Animal Nutrition 7 (2021) 801–811

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Review Article

Current experimental models, assessment and dietary modulations of intestinal permeability in broiler chickens

Saad Gilani [a], Peter V. Chrystal [b], Reza Barekatain [c, d, *]

[a] Danisco Animal Nutrition (IFF), Oegstgeest, the Netherlands
[b] Baiada Poultry Pty Ltd, Pendle Hill, NSW, Australia
[c] South Australian Research and Development Institute, Roseworthy Campus, Roseworthy, SA, Australia
[d] School of Animal and Veterinary Sciences, Roseworthy Campus, University of Adelaide, Roseworthy, SA, Australia

ARTICLE INFO

Article history:
Received 27 August 2020
Received in revised form
26 February 2021
Accepted 5 March 2021
Available online 12 May 2021

Keywords:
Intestinal barrier function
Permeability
Tight junction protein
Gut leakage model

ABSTRACT

Maintaining and optimising the intestinal barrier (IB) function in poultry has important implications for the health and performance of the birds. As a key aspect of the IB, intestinal permeability (IP) is mainly controlled by complex junctional proteins called tight junction proteins (TJ) that link enterocytes together. The disruption of TJ is associated with increased gut leakage with possible subsequent implications for bacterial translocation, intestinal inflammation, compromised health and performance of the birds. Despite considerable data being available for other species, research on IP in broiler chickens and in general avian species is still an understudied topic. This paper reviews the available literature with a specific focus on IP in broiler chickens with consideration given to practical factors affecting the IP, current assessment methods, markers and nutritional modulation of IP. Several experimental models to induce gut leakage are discussed including pathogens, rye-based diets, feed deprivation and stress-inducing agents such as exogenous glucocorticoids and heat stress. Although various markers including fluorescein isothiocyanate dextran, expression of TJ and bacterial translocation have been widely utilized to study IP, recent studies have identified a number of excreta biomarkers to evaluate intestinal integrity, in particular non-invasive IP. Although the research on various nutrients and feed additives to potentially modulate IP is still at an early stage, the most promising outcomes are anticipated for probiotics, prebiotics, amino acids and those feed ingredients, nutrients and additives with anti-inflammatory properties. Considerable research gaps are identified for the mechanistic mode of action of various nutrients to influence IP under different experimental models. The modulation of IP through various strategies (i.e. nutritional manipulation of diet) may be regarded as a new frontier for disease prevention and improving the health and performance of poultry particularly in an antibiotic-free production system.

1. Introduction

Intestinal barrier (IB) function and intestinal permeability (IP) are terms often used to refer to intestinal health. However, unambiguous definitions of both are required to accurately assess their roles in disease and disease prevention (Bischoff et al., 2014). The changes in IP are not necessarily accompanied by changes in the component of IB, such as the production of mucus IgA or antimicrobial compounds (Wells et al., 2017). The IB broadly consists of 3 components: firstly, a physical barrier (mucin, intestinal epithelial cells lining and tight junctions [TJ]); secondly, chemical component (cytokines, immune cells and digestive secretions etc.); and thirdly, modulation by gastrointestinal tract (GIT) microbiota (Bischoff et al., 2014). Goblet cells in the intestinal epithelium secrete mucin, which are important physical barriers (Wells et al., 2017). Moreover, intestinal cells are linked together with

* Corresponding author. South Australian Research and Development Institute, Roseworthy Campus, Roseworthy, SA, Australia.
E-mail address: Reza.Barekatain@sa.gov.au (R. Barekatain).

Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2021.03.001

structural proteins providing a physical barrier as explained later specifically. The chemical barrier includes mesenchymal cells, dendritic cells, macrophages and lymphocytes (Celi et al., 2017) that also interact with microbiota (Wells et al., 2017). The chemical barrier also includes digestion secretions, including enzymes which are further affected by diet form, fiber, protein, starch and feed supplements (Celi et al., 2017). There are in general strong interactions between these barriers making it a complex structure to influence. The microbial barrier is particularly influenced by many factors including nutrition and health of the host and interacts with the immune system (chemical barrier), strongly impacting digestion and health of the host (Celi et al., 2017; Salzman, 2011). Intestinal permeability, resulted from disruption of physical barrier, refers to epithelial permeability as described by electrophysiologists using Ussing Chambers for in vitro tissue research and is thus a measurable feature of the IB (Clarke, 2009; Hering et al., 2012).

Special adhesive protein complexes, comprising desmosomes, adherens junctions (AJ) and TJ, join intestinal cells and allow selective IP through paracellular and transcellular pathways. Desmosomes and AJ link cells together mechanically whilst TJ, including occludin, zonula occludens (ZO) and junctional adhesive molecules link epithelial cells apically (Groschwitz and Hogan, 2009). In contrast to many poultry studies on TJ, there is a paucity of reported data on desmosomes and AJ in avian species. Molecules and solutes can be transferred from the gut lumen to the blood via transcellular pathway (through various transporters) or paracellular pathway through TJ, desmosomes and AJ. Where this barrier is compromised, the passage of specific molecules such as sugars or electrolytes across the epithelium to submucosal sites or the portal blood may increase, which is described as increased IP (Gilani et al., 2016). Thus, increased IP may facilitate the passage of harmful substances from the gut lumen via portal blood to target organs such as liver or spleen. Furthermore, bacterial chondronecrosis with osteomyelitis most commonly caused by *Staphylococcus aureus*, may be a result of the adherence of blood-borne bacteria to exposed cartilage at the growth plates of bones and is estimated to be the most common cause of lameness in broiler chickens (McNamee and Smyth, 2000). Subsequently, increased inflammation and compromised health and performance may occur in birds when IP is increased. Therefore, maintaining the IB and optimal IP can be regarded as a new frontier for disease resistance and influencing the health and performance of animals (Vancamelbeke and Vermeire, 2017). Interestingly, some reports in humans suggest that IP can be increased without side-effects on health, as it happens just after exercise, as reviewed by González-González et al. (2019). However, in most animal studies and models, IP is indeed induced with a stressor, therefore increasing IP under a healthy situation requires further elucidation.

It is important to note that an increased IP does not necessarily lead to an increased bacterial translocation. In human research, Quigley (2016) suggested that there are no data to prove that increased IP can lead to the elevated passage of bacteria and subsequent series of events such as celiac disease, irritable bowel syndrome and other digestive disorders. However, there have been many reports on bacterial translocation and various markers including fluorescein isothiocyanate dextran (FITC-d) with a molecular weight of 3,000 to 5,000 Da; some of these studies have been summarized in Table 1. The exact pathogenesis of passing bacteria, toxins or molecules like FITC-d to blood and internal organs such as liver is not clearly understood and is still under investigation. This is not only critical in production animals, where humans may be exposed directly via ingestion (*Campylobacter* and *Salmonella* related health issues) (Salmonella and Campylobacter in chicken meat: meeting report, 2009) but also important in wildlife where viruses may cause zoonotic diseases as suggested by recent work on avian viruses (François and Pybus, 2020) and welfare (Wideman Jr, 2016). There has been substantial interest in the intestinal health of production animals as recently reviewed (Oviedo-Rondón, 2019). However, intestinal health is a vast research subject, including abundant literature on the impact of GIT microbiota composition on bird health and performance. Because IP is the last physical defence mechanism in the GIT for animals, this review focuses only on permeability, with the main emphasis on broiler chickens. The main objectives of this review are to summarise recent literature, understanding of increased IP, experimental factors affecting IP and selected studies suggesting possible nutritional modulation of IP.

2. Methods to evaluate intestinal permeability

Various ex vivo, histological or marker methods have been used to assess IP in chickens. Markers of IP in chickens have been summarized by Gilani et al. (2016). A complete list of potential assays for permeability and GIT barrier function for use in poultry and some other domesticated animals have already been reviewed and are not intended to be repeated herein (Celi et al., 2019; Ducatelle et al., 2018). Transepithelial electrical resistance (TEER) is measured by utilising an Ussing chamber and is an effective and sensitive ex vivo method to assess IP on intestinal tissues harvested from animals. The method is based on measuring the short-circuit current as an indicator of ion transport between the apical and basolateral sides of the epithelium. However, the ex vivo method poses limitations in animal experiments as fresh intestinal tissues must be used (Wells et al., 2017), and it may not fully reflect the real-time changes in IP.

As for in vivo techniques, orally administered large molecular size sugars, including lactulose (382 Da), mannitol (182 Da), rhamnose (164 Da) and their ratios can be utilized to measure the increased passage of these sugars via transcellular and paracellular pathways for assessment of changes in IP. These sugars remain intact in the GIT and their presence and concentrations in blood may be measured by ionic chromatography. Higher sugars in the blood indicate compromised IP, although healthy animals still have some permeability as cell lining is not a sealed wall structure (Tooley et al., 2009). Similarly, FITC-d (3,000 to 5,000 Da), which passes through paracellular pathways, has been the most widely used assay to measure IP in chickens. The basic principle is the same as for dual sugars assays. However, FITC-d is measured spectrophotometrically instead of chromatographically (Gilani et al., 2018a; Vicuña et al., 2015b). The FITC-d assay measures IP throughout the GIT and is not only specific to the small intestine. Interestingly, FITC-d in the duodenum was significantly higher in fasting, dextran sodium sulphate (DSS) and rye diet compared to control. However, FITC-d in the ileum and ceca was only significantly higher in fasting treatment (Vicuña et al., 2015b). This may suggest that major uptake of FITC-d is in the duodenum with variation depending on the treatment. The methodology of FITC-d has been criticised by Hollemans et al. (2020) suggesting it requires further validation and accounting for intestinal feed residue by introducing a period of short-term fasting. This suggestion may be questionable as Gilani et al. (2018a) found that short-term fasting (4.5 h) itself indeed increased IP, therefore potentially masking or confounding the effects of dietary factors or study parameters for which the permeability assay is conducted. Additionally, Hollemans et al. (2020) used a higher concentration of FITC-d (3.8 vs. 2.2 mg), a lower solution volume (0.35 vs. 1 mL), and a different sampling time (120 vs. 150 min) compared to the previous studies. Nevertheless, FITC-d remains the most widely used in chicken experiments. Any further adjustment of the FITC-d assays requires establishing the significance or otherwise of the intestinal

Table 1
Summary of studies for models, method of evaluation and nutritional modulation of intestinal permeability (IP) in chickens.[1]

Models	Method of IP assessment	Dietary changes affecting IP	References
Aflatoxins	(1) Bacterial translocation (BT); (2) FITC-d + BT; (3) Lactulose	(1 to 2) NA; (3) Increased protein reduced IP	(1) Galarza-Seeber et al., 2016; (2) Hernández-Ramírez et al. (2020); (3) Chen et al. (2016)
Campylobacter	(1) *Escherichia coli* in liver; (2) Bacterial translocation	(1) NA; (2) Bacterial translocation (reduced with probiotics and enzymes)	(1) Awad et al. (2016); (2) Gibbs et al., 2016
Coccidiosis	(1) TEER; (2) Fecal ovo-transferrin; (3) FITC-d; (4) FITC-d + TJ; (5) FITC-d + *ZO-1*, *JAM*, occludin; (6) FITC-d; (7) FITC-d and BT; (8) *Escherichia coli*; (9) FITC-d	(1 to 5) NA; (6) Zn reduced IP; (7) Organic acid reduced IP; (8) IP reduced by coated Sodium butyrate. (9) IP reduced by 0.5% glutamine	(1) Murugesan et al. (2014); (2) Goossens et al., 2018; (3) Chadwick et al. (2020); (4) Teng et al. (2020); (5) Schneiders et al. (2020) (6) dos Santos et al., 2020; (7) Pham et al. (2020); (8) Song et al. (2017); (9) Oxford and Selvaraj, (2020)
Dexamethasone	(1) FITC-d + BT; (2) FITC-d; (3 to 4) FITC-d	(1 to 2) NA; (3) Protein tended to reduce IP; (4) Arginine reduced IP	(1) Vicuña et al. (2015a); (2) (Macalintal et al., 2019); (3) Barekatain et al. (2019a); Barekatain et al. (2019b)
Dextran sodium sulphate	(1) FITC-d; (2) FITC + Lactulose sugars; (3) FITC-d	(1) Increased by Rye diet; (2) NA; (3) Reduced by paddy rice	(1) Vicuña et al. (2015b); (2) Gilani et al. (2017b); (3) Murai et al. (2018)
Fasting	(1) FITC-d; (2) FITC-d; (3) Iohexol; (4) Lactulose sugars; (5) FITC-d; (6) FITC-d + BT	(1 to 3) NA; (4) Olive pomace had no effect; (5) IP increased by glutamine/Sodium butyrate; (6) IP reduced by humic acid	(1) Vicuña et al. (2015b); (2) Baxter et al. (2017); (3) Wilhelm et al., 2020; (4) Herrero-Encinas et al. (2020); (5) Gilani et al. (2018b); (6) Maguey-Gonzalez et al. (2018)
Heat stress	(1) TEER; (2) FITC-d + TJ; (3) FITC-d; (4) TEER + FITC-d; (5) FITC-d; (6) FITC-d + TJ; (7) TEER; (8) TEER	(1 to 3) NA; (4) Prebiotics reduced FITC-d; (5) IP reduced by spray dried plasma; (6) Some effect of Resveratrol; (7) IP improved by betaine.	(1) Goo et al. (2019a); (2) Tabler et al. (2020); (3) Ruff et al. (2020); (4) Song et al. (2013); Song et al. (2014); (5) Ruff et al. (2020); (6) Zhang et al. (2017); (7) Shakeri et al. (2018) & (8) Shakeri et al. (2020)
Lipopolysaccharide	(1) Lactulose, FITC-d; (2) Diamine oxidase + D-lactate	(1) NA; (2) IP reduced by clinoptilolite clay	(1) Gilani et al. (2016); (2) Wu et al. (2013)
Clostridium perfringens	FITC-d + Endotoxins	Reduced by a combination of chelated and inorganic zinc	Zhang et al. (2019)
Rye	(1) FITC-d + BT; (2) Endotoxins and fatty acid binding protein gene; (3) BT; (4) FITC-d; (5) FITC-d + BT	(1 to 3) NA; (4) Reduced by salinomycin; (5) IP impacted by breed line and corn or rye diets	(1) Tellez et al. (2015); (2) Chen et al. (2015); (3) Latorre et al. (2015); (4) Naghizadeh et al. (2019); (5) Baxter et al., 2019
(1) Sal + Heat stress; (2) Sal + Coccidiosis; (3) Sal; (4) Sal + Coccidiosis; (5) Sal; (6) Sal	(1) *Salmonella* proliferation increased in liver; (2) FITC-d + BT; (3) *Clostridium perfringens*; (4) FITC-d; (5) FITC-d; (6) mRNA of occludin and claudin	(1 to 2) NA; (3) Prebiotics reduced translocation; (4) IP reduced by probiotics; (5) IP reduced by boric acid; (6) mRNA expressions increased by Zn.	(1) Quinteiro-Filho et al. (2012); (2) Latorre et al. (2018); (3) Shao et al. (2013); (4) Hernandez-Patlan et al. (2019b); (5) Hernandez-Patlan et al. (2019a); (6) Zhang et al. (2012)
(1) Stocking density; (2) feeding fines	(1) lipopolysaccharide in blood; (2) FITC-d	(1) Plasma lipopolysaccharide increased by stocking density; (2) IP increased by feeding fines	(1) Goo et al. (2019b); (2) Kenny (2019)
Used litter	(1) FITC-d; (2) Endotoxins	(1) IP reduced by phytogenic; (2) IP reduced by probiotics	(1) Vieira et al. (2020); (2) Murugesan et al. (2014)
No challenge	FITC-d	IP increased by formaldehyde and Amasil but reduced by silo health compounds	Feye et al. (2019)

FITC-d = fluorescence isothiocyanate dextran; BT = bacterial translocation; NA = not applicable; TJ = tight junction proteins; *ZO-1* = zonula occludens-1; *JAM* = junctional adhesion molecule. TEER = transepithelial electrical resistance; Sal = *Salmonella*.

[1] Studies are given a number from 1 to 9 in parentheses within each row of the table in the order of given references.

feed residue and the extent of the effect of body weight on FITC-d passage through the intestine in chickens.

Recently, a study in laying hens utilized a radiographic (not radioactive) marker, Ioxhale (Wilhelm et al., 2020). Whilst the basic principle is the same as previously mentioned, it could potentially provide more insight for tissue-specific leakage given its radiographic nature. The expression of TJ is also an indirect qualitative assessment of IP due to their pivotal roles in controlling permeability. Nevertheless, there is a general consensus that applying a suite of markers is superior to using a solitary one for reliable assessment of IP. For example, this is highlighted in some studies where TEER or FITC-d is affected and no disruption of TJ was observed (Goo et al., 2019b; Pham et al., 2020). Biomarkers specifically originated from biological samples include but not limited to intestinal fatty acid binding protein, α-antitrypsin inhibitor, diamine oxidase, D-lactate and lipopolysaccharide in blood. These biomarkers can be measured in blood and provide a good indication of GIT health. However, results have been contradictory with different models (Gilani et al., 2016, 2017a, 2017b) hence require further research. Collectively, most of the assessment methods currently utilised are invasive and require blood collection or euthanizing animals for tissue collection. Therefore, there is an ongoing effort to identify biomarkers that can be detected in excreta or faecal material to facilitate on-farm assessment of GIT leakage (De Meyer et al., 2019). Noteworthy, two recent studies investigated faecal biomarkers in chickens. Goossens et al. (2018) discovered faecal ovotransferrin can be released under coccidiosis and necrotic enteritis stress models and can be detected in excreta. They, however, cautioned that ovotransferrin is heat-labile and thus requires testing fresh excreta. More recently, Barekatain et al. (2020b) documented changes in concentrations of intestinal alkaline phosphatase, ovotransferrin and fibronectin in excreta samples of birds subjected to a leaky gut model suggesting that these proteins can be regarded as non-invasive biomarkers of IP when assessed in excreta. These authors also observed a tendency for elevated lipocalin-2 in excreta as a potential biomarker. For practical and field application of IP, various validation studies are required, and the excreta samples have the potential to be used as a non-invasive method of IP assessment. Nevertheless, the type of marker utilized to assess the IP remains as a significant cause of the discrepancy between the studies and therefore comprehensive validation studies of different assays are warranted.

3. Factors affecting intestinal permeability investigated in broilers

Intestinal permeability is fundamentally influenced by numerous cellular and molecular factors in addition to the interaction between intestinal microbiota and the host metabolism and their manifestation on IB and resulting changes in IP (Barekatain et al., 2021; Ren et al., 2020). The review of all these factors is outside of the scope of this paper and thus this section only focuses on practical factors based on available published literature specific to poultry.

3.1. Gender, genetics and age

Although most studies for IP have been conducted on male chickens, it is important to note that gender did not appear to have a significant impact on IP when measured by TEER and lipopolysaccharide (LPS) in the blood (Goo et al., 2019a).

It is known that the rate of intestinal development in chickens rapidly increases post–hatch, peaks at 6 to 10 d of age and declines in relative growth term after 21 d (Lilburn and Loeffler, 2015). However, only recent observations in birds suggest IP increases in the first 2 weeks and subsequently decreases in weeks 3 and 4 post–hatch (Duff et al., 2020). Similar trends were observed in another study in young chickens at 2, 5 and 7 d post–hatch (Gilani et al., 2018b). In both studies, FITC-d was used as a biomarker in the treatment groups. Accordingly, it is prudent to conclude that age could play an important role in IP determination and comparisons should be drawn with caution when comparing different studies even within the same breed. Additionally, it also suggests that young chickens may have higher IP in general, resulting in improved pathogen success rates in inflammation and mortality.

Two studies investigated leaky gut in jungle fowl and 2 Cobb lines (1995 vs. 2015 as slow or modern fast-growing lines) (Tabler et al., 2020). Jungle fowls were observed to have less gut leakage at 10 d of age compared to two modern lines offered corn or rye-based diets. However, when measured at 20 d post–hatch using corn-based diets, Jungle fowl exhibited higher IP than the 2 modern lines (Tabler et al., 2020). This suggests that some breeding lines may be inherently different in their intestinal functionality resulting in different IP. Additionally, the effect of age for one strain may not be comparable to another strain. Confirmation of an age effect in 29-d-old Jungle fowl (higher IP) compared to other 2 lines (lower IP) has been observed (Baxter et al., 2019). Furthermore, there can be interactions between breed, ambient temperature, and diet (Baxter et al., 2019; Tabler et al., 2020). Hence, it is important to consider birds' strain and age when investigating IP in broilers.

3.2. Nutritional factors

Drastic changes in diet composition from rye to corn and vice versa have been shown to affect IP. Corn-to rye-based dietary changes exhibited increased IP compared to rye–rye changes (Baxter et al., 2019). This may suggest that timing of diet changes from starter to grower or finisher may also impact IP. Thus, recording details of these parameters in the future may help to understand some additional commercial challenges in poultry production in relation to IB and enteric disorders. Similarly, the impact of rye-compared to corn-based diets on IP illustrated by Tabler et al. (2020) has also been observed in other studies as shown in Table 1.

Gharib Naseri et al. (2018) observed that FITC-d in birds fed rye-based diet was higher compared to birds fed wheat or barley diets. However, there was no significant difference between birds offered wheat or barley-based diets. Rye contains high levels of non-starch polysaccharides (NSP), which increases the viscosity of the digesta and can lead to intestinal inflammation and an elevated IP (Tellez et al., 2015). This suggests that NSP may play a bigger role in IP increment than cereal type. Furthermore, there may also be an interaction between cereal type (or NSP) and different genotypes, as shown by Tabler et al. (2020) and Baxter et al. (2019). This may suggest that compositional changes when switching feed between starter, grower and finisher may also affect IP in modern broilers, but this has not been fully investigated.

Similar to NSP, reducing dietary crude protein has shown a trend towards increased IP in chickens compared with birds fed higher concentrations of amino acids (Barekatain et al., 2019b). The composition and source of protein such as meat and bone meal may also impact IP (Zanu et al., 2020). Protein or amino acids are required for body functions and growth; hence deficiency or excess availability of protein or amino acids can impact intestinal function. This requires further research, including the sources of protein (vegetable or alternative protein sources). Additionally, fats (saturated and unsaturated), energy and its ratio to the protein on increasing IP in healthy and stressed birds warrant future studies. These factors will be discussed further in the paper as part of dietary interventions to influence IP.

3.3. Stocking density

High stocking density can create stress through either moderate heat stress (Feddes et al., 2002) or stress from feeding competition (Cengiz et al., 2015) due to limited space in meat-type intensive chicken production. Increasing stocking density to 30 birds/m^2 (39.6 kg/m^2) has been shown to significantly increase IP, but no significant effect was observed when stocking density of 25 birds/m^2 (33 kg/m^2) was applied (Goo et al., 2019a). This may have an implication on different rearing standards e.g. in the UK conventional vs. free-range guides (33 to 39 kg/m^2 vs. 25 kg/m^2) (UK farmed animal regulation, 2007).

4. Experimental models to increase intestinal permeability based on disease challenges and various stress factors

4.1. Protozoa, bacteria or toxins

In addition to the nutritional factors discussed in the previous section, a number of models have been developed for investigating GIT health in animals other than chickens, as reviewed by González-González et al. (2019). However, until recently, a limited number of models have been developed in chickens to induce IP. In this review, studies were selected where markers were studied in vivo, including FITC-d, lactulose-to-rhamnose (L:R) and/or lactulose-to-mannitol (L:M) ratios, or expressions of TJ, i.e., *ZO*, occludin. Table 1 shows selective studies on different experimental models developed in chickens. This includes pathogens such as protozoa, *Eimeria* or coccidiosis, bacteria (*Campylobacter*, *Clostridium perfringens* and *Salmonella* spp.), toxins (LPS or aflatoxin), stress-inducing agents (dexamethasone (DEX) and DSS, reuse of bedding materials and feed withdrawal.

Coccidiosis in either clinical or subclinical form results in performance losses to the poultry industry and compromises bird health and welfare (Williams, 2005). Being a field-relevant model, this may have been the reason that coccidiosis was utilized as a model to induce gut leakage in chickens. Models can be divided into 2 major categories. In the first category, coccidiosis causative agents like *Eimeria maxima, tenella* and *acervulina* were used as a sole agent to increase IP (referred hereafter as the Cocci model) (dos Santos et al., 2020; Goossens et al., 2018; Murugesan et al., 2014; Teng et al., 2020). These models utilized Cobb and Ross birds and showed a significant increment in IP measured by FITC-d (dos Santos et al., 2020), ovotransferrin in feces (Goossens et al., 2018) or TEER in intestinal tissue (Murugesan et al., 2014). In the second category, additional stress or agent was utilized (referred hereafter as the Cocci plus). In the Cocci plus models, mainly *C. perfringens* was also inoculated in birds after the coccidiosis challenge (Goossens et al., 2018; Pham et al., 2020; Song et al., 2017) where increased IP was either measured by FITC-d in serum or fecal ovotransferrin in birds of 4 weeks of age or older. Coccidiosis has been known to increase intestinal inflammation, reduce villi length, impair nutrient absorption and increase plasma proteins in chickens (Williams, 2005). A recent study showed that IP was increased in the Cocci plus heat stress (HS) model when FITC-d was measured in serum. However, in the same study there was no correlation between FITC-d and mRNA expression of *ZO-1*, junctional adhesion molecule −2, occludin and claudin-1 (Schneiders et al., 2020). It is possible that broiler strain (Ross-708 vs. Ross 308 and Cobb) or *Eimeria* dose (single *E. maxima* vs. cocktails of *E. maxima, E. tenella and E. acervulina*) or combination (coccidiosis with HS vs. *C. perfringens*) may have played a role in different results.

Food safety has been a major focus from human health perspectives where *Campylobacter* and *Salmonella* have caused foodborne diseases (Salmonella and Campylobacter in chicken meat: meeting report, 2009). Chickens with increased IP can increase incidences of *Campylobacter* and *Salmonella* as these pathogens may be translocated from GIT lumen to liver or edible portions leading to increased incidence of food poisoning in humans. *Campylobacter* and *Salmonella* both have been shown to impact GIT villi structure and increase intestinal inflammation (Awad et al., 2016; Shao et al., 2013). *Campylobacter* has been shown to increase IP measured by the bacterial translocation of either *Campylobacter* and/or *Escherichia coli* in the liver (Awad et al., 2016). Similarly, the *Salmonella* model has been utilized to increase IP either alone (Hernandez-Patlan et al., 2019a; Shao et al., 2013; Zhang et al., 2012), in combination with coccidiosis (Hernandez-Patlan et al., 2019b; Latorre et al., 2018) or with HS (Quinteiro-Filho et al., 2012). All these models have been shown to increase IP either measured by FITC-d or increased bacterial translocation. An interesting observation to note in *Salmonella* models is that these studies utilized Arbor Acre or Cobb birds (not Ross birds). Additionally, in models with Cocci, *Campylobacter* and *Salmonella*, birds were offered mainly corn-based diets although in some cases, wheat-based diets have also been used (Goossens et al., 2018). This suggests that more studies with Ross birds and wheat as the main cereal (or mixed type of diets) is required to understand increased IP and its impact on food safety in nutritional studies.

Similar to bacterial models that can induce intestinal inflammation and increase IP, bacterial toxins or outer coats like LPS may induce stress in the GIT. LPS has been demonstrated to increase IP in chickens when measured by D-lactate and diamine oxidase (DAO) (Wu et al., 2013). However, contrary to this, Gilani et al. (2017a) have shown in a series of experiments that LPS did not impact IP in chickens assessed by different markers. Although LPS (*E. coli* serotype 055) utilized in both studies was the same, birds, sampling time and markers utilized were different. In the earlier study, sampling was done within 2 h of final injection, but in the latter study, sampling was done 24 h after the final injection of LPS. It is possible that IP recovers quickly after the stressor is removed and hence sampling lag time and different broiler strains may have contributed to differences in responses to LPS. Whilst this model may be safer to utilize compared to introducing live pathogens into birds, there is no conclusive evidence to suggest that LPS can increase IP in broiler chickens and the suitability of LPS for use as a GIT permeability model is currently questionable.

4.2. Stress models

Stress can cause physiological changes including elevated heat shock proteins, acute phase proteins and interleukin IL-6 (Zulkifli et al., 2014). Stress can be applied to birds via different means including HS directly in an animal's environment (HS models), via synthetic glucocorticoids like DEX, which mimics the stress in animals, or inducing GIT stress by DSS. DEX has been successfully applied as a model to increase IP in chickens recently in several studies (Barekatain et al., 2019a, 2019b; Duff et al., 2020; Vicuña et al., 2015a). These studies confirm that DEX increases IP in Cobb or Ross birds fed corn or wheat-based diets, in the feed (0.577 mg/kg feed) or repeated intraperitoneal injections (0.5 to 2 mg/kg body weight) and in 10- vs. 21-d-old birds, suggesting that stress can induce leaky gut. This is relevant in modern poultry production because stress is present in various stages of the bird's life and production, including transportation of chicks to farm, exposure to diseases, environmental temperature and humidity, phase feeding, overcrowding, pecking disorders, catching and transportation to slaughterhouses. Caveats in the use of DEX are, however, significant retardation of growth and disinhibition of any underlying health issues in the birds due to being a strong immunosuppressant (Barekatain et al., 2019b; Wideman Jr and Pevzner, 2012).

Heat stress has been observed to induce a wide range of physiological changes, i.e. increase of oxidation in the GIT that may result in increased IP, but may also impact the health and welfare of birds negatively influencing performance and causing production losses (Lara and Rostagno, 2013). The impact of HS on IP has been demonstrated in some studies assessed by FITC-d in serum (Goo et al., 2019b; Zhang et al., 2017). Goo et al. (2019b) also observed increased endotoxin in blood attributed to increased IP. Some other studies also investigated TEER and FITC-d in intestinal tissues of birds subjected to HS (Song et al., 2013, 2014). These studies utilized HS for 10 h during the day for 2 to 3 weeks with a temperature around 30 to 33 °C before measuring IP. Interestingly, a recent study showed that HS for as little as 2 h at 36 °C also increased IP in modern broiler chicken lines (Tabler et al., 2020). There was no effect of HS on IP in jungle fowl, suggesting that these strains can cope with HS better than modern broiler types (Tabler et al., 2020), perhaps due to slower growth and thus reduced metabolic heat production.

Dextran sodium sulphate is a heparin-like polysaccharide that may induce serious damage to GIT epithelium and increase IP, as shown by Vicuña et al. (2015b) and Murai et al. (2018). However, Gilani et al. (2017b) observed that DSS did not increase IP in chickens. Although dose and route of administration of DSS (as 0.75% in drinking water) and markers (FITC-d) used in Vicuña et al. (2015b) and Gilani et al. (2017b) were identical, sampling age was different (being 7 to 14 vs. 21 d). Additionally, diet composition differed (corn-based vs. wheat-based) between the experiments. Bird strain was not known in earlier studies, which along with other factors might explain the differences between studies. Contrarily, Murai et al. (2018) observed increased IP by FITC-d in intestinal tissues in corn-based diets in layer-type chickens at 23 d post–hatch, however they utilized DSS at a dose of 2% instead of 0.75%. Limited data suggest that further research on the DSS model may be needed to ascertain if this model can be utilized to induce leaky gut.

4.3. Fasting

Modern meat-type chickens have been bred for high feed intake and growth, therefore keeping them off-feed can also induce stress with consequences on intestinal function including an increased IP. Fasting has been shown to increase IP in chickens measured by L:M, L:R ratios or FITC-d. Fasting of 4.5 and 9 h (Gilani et al., 2018a), 15.5 h (Herrero-Encinas et al., 2020) and 24 h (Vicuña et al., 2015b) have shown to induce a GIT leakage. In addition to FITC-d and L:M ratio markers, claudin-3 and fatty acid binding protein (FABP) were significantly reduced in 4.5 and 9 h fasted chickens suggesting TJ modulation as well (Gilani et al., 2018c). In all the above studies, birds from Cobb or Ross and different ages (7 to 38 d post–hatch) were utilized, suggesting that fasting is a robust stressor and can increase IP in most circumstances. Feed deprivation also has a practical implication considering that birds may require a period of fasting prior to administration of medication (when the need arises) and during depopulation and transportation to the abattoir. Additionally, young chicks can be without feed for an extended period post–hatch until they arrive at the farm. However, the latter is less likely as shown by Gilani et al. (2018b), where 24 h feed deprivation post–hatch did not increase IP. A possible explanation is residual yolk reserves in chicks may mitigate against this stress enabling chicks to maintain the IP at normal levels.

4.4. Other models (used litter, aflatoxin and laminitis models)

Other models have been utilized to increase IP in chickens, including used litter to induce microbial challenge in birds as well as mycotoxins that may be present in the feed. Used litter has been shown to increase IP in 21- and 28-d-old birds measured either by TEER in intestinal tissues or FITC-d in the blood (Vieira et al., 2020). This may also highlight the need to record litter observations in IP studies, because in a commercial production setting, some countries utilize used litter although others use fresh litter. Aflatoxins, on the other hand, have been shown to increase IP measured by L:R ratio (Chen et al., 2016) whereas Galarza-Seeber et al. (2016) suggested that mycotoxins did not increase IP. Both studies utilized aflatoxin B1 at the same dose of 1.5 mg/kg at similar ages of 20-21 d post–hatch, respectively. However, bird strain and method to produce aflatoxins were different (Cobb or Ross 708 and aflatoxins produced in either rice or corn). Additionally, Galarza-Seeber et al. (2016) utilized bacterial translocation and FITC-d which is a bigger size molecule (3,000 to 5,000 Da) compared to Chen et al. (2016) who utilized lactulose sugars which is only 382 Da. Additionally, Hernández-Ramírez et al. (2020) utilized an additional challenge of *Salmonella enteritidis*; hence it was possible that this combination exacerbated the gut leakage. These fundamentally important factors may explain contradictory reports on aflatoxins as a model to induce IP in chickens.

Another stress model has been developed and summarized by Wideman Jr (2016). This model includes the stress from the DEX as mentioned above, but it also added physical stress on bones with angled wire-mesh for birds. In a series of studies, they were able to show bacterial translocation from the intestine to the proximal growth plates of the femur resulting in acute bacterial osteomyelitis chondronecrosis.

With the above information, it seems coccidiosis models and stress models can alter IP significantly and are also relevant to field challenges.

5. Dietary interventions to reduce intestinal permeability

Recently, many studies have investigated the nutritional modulation of IP in chickens. However, most studies have not been repeated except a few as shown in Table 1. Some of these factors have been discussed below in the light of models, assessment method and their relevance in poultry production.

5.1. Macro- and micronutrients

Dietary protein in particular amino acids play pivotal roles in maintaining the GIT functions and permeability as well as regulating intestinal inflammation (He et al., 2018). In other species, the evidence is mounting that the expression and abundance of TJ are affected by the dietary concentration of amino acids (Ren et al., 2020). Glutamine, glutamate, cysteine, arginine and glycine are amongst the amino acids that enhance protein synthesis in epithelial cells via different signaling pathways and therefore they may be involved in intestinal defensive responses and functions of intercellular junctions (Ren et al., 2020). Comparatively, literature is scarce regarding the specific role of amino acids on IP in poultry. Dietary amino acid concentration and reduction in dietary crude protein offered to broiler chickens may modulate IP accompanied with differential gene expression of various TJ (Barekatain et al., 2019b). Accordingly, reducing dietary protein from 202 to 170 g/kg led to increased leakage of FITC-d into serum compared with a diet containing 10% extra essential amino acids above Ross 308 specifications (Barekatain et al., 2019b). A similar trend was observed in broilers offered diets containing 260 g/kg crude protein with an aflatoxin challenge, resulting in a tendency for reduced IP (Chen et al., 2016). Specific to individual amino acids, glycine supplementation did not impact IP, but arginine supplementation showed a trend towards reduced IP and increased expression of

claudin-1 in the ileum (Barekatain et al., 2019a). In the same study by Barekatain et al., 2021, arginine did not influence IL-8 and IL-1β that may partly explain why there was only a trend towards increased IP. Additionally, this may suggest that some non-essential amino acids play a role in ameliorating increased IP, which has prompted interest to investigate the role of glutamine in improving GIT integrity (Barekatain and Toghyani, 2019). However, limited studies exist on the positive impact of glutamine on reducing IP in poultry. Gilani et al. (2018b) and Barekatain et al. (2019a) have shown that 10 g/kg glutamine supplementation of feed did not decrease IP (when measured by FITC-d) in fasted and DEX challenged birds, respectively. However, in the latter study, a trend to upregulate claudin-1 mRNA expression occurred in broilers offered glutamine supplemented diets. Contrary to that, Oxford and Selvaraj (2020) have recently shown that 5 g/kg dietary glutamine supplementation reduced IP accompanied with an increase in IL-10, suggesting that glutamine may reduce intestinal inflammation. In the previous studies, wheat-based diets were utilized, which suggests that glutamine may not have been limiting in these diets.

Deficiency of threonine has also been linked to an increased IP in broiler chickens (Zhang et al., 2016). Chen et al. (2018) also observed that supplementation of threonine increased gene expression of claudin-3 and *ZO-1* in broilers under LPS challenge, although IP remained unaffected by changing dietary threonine when assessed by serum diamine oxidase and D-lactic acid.

Given the paramount importance of amino acids and dietary protein on bird performance, intestinal integrity, bird welfare and cost efficiency of production, the research on the mechanistic and practical application of amino acids for IB function and IP in particular as a key criterion remains active under various challenge conditions. Furthermore, with different models, markers, birds, crude protein supplied, the source of protein being animal by-products or vegetable protein and synthetic amino acids supplementation make it difficult to compare various studies.

Apart from amino acids and the protein content, there are still significant gaps in the literature for the effects of other major nutrients such as energy, fat, starch, calcium and phosphorus on IP and TJ integrity in chickens. For example, research in other species suggests that a high level of dietary energy and high-fat diets can lead to an increased IP through various mechanisms including direct upregulation of proinflammatory signaling cascades, indirect stimulation of cytokines, disruption of TJ, shifting the microbial composition and mucin production in the intestine (Rohr et al., 2020).

With no available data in poultry, studies in pigs have shown that increasing dietary calcium concentration relative to phosphorus downregulated expression of several TJ in the duodenum implying possible negative impact on gut integrity (Lagos et al., 2019). These negative impacts on TJ may be related to the strong passive absorption and intestinal availability of calcium as discussed by Metzler-Zebeli et al. (2015). Therefore, it is of both scientific and commercial interest to investigate the effect of calcium and phosphorus on IP and TJ integrity in poultry.

The beneficial effect of micro-minerals in particular, zinc (Zn), on IB function and IP is documented in other species (Shao et al., 2017). In broilers, compared with ZnSO4, organic Zn has been shown to reduce IP when measured by FITC-d in birds under *C. perfringens* and coccidiosis challenge (Bortoluzzi et al., 2019). In contrast, Zhang et al. (2012) showed there was no effect of Zn on permeability (with *S. Typhimurium* model) when measured by endotoxins but mRNA expressions of TJ, claudin-1 and occludin were upregulated. Another study showed that dietary Zn supplementation decreased IP (in the *Eimeria* challenge model) whereas copper failed to reduce the blood concentration of FITC-d (dos Santos et al., 2020). Different models, lack of consistency and limited available literature, warrants further research for the role of various micronutrients on IB function in poultry.

5.2. Additives

5.2.1. Probiotics and prebiotics

The positive effect of probiotics on intestinal integrity is likely driven by competitive exclusion mechanisms or suppression of proliferation of harmful species (Edens et al., 1997; Meyer et al., 2020), although the exact mechanisms by which the IP is affected remains elusive. Probiotics have been observed to ameliorate the effects of increased IP (Hernandez-Patlan et al., 2019b; Latorre et al., 2015; Murugesan et al., 2014). Probiotics *Lactobacillus reuteri* and *Lactobacillus plantarum*, have been shown to reduce IP in 12-h fasted chickens (Meyer et al., 2020). In another study translocation of campylobacter from the gut to the liver was significantly reduced by using a probiotic (Gibbs et al., 2016). As reviewed by (Wideman, 2016), in series of experiments, studies have shown that probiotics helped to reduce the bacterial translocation from the GIT to bones by changing the microbiome and potentially reducing immune stress in birds.

Contrarily, no significant effect of probiotics was found in a study by Song et al. (2014) in which IP was assessed by TEER and mRNA expression of TJ. In this study, the measurements were taken in older birds subjected to HS. Noteworthy is the probiotic strains (*Bacillus licheniformis, Bacillus subtilis* and *L. plantarum*) that may not have an impact under HS but possibly be effective when there is bacterial/cocci challenge. Interestingly, in 2 studies where *Bacillus amyloliquefaciens* were utilized, there was a reduction in IP. One of these studies utilized rye as a starch source (Latorre et al., 2015) whereas the other study used corn (Hernandez-Patlan et al., 2019b). The strains of *Bacillus amyloliquefacien* were chosen based on their characteristics to produce xylanase, which suggests the importance of different NSP sources, exogenous NSP enzymes, probiotic strains and the nature of the challenge before applying probiotics to decrease GIT leakage. A recent study by Barekatain et al. (2020) also demonstrated no changes in IP attributed to a *Bacillus*-based probiotic in birds subjected to DEX and a rye-based diet.

Prebiotics are also able to promote GLP-2 production and restore TJ protein expression which consequently can reduce IP (Cani et al., 2009). In chickens, prebiotics have been shown to reduce IP measured by bacterial translocation, claudin-1 and occludin mRNA expression under a *Salmonella* challenge (Shao et al., 2013). In another study, cello-oligosaccharide as prebiotics, reduced IP assessed by FITC-d but no change was observed when measured by TEER in intestinal tissues (Song et al., 2013). The effectiveness of prebiotics on the digestive ecosystem and GIT barrier function may largely differ depending upon the type of products, concentration and changes in microbiota, stimulation of fermentation process, various metabolites and mechanistic and immune pathways in the GIT (Teng and Kim, 2018). Noteworthy, if the fermentation is rapidly increased in the intestine, the unusually elevated production of short-chain fatty acids (SCFA) can result in damage to epithelial cells with a concomitant increased IP (Ten Bruggencate et al., 2005; Teng and Kim, 2018).

Nevertheless, rather contradicting results observed for probiotics and prebiotics on IP warrant further studies under various stressors and different types of strains and products in order to understand the mechanisms by which probiotic and, in general, GIT microbiota composition can affect IP.

5.2.2. Sodium butyrate

Amongst SCFA, the microbial metabolites in the intestine, butyrate is preferentially used by intestinal epithelial cells as a

source of energy and therefore promotes the growth of epithelial cells (Wu et al., 2018). Additionally, butyrate can exert its benefits through modulating microbial community activity, expression of TJ, mucin production and anti-inflammatory properties (Wu et al., 2018). Sodium butyrate (SB) with an active butyrate component has been investigated extensively for improving GIT health in chickens (Moquet, 2018) with reports on reducing IP. Additionally, SB has been observed to reduce bacterial translocation to the liver and, by implication, decreased IP (Song et al., 2017). Contrary to that, Naghizadeh et al. (2019), found no effect of SB on IP where the sampling time can impact IP measurements. In the study by Song et al. (2017), there was a longer time difference between the challenge and sampling (12 d) than Naghizadeh et al. (2019) who conducted the sampling only 6 d after the challenge. This has also been shown by Teng et al. 2020 where they utilized coccidiosis challenge in which the IP increased until 7 d post−challenge and then returned to normal at day 9 post−challenge. Additionally, Gilani et al. (2018b) showed that SB increased IP in young chickens at 4 and 7 d post−hatch. It is important to note that in the aforementioned studies, coated sodium butyrate in feed was utilized, but Gilani et al. (2018b) utilised non-coated sodium butyrate in drinking water. Similarly, the sampling was conducted shortly after the challenge (7 d), which may explain discrepancies between the studies.

5.2.3. Boric acid and clays

Inclusion of boric acid in a corn-based diet at 1 g/kg reduced IP and IgA in chickens subjected to a *Salmonella* challenge model (Hernandez-Patlan et al., 2019a). It was also able to significantly reduce *Salmonella* colonisation at the cecal tonsil, but there was no difference in lactic acid bacteria proliferation. Hernandez-Patlan et al. (2019a) suggested that it is the antibacterial and anti-inflammatory activity of boric acid that led to reduced IP. Antibacterial activity was not shown in lactic acid bacteria, whereas attachment or presence of *Salmonella* was significantly reduced. Additionally, being anti-inflammatory, boric acid decreased IgA, however, there was no negative control (without *Salmonella* challenge) in the study for comparisons. Similarly, modified clay has been shown to reduce IP in chickens assessed by DAO and D-lactate biomarkers and anti-inflammatory cytokines (IL-1β, IL-2, IL-4, IL-6 and IL- 10), suggesting that anti-inflammatory properties of the supplement may facilitate reducing IP (Wu et al., 2013). However, it should be noted that DAO and D-lactate were measured within few hours of treatment; whether this effect lasts longer remains to be elucidated. Additionally, iso-quinoline alkaloids have been shown to reduce inflammatory cytokines IL-6, tumor necrosis factor (TNF) and inducible nitric oxide synthase in heat-stressed chickens and reduced FITC-d in serum (Kikusato et al., 2021). The above studies suggest that nutrients or chemicals that have anti-inflammatory properties may be able to reduce IP in chickens under pathogenic models.

5.2.4. Betaine

Betaine has been known to improve GIT health through various mechanisms, including but not limited to improving intestinal morphology, impacting osmoregulation and acting as a methyl donor. In addition to improving GIT morphology, betaine supplementation also reduced IP when measured by TEER under HS (Shakeri et al., 2020). These authors also found that the concentration of betaine in the intestine, kidney and spleen was significantly increased which may have contributed to improving the GIT integrity. Contrarily, Shakeri et al. (2018) showed that betaine did not influence the TEER in HS conditions, but the FITC-d marker in the jejunum was increased in an Ussing chamber showing increased permeability. Given that TEER was utilized in both studies, these differences could be due to the HS applied (10 vs. 1 to 42 d post−hatch). Collectively, betaine possesses the potential to reduce IP in chickens under HS, but further investigations are required using other stress models to verify such potential.

5.3. Miscellaneous

A wide range of ingredients and additives have been demonstrated to have an impact on IP, but less is known about their mechanistic mode of action. In most cases, the anti-inflammatory properties of ingredients or feed additives have been associated with reduction in IP and intestinal inflammation. For example, decreased IP has been documented for dietary inclusion of paddy rice compared with corn under a DDS model (Murai et al., 2018), humic acid under 24 h fasting model (Maguey-Gonzalez et al., 2018), and resveratrol under the HS model (Zhang et al., 2017). A significant increase in goblet cells and *MUC-2* expression in birds offered paddy rice was observed by Murai et al. (2018). A similar trend was observed by Zhang et al. (2017) where resveratrol increased *MUC-2* expression and goblet cell concentrations. A lack of effect on IP has also been observed for olive pomace under the 15.5 h fasting model (Herrero-Encinas et al., 2020). It would have been interesting to study the effect of olive pomace feeding after fasting, but it was not studied.

By contrast, formaldehyde increased IP, and medium-chain fatty acids reduced IP when compared to control diets whereas formic acid supplementation tended to increase IP compared to control treatment (Feye et al., 2019).

Phytogenic additives have also been observed to reduce IP in chickens (Vieira et al., 2020). A combination of essential oils and organic acid has been shown by Pham et al. (2020) to reduce IP when measured by FITC-d after 1-h gavage but not when measured after 2.5 h, accompanied with differential effect on gene expression of TJ. Pham et al. (2020) also observed that *TNFSF-15* (Tumor necrosis factor super family) which is one of the pro-inflammatory cytokines was significantly reduced in the treatment group. Additionally, *Tollip* (Toll interacting protein), was downregulated. *Tollip* and *TNFSF-15* are involved in *TLR* (Toll Like receptor) mediated pathways suggesting that reducing inflammation may help decrease IP in chickens. Vieira et al. (2020) also observed that TNF-α expression was significantly reduced in birds fed phytogenic additive suggesting their anti-inflammatory role and hence reducing IP. However, in the same study, an anti-inflammatory cytokine (*IL-10*) was also markedly reduced, which may underline the involvement of other mechanisms affecting IP in addition to cytokines. Similarly, inflammatory cytokines *IL-6*, *TNF* and inducible nitric oxide synthase were reduced by iso-quinoline alkaloids and were able to reduce IP when measured by FITC-d in serum (Kikusato et al., 2021).

Finally, there is some indication that feed presentation and particle size (excessive fines 50%) can also increase IP in turkeys (Kenny, 2019). It is possible that turkeys may be more sensitive than chickens, however, further research is needed in this area to understand the impact of feed presentation in chickens, especially when the percentage of fine particles in the feed is not recorded.

6. Conclusions

Although an increasing number of studies are being conducted on IP as a major criterion to assess IB function in poultry, and broiler chickens in particular, this research area in poultry is still not fully developed and defined. The main objectives have been to facilitate optimization of intestinal integrity, reduce the chance of bacterial translocation and intestinal inflammation and therefore avoid loss of performance which otherwise would cause significant economic

loss to the industry. The research on IP in poultry is likely to receive considerably more attention as the industry moves towards antibiotic-free production systems, as is the case in the EU. In an experimental setting, inducing GIT leakage has been achieved using various pathogenic models with or without a second challenge or stressor. Stress models including synthetic glucocorticoids and HS, elevated NSP diets and fasting have shown to increase IP in broiler chickens. It is noted that some of the well-established gut barrier dysfunction models in other species such as DSS and LPS have not been conclusive in broiler chickens. Commonly used assays of IP include sugar markers such as lactulose, rhamnose and mannitol, FITC-d, bacterial translocation, and TJ expressions. However, these assays often require invasive techniques and non-invasive methods, in particular excreta biomarkers could be utilized pending validation studies and real-time methods that can be applied on farms. Inconsistent results have been obtained with nutritional modulation of IP in poultry. This is mainly because most currently available studies vary in the basic nutrition, birds' strain, age, models applied, and markers utilized to assess IP. Significant gaps are evident in the literature on the holistic understanding of the effect of various nutrients on IP and complex mechanisms underpinning such effects. Thus, further studies are required to investigate the possibility of modulating IP in poultry with consideration given to the mode of action and mechanistic understanding of IB function at systemic, cellular and molecular levels.

Author contributions

Saad Gilani: Conceptualization; Investigation, Writing - Original Draft. **Peter V. Chrystal:** Investigation, Writing - Review & Editing. **Reza Barekatain:** Conceptualization; Investigation, Resources, Writing - Original Draft, Writing - Review & Editing.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that might inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

References

Barekatain R, Toghyani M. High dietary zinc and glutamine do not improve the performance or reduce excreta moisture of broiler chickens fed diets with and without magnesium supplementation. Poultry Sci 2019;98(9):4066–72.

Barekatain R, Chrystal PV, Howarth G, McLaughlan C, Gilani S, Nattrass G. Performance, intestinal permeability, and gene expression of selected tight junction proteins in broiler chickens fed reduced protein diets supplemented with arginine, glutamine, and glycine subjected to a leaky gut model. Poultry Sci 2019a;98(12):6761–71.

Barekatain R, Nattrass G, Tilbrook A, Chousalkar K, Gilani S. Reduced protein diet and amino acid concentration alter intestinal barrier function and performance of broiler chickens with or without synthetic glucocorticoid. Poultry Sci 2019b;98(9):3662–75.

Awad WA, Dublecz F, Hess C, Dublecz K, Khayal B, Aschenbach JR, et al. Campylobacter jejuni colonization promotes the translocation of Escherichia coli to extra-intestinal organs and disturbs the short-chain fatty acids profiles in the chicken gut. Poultry Sci 2016;95(10):2259–65.

Barekatain R, Chrystal PV, Gilani S, McLaughlan CJ. Expression of selected genes encoding mechanistic pathways, nutrient and amino acid transporters in jejunum and ileum of broiler chickens fed a reduced protein diet supplemented with arginine, glutamine and glycine under stress stimulated by dexamethasone. J Anim Physiol Anim Nutr 2021;105:90–8.

Barekatain R, Howarth G, Willson N-L, Cadogan D, Wilkinson S. Excreta biomarkers in response to different gut barrier dysfunction models and probiotic supplementation in broiler chickens. PloS One 2020;15(8):e0237505.

Baxter MFA, Dridi S, Koltes DA, Latorre JD, Bottje WG, Greene ES, et al. Evaluation of intestinal permeability and liver bacterial translocation in two modern broilers and their jungle fowl ancestor. Front Genet 2019;10:480.

Baxter MFA, Merino-Guzman R, Latorre JD, Mahaffey BD, Yang Y, Teague KD, et al. Optimizing fluorescein isothiocyanate dextran measurement as a biomarker in a 24-h feed restriction model to induce gut permeability in broiler chickens. Front Vet Sci 2017;(4):1–6.

Bischoff SC, Barbara G, Buurman W, Ockhuizen T, Schulzke J-D, Serino M, et al. Intestinal permeability–a new target for disease prevention and therapy. BMC Gastroenterol 2014;14(1):189.

Bortoluzzi C, Lumpkins B, Mathis G, França M, King W, Graugnard D, et al. Zinc source modulates intestinal inflammation and intestinal integrity of broiler chickens challenged with coccidia and Clostridium perfringens. Poultry Sci 2019;98(5):2211–9.

Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 2009;58(8):1091–103.

Celi P, Cowieson AJ, Fru-Nji F, Steinert RE, Kluenter AM, Verlhac V. Gastrointestinal functionality in animal nutrition and health: new opportunities for sustainable animal production. Anim Feed Sci Technol 2017;234:88–100.

Celi P, Verlhac V, Calvo EP, Schmeisser J, Kluenter A-M. Biomarkers of gastrointestinal functionality in animal nutrition and health. Anim Feed Sci Technol 2019;250:9–31.

Cengiz Ö, Köksal BH, Tatlı O, Sevim Ö, Ahsan U, Üner AG, et al. Effect of dietary probiotic and high stocking density on the performance, carcass yield, gut microflora, and stress indicators of broilers. Poultry Sci 2015;94(10):2395–403.

Chadwick E, Rahimi S, Grimes J, Pitts J, Beckstead R. Sodium bisulfate feed additive aids broilers in growth and intestinal health during a coccidiosis challenge. Poultry Sci 2020;99(11):5324–30.

Chen J, Tellez G, Richards JD, Escobar J. Identification of potential biomarkers for gut barrier failure in broiler chickens. Front Vet Sci 2015;2:14. https://doi.org/10.3389/fvets.2015.00014.

Chen X, Naehrer K, Applegate T. Interactive effects of dietary protein concentration and aflatoxin B1 on performance, nutrient digestibility, and gut health in broiler chicks. Poultry Sci 2016;95(6):1312–25.

Chen Y, Zhang H, Cheng Y, Li Y, Wen C, Zhou Y. Dietary l-threonine supplementation attenuates lipopolysaccharide-induced inflammatory responses and intestinal barrier damage of broiler chickens at an early age. Br J Nutr 2018;119(11):1254–62.

Clarke LL. A guide to Ussing chamber studies of mouse intestine. Am J Physiol Gastrointest Liver Physiol 2009;296(6):G1151–66.

De Meyer F, Eeckhaut V, Ducatelle R, Dhaenens M, Daled S, Dedeurwaerder A, et al. Host intestinal biomarker identification in a gut leakage model in broilers. BMC Vet Res 2019;50(1):46.

dos Santos TS, Teng P-Y, Yadav S, de Souza Castro FL, Gould RL, Craig SW, et al. Effects of inorganic Zn and Cu supplementation on gut health in broiler chickens challenged with Eimeria spp. Front Vet Sci 2020;7:230.

Ducatelle R, Goossens E, De Meyer F, Eeckhaut V, Antonissen G, Haesebrouck F, et al. Biomarkers for monitoring intestinal health in poultry: present status and future perspectives. BMC Vet Res 2018;49(1):1–9.

Duff AF, Chasser K, Briggs W, Rodrigues D, Bielke LR. Age-related changes in gut permeability and optimal timing for experimentally induced gut permeability. Int Poultry Scientific Forum 2020. Abstract No. M33, https://poultryscience.org/files/galleries/2020_IPSF_Abstracts.pdf.

Edens F, Parkhurst C, Casas I, Dobrogosz W. Principles of ex ovo competitive exclusion and in ovo administration of Lactobacillus reuteri. Poultry Sci 1997;76(1):179–96.

Feddes J, Emmanuel E, Zuidhoft M. Broiler performance, body weight variance, feed and water intake, and carcass quality at different stocking densities. Poultry Sci 2002;81(6):774–9.

Feye K, Baxter MF, Jendza J, Tellez G, Owens C, Kidd M, Ricke S. The effects of Amasil NA, SILO Health 104L, and formaldehyde on the gut permeability of d 42 Cobb 700 broilers. Poultry Science Association 108th Annual Meeting 2019.

François S, Pybus OG. Towards an understanding of the avian virome. J Gen Virol 2020:001447.

Galarza-Seeber R, Latorre JD, Bielke LR, Kuttappan VA, Wolfenden AD, Hernandez-Velasco X, et al. Leaky gut and mycotoxins: aflatoxin B1 does not increase gut permeability in broiler chickens. Front Vet Sci 2016;3:10.

Gharib Naseri K, Swick R, Choct M, Morgan N, Wu S. Impact of grain type on performance and gut microbiota composition in broilers under necrotic enteritis challenge. Australian Poultry Science Symposium 2018;29:176–8.

Gibbs KA, Lacharme-Lora L, Chaloner G, Wealleans A, Dersjant-Li Y, Wigley P. A combination of direct-fed microbial and enzymes reduces intestinal inflammation and extraintestinal spread in Campylobacter jejuni-challenged broilers. Poultry Science Association 105th Annual Meeting Abstracts. Poultry Sci 2016;95(E-Suppl. 1). No. 227.

Gilani S, Howarth G, Kitessa S, Tran C, Forder R, Hughes R. Intestinal permeability induced by lipopolysaccharide and measured by lactulose, rhamnose and mannitol sugars in chickens. Animal 2017a;11(7):1174–9.

Gilani S, Howarth G, Kitessa S, Tran C, Forder R, Hughes R. New biomarkers for increased intestinal permeability induced by dextran sodium sulphate and fasting in chickens. J Anim Physiol Anim Nutr 2017b;101(5):e237–45.

Gilani S, Howarth G, Tran C, Barekatain R, Kitessa S, Forder R, et al. Reduced fasting periods increase intestinal permeability in chickens. J Anim Physiol Anim Nutr 2018a;102(1):e486–92.
Gilani S, Howarth GS, Tran CD, Kitessa SM, Forder RE, Barekatain R, et al. Effects of delayed feeding, sodium butyrate and glutamine on intestinal permeability in newly-hatched broiler chickens. J Appl Anim Res 2018b;46(1):973–6.
Gilani S, Howarth GS, Kitessa SM, Forder REA, Tran CD, Hughes RJ. New biomarkers for intestinal permeability induced by lipopolysaccharide in chickens. Anim Prod Sci 2016;56(12):1984–97.
Gilani S, Howarth GS, Nattrass G, Kitessa SM, Barekatain R, Forder REA, et al. Gene expression and morphological changes in the intestinal mucosa associated with increased permeability induced by short-term fasting in chickens. J Anim Physiol Anim Nutr 2018c;102:e653–61.
González-González M, Díaz-Zepeda C, Eyzaguirre-Velásquez J, González-Arancibia C, Bravo JA, Julio-Pieper M. Investigating gut permeability in animal models of disease. Front Physiol 2019;9:1962.
Goo D, Kim J, Choi H, Park G, Han G, Kil D. Effect of stocking density and sex on growth performance, meat quality, and intestinal barrier function in broiler chickens. Poultry Sci 2019a;98(3):1153–60.
Goo D, Kim JH, Park GH, Delos Reyes JB, Kil DY. Effect of heat stress and stocking density on growth performance, breast meat quality, and intestinal barrier function in broiler chickens. Animals 2019b;9(3):107.
Goossens E, Debyser G, Callens C, De Gussem M, Dedeurwaerder A, Devreese B, et al. Elevated faecal ovotransferrin concentrations are indicative for intestinal barrier failure in broiler chickens. BMC Vet Res 2018;49(1):51.
Groschwitz KR, Hogan SP. Intestinal barrier function: molecular regulation and disease pathogenesis. J Allergy Clin Immunol 2009;124(1):3–20.
He F, Wu C, Li P, Li N, Zhang D, Zhu Q, et al. Functions and signaling pathways of amino acids in intestinal inflammation. BioMed Res Int 2018:9171905.
Hering NA, Fromm M, Schulzke JD. Determinants of colonic barrier function in inflammatory bowel disease and potential therapeutics. J Physiol 2012;590(5):1035–44.
Hernandez-Patlan D, Solis-Cruz B, Adhikari B, Pontin KP, Latorre JD, Baxter MF, et al. Evaluation of the antimicrobial and intestinal integrity properties of boric acid in broiler chickens infected with Salmonella enteritidis: proof of concept. Res Vet Sci 2019a;123:7–13.
Hernandez-Patlan D, Solis-Cruz B, Pontin KP, Hernandez-Velasco X, Merino-Guzman R, Adhikari B, et al. Impact of a Bacillus direct-fed microbial on growth performance, intestinal barrier integrity, necrotic enteritis lesions, and ileal microbiota in broiler chickens using a laboratory challenge model. Front Vet Sci 2019b;6:108.
Hernández-Ramírez JO, Nava-Ramírez MJ, Merino-Guzmán R, Téllez-Isaías G, Vázquez-Durán A, Méndez-Albores A. The effect of moderate-dose aflatoxin B 1 and Salmonella Enteritidis infection on intestinal permeability in broiler chickens. Mycotoxin Res 2020;36(1):31–9.
Herrero-Encinas J, Blanch M, Pastor JJ, Menoyo D. Diet supplementation with a bioactive pomace extract from Olea europaea partially mitigates negative effects on gut health arising from a short-term fasting period in broiler chickens. Animals 2020;10(2):349.
Hollemans MS, van-Baal J, Reilingh GdV, Kemp B, Lammers A, Vries Sd. Intestinal epithelium integrity after delayed onset of nutrition in broiler chickens. Poult Sci 2020;99(12):6818–27.
Kenny M. Effect of feed form on Turkey performance and enteric condition. Nordic Poultry Conference 2019. 5th to 7th November 2019 (Oral presentation).
Kikusato LV, Xue G, Pastor A, Niewold TA, Toyomizu M. Effects of plant-derived isoquinoline alkaloids on growth performance and intestinal function of broiler chickens under heat stress. Poult Sci 2021;100(2):957–63.
Lagos LV, Lee SA, Fondevila G, Walk CL, Murphy MR, Loor JJ, et al. Influence of the concentration of dietary digestible calcium on growth performance, bone mineralization, plasma calcium, and abundance of genes involved in intestinal absorption of calcium in pigs from 11 to 22 kg fed diets with different concentrations of digestible phosphorus. J Anim Sci Biotechnol 2019;10. https://doi.org/10.1186/s40104-019-0349-2. Article No 47.
Lara LJ, Rostagno MH. Impact of heat stress on poultry production. Animals 2013;3(2):356–69.
Latorre J, Hernandez-Velasco X, Bielke L, Vicente J, Wolfenden R, Menconi A, et al. Evaluation of a Bacillus direct-fed microbial candidate on digesta viscosity, bacterial translocation, microbiota composition and bone mineralisation in broiler chickens fed on a rye-based diet. Br Poultry Sci 2015;56(6):723–32.
Latorre JD, Adhikari B, Park SH, Teague KD, Graham LE, Mahaffey BD, et al. Evaluation of the epithelial barrier function and ileal microbiome in an established necrotic enteritis challenge model in broiler chickens. Front Vet Sci 2018;5:199.
Lilburn MS, Loeffler S. Early intestinal growth and development in poultry. Poultry Sci 2015;94(7):1569–76.
Macalintal L, Brennan K, Pescatore T, Ao T, Ford M, Dawson K. Repeated intraperitoneal exposure to dexamethasone overtly retards growth, gut integrity, blood chemistry and immune organ development in young broiler chicks. 108th Poultry Science Association Meeting 2019;98(E-Supplement 1):459P.
Maguey-Gonzalez JA, Michel MA, Baxter MF, Tellez Jr G, Moore Jr PA, Solis-Cruz B, et al. Effect of humic acids on intestinal viscosity, leaky gut and ammonia excretion in a 24 hr feed restriction model to induce intestinal permeability in broiler chickens. Anim Sci J 2018;89(7):1002–10.
McNamee PT, Smyth JA. Bacterial chondronecrosis with osteomyelitis ('femoral head necrosis') of broiler chickens: a review. Avian Pathol 2000;29(5):477–95.
Metzler-Zebeli B, Mann E, Ertl R, Schmitz-Esser S, Wagner M, Klein D, et al. Dietary calcium concentration and cereals differentially affect mineral balance and tight junction proteins expression in jejunum of weaned pigs. Br J Nutr 2015;113(7):1019–31.
Meyer MM, Fries-Craft KA, Bobeck EA. Composition and inclusion of probiotics in broiler diets alter intestinal permeability and spleen immune cell profiles without negatively affecting performance. J Anim Sci 2020;98(1):skz383.
Moquet PC. Butyrate in broiler diets: impact of butyrate presence in distinct gastrointestinal tract segments on digestive function, microbiota composition and immune responses. Wageningen University; 2018.
Murai A, Kitahara K, Terada H, Ueno A, Ohmori Y, Kobayashi M, et al. Ingestion of paddy rice increases intestinal mucin secretion and goblet cell number and prevents dextran sodium sulfate-induced intestinal barrier defect in chickens. Poultry Sci 2018;97(10):3577–86.
Murugesan G, Gabler N, Persia M. Effects of direct-fed microbial supplementation on broiler performance, intestinal nutrient transport and integrity under experimental conditions with increased microbial challenge. Br Poultry Sci 2014;55(1):89–97.
Naghizadeh M, Dalgaard TS, Engberg RM. Encapsulated butyrate in the maintenance of broiler gut barrier function applying a dysbacteriosis model. 6th Conference on Poultry Intestinal Health. 2019. Rome, Italy April 3-5.
Oviedo-Rondón EO. Holistic view of intestinal health in poultry. Anim Feed Sci Technol 2019;250:1–8.
Oxford J, Selvaraj R. Effects of glutamine supplementation on broiler performance and intestinal immune parameters during an experimental necrotic enteritis infection. Int Poultry Scientific Forum 2020. Abstract No. M36, https://poultryscience.org/files/galleries/2020_IPSF_Abstracts.pdf.
Pham VH, Kan L, Huang J, Geng Y, Zhen W, Guo Y, et al. Dietary encapsulated essential oils and organic acids mixture improves gut health in broiler chickens challenged with necrotic enteritis. J Anim Sci Biotechnol 2020;11(1):1–18.
Quigley EMM. Leaky gut—concept or clinical entity? Curr Opin Gastroenterol 2016;32(2):74–9.
Quinteiro-Filho W, Gomes A, Pinheiro M, Ribeiro A, Ferraz-de-Paula V, Astolfi-Ferreira C, et al. Heat stress impairs performance and induces intestinal inflammation in broiler chickens infected with Salmonella Enteritidis. Avian Pathol 2012;41(5):421–7.
Ren W, Bin P, Yin Y, Wu G. Impacts of amino acids on the intestinal defensive system. Amino Acids in Nutrition and Health. Springer; 2020. p. 133–51.
Rohr MW, Narasimhulu CA, Rudeski-Rohr TA, Parthasarathy S. Negative effects of a high-fat diet on intestinal permeability: a review. Adv in Nutr 2020;11:77–91.
Ruff J, Barros T, Campbell J, González-Esquerra R, Vuong C, Dridi S, et al. Effect of feeding spray-dried plasma in broilers raised under heat stress conditions. Int Poultry Scientific Forum 2020. Abstract No. P248, https://poultryscience.org/files/galleries/2020_IPSF_Abstracts.pdf.
Salzman NH. Microbiota—immune system interaction: an uneasy alliance. Curr Opin Microbiol 2011;14:99–105.
Schneiders G, Foutz J, Rivera A, Milfort M, Fuller A, Rekaya R, et al. Assessing intestinal permeability: FITC-d versus mRNA expression of tight junction genes. Int Poultry Scientific Forum 2020. Abstract No. P231, https://poultryscience.org/files/galleries/2020_IPSF_Abstracts.pdf.
Shakeri M, Cottrell JJ, Wilkinson S, Ringuet M, Furness JB, Dunshea FR. Betaine and antioxidants improve growth performance, breast muscle development and ameliorate thermoregulatory responses to cyclic heat exposure in broiler chickens. Animals 2018;8(10):162.
Shakeri M, Cottrell JJ, Wilkinson S, Zhao W, Le HH, McQuade R, et al. Dietary betaine improves intestinal barrier function and ameliorates the impact of heat stress in multiple vital organs as measured by evans blue dye in broiler chickens. Animals 2020;10(1):38.
Shao Y, Guo Y, Wang Z. β-1, 3/1, 6-Glucan alleviated intestinal mucosal barrier impairment of broiler chickens challenged with Salmonella enterica serovar Typhimurium. Poultry Sci 2013;92(7):1764–73.
Song J, Jiao L, Xiao K, Luan Z, Hu C, Shi B, et al. Cello-oligosaccharide ameliorates heat stress-induced impairment of intestinal microflora, morphology and barrier integrity in broilers. Anim Feed Sci Technol 2013;185(3–4):175–81.
Song J, Xiao K, Ke Y, Jiao L, Hu C, Diao Q, et al. Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. Poultry Sci 2014;93(3):581–8.
Shao Y, Wolf P, Guo S, Guo Y, Gaskins HR, Zhang B. Zinc enhances intestinal epithelial barrier function through the PI3K/AKT/mTOR signaling pathway in Caco-2 cells. J Nutr Biochem 2017;43:18–26.
Song B, Li H, Wu Y, Zhen W, Wang Z, Xia Z, et al. Effect of microencapsulated sodium butyrate dietary supplementation on growth performance and intestinal barrier function of broiler chickens infected with necrotic enteritis. Anim Feed Sci Technol 2017;232:6–15.
Tabler TW, Greene ES, Orlowski SK, Hiltz JZ, Anthony NB, Dridi S. Intestinal barrier integrity in heat-stressed modern broilers and their ancestor wild jungle fowl. Front Vet Sci 2020;7.
Tellez G, Latorre JD, Kuttappan VA, Hargis BM, Hernandez-Velasco X. Rye affects bacterial translocation, intestinal viscosity, microbiota composition and bone mineralization in Turkey poults. PloS One 2015;10(4). e012239.0.
Ten Bruggencate SJ, Bovee-Oudenhoven IM, Lettink-Wissink ML, Van der Meer R. Dietary fructooligosaccharides increase intestinal permeability in rats. J Nutr 2005;135(4):837–42.

Teng P, Kim W. Review: roles of prebiotics in intestinal ecosystem of broilers. Front Vet Sci 2018;5:245.
Teng P-Y, Yadav S, de Souza Castro FL, Tompkins YH, Fuller AL, Kim WK. Graded eimeria challenge on broiler gastrointestinal health parameters. Poultry Sci 2020;99(9):4203–16.
Tooley KL, Howarth GS, Butler RN. Mucositis and non-invasive markers of small intestinal function. Canc Biol Ther 2009;8(9):753–8.
Vancamelbeke M, Vermeire S. The intestinal barrier: a fundamental role in health and disease. Expert Rev Gastroent 2017;11(9):821–34.
Vicuña E, Kuttappan V, Galarza-Seeber R, Latorre J, Faulkner O, Hargis B, et al. Effect of dexamethasone in feed on intestinal permeability, differential white blood cell counts, and immune organs in broiler chicks. Poultry Sci 2015a;94:2075–80.
Vicuña EA, Kuttappan VA, Tellez G, Hernandez-Velasco X, Seeber-Galarza R, Latorre JD, et al. Dose titration of FITC-D for optimal measurement of enteric inflammation in broiler chicks. Poultry Sci 2015b;94(6):1353–9.
Vieira S, Kindlein L, Favero A, Pastor A, Rostagno M, Drebes D. Effects of in-feed phytogenic additive in broilers vaccinated for coccidiosis. Int Poultry Scientific Forum 2020. Abstract No. T177, https://poultryscience.org/files/galleries/2020_IPSF_Abstracts.pdf.
Wells JM, Brummer RJ, Derrien M, MacDonald TT, Troost F, Cani PD, et al. Homeostasis of the gut barrier and potential biomarkers. Am J Physiol Gastrointest Liver Physiol 2017;312:G171–93.
Wideman Jr RF. Bacterial chondronecrosis with osteomyelitis and lameness in broilers: a review. Poultry Sci 2016;95(2):325–44.
Wideman Jr R, Pevzner I. Dexamethasone triggers lameness associated with necrosis of the proximal tibial head and proximal femoral head in broilers. Poultry Sci 2012;91(10):2464–74.
Wilhelm FR, Krautwald-Junghanns M-E, Piqueras VO, Junilla J, Cramer K, Forsgård RA, et al. Iohexol-based measurement of intestinal permeability in birds. J Exot Pet Med 2020;34:18–23.
Williams R. Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. Avian Pathol 2005;34(3):159–80.
Wu QJ, Zhou YM, Wu YN, Zhang LL, Wang T. The effects of natural and modified clinoptilolite on intestinal barrier function and immune response to LPS in broiler chickens. Vet Immunol Immunopathol 2013;153(1–2):70–6.
Wu W, Xiao Z, An W, Dong Y, Zhang B. Dietary sodium butyrate improves intestinal development and function by modulating the microbial community in broilers. PloS One 2018;13(5):e0197762.
Zanu H, Keerqin C, Kheravii S, Morgan N, Wu S-B, Bedford M, et al. Influence of meat and bone meal, phytase, and antibiotics on broiler chickens challenged with subclinical necrotic enteritis: 2. intestinal permeability, organ weights, hematology, intestinal morphology, and jejunal gene expression. Poultry Sci 2020;99(5):2581–9.
Zhang B, Shao Y, Liu D, Yin P, Guo Y, Yuan J. Zinc prevents Salmonella enterica serovar Typhimurium-induced loss of intestinal mucosal barrier function in broiler chickens. Avian Pathol 2012;41(4):361–7.
Zhang Q, Chen X, Eicher SD, Ajuwon KM, Applegate TJ. Effect of threonine deficiency on intestinal integrity and immune response to feed withdrawal combined with coccidial vaccine challenge in broiler chicks. Br J Nutr 2016;116(12):2030–43.
Zhang C, Rebollo MA, Torres C, Ward TL, Zhang B. Zinc amino acid complex alleviates the loss of intestinal mucosal barrier function induecd by C.perfringens challenge. Int. Conference on Poultry Intestinal Health 2019;104:156–7.
Zhang C, Zhao X, Yang L, Chen X, Jiang R, Jin S, et al. Resveratrol alleviates heat stress-induced impairment of intestinal morphology, microflora, and barrier integrity in broilers. Poultry Sci 2017;96(12):4325–32.
Zulkifli I, Najafi P, Nurfarahin A, Soleimani A, Kumari S, Aryani AA, et al. Acute phase proteins, interleukin 6, and heat shock protein 70 in broiler chickens administered with corticosterone. Poultry Sci 2014;93(12):3112–8.
FAO. World Health Organization. Salmonella and Campylobacter in chicken meat: meeting report. World Health Organization; 2009. http://www.fao.org/3/i1133e/i1133e.pdf.