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禽源乳杆菌联合酵母发酵豆粕对肉鸡生长和肠道健康的影响

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摘要：【目的】益生菌发酵豆粕能够提高豆粕的营养价值、蛋白质消化率和利用率，其中发酵菌株是影响豆粕发酵效果的关键。研究旨在探究禽内源乳杆菌和酵母菌联合发酵豆粕对黄羽肉鸡相关指标的影响，为复合菌发酵豆粕在肉鸡生产应用提供科学依据。【方法】试验选取初始体质量相近的37 d黄羽肉鸡90羽，随机分为3组，每组15个重复，每个重复2羽。试验组分别为对照组（饲喂基础日粮）、鸡内源乳杆菌组（添加0.5%单克隆乳杆菌发酵豆粕）和复合菌组（添加0.5%乳杆菌和酵母菌联合发酵豆粕）。预试期3 d，正试期28 d。试验结束测定各组别肉鸡生长性能；选取对照组、复合菌组肉鸡的法氏囊、肝脏、胰腺和脾脏指数，以及肠道长度、质量、pH进行测定；比较分析HE染色后十二指肠的绒毛高度、隐窝深度、绒毛隐窝比例；采用qPCR测定对照组、复合菌组肉鸡盲肠黏膜炎症因子mRNA的相对表达量。【结果】（1）日粮添加复合菌发酵豆粕组肉鸡平均日体增重（ADG）显著提高（$P<0.05$），平均日采食量（ADFI）有提高的趋势（$P=0.053$），整体效果优于单独鸡内源乳杆菌发酵豆粕组，但两组料重比（F/G）与对照组间均无显著差异（$P>0.05$）；（2）与对照组相比，复合菌发酵豆粕组显著提高了肉鸡腹脂率（$P<0.05$），具有提高法氏囊指数的趋势（$P=0.059$），但对肝脏指数、胰腺指数和脾脏指数没有显著影响（$P>0.05$）；（3）复合菌发酵豆粕组肉鸡回肠内容物pH有降低的趋势（$P=0.081$），盲肠质量/长度比有提高的趋势（$P=0.084$），盲肠长度、盲肠指数、结直肠长度、十二指肠内容物pH、盲肠内容物pH没有显著影响（$P>0.05$）；（4）与对照组相比，复合菌组肉鸡的十二指肠绒毛高度、隐窝深度和绒隐比没有显著影响（$P>0.05$）；（5）与对照组相比，复合菌组肉鸡的盲肠黏膜*IL-6* mRNA相对表达量显著提高（$P<0.05$），*IL-10* mRNA相对表达量显著降低（$P<0.05$），*IL-8*的mRNA相对表达量有提高的趋势（$P=0.064$），但*IL-1β*、*IL-2*、*IL-4*、*IFN-α*、*IFN-β*、*IFN-γ*、*TNF-α*的mRNA相对表达量没有显著影响（$P>0.05$）。【结论】饲料添加0.5%鸡内源乳杆菌联合酵母发酵豆粕能够显著提高肉鸡平均日增重和腹脂率，有提高肉鸡法氏囊指数、降低回肠内容物pH的趋势。

关键词：发酵豆粕；肉鸡；生长性能；免疫器官指数；肠道健康
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Effects of soybean meal fermented by endogenous lactic acid bacteria in birds and yeast on growth performance, immune organ index and intestinal health of broilers

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Abstract: [**Objective**] Soybean meal fermented by probiotics can improve the nutritional value, protein digestibility and utilization rate of soybean meal.Fermentation strain is the key factor affecting the fermentation effect of soybean meal. The purpose of this study is to explore the effects of soybean meal fermented by endogenous lactic acid bacteria in birds on related indexes of yellow-feather broilers, which can provide a theoretical basis for the development and use of compound bacteria fermented soybean meal in animal husbandry. [**Method**] 90 37-day-old yellow-feathered broilers with similar beginning weights were randomly assigned to three groups, with 15 replicates in each group and 2 broilers in each replicate.The experimental groups included the control group (fed with the basal diet), chicken endogenous lactobacillus group (the basal diet added with 0.5% monoclonal lactobacillus fermented soybean meal) and the group of compound bacterial (the basal diet added with 0.5% lactobacillus and yeast combination fermented soybean meal).The preliminary test period was 3 days, and the formal test period was 28 days.During the experiment, the growth performance of all groups was measured.The bursa of fasciola, liver, pancreas, and spleen index, as well as intestinal length, weight, and pH, were measured for both the control group and the compound bacteria group. HE staining was performed on the duodenum, then the height of the duodenal villi, the depth of the crypts, as well as the villus-crypt ratio were compared and analyzed. qPCR was used to determine the relative expression of inflammatory factors mRNA in the cecum mucosa. [**Result**] (1) Compared with the control group, the average daily weight gain (ADG) of broilers in the fermented soybean meal group with compoound bacteria significantly increased (P<0.05), the average daily feed intake (ADFI) showed a tendency to increase (P=0.053), and the overall effect was better than that of the group using soybean meal fermented by chicken endogenous Lactobacillus alone., However, there were no significant differences in the feed conversion ratio (F/G) between the two groups and the control group (P>0.05).(2) Compared with the control group, the group fed with soybean meal fermented by compound bacteria significantly increased the abdominal fat rate of broiler chickens (P<0.05), and there was a tendency to increase the bursa of Fabricius index (P=0.059). However, it had no significant effects on the liver index, pancreas index and spleen index (P>0.05). (3) The pH of the ileum contents was decreasing (P=0.081), and the weight/length of the cecum had the tendency to rise (P=0.084). There was no significant effects on the length of the cecum, cecum index, colorectal length, duodenal content pH, or cecal content pH (P>0.05).(4) There was no significant effects on the height of the duodenal villi, the depth of the crypt, or the villi to crypt ratio (P>0.05).(5) The relative expression level of *IL-6* mRNA in the cecal mucosa was significantly increased (P<0.05), the relative expression level of *IL-10* mRNA was significantly decreased (P<0.05), and there was a trend of increased relative expression level of *IL-8* mRNA (P=0.064).However, the relative expression levels of *IL-1β*, *IL-2*, *IL-4*, *IFN-α*, *IFN-β*, *IFN-γ* and *TNF-α* were not significantly affected (P>0.05). [**Conclusion**] Adding 0.5% compound bacteria fermented soybean meal to the feed can significantly increase the average daily weight gain and abdominal fat rate of broilers.It also has the tendency to lower the pH of ileum contents and increase the bursa index of broilers.

Keywords:fermented soybean meal;broiler;growth performance;immune organ index;intestinal health

【研究意义】微生物发酵豆粕已经广泛应用于畜禽养殖生产。研究表明,日粮中添加发酵豆粕不仅能够提高畜禽生长性能[1-3],而且在调节畜禽肠道健康、改善免疫状况方面具有重要作用[4-6]。因此,优化微生物发酵豆粕的菌种组合和发酵工艺,对于进一步提升发酵豆粕应用效果具有重要意义。【前人研究进展】发酵不仅能提高原料的营养价值,而且其中活的益生菌可在畜禽肠道定植,调节肠道炎症因子分泌、增强肠道屏障[7]。而且,灭活的益生菌菌体结构还能够黏附于肠道上皮细胞,并与微生物代谢产物一起共同调节肠道炎症和增强免疫应答[8]。但目前生产应用过程中,菌种选择仍是决定发酵豆粕品质的核心。常用于发酵豆粕的菌种包括枯草芽孢杆菌、地衣芽孢杆菌、乳杆菌等细菌和酵母菌、黑曲霉、米曲霉等真菌[9-11]。乳杆菌广泛存在于植物、饲料、动物胃肠道和环境中[12]。大多数从动物胃肠道分离出来的乳杆菌具有耐酸、耐胆盐、特异性地黏附于宿主肠道上皮细胞、抗病原菌、调控肠道免疫等优良的益生菌特性[13-14]。本实验室前期研究发现,禽内源干酪乳杆菌发酵豆粕能够改善肉鸡肠道发育、肠道炎症状况和盲肠微生物菌群平衡,进而改善肠道健康,提高肉鸡生长性能[15]。豆粕发酵通常采用菌种联合发酵的方式,有利于提高发酵效率[16-17]。多数情况下,乳杆菌、酵母菌以互利共生的方式进行发酵[18]。一方面,酵母菌能够为乳杆菌提供厌氧环境和生长所必需的维生素、氨基酸和嘌呤等营养成分[19-21]。另一方面,乳杆菌能够大量产生乳酸,降低pH,抑制病原菌生长,从而促进酵母菌生长[22-23]。本实验室前期对禽内源乳酸菌进行筛选和鉴定,发现所得菌株均为乳杆菌[15]。由于乳杆菌需要在厌氧环境下进行发酵[24-25],因此发酵初期速度较慢,而酵母菌能够迅速耗竭氧气,从而触发厌氧发酵过程[26]。有研究[27]发现,嗜酸乳杆菌、酿酒酵母与解淀粉芽孢杆菌联合发酵豆粕能够调节肉鸡盲肠微生物菌群,改善机体免疫状况,进而提高肉鸡生长性能。

【本研究切入点】实验室前期利用单一禽源乳杆菌制备发酵豆粕,发现其对肉鸡生长和肠道健康具有明显改善作用。但目前尚不清楚禽源益生菌与酵母菌联合发酵豆粕能否进一步提高其改善肉鸡生长性能方面的效果。【拟解决的关键问题】试验利用固态发酵豆粕工艺,测定分析鸡内源乳杆菌和酵母菌联合发酵豆粕对肉鸡生长性能、免疫器官指数及肠道健康的综合影响,探究益生菌豆粕营在肉鸡肠道健康和免疫调控方面的潜力,为鸡内源乳杆菌和酵母菌联合发酵豆粕技术潜力的挖掘及其在养禽生产中的应用提供参考依据。

1　材料与方法

1.1　发酵豆粕的制备

发酵豆粕的制备方法参考先前的研究并进行修改[28]。试验所使用的谷糠乳杆菌(F1/2/3/4/5/6)由本实验室预先从鸡肠道内容物中筛选出并储存在-80 °C冰箱。酵母菌由广东省科学院微生物研究所提供。将谷糠乳杆菌、酵母菌分别置于MRS肉汤(027312,广东环凯微生物科技有限公司)液体培养基中,37 °C静置培养24 h。发酵原料和水的配比为豆粕(46%):蔗糖:水＝18:15:67和10:15:75。豆粕由华洋饲料有限公司提供,蔗糖由天津市大贸化学试剂厂提供。复合菌配比为乳杆菌:酵母菌=2:1。将乳酸杆菌或复合菌按照 $1×10^5$ cfu/g的接种量接种于发酵原料,厌氧发酵7 d,置于83 ℃烘箱中烘干,研磨粉碎,室温储存以备使用。

1.2　试验动物与试验设计

快速型黄羽肉鸡购于广东省佛山市三水温氏家禽有限公司。试验选取初始体质量相近的37 d黄羽肉鸡90羽,随机分为3组,每组15个重复,每个重复2羽。试验组分别为对照组(饲喂基础日粮)、鸡内源乳杆菌组(添加0.5%单克隆乳杆菌发酵豆粕)和复合菌组(添加0.5%乳杆菌和酵母菌联合发酵豆粕)。预试期3 d,正试期28 d。基础日粮由广东省佛山市华洋饲料有限公司提供,日粮组成及营养水平见表1。

1.3　饲养管理

试验动物于鸡舍的四层立体笼中进行单笼饲养,饲养密度12羽/m^2。试验期间自由采食与饮水。采用24 h光照制度,正常通风。试验前对饲养场所进行卫生消毒。按照正常免疫程序进行免疫接种。

表 1 基础日粮组成及营养水平(干物质基础)

Tab.1 Dietary composition and nutritional level(dry matter basis)

项目 Items	含量 Contents	营养水平[2)] Nutrition level[2)]	含量 Contents
原料组成/% Ingredients		代谢能/(Kcal·kg^{-1})Metabolic energy	3 105.32
玉米/% Corn	64.60	粗蛋白/% Crude protein	17.06
豆粕/% Soybean meal	28.00	钙/% Calcium	0.73
大豆油/% Soybean oil	4.5	有效磷/% Available phosphorus	0.22
石粉/% Limestone powder	1.2	赖氨酸/% Lysine	0.91
磷酸氢钙/% Calcium hydrogen phosphate	0.8	蛋氨酸/% Methionine	0.38
氯化钠/% Sodium chloride	0.26	蛋氨酸+胱氨酸/% Methionine+cystine	0.68
预混料[1)]/% Premix[1)]	0.13	苏氨酸/% Threonine	0.72
L-赖氨酸盐酸盐/% L-lysine hydrochloride	0.05		
DL-蛋氨酸/% DL-methionine	0.11		
氯化胆碱/% Choline chloride	0.06		
合计/% Total	100		

[1)].预混料为每kg饲粮提供:维生素A 10 000 IU,维生素D_3 2 500 IU,维生素E 25 IU,维生素K_3 2 mg,维生素B_1 1 mg,维生素B_2 4 mg,维生素B_6 2 mg,维生素B_{12} 0.01 mg,烟酸20 mg,泛酸钙8 mg,叶酸0.95 mg,生物素0.05 mg,铁80 mg,铜8 mg,锰100 mg,锌75 mg,碘2.5 mg,硒0.35 mg。[2)].营养水平为计算值。

[1)].The premix supplied the following per kg of the feed:vitamin A 10 000 IU,vitamin D_3 2 500 IU,vitamin E 25 IU,vitamin K_3 2 mg,vitamin B_1 1 mg,vitamin B_2 4 mg,vitamin B_6 2 mg,vitamin B_{12} 0.01 mg,nicotinic acid 20 mg,calcium pantothenate 8 mg,folic acid 0.95 mg,biotin 0.05 mg,iron 80 mg,copper 8 mg,manganese 100 mg,zinc 75 mg,iodine 2.5 mg and selenium 0.35 mg per kg.[2)]Nutrition levels were the calculated values.

1.4 生长性能

试验期间观察黄羽肉鸡的生长状况。按每重复单位记录黄羽肉鸡的采食量,分别在正式试验第1天和第28天对黄羽肉鸡进行称重,记录黄羽肉鸡的初始体质量和末重,计算分析平均日采食量(average daily feed intake,ADFI)、平均日增重(average daily gain,ADG)和料重比(feed/gain,F/G)。本试验条件下,对比各处理组发现,复合菌发酵豆粕组肉鸡生长性能的改善作用最明显,因此选取对照组、复合菌组肉鸡进行样品采集,以探究禽内源乳杆菌和酵母菌联合发酵豆粕影响肉鸡生长性能的可能机制。

1.5 样品采集与方法

正式试验第31天,分别从对照组、复合菌组中选取10羽接近该组平均体质量的健康黄羽肉鸡,称量活体质量,屠宰后分离肝脏、脾脏、法氏囊、胰腺、腹脂,称量质量,计算免疫器官指数和腹脂率:脏器指数=脏器质量(g)/活体质量(g)×100%;腹脂率=腹部脂质量(g)/活体质量(g)×100%。

屠宰后分离十二指肠、盲肠、回肠、结直肠[29]。从十二指肠中部剪取2段1 cm左右的肠段,浸泡于4%多聚甲醛通用型组织固定液(BL539A,广州科源生物科技有限公司)中。用直尺测量盲肠和结直肠长度。将十二指肠、盲肠、回肠内容物挤出,测定不同肠段内容物的pH。剪开盲肠,用生理盐水清洗2~3遍,吸水纸擦干后取一侧盲肠称取质量,计算盲肠指数:盲肠指数=盲肠质量(g)/活体质量(g)×100 %。取另一侧盲肠刮取黏膜,液氮速冻,保存于−80 ℃。

1.6 十二指肠组织形态测定

将浸泡于4%多聚甲醛通用型组织固定液(BL539A,广州科源生物科技有限公司)中的十二指肠室温固定24 h,送至武汉塞维尔生物科技有限公司进行石蜡包埋、切片及HE染色[30]。在光学显微镜(广州明美科技有限公司)下观测十二指肠绒毛高度(VH)和隐窝深度(CD),用Image J软件统计绒隐比(V/C):绒隐比(V/C)=绒毛高度(μm)/隐窝深度(μm)。

1.7 盲肠黏膜炎症因子mRNA相对表达量

使用RNA提取试剂盒(R4130-02,广州美基生物科技有限公司)从肉鸡盲肠黏膜提取总RNA,然后

使用微量分光光度计(赛默飞世尔科技公司)测定总RNA浓度。按照反转录试剂盒(A0010CGQ,美国EZBioscience公司)的操作步骤将1 μg RNA反转录为cDNA。根据2×Color SYBR Green qPCR Master Mix试剂(ROX2 plus)(A0012-R2,美国EZBioscience公司)的操作说明稀释cDNA和配制反应体系,并在荧光定量PCR仪(美国Stratagene公司)上按照95 °C 30 s(1个循环),95 °C 15 s、60 °C 10 s和72 °C 30 s(40个循环)的反应程序进行检测。实时荧光定量PCR(qPCR)的反应体系为:上游引物、下游引物各0.2 μL,1 μL cDNA,5 μL SYBR Green Master Mix,4.6 μL ddH_2O。为探究发酵豆粕对肠道炎症状况的影响,选取白介素(Interleukin,*IL-1*)基因中*IL-1β*、*IL-2*、*IL-4*、*IL-6*、*IL-8*、*IL-10*,以及干扰素(Interferon,*IFN*)基因中*IFN-α*、*IFN-β*、*IFN-γ*与肿瘤坏死因子α(Tumor necrosis factor,*TNF-α*)等炎症因子相关基因进行盲肠黏膜炎症因子mRNA相对表达量的检测[31-32]。各炎症因子相关基因的mRNA相对表达量均使用β-肌动蛋白(*β-actin*)作为内参基因,用$2^{-\triangle\triangle Ct}$值表示,相关的引物序列见表2。

表2 相关基因与引物信息

Tab.2 Related genes and primer information

基因 Genes	引物序列(5'-3') Primer sequence	PCR产物长度/bp PCR product length	登录号 Accession numbers	退火温度/℃ T_m	参考文献 References
β-actin	F-AAGATCATTGCCCCACCTGA R-AAAGCCATGCCAATCTCGTC	232	NM_205518.1	60.0	-
IL-1β	F-GCTTCATCTTCTACCGCCTG R-ACTTAGCTTGTAGGTGGCGA	161	XM_046931582.1	60.0	[15]
IL-2	F-CAAGAGTCTTACGGGTCTAAATCAC R-GTTGGTCAGTTCATGGAGAAAATC	100	NM_204153.2	60.0	[15]
IL-4	F-GTGCCCACGCTGTGCTTAC R-AGGAAACCTCTCCCTGGATGTC	82	NM_001007079.1	60.0	[15]
IL-6	F-AAATCCCTCCTCGCCAATCT R-CCCTCACGGTCTTCTCCATAAA	106	NM_204628.2	60.0	[15]
IL-8	F-GGCTTGCTAGGGGAAATGA R-AGCTGACTCTGACTAGGAAACTGT	200	NM_205498.2	60.0	[33]
IL-10	F-CAGACCAGCACCAGTCATCA R-TCCCGTTCTCATCCATCTTCTC	163	NM_001004414.2	60.0	[34]
IFN-α	F-TTCAGCTGCCTCCACACCTT R-TTGTGGATGTGCAGGAACCA	101	XM_046936231.1	60.0	[15]
IFN-β	F-TGCAACCATCTTCGTCACCA R-GGAGGTGGAGCCGTATTCTG	77	NM_001024836.2	60.0	[35]
IFN-γ	F-ACACTGACAAGTCAAAGCCGC R-AGTCGTTCATCGGGAGCTTG	129	NM_205149.2	60.0	[35]
TNF-α	F-TGTTCTATGACCGCCCAGTT R-AGCATCAACGCAAAAGGGAA	164	XM_046927265.1	60.0	[15]

1.8 数据统计与分析

试验数据使用Excel 2020进行初步处理,分别使用SPSS Statistics 26.0进行独立样本*t*检验、GraphPad prism 8.30分析及作图。

2 结果与分析

2.1 禽内源乳杆菌及其与酵母菌联合发酵豆粕对黄羽肉鸡生长性能的影响

由表3可知,与对照组相比,添加0.5%复合菌发酵豆粕能显著提高肉鸡平均日增重(*P*<0.05),有提

高采食量的趋势(P=0.053),但对料重比无显著影响(P>0.05)。与对照组相比,日粮添加0.5%鸡内源乳杆菌发酵豆粕有提高肉鸡采食量(P=0.051)和增重(P=0.102)的趋势,但料重比影响不大(P>0.05)。

表3 日粮添加禽内源乳杆菌与酵母菌联合发酵豆粕对黄羽肉鸡生长性能的影响

Tab.3 Effects of dietary addition of endogenous *Lactobacillus* in birds and the co-fermentation with yeast of soybean meal on the growth performance of yellow-feathered broilers

项目 Items	组别 Groups			均值标准误 SEM	P值 P-value	
	对照组 Control	鸡内源乳杆菌组 Chicken endogenous *Lactobacillus* group	复合菌组 Composite bacterial group		鸡内源乳杆菌组 Chicken endogenous *Lactobacillus* group	复合菌组 Composite bacterial group
平均日采食量/($g \cdot d^{-1}$) ADFI	99.44	105.50	105.50	1.228	0.051	0.053
平均日增重/($g \cdot d^{-1}$) ADG	36.94	39.42	40.28	0.596	0.102	0.028
料重比 F/G	2.73	2.69	2.63	0.024	0.616	0.121

P<0.05表示差异显著,0.05<P<0.1表示有趋势,P>0.05表示差异不显著。

P<0.05 indicates significant difference, 0.05<P<0.1 indicates a trend, and P>0.05 indicates no significant difference.

2.2 禽内源乳杆菌和酵母菌联合发酵豆粕对黄羽肉鸡脏器指数的影响

由表4可知,与对照组相比,饲料添加0.5%鸡内源乳杆菌和酵母菌联合发酵豆粕能显著提高肉鸡的腹脂率(P<0.05),有提高肉鸡法氏囊指数的趋势(P=0.059),但对脾脏指数、胰腺指数和肝脏指数无显著影响(P>0.05)。

表4 日粮添加复合菌联合发酵豆粕对黄羽肉鸡脏器指数的影响

Tab.4 Effects of dietary addition of complex bacteria co-fermented soybean meal on the organ indexes of yellow-feathered broilers

项目 Items	对照组 Control	复合菌组 Composite bacterial group	均值标准误 SEM	P值 P-value
肝脏指数/% Liver index	2.29	2.31	0.061	0.833
胰腺指数/% Pancreatic index	0.20	0.21	0.008	0.548
法氏囊指数/% Bursa of fasciola index	0.05	0.10	0.012	0.059
脾脏指数/% Spleen index	0.33	0.29	0.028	0.504
腹脂率/% Abdominal fat rate	0.79	1.18	0.098	0.046

P<0.05表示差异显著,0.05<P<0.1表示有趋势,P>0.05表示差异不显著。

P<0.05 indicates significant difference, 0.05<P<0.1 indicates a trend, and P>0.05 indicates no significant difference.

2.3 禽内源乳杆菌和酵母菌联合发酵豆粕对黄羽肉鸡肠道指数和内容物pH的影响

由表5可知,与对照组相比,饲料添加0.5%复合菌联合发酵豆粕有降低回肠内容物pH(P=0.081)和提高盲肠质量长度比的趋势(P=0.084),对盲肠长度、盲肠指数、结直肠长度、十二指肠内容物pH、盲肠内容物pH均无显著影响(P>0.05)。

2.4 禽内源乳杆菌和酵母菌联合发酵豆粕对黄羽肉鸡十二指形态的影响

由图1可知,与对照组相比,饲料添加0.5%鸡内源乳杆菌和酵母菌联合发酵豆粕对肉鸡十二指肠绒毛高度、隐窝深度和绒隐比无显著影响(P>0.05)。

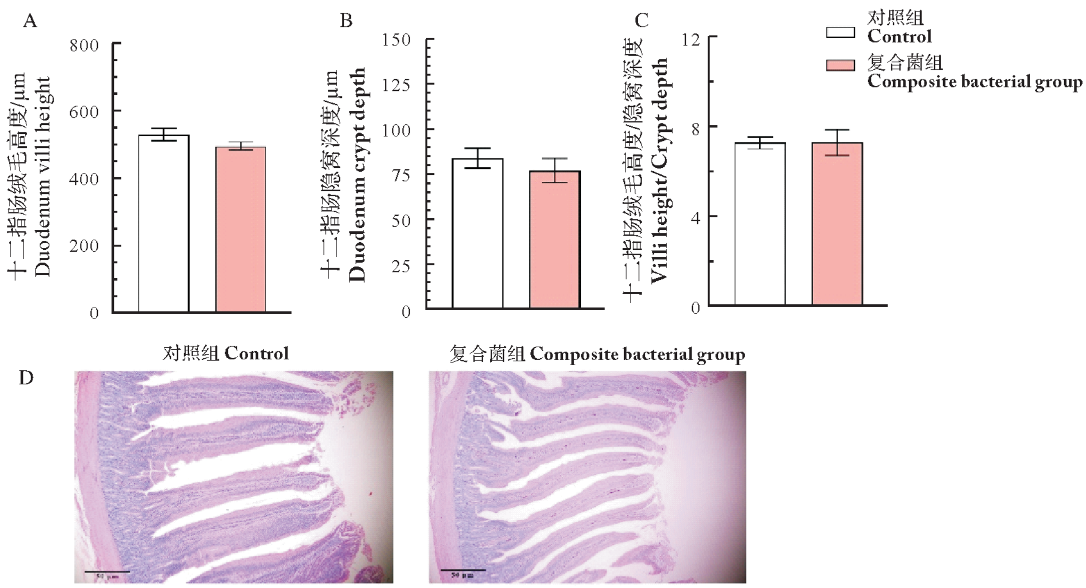
表 5　日粮添加复合菌联合发酵豆粕对黄羽肉鸡肠道指数和内容物 pH 的影响

Tab.5　Effects of dietary addition of complex bacteria co-fermented soybean meal on intestinal indexes and pH of contents of yellow-feathered broilers

项目 Items	对照组 Control	复合菌组 Composite bacterial group	均值标准误 SEM	*P* 值 *P*-value
盲肠长度/cm Cecal length	17.01	15.84	0.407	0.158
结直肠长度/cm Colorectal length	5.61	5.39	0.160	0.505
盲肠指数/% Cecum index	0.36	0.41	0.016	0.131
盲肠质量长度比/($g·cm^{-1}$) Cecum weight/length	0.19	0.23	0.012	0.084
十二指肠内容物 pH Duodenal content pH	5.60	5.56	0.052	0.742
回肠内容物 pH Ileal content pH	6.59	6.40	0.055	0.081
盲肠内容物 pH Cecal content pH	5.61	5.39	0.116	0.505

$P<0.05$ 表示差异显著，$0.05<P<0.1$ 表示有趋势，$P>0.05$ 表示差异不显著。

$P<0.05$ indicates significant difference, $0.05<P<0.1$ indicates a trend, and $P>0.05$ indicates no significant difference.



(A)十二指肠绒毛高度(B)十二指肠隐窝深度(C)十二指肠绒毛高度/隐窝深度(D)对照组和复合菌组十二指肠(比例尺:50 μm)。

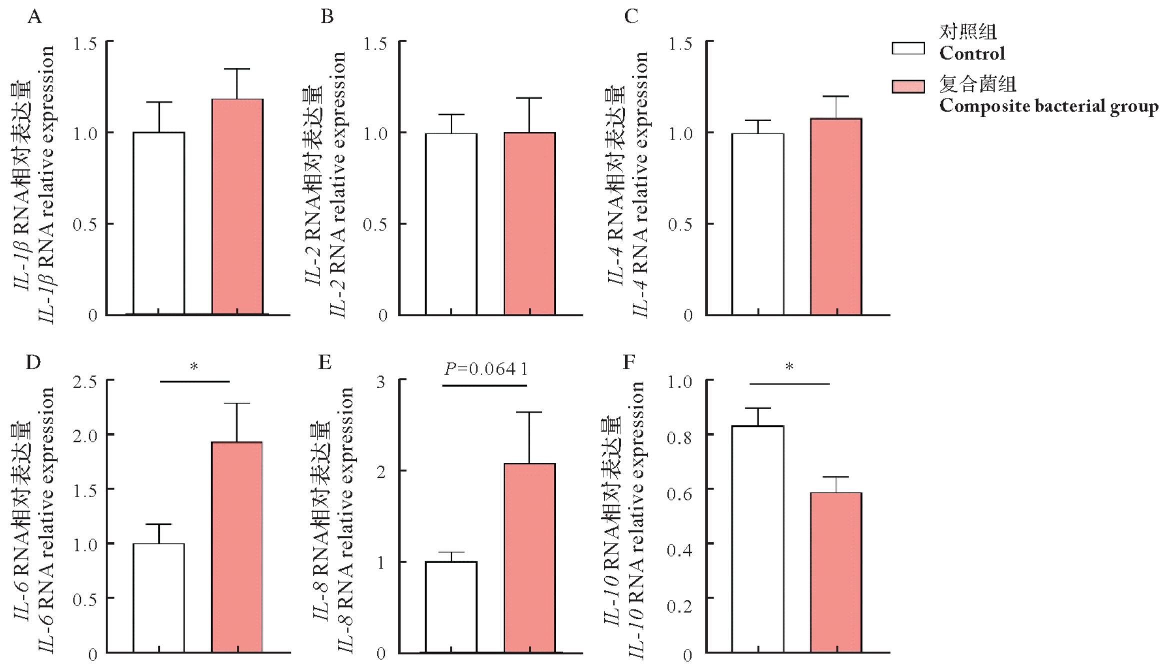
(A)Duodenum villi height of broilers(B)Duodenum crypt depth of broilers(C)Duodenum villi height/crypt depth of broilers (D)Duodenum of control group and compound bacteria group(scale:50 μm).

图 1　日粮添加复合菌联合发酵豆粕对黄羽肉鸡十二指肠形态的影响

Fig.1　Effects of dietary addition of complex bacteria co-fermented soybean meal on duodenal morphology of yellow-feathered broilers

2.5　禽内源乳杆菌和酵母菌联合发酵豆粕对肉鸡盲肠黏膜炎症因子相关基因 mRNA 相对表达量的影响

由图 2 和图 3 可知，与对照组相比，饲料添加 0.5% 鸡内源乳杆菌和酵母菌联合发酵豆粕显著提高 *IL-6* mRNA 相对表达量，显著降低 *IL-10* mRNA 相对表达量，盲肠黏膜 *IL-8* 的 mRNA 相对表达量有提高趋势($P=0.064$)，但对 *IL-1β*、*IL-2*、*IL-4*、*IFN-α*、*IFN-β*、*IFN-γ*、*TNF-α* 的 mRNA 相对表达量没有显著影响($P>0.05$)。



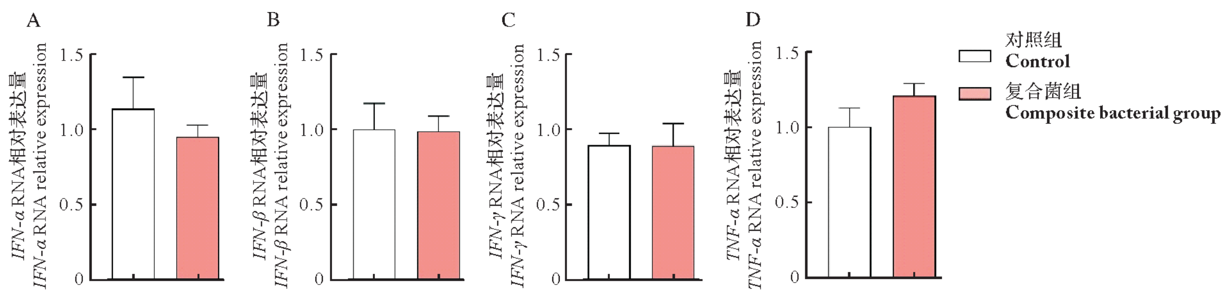
(A)*IL-1β*(B)*IL-2*(C)*IL-4*(D)*IL-6*(E)*IL-8*(F)*IL-10*。*表示差异显著($P<0.05$)。

(A)*Interleukin 1β*(B)*Interleukin* 2(C)*Interleukin* 4(D)*Interleukin* 6(E)*Interleukin* 8(F)*Interleukin* 10.

* indicates significant difference($P<0.05$).

图2 日粮添加复合菌联合发酵豆粕对黄羽肉鸡盲肠黏膜白介素相关基因mRNA相对表达量的影响

Fig.2 Effects of dietary addition of complex bacteria co-fermented soybean meal on mRNA relative expressions of interleukin factor-related genes in cecum mucosa of yellow-feathered broilers



(A)*IFN-α*(B)*IFN-β*(C)*IFN-γ*(D)*TNF-α*(A)*Interferon-α*(B)*Interferon-β*(C)*Interferon-γ*(D)Tumor necrosis factor-α.

图3 日粮添加复合菌联合发酵豆粕对黄羽肉鸡盲肠黏膜干扰素和肿瘤坏死因子相关基因mRNA相对表达量的影响

Fig.3 Effects of dietary addition of complex bacteria co-fermented soybean meal on mRNA relative expressions of interferon and tumor necrosis factor-related genes in cecum mucosa of yellow-feathered broilers

3 讨 论

3.1 禽内源乳杆菌和酵母菌联合发酵豆粕对黄羽肉鸡生长性能的影响

已有大量研究[2,27,36]表明，饲料添加益生菌和酵母菌联合发酵豆粕对畜禽生长性能有改善作用。益生菌通过产生生物活性物质、减少致病菌的数量、改善鸡的胃肠道健康和免疫状态影响肉鸡生长性能[5]。益生菌豆粕发酵过程中可以产生可溶性肽、游离氨基酸、有机酸等生物活性物质，并刺激肠道免疫，促进肠道屏障的建立[7,37-38]。灭活的益生菌、益生菌细胞内组分和裂解产物通过调节机体免疫系统、增加有益菌对肠道细胞黏附维持肠道健康，从而影响动物生长性能[7,39]。本研究显示，与对照组相比，0.5%鸡内源乳杆菌、酵母菌联合发酵豆粕显著提高了肉鸡ADG，有提高ADFI的趋势，0.5%鸡内源乳杆菌发酵豆粕组肉鸡有ADFI提高的趋势，但ADG、F/G均没有显著影响。因此，本试验条件下，鸡内源乳杆菌、酵母菌联合发酵豆粕在肉鸡生长性能方面的应用效果优于单一益生菌发酵豆粕。不同菌种、发酵基质和发酵工艺是影响发酵效果的重要因素，其中不同菌种之间的互作效应是影响发酵豆粕成分的关键因素[6,39-40]。

有研究[41]发现,不同菌株协同发酵效果优于单一菌株,所以菌种互作导致的代谢产物的改变可能是影响本试验复合菌和单一菌发酵豆粕应用效果差别的主要因素。

3.2 禽内源乳杆菌和酵母菌联合发酵豆粕对黄羽肉鸡腹脂率的影响

本研究发现,日粮添加0.5%鸡内源乳杆菌和酵母菌联合发酵豆粕显著提高了肉鸡的腹脂率,但整体腹脂率不超过1.2%,并不影响肉鸡胴体品质。腹部脂肪广泛用于评估肉鸡总脂肪含量[42]。有研究[2]显示,日粮添加3%和6%乳杆菌发酵豆粕有降低肉鸡腹部脂肪质量的趋势,这与本研究结果不一致,推测可能与发酵体系中豆粕的添加量有关。微生物发酵饲料调控宿主脂代谢的机制比较复杂。有研究[42]认为微生物能够参与并调控脂质合成与脂肪酸分解,从而调控腹部脂肪沉积状况。也有研究[36,43]发现,灭活益生菌的发酵豆粕能够调节肠道微生物区系,通过菌群与肠道细胞互作调节脂代谢。肠道微生物一方面通过为宿主提供短链脂肪酸提供能量[44],另一方面也刺激肠道黏膜氧化损伤导致能量浪费[45-46]。这些结果提示本研究所用菌株可能通过改善肠道菌群区系,减少了肠道能量损失,进而使得多余的能量以腹脂形式储存。后续可以考虑在添加发酵豆粕基础上降低日粮能量水平,可以进一步节约日粮成本,研制新型能量节约型日粮。

3.3 禽内源乳杆菌和酵母菌联合发酵豆粕对黄羽肉鸡免疫功能和肠道健康的影响

在机体稳态条件下,白介素在调节机体免疫应答方面起着重要作用[47]。*IL-6*、*IL-8*、*IL-10*分别具有活化T细胞与激活急性免疫的作用、促进中性粒细胞与T细胞趋化和免疫刺激与免疫抑制的作用[48-49]。有研究[50]发现,饲料添加短乳杆菌发酵豆粕显著上调肉鸡空肠*NF-κB*、*IL-1β*、*IL-6*、*IL-10*、*IFN-γ*的mRNA相对表达量。本研究显示,与对照组相比,0.5%鸡内源乳杆菌、酵母菌联合发酵豆粕显著提高盲肠黏膜*IL-6* mRNA相对表达水平,显著降低*IL-10* mRNA相对表达水平,有提高*IL-8* mRNA相对表达水平的趋势,这说明复合菌发酵豆粕可能通过调节肉鸡肠道免疫应答影响机体免疫。不一致的是,本研究中,复合菌发酵组肉鸡盲肠黏膜*IL-10* mRNA相对表达水平显著降低。在肠道中,*IL-10*主要由巨噬细胞、T细胞等细胞产生,其具有双向免疫调节的作用[51-52]。有研究[53]显示,酵母菌β-葡聚糖通过调节回肠微生物菌群组成和代谢,抑制*TLR-NF-κB*信号通路下调蛋鸡回肠*IL-10* mRNA表达量,进而调节肠道免疫状态。因此,灭活的酵母菌可能能够通过细胞壁的β-葡聚糖结构,调节肠道微生物组成与代谢,进而影响肠道对*IL-10*的免疫调控。

法氏囊指数是反映禽类机体免疫功能的重要指标[54-55]。法氏囊是家禽特有的中枢淋巴器官,主要负责机体体液免疫,即B淋巴细胞的成熟和分化[56]。与潘氏结类似的是,法氏囊也属于肠道淋巴相关组织,且涉及抗原的捕获、处理和呈递,以及免疫应答的启动[57]。此外,白细胞介素连接肠道免疫和法氏囊免疫,在机体免疫反应调节的过程中起着重要作用[48,58]。其中*IL-2*、*IL-12*、*IL-15*、*IL-18*、*IL-23*主要影响细胞免疫,*IL-4*、*IL-5*、*IL-6*、*IL-10*、*IL-21*主要影响体液免疫[48,59-60]。研究[60]发现,饲料添加15%、20%、25%植物乳杆菌、枯草芽孢杆菌和酵母菌联合发酵饲料显著提高肉鸡法氏囊指数。本研究显示,饲料添加0.5%鸡内源乳杆菌、酵母菌联合发酵豆粕有提高肉鸡法氏囊指数的趋势,这说明其具有提高肉鸡法氏囊免疫功能的作用。目前,推测发酵豆粕可能存在2种影响机体免疫的机制。一是发酵豆粕通过改善肠道环境和微生物平衡对法氏囊免疫和肠道免疫发挥积极作用,进而影响机体免疫功能[61-62],二是可能是通过细菌本身或其细胞壁组分影响血液免疫球蛋白水平,促进T淋巴细胞和B淋巴细胞的增殖,影响T细胞极化和细胞因子表达,通过激活体液和细胞免疫影响机体免疫功能[63-65]。

肠道长度和相对质量反映了肠道炎症和健康状态[66]。发酵豆粕中含有大量的益生菌或益生元和微生物代谢产物,可以直接或间接地改变肠道微生物菌组成,进而影响肠道内容物pH,并激活肠道免疫[67]。除此之外,肠道pH也与肠道健康状况有关,肠道pH的降低能够抑制肠道内有害菌生长,促进有益菌生长[68]。有研究[54]发现,植物乳杆菌发酵物能显著增加肉鸡肠道乳酸杆菌和双歧杆菌的数量,通过产生乳酸降低肉鸡肠道pH。白细胞介素作为最重要的一类细胞因子,通过参与肠道炎症相关信号通路的激活、影响免疫细胞活性、影响肠道细胞连接、调节肠道上皮细胞分化和黏蛋白分泌等过程,与肠道免疫细胞共同参与炎症反应的调控[60]。本研究中,饲喂0.5%鸡内源乳杆菌、酵母菌联合发酵豆粕有提高肉鸡盲

肠质量长度比、降低回肠内容物 pH 的趋势。这说明乳杆菌发酵豆粕可能通过改变肠道菌群，缓解肠道炎症，进而影响肠道健康。

4 结 论

日粮添加 0.5% 鸡内源乳杆菌和酵母菌联合发酵豆粕在改善肉鸡生产性能方面优于单独鸡内源乳杆菌发酵，且能调节肉鸡机体免疫和肠道健康状况。本研究为禽源益生菌和酵母菌联合发酵豆粕的应用提供了试验依据。

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