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微生态制剂对家禽肠道健康影响的研究进展

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摘　要: 近年来,微生态制剂的研发和应用受到国内外学者的广泛关注。肠道作为家禽消化吸收营养物质的重要场所,其健康状况与家禽的生产性能息息相关。微生态制剂不易产生抗性、对环境友好且无毒副作用,包含益生菌、益生元和合生元3类物质,其在维持肠道健康等方面具有显著作用,可促进营养物质消化吸收,有利于家禽生产出高品质产品。因此,本文总结了国内外关于微生态制剂对家禽肠道物理屏障、肠道免疫功能和肠道菌群影响的研究进展,以期为微生态制剂在畜牧业中开发和应用提供理论依据。

关键词: 微生态制剂;家禽;肠道健康

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家禽的生产性能直接影响家禽养殖业的经济效益,而肠道作为家禽消化和吸收营养物质的主要场所,其健康程度关系着家禽的健康水平和生产性能[1]。肠道不仅是营养物质消化吸收的主要场所,还具有十分重要的防御功能。肠道内环境失衡会导致一系列肠道疾病,肠腔内细菌和毒素潜入肠道的几率升高,感染、炎症等问题加剧,阻碍营养物质的消化吸收,引起动物生产性能降低甚至死亡[2]。为了维持家禽健康和提高其产品的品质,生产中往往会通过改良饲粮配比的方式促进家禽对营养物质的消化吸收及转化合成。微生态制剂(microecological agents)作为一种绿色环保的饲料添加剂,其改善肠道健康效果明显,具有巨大的利用价值。本文将综述微生态制剂在改善家禽肠道健康方面的研究进展,以期为微生态制剂的深入研究提供参考。

1　微生态制剂

微生态制剂是指被摄入动物体内并且能够有效参与肠道微生态平衡、调节微生物区系、促进肠道消化吸收和转化等生理功能,可以提高动物生产性能的一类活的微生物培养物的总称。微生态制剂被分为3类:益生菌(probiotics)、益生元(prebiotics)和合生元(synbiotic)。

益生菌是指被摄入体内后对宿主(如动物或人类)有正面效益的具有生物活性的微生物,其中广泛应用于家禽养殖业的有乳酸菌类、丁酸梭菌类、芽孢杆菌类等。研究表明,益生菌能够有效促进肠道健康,加强肠道免疫功能;益生菌作为免疫系统的调节物质,在炎症和抗炎反应时维持肠道内平衡[3]。

益生元是指一类不被宿主消化吸收却能够选择性地促进体内有益菌的代谢和增殖,从而改善宿主健康的碳水化合物的小片段,被认为是肠道微生物菌群的“肥料”,最常用的益生元有低聚果糖(fructo-oligosaccharide,FOS)、低聚反式半乳糖(transgalacto-sacchorides,TOS)、菊粉、低聚木糖(xylooligosaccharides,XOS)等。益生元通过选择性刺激肠道内微生物的活性对宿主产生有益的影响,从而改善肠道健康[4]。

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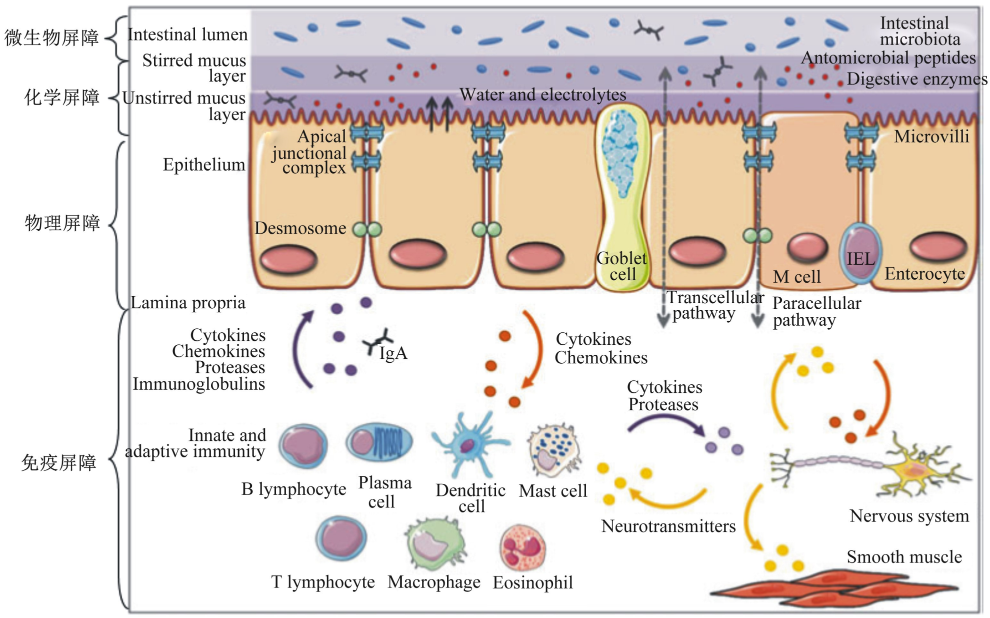
合生元是将益生菌和益生元联合使用,共同发挥二者益生作用的一类复合微生态制剂[5]。

2　微生态制剂对家禽肠道健康的影响

2.1　微生态制剂对家禽肠道物理屏障的影响

肠黏膜屏障包含物理屏障、化学屏障、免疫屏障和微生物屏障(图 1)。肠道物理屏障是肠腔与内环境之间的第 1 道防线,由黏液凝胶层、肠上皮细胞(intestinal epithelial cell,IEC)和细胞间的连接所组成。细胞间的连接从顶端到基膜依次为紧密连接(tight junction)、黏附连接(adherence junction)、桥粒(desmosome)和缝隙连接(gap junction)。紧密连接是细胞间最基本的结构形式。紧密连接蛋白主要有闭锁蛋白(Occludin)、闭合蛋白(Claudin)和连接黏附分子(junctional adhesion molecule,JAM)3 种完整的跨膜蛋白和闭合小环蛋白(zonula occludens,ZO)等。在家禽中,关于 Claudin-1、Claudin-2、Claudin-3、Claudin-5、Claudin-16、ZO-1 和 ZO-2 以及 Occludin 等的研究较多,研究中多以十二指肠、空肠、回肠的形态指标来反映肠道物理屏障的完整情况[6],如绒毛高度(villus height)、隐窝深度(crypt depth)以及绒毛高度与隐窝深度的比值(villus height/crypt depth,V/C)。绒毛高度越高,隐窝深度越浅,表明小肠对营养物质的利用率越高,越有利于家禽的生长发育[7]。



Intestinal lumen:肠腔;Stirred mucus layer:外黏液层;Unstirred mucus layer:内黏液层;Epithelium:上皮细胞;Water and electrolytes:水和电解质;Apical junctional complex:紧密连接复合体;Desmosome:桥粒;Lamina propria:固有层;Cytokines:细胞因子;Chemokines:趋化因子;Proteases:蛋白酶;Immunoglobulins:免疫球蛋白;Innate and adaptive immunity:先天免疫和适应性免疫;B lymphocyte:B 淋巴细胞;Plasma cell:等离子体细胞;Dendritic cell:树突状细胞;Mast cell:肥大细胞;T lymphocyte:T 淋巴细胞;Macrophage:巨噬细胞;Eosinophil:嗜酸性粒细胞;Goblet cell:杯状细胞;Transcellular pathway:(跨)细胞通路;Neurotransmitters:神经递质;Intestinal microbiota:肠道菌群;Antomicrobial peptides:抗菌肽;Digestive enzymes:消化酶;Microvilli:微绒毛;M cell:微褶皱细胞;Paracellular pathway:细胞旁通路;IEL:肠道上皮内淋巴细胞 intraepithelial lymphocyte;Enterocyte:肠细胞;Nervous system:神经系统;Smooth muscle:平滑肌;Intestinal microbiota:肠道微生物区系。

图 1　肠黏膜屏障结构

Fig.1　Structure of intestinal mucosal barrier[8]

2.1.1　益生菌对家禽肠道物理屏障的影响

大量研究证实,益生菌有利于维护家禽肠道物理屏障的完整性,改善肠道形态。Xu 等[9]发现饲粮中添加 200 mg/kg 凝结芽孢杆菌增加了肉鸡

十二指肠 V/C。解淀粉芽孢杆菌制剂也能够增加十二指肠、空肠和回肠的 V/C,维持肠道物理屏障的完整性。Jiang 等[10]报道,饲粮中添加益生菌能够显著提高肉鸡十二指肠、回肠绒毛高度。酵母培养物(yeast culture,YC)是指在特定工艺条件控制下由酵母菌在特定的培养基上经过充分的厌氧发酵后形成的微生态制剂。饲粮中添加 0.3% YC 后,蛋鸡肠道中紧密连接蛋白 *Occludin* 和 *Claudin*-1表达量增加[11]。此外,乳酸菌对乳鸽肠道具有类似的作用效果。研究表明,饲喂乳酸菌后乳鸽回肠和十二指肠绒毛高度、V/C 增加[12]。袁文华等[13]在亲鸽饲粮中添加了丁酸梭菌和乳酸菌,结果发现亲鸽和乳鸽肠道形态都得到改善,该结果可以解释为丁酸梭菌的代谢产物——丁酸,有利于肠道上皮细胞的增殖,并构成了适宜乳酸菌生存的物理环境,进而维持有益菌群平衡,保持肠道屏障完整。与其研究结果相类似,谢鹏等[14]向亲鸽饲粮中添加枯草芽孢杆菌后有效提高了乳鸽肠道绒毛高度,降低了隐窝深度,增大绒毛表面积,说明枯草芽孢杆菌能够间接促进乳鸽肠道绒毛的发育,提高其吸收营养物质的效率。

益生菌制剂能够修复肠道因外界因素所造成的损伤。研究显示,添加不同种类的枯草芽孢杆菌能够修复感染鸡球虫病的蛋鸡空肠黏膜的损伤,促使蛋鸡空肠中连接附着分子 2(junctional adhesion molecule 2,*JAM* 2)和 *Occludin* 表达量升高[15]。夏季高温环境导致家禽热应激的发生,热应激破坏家禽肠道形态结构,包括绒毛断裂、绒毛高度降低、绒毛面积减小、隐窝加深和黏膜上皮细胞脱落[16]。发生热应激时,添加益生菌后 7 和 21 日龄肉鸡十二指肠绒毛高度增加,7 日龄肉鸡回肠 V/C 升高;7 日龄肉鸡回肠黏膜中 *Occludin* 的 mRNA表达量升高,结果证明益生菌能够有效缓解热应激对肠道物理屏障造成的负面影响[17]。Jiang 等[10]报道,益生菌能够修复因热应激而导致的肉鸡肠道功能受损,通过调节肠道黏蛋白 mRNA 和蛋白的表达并加速杯状细胞的分化来增加杯状细胞的数量。另一种解释称,益生菌是通过调控皮质酮水平降低过量的促炎因子,从而修复肠道物理屏障损伤的[18]。此外,添加微生态制剂还能缓解脂多糖(lipopolysaccharides,LPS)对肉鸡肠道造成的损伤。研究表明,饲粮中添加枯草芽孢杆菌可使注射 LPS 的肉鸡的肠道紧密连接蛋白的基因表达量升高,表明肠道屏障功能得以修复[19]。

2.1.2　益生元对家禽肠道物理屏障的影响

有关益生元的研究大多数体现在不同种类的寡糖如纤维寡糖(cello-oligosaccharide,COS)、多聚木糖和菊粉等对于肠道的影响。Song 等[20]研究显示纤维寡糖增加了肉鸡空肠绒毛高度和V/C。一类富含多糖的海藻——浒苔,被证明可对蛋鸡的空肠绒毛高度和 V/C 产生积极的影响[21]。菊粉通过直接降低鸡的促炎反应和增强黏膜免疫来减轻硒引起的肠道损伤,具体表现为减轻十二指肠、空肠和回肠的绒毛萎缩和脱落现象[22]。还有研究揭示了菊粉通过提高紧密连接蛋白的基因表达量来增强肠道屏障功能,从而减轻硒对肠道造成的损伤[23]。在蛋鸡饲粮中添加低聚木糖,空肠绒毛高度和 V/C 呈线性增加,十二指肠绒毛高度呈二次增加[24]。

2.1.3　合生元对家禽肠道物理屏障的影响

益生菌和益生元联合使用往往会产生更好的效果,有效促进肠道健康。洪奇华等[25]发现由益生菌和纤维寡糖组成的合生元可有效缓解热应激对肉鸡肠道造成的损伤,结果显示空肠绒毛高度和 V/C 升高,空肠跨上皮细胞电阻抗值(trans-epithelium electrical resistant,TER)也升高,TER 是评价肠上皮屏障完整性的指标,在一定范围内,TER越大,表明肠上皮屏障完整性越好。Wu 等[26]研究表明,饲粮中添加合生元(低聚果糖和其他有益微生物的混合物)后,能够有效修复热应激导致的肉鸡十二指肠和回肠形态受损。

2.2　微生态制剂对家禽肠道免疫功能的影响

肠相关淋巴样组织(gut-associated lymphoid tissue,GALT)即免疫屏障,分为组织性淋巴组织及散在于整个肠壁中的淋巴细胞,前者包括肠集合淋巴结和肠系膜淋巴结及较小的孤立淋巴滤泡,后者主要是指微皱褶细胞及散在于黏膜固有层及上皮细胞层内的各种淋巴细胞、树突状细胞、肥大细胞等(图 1)。GALT 可以分泌多种细胞因子,如干扰素(interferon,IFN)、集落刺激因子(colony stimulating factor,CSF)、白细胞介素(interleukin,IL)、肿瘤坏死因子(tumor necrosis factor,TNF)等,它们能够杀死病原体并调节肠道黏膜的免疫功能[27]。肠上皮细胞通过表达免疫识别受体如 Toll 样受体(Toll-like receptors,TLR)和 NOD样受体(NOD-like receptor,NLR)等,激活核转录

因子-κB(nuclear factor kappa-B,NF-κB)和促分裂原活化蛋白激酶(mitogen-activated protein kinase,MAPK)等产生免疫应答[28]。

2.2.1 益生菌对家禽肠道免疫功能的影响

肠道中免疫相关细胞的数量和分布能够反映肠道免疫功能。研究证明,益生菌通过激活巨噬细胞,增加肠道上皮内淋巴细胞(intraepithelial lymphocyte,IEL)产生的细胞因子和免疫球蛋白(immunoglobulins,Ig)(尤其是IgA)来增强肠道免疫性能[4]。饲喂细菌凝结物后,肉仔鸡肠道中IgA阳性细胞数量增多[9]。饲粮中添加地衣芽孢杆菌(*Bacillus licheniformis*)后,蛋鸡肠道中肥大细胞的数量增加,回肠和盲肠中IEL的数量增加[29]。Zhang等[11]研究表明,饲粮中添加YC后,蛋鸡十二指肠和空肠中的β-防御素和抗菌肽基因表达上调,可改善蛋鸡肠道免疫功能。Munyaka等[30]研究了YC对肉鸡肠道免疫功能的影响,结果表明,肉鸡肠道中*TLR*4、*IL*-12、*IL*-10和*IFN*-*γ*表达下调。饲粮中添加200 mg/kg凝结芽孢杆菌后,肉仔鸡十二指肠中IFN-α、TLR3和黑色素瘤分化相关基因-5(melanoma differentiation-associated gene-5,MDA-5)的水平提高,分泌型免疫球蛋白A(secreted immunoglobulin,sIgA)阳性细胞的数量增多[9]。

饲粮中补充益生菌可以增强与免疫相关基因的表达。枯草芽孢杆菌可以修复由LPS刺激引起的肉鸡肠道损伤,从而提高黏蛋白(mucin 2,*MUC*2)的表达,以及在炎症状态下提高紧密连接蛋白和*MUC*2的mRNA表达。Chen等[31]报道,饲粮中添加枯草芽孢杆菌可降低蛋鸡肠道中*TLR*4、*MyD*88、*NF*-*κB p*65、*IL*-1*β*和*TNF*-*α*的mRNA表达水平,推测可能通过TLR4、MyD88、NF-κB信号通路抑制促炎性细胞因子释放,进而维持肠道免疫系统的稳定性。

2.2.2 益生元对家禽肠道免疫功能的影响

益生元附着在病原体上,充当发酵底物,增加肠腔的渗透作用,间接刺激巨噬细胞的反应和短链脂肪酸(short-chain fatty acid,SCFA)的产生,从而调节免疫系统[32]。菊粉可促进家禽肠道中有益菌的增殖、抑制病原菌的生长、刺激SCFA增多和肠道免疫反应[33]。此外,菊粉可通过激活免疫过程中涉及的基因和细胞因子的表达来调节免疫反应[34]。

2.2.3 合生元菌对家禽肠道免疫功能的影响

甘露寡糖与益生菌的混合物可提高慢性热应激肉鸡的肠道免疫功能[35]。Ashraf等[36]报道,饲粮中添加甘露寡糖和益生菌的混合物改善了热应激肉鸡IEL和杯状细胞的数量及分布。

2.3 微生态制剂对家禽肠道菌群的影响

附着于肠黏膜上的各类肠道菌群共同组成了肠道微生物屏障(图1)。肠道微生物屏障的稳定性有助于家禽肠道内环境的稳态和家禽健康。常见的肠道有益菌主要包含乳酸菌和双歧杆菌等,有害菌以大肠杆菌和沙门氏菌最为多见。盲肠最具菌群多样性,有利于其消化吸收富含纤维素、淀粉等多糖的饲粮[37]。

2.3.1 益生菌对家禽肠道菌群的影响

饲粮中添加益生菌能够直接或者间接影响家禽肠道中有益菌和有害菌的数量及肠道微生物的代谢产物等,维持肠道健康。研究表明,枯草芽孢杆菌的代谢产物通过降低肠道pH和组织病原体增殖,刺激与肠道相关的免疫系统,从而维持肠道健康[38]。以肉鸽为研究对象,在亲鸽饲粮中添加枯草芽孢杆菌能显著降低乳鸽盲肠中大肠杆菌的数量并提高乳酸杆菌的数量,从而维持乳鸽肠道菌群平衡,有效避免肠黏膜受到病原菌毒素的侵害,维持肠道健康[12]。Yang等[39]报道,饲喂枯草芽孢杆菌后能够增加肉鸡肠道中乳酸菌和双歧杆菌的数量,减少大肠杆菌和沙门氏菌的数量;维持肠道菌群多样性,减少致病菌的附着,提高肠道免疫功能。枯草芽孢杆菌具有提高肉鸡肠道菌群香农-维纳指数(Shannon-wiener index)的作用,表明肠道中菌群多样性增加[40]。Forte等[41]报道,嗜酸乳杆菌和枯草芽孢杆菌联合使用会影响肠道微生物组成,促进有益菌的生长繁殖,减少有害菌的存活。饲喂芽孢杆菌的蛋鸡盲肠中需氧菌(芽孢杆菌、乳酸杆菌和双歧杆菌)的数量升高,大肠杆菌和沙门氏菌的数量降低[19]。Rawski等[42]证明了饲粮中补充益生菌可减少有害菌的繁殖。丁酸梭菌是一类常见的益生菌,Zhan等[43]研究表明,饲粮中添加丁酸梭菌可增加蛋鸡盲肠双歧杆菌的数量,同时降低大肠杆菌的数量。推测这是由于丁酸梭菌通过自身代谢产生大量SCFA,与病原体竞争肠道表面的附着位点,抑制病原体的黏附[14]。

2.3.2 益生元对家禽肠道菌群的影响

大量研究证实,益生元不仅有利于肠道中有

益菌的增殖,还可提高肠道中微生物代谢物——SCFA 的含量,进而保护肠道健康。Cengiz 等[44]研究了持续 14 d 饲喂益生元对雏鸡肠道菌群的影响,在试验第 7 天时发现,雏鸡肠道中大肠杆菌数量减少、乳酸菌数量增加。聚甘露糖醛酸盐(polymannuronate,PM)是一种从海藻褐藻中分离出来的藻酸盐,被认为是较具潜力的益生元。随着饲粮中 PM 添加量的升高,雏鸡肠道中大肠杆菌数量减少,乳酸菌数量增加,乙酸含量升高,肠道内环境的 pH 降低,有效阻碍了有害菌的定植[45]。此外,Li 等[46]表明,饲粮中添加壳寡糖可以调节肉鸡盲肠菌群组成,增加乳酸菌数量,降低大肠杆菌数量,低聚木糖对蛋鸡肠道具有相同的作用[24]。

2.3.3 合生元对家禽肠道菌群的影响

合生元对于肠道菌群的作用主要体现在提高有益菌数量、降低有害菌定植方面,从而实现维护肠道微生态平衡。饲粮中添加枯草芽孢杆菌和菊粉的混合制剂会降低 65~75 周龄蛋鸡肠道中大肠杆菌的数量,增加双歧杆菌和乳酸菌的数量[47]。由低聚寡糖和枯草芽孢杆菌组成的合生元使肉用鹌鹑肠道中大肠杆菌和沙门氏菌的数量减少,乳酸菌的数量增加[48]。Mookiah 等[49]报道,饲粮中添加合生元后,21 日龄肉鸡肠道中乳酸杆菌和双歧杆菌的数量增加,大肠杆菌的数量减少。

3 小结与展望

目前,关于微生态制剂在家禽养殖业中的应用研究已取得很大进展,大量研究已证明了不同种类的益生菌、益生元和合生元的优良饲喂效果,文中所提及的微生态制剂能够从保持家禽维持肠道屏障完整、提高肠道免疫功能、维持肠道微生物区系的相对平衡以及对饲粮的消化吸收和转换利用等多方面保护家禽肠道健康和保持肠道内环境稳定,从而提高家禽的生产性能,有利于家禽养殖业的发展和经济效益的提高。然而,微生态制剂在家禽养殖业中的利用现状仍存在局限性,例如,对于益生菌制剂种类的选择过于局限,测定指标较为常规;有关微生态制剂在家禽肠道健康的作用机制方面的研究仍需不断深入,如对于信号通路的研究和相关蛋白表达情况以及在影响微生物多样性方面仍需探索;微生态制剂和家禽品种的选择可多样化,大量研究都集中在枯草芽孢杆菌、乳酸菌、各类寡糖等上,对于微生态制剂的探索和创新较少;此外,除鸡、鸭外,在家鸽和鹌鹑上的研究较少,应加强研究。相信未来关于作用机制方面的研究会不断深入,对于动物品种的选取更加多样化,以期为微生态制剂应用于家禽业提供全面的科学理论依据。

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Research Progress on Effects of Microecological Agents on Intestinal Health of Poultry

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Abstract: Recently, domestic and foreign scholars have paid much attention to the development and application of microecological agents. Intestine is an important organ for the digestion and absorption of nutrients in poultry. Intestinal health is closely related to the performance of poultry. Microecological agent is a type of no resistance, environmentally friendly and non-toxic feed addictive, which contains probiotics, prebiotics and synbiotics. Their effects on poultry are to maintain intestinal health, promote the digestion and absorption of nutrients and improve product quality of poultry. Therefore, this paper reviewed the domestic and foreign research progress on the effects of microecological agents on poultry intestinal physical barrier, intestinal immune function and intestinal microflora, in order to provide reference for the development and wide application of microecological agents in husbandry. [***Chinese Journal of Animal Nutrition***, **2021**, **33**(**4**):**1851-1858**]

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