the exact mechanism of action of various PFAs at the molecular level is necessary to assess their potential for use in poultry production. Furthermore, as most of the problems that threaten animal health are multifactorial, there is no single solution to all the issues. This results in the need for a holistic approach to poultry production and possibly for the implementation of a combination of different types of feed additives to break the vicious cycle of disease and improve the overall performance of animals.

Contributors' Statement

Drafting the manuscript: U. Latek, M. Mendel; design of the review: U. Latek, W. Karlik; critical revision of the manuscript: U. Latek, M. Chłopecka, W. Karlik, M. Mendel. All authors read and approved the final version of the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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