

Research Article



Enhancing Growth Performance and Slaughtering Quality in Broiler Chickens through Co-Administration of Distiller's Grains and Chinese Medicinal Herbs

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Abstract:

In pursuit of sustainable livestock practices and the valorization of agro-industrial by-products, this study innovatively combines the utilization of distiller's grains with advancements in herbal agriculture. We investigate the impacts of distiller's grains and Chinese medicinal herbs on the growth performance and slaughter quality of broiler chickens. Our exploration delves into the synergistic relationships between distiller's grains and specific classes of Chinese medicines herbs: Digestive-Promoting Herbs (D-CMHs), Antibacterial & Anti-inflammatory (A-CMHs), Typhoid and Miscellaneous Disease-Fighting (T-CMHs) and Immune-Enhancing (I-CMHs) Herbs, in enhancing feed value. Results indicate that, compared to conventional feed controls, distiller's grains significantly enhance the average weight gain in broiler chickens and have a positive impact on slaughter performance. The efficacy of herbal supplements is contingent upon their specific functional types. D-CMHs and T-CMHs benefits markedly improve production metrics and meat quality. Importantly, there is a synergistic effect between distiller's grains and T-CMHs, while an additive effect is noted with D-CMHs. Moreover, the addition of Chinese medicines herbs across all types does not undermine the positive nutritional effects of distiller's grains. These results underscore the potential for cooperative enhancement of poultry nutrition through the strategic supplementation of distiller's grains and Chinese herbal additives, which is dependent on the herb type. Precise formulation of herb-grain combinations can effectively boost poultry production efficiency and meat quality, driving the resourceful utilization of distiller's grains and the evolution of herbal agriculture.

Keywords: Distiller's Grains; Chinese Medicinal Herbs; Herbal Agriculture; Synergistic Effect; Broiler

Introduction

In the context of global sustainable agriculture, where efficient resource utilization garners increasing attention (Andile et al., 2021), distiller's grains, a byproduct of the fermentation industry with vast global production (Ortiz et al., 2019), emerge as a significant resource. These grains are rich in proteins, fiber, minerals, and B-vitamins (Roth et al., 2019), particularly abundant in lysine and methionine. This composition makes them a valuable protein source for animal feed

(Belyea et al., 2004; Lim et al., 2011; Kitaw et al., 2020). Studies indicate that their judicious inclusion in animal diets can significantly enhance performance. For ruminants, supplementing diets with distiller's grains improves digestibility, production attributes, and beef quality in cattle (Kellen et al., 2019; Yizhao et al., 2019; Viviane et al., 2020). They also boost growth and carcass quality in sheep (Hodges et al., 2019). In monogastric animals, moderate inclusion

enhances slaughter traits (Benz et al., 2010) and offers a viable alternative protein source (Garavito-Duarte et al., 2023) for swine. In poultry, replacing conventional feeds with distiller's grains does not impair daily weight gain or feed efficiency, yet it improves gut health and reduces disease incidence (Zhai et al., 2020). However, high moisture content, susceptibility to spoilage, presence of anti-nutritional factors, and residual alcohol constrain their use in animal feeds (Shad et al., 2021; Sun et al., 2021). Particularly in monogastrics, feeding distiller's grains might offer negligible or even negative effects on performance (Xu et al., 2010). Although efforts are being made to develop effective pretreatment methods like fermentation and enzymatic hydrolysis (Cordeiro et al., 2022; Hayder et al., 2024; Wang et al., 2024) to optimize nutrient content, few studies have explored enhancing the nutritive value of distiller's grains through optimizing feed formulations with natural products.

In the expanding domain of herbs agriculture, Chinese medicinal herbs (CMHs), bearing their intrinsic bioactive constituents and pharmacological attributes, have risen as a crucial element in livestock nutrition (Lin et al., 2020). The integration of CMHs and their extracts into animal diets has been evidenced to substantially augment growth performance and product quality in poultry (Vlaicu et al., 2021; Zoidis et al., 2022), swine (Lin et al., 2020), and (Chen et al., 2003). This enhancement is largely attributable to the multifunctional bioactivities of CMH components. Primarily, CMH bioactives modulate nutrient metabolism in livestock. As an illustration, ultrafine Chinese herbal formulas (CHFAs) and *Astragalus* polysaccharides elevate circulating levels of functional amino acids in pigs (Kong et al., 2007), while arginine found in CMHs catalyzes muscle protein synthesis in broilers and piglets (Tan et al., 2009; Castro et al., 2019). Secondly, CMH supplementation significantly bolsters innate and adaptive immune responses in pigs (Chen et al., 2003; Lin et al., 2020), thereby enhancing resistance to pathogens and overall health. Amidst escalating concerns over antibiotic misuse and environmental pollution in livestock farming, the potential of CMHs as alternatives to antibiotics has gained increased recognition, with their efficacy progressively validated (Abdallah et al., 2018). Lastly, CMHs foster growth and

maintain gut health by strengthening intestinal barrier function, moderating inflammation, and shaping microbiota composition (Zhou et al., 2023). Moreover, CMHs display noteworthy antiparasitic effects in livestock (Jamil et al., 2022). Nonetheless, a lingering question is whether CMHs can amplify the nutritional worth of distiller's grains, a byproduct abundant in proteins, fibers, and trace elements. Supplementing distiller's grains with CMHs possessing specific pharmacological actions could potentially enrich their nutritive profile and health benefits. However, existing research predominantly spotlights a singular or a narrow spectrum of CMHs, neglecting the synergistic effects of complex formulations and the intricacy of CMH interactions (Wang et al., 2012). Identifying suitable combinations or functional categories from the extensive repertoire of CMHs poses a significant challenge that demands further exploration.

Distiller's grains and medicinal herbs (CMHs) abound globally, offering unique prospects for efficient resource cycling and sustainable agriculture, especially in Southwest China. Here, a robust liquor industry churns out massive volumes of distiller's grains (Liu et al., 2022; Ma et al., 2023). Taking Guizhou Province as a case study, where in 2022, standardized baijiu manufacturers produced roughly 290,000 tons of liquor, this translates to an annual distiller's grains output surpassing million tons considering the 3-to-5-ton byproduct ratio per ton of liquor produced. Prudent utilization of these distiller's grains is crucial for the sustainability of the local liquor industry, notably the famed soy sauce-flavored baijiu sector. Concurrently, Southwest China's mountainous landscape, paired with its eclectic climate and soil diversity, nurtures a rich trove of CMHs. Guizhou alone harbors over 7,300 medicinal plant species. In 2022, the province dedicated 529,300 hectares to CMH cultivation, ranking it second in China. The production volume of medicinal herbs amounted to 2.9 million tons, generating a revenue of \$4.0 billion, reflecting a rapidly advancing industry. Beyond sheer quantity, the region emphasizes standardized farming and quality enhancement, setting up numerous Good Agricultural Practice (GAP)-compliant CMH plantations to ensure quality control and safety. In the domain of CMH-facilitated green livestock farming, given the

demonstrated benefits of CMHs in augmenting animal immunity, disease resilience, enhancing meat and egg quality, and minimizing antibiotic dependence, cultivating CMH agriculture, particularly in livestock husbandry, holds substantial potential for propelling eco-friendly livestock product production in this region. The amalgamation of distiller's grains and CMHs opens new avenues for optimizing livestock nutrition, bolstering animal health, and advancing sustainable agricultural practices.

The spotlight of this research is on integrating distiller's grains and Chinese medicinal herbs (CMHs) within the context of broiler chicken husbandry. By focusing on CMHs that serve as digestive aids, inflammation reducers, infection fighters, and immunity boosters, we embark on feeding trials. These trials assess how distiller's grains and CMHs influence the growth and carcass quality of broilers. Underpinning our investigation is the hypothesis that CMHs and distiller's grains, when used together in poultry diets, exhibit a synergistic effect. However, this synergy is conditional upon the functional categories of the CMHs incorporated. The outcomes will serve as a cornerstone for pinpointing efficacious feed compositions that capitalize on the joint advantages of distiller's grains and CMHs for optimized poultry productivity.

1. Materials and Methods

2.1 Experimental Design

The experimental phase was conducted from September 1 to October 11, 2021, at Moutai institute in Guizhou, China. We employed a standard feed (61.5% cornmeal, 32% cooked soybean meal, 2.5% soybean oil, 1.5% limestone, 1.5% dicalcium phosphate, 0.3% sodium chloride, 0.1% choline chloride [50%], 0.1% methionine [98%], 0.5% premix [0.5%]) as the baseline (CK). Variations included the addition of 25% distiller's grains (DG), 1% Digestive-Promoting CMHs (25% tangerine peel, 25% hawthorn, 20% licorice, 20% malt, 10% codonopsis)(D-CMHs), Antibacterial & Anti-inflammatory CMHs (25% woad root, 25% dandelion, 25% wild chrysanthemum, 12.5% honeysuckle, 12.5% astragalus)(A-CMHs), Typhoid & Miscellaneous Disease-Fighting CMHs (25% dandelion, 20% platycodon, 20% sophora root, 20% atractylodes,

15% licorice)(T-CMHs), and Immunity-Boosting CMHs (30% astragalus, 25% purple coneflower, 15% angelica, 10% rehmannia, 10% kudzu, 10% plantain grass)(I-CMHs). Combinations included distiller's grains plus each of the aforementioned CMH categories, including the DG+D-CMHs, DG+A-CMHs, DG+T-CMHs, DG+I-CMHs. In total, there were ten feed formulations. Feed ingredients were purchased commercially, and the sauce-flavored baijiu distiller's grains were sourced from Moutai Industrial Recycling Company, derived from sorghum and wheat fermentation. Ingredients were mixed according to the recipe, pelletized, dried, and stored for feeding. sixteen local Cishui black-boned broiler chicks (males, 20 days old, vaccinated, healthy, weighing 348 ± 40 g) per feed formulation were acquired from the local market for the feeding trial, totaling 80 chicks. Prior to the treatment, all groups were fed the standard diet. From day 25, treatments commenced with feed adjusted to 120g per chick, increasing by 5g every other day, divided equally into three daily feedings at 9 am, 3 pm, and 8 pm. Each chick was tagged and housed on stainless steel wire mesh platforms in individual cages measuring 70 x 70 x 30 cm, equipped with water dispensers and feed troughs. The feeding trials took place in an open veranda-like space that was sheltered from rain but not temperature-controlled. Fresh water was provided daily, and waste and leftover feed were removed regularly. The feeding regime lasted for 35 days.

2.2 Samples and Measurements

Post-initiation of feeding, each bird was weighed and recorded every five days before the morning feed, allowing for the calculation of average daily weight gain based on the elapsed feeding period. Throughout the trial, continuous observation of the birds' activity levels was conducted, and any mortality was promptly addressed: deceased birds were immediately removed from the pens and their numbers documented for mortality rate calculations. At the conclusion of the feeding intervention, humane euthanasia was carried out to ensure a compassionate slaughter. Following rapid scalding and defeathering, along with removal of feet, toes, and beak, the carcasses were drained and weighed to obtain the dressed weight. Subsequent to the excision of the esophagus, intestines, trachea, spleen, pancreas, gallbladder, and reproductive organs, the eviscerated carcasses

were weighed to determine the semi-eviscerated weight. Further, after removing the heart, liver, proventriculus, gizzard, fat, head, and feet, the fully eviscerated carcasses were weighed to ascertain the eviscerated weight. Finally, weights of the wings, legs, leg muscles, and breast muscles were taken, and ratios of wing weight, leg muscle weight, breast muscle weight, and lean meat percentage relative to the eviscerated weight were calculated.

2.3 Data Analysis

Data were analyzed using SPSS statistical software (version 21). One-way analysis of variance (ANOVA) with treatment as fixed factor was performed to compare the differences in body weight before slaughter, average daily gain and carcass quality among treatments. Furthermore, to explore the interactive effects of distiller's grains supplementation and CMH inclusion, a two-factor ANOVA was conducted for all measured indicators, with the four types of CMH additions and the distiller's grains supplementation as fixed factors. The normality and homogeneity of

variances were previously verified; if necessary, natural logarithms (log) of raw data were taken to meet homoscedasticity and normality requirements. Post-hoc multiple comparisons were conducted using Duncan's test following ANOVA.

2. Results

3.1 Growth Performance

Tables 1 reveal that throughout the feeding period, compared to the baseline diet (CK), the inclusion of distiller's grains significantly boosted the average daily weight gain in broiler chickens. Similarly, the addition of Chinese medicinal herbs (CMHs) also led to a significant increase in average daily weight gain, though this was only pronounced for Digestive-Promoting CMHs (D-CMHs) and Typhoid & Miscellaneous Disease-Fighting CMHs (T-CMHs). The weight gain effect of distiller's grains (DG) was notably superior to that of Antibacterial & Anti-inflammatory CMHs (A-CMHs) and Immunity-boosting CMHs (I-CMHs), yet did not differ significantly from D-CMHs and T-CMHs.

Table 1. Effects of Distillery Grains and Chinese Medicines Herbal Additives on Daily Weight Gain (g) in Broiler Chickens. Treatments are Standard Feed (CK), Standard Feed with Distillery Grains (DG), and Standard Feed with Digestive-Promoting Chinese Medicines Herbs (D-CMHs), Antibacterial & Anti-inflammatory Chinese Medicines Herbs (A-CMHs), Typhoid and Miscellaneous Disease-Fighting Chinese Medicines Herbs (T-CMHs), Immune-Boosting Chinese Medicines Herbs (I-CMHs), and the combinations of DG with D-CMHs (DG+D-CMHs), A-CMHs (DG+A-CMHs), T-CMHs (DG+T-CMHs), I-CMHs (DG+I-CMHs). Values (mean $\pm$ S. D.) in a column with different lowercase letters indicate significant differences at $P < 0.05$ level by Duncan.

Treatments	Days after feed treatment						
	10	15	20	25	30	35	Average
CK	2.4 $\pm$ 5.6c	3.4 $\pm$ 6.0bc	-2.2 $\pm$ 6.6bcd	-4.7 $\pm$ 7.8d	-15.2 $\pm$ 29.6c	5.2 $\pm$ 1.6cde	1.4 $\pm$ 3.0e
DG	12.1 $\pm$ 4.9ab	12.5 $\pm$ 5.3a	12.2 $\pm$ 4.7a	1.2 $\pm$ 8.8cd	6.3 $\pm$ 6.0b	-3.1 $\pm$ 10.4de	6.9 $\pm$ 3.1cd
D-CMHs	10.1 $\pm$ 6.5ab	-1.0 $\pm$ 5.4b	-9.6 $\pm$ 25.5d	-4.7 $\pm$ 9.8d	4.6 $\pm$ 13.7b	21.0 $\pm$ 17.2ab	3.7 $\pm$ 2.0de
A-CMHs	3.3 $\pm$ 4.4c	3.9 $\pm$ 4.6bc	-3.3 $\pm$ 4.2cd	4.7 $\pm$ 7.1bc	-0.7 $\pm$ 14.5b	-7.1 $\pm$ 6.6e	0.9 $\pm$ 2.1e
T-CMHs	10.6 $\pm$ 2.9ab	9.1 $\pm$ 4.3ab	0.7 $\pm$ 5.9bcd	4.0 $\pm$ 6.1bc	3.6 $\pm$ 6.9b	9.4 $\pm$ 13.6bcd	6.2 $\pm$ 2.2cd
I-CMHs	5.8 $\pm$ 4.2bc	2.7 $\pm$ 4.6bc	-7.7 $\pm$ 6.3d	4.8 $\pm$ 7.1bc	1.8 $\pm$ 13.3b	-3.1 $\pm$ 5.6de	0.8 $\pm$ 2.4e
DG+D-CMHs	6.5 $\pm$ 7.4bc	14.0 $\pm$ 9.4a	12.4 $\pm$ 8.6a	12.3 $\pm$ 9.1ab	23.9 $\pm$ 7.2a	14.5 $\pm$ 12.8abc	13.9 $\pm$ 4.5b
DG+A-CMHs	12.2 $\pm$ 5.9ab	14.5 $\pm$ 3.6a	7.4 $\pm$ 6abc	5.8 $\pm$ 6.7bc	4.7 $\pm$ 5.4b	6.2 $\pm$ 4.8cde	8.5 $\pm$ 3.1c
DG+T-CMHs	11.6 $\pm$ 7.5ab	13.7 $\pm$ 10.4a	13.6 $\pm$ 5.1a	18.5 $\pm$ 4.9a	22.3 $\pm$ 17.2a	25.5 $\pm$ 22.7a	17.5 $\pm$ 4.7a
DG+I-CMHs	15.5 $\pm$ 4.2a	12.6 $\pm$ 5.5a	8.1 $\pm$ 5.6ab	10.8 $\pm$ 6.9ab	7.4 $\pm$ 5b	-1.3 $\pm$ 3.3de	8.8 $\pm$ 2.8c

<i>F</i>	4.655*	6.337*	5.978*	6.931*	4.968*	5.885*	22.215*
<i>P</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

When distiller's grains were supplemented alongside Digestive-Promoting (DG+D-CMHs) or Typhoid & Miscellaneous Disease-Fighting (DG+T-CMHs) CMHs, the average daily weight gain saw a marked uplift compared to DG alone, suggesting an additive effect between these CMHs and distiller's grains for weight gain promotion. However, no interaction effect was observed between distiller's grains and Digestive-Promoting CMHs, while a significant mutual promotion effect was detected when distiller's grains were combined with Typhoid & Miscellaneous Disease-Fighting CMHs. In contrast, the average daily weight gain of chickens fed distiller's grains in conjunction with Anti-inflammatory CMHs (DG+A-CMHs) or Immunity-boosting CMHs (DG+I-CMHs) did not differ significantly from those fed distiller's grains alone, indicating no additive or interactive effect between distiller's grains and these CMHs. Consequently, at the end

of the feeding period, broilers that received distiller's grains in combination with digestive enhancers or fever-reducing herbs showed a significant weight increase of 81.48% and 87.67%, respectively ($F = 11.750$, $P < 0.001$; Fig. 1A). Notably, the weight gain effect of distiller's grains was more pronounced during the initial feeding phase (within the first 20 days) and diminished or became non-significant in the latter half (days 20-35). The impact of CMH addition, however, did not show a clear pattern over time. Remarkably, the concurrent addition of digestive enhancers or fever-reducing herbs with distiller's grains consistently exhibited a significant weight gain effect across all feeding stages. Beyond weight gain, the incorporation of distiller's grains and CMHs also reduced mortality rates. At the feeding period's end, the baseline diet group had a mortality rate of 37.5%, whereas other treatments maintained mortality below 25% (Fig. 1B).

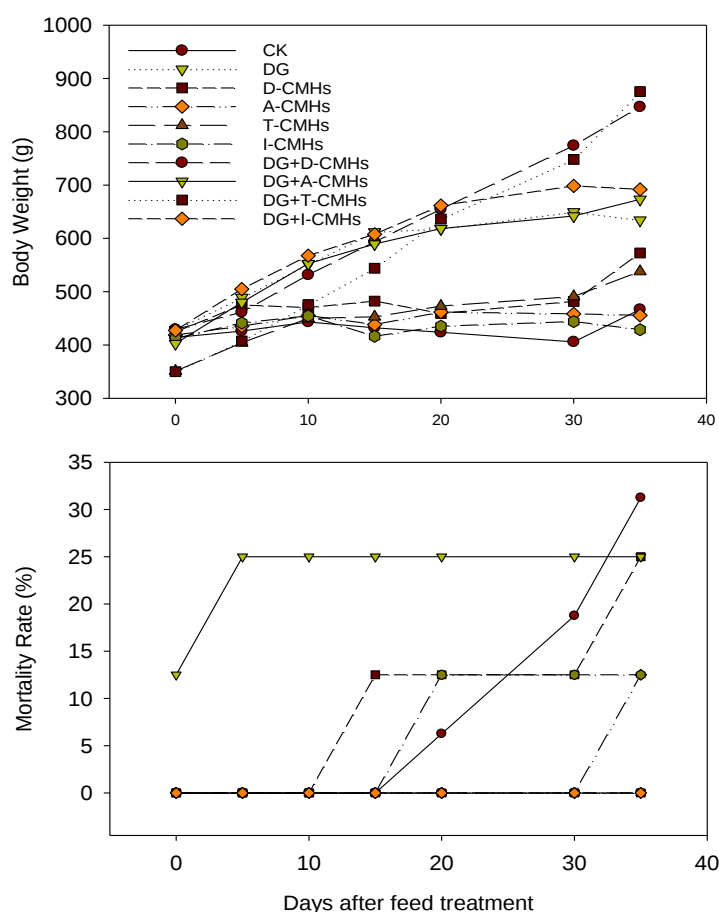


Figure 1 Effects of distillery grains and chinese herbal additives on the growth dynamics (A) and mortality rate (B) of broiler chickens. Treatments are Standard Feed (CK), Standard Feed with Distillery Grains (DG), and Standard Feed with Digestive-Promoting Chinese Medicines Herbs (D-

CMHs), Antibacterial & Anti-inflammatory Chinese Medicines Herbs (A-CMHs), Typhoid and Miscellaneous Disease-Fighting Chinese Medicines Herbs (T-CMHs), Immune-Boosting Chinese Medicines Herbs (I-CMHs), and the combinations of DG with D-CMHs (DG+D-CMHs), A-CMHs (DG+A-CMHs), T-CMHs (DG+T-CMHs), I-CMHs (DG+I-CMHs). Values are mean $\pm$ S.D. for body weight and only mean for mortality.

3.2 Carcass Quality

Tables 2 and 3 illustrate the impacts of distiller's grains and CMH supplementation on the slaughtering quality of broiler chickens. Compared to the control, the addition of distiller's grains (DG) significantly enhanced the dressed weight, semi-eviscerated weight, eviscerated weight, leg weight, leg muscle weight, and eviscerated weight ratio, with no significant effect on wing weight, breast muscle weight, slaughter yield, wing ratio, leg muscle ratio, breast muscle ratio, and lean meat ratio. Meanwhile, CMH inclusion did not significantly affect dressed weight, semi-eviscerated weight ratio, eviscerated weight ratio, wing weight, leg weight, leg muscle weight, breast muscle weight, slaughter yield, and breast muscle ratio. However, the addition of Digestive-Promoting CMHs (D-CMHs) significantly increased the semi-eviscerated weight ratio, wing ratio, and leg muscle ratio, and Typhoid & Miscellaneous Disease-Fighting CMHs (T-CMHs) significantly improved the semi-eviscerated weight ratio. The dressed weight, semi-eviscerated weight, eviscerated

weight, leg weight, leg muscle weight, eviscerated weight ratio, and leg muscle ratio of broilers fed distiller's grains did not differ significantly from those fed digestive enhancers or fever-reducing herbs, but were significantly higher than those fed antimicrobial or immune-boosting herbs (except for wing ratio, leg muscle ratio, and breast muscle ratio). Crucially, no interaction effects were observed between distiller's grains and the four types of CMHs on all slaughtering quality-related metrics. Yet, when distiller's grains were combined with Digestive-Promoting CMHs (DG+A-CMHs) or Typhoid & Miscellaneous Disease-Fighting CMHs (DG+T-CMHs), the dressed weight, semi-eviscerated weight, eviscerated weight, wing weight, leg weight, leg muscle weight, and eviscerated weight ratio were significantly greater than when either was administered alone, indicating an additive effect on slaughtering quality. These findings underscore that the addition of distiller's grains in conjunction with digestive enhancers and fever-reducing herbs can elevate slaughtering quality, with a cumulative benefit when applied concurrently.

Table 2. Effects of distillery grains and chinese herbal medicine addition on broiler chicken carcass quality. Treatments are Standard Feed (CK), Standard Feed with Distillery Grains (DG), and Standard Feed with Digestive-Promoting Chinese Medicines Herbs (D-CMHs), Antibacterial & Anti-inflammatory Chinese Medicines Herbs (A-CMHs), Typhoid and Miscellaneous Disease-Fighting Chinese Medicines Herbs (T-CMHs), Immune-Boosting Chinese Medicines Herbs (I-CMHs), and the combinations of DG with D-CMHs (DG+D-CMHs), A-CMHs (DG+A-CMHs), T-CMHs (DG+T-CMHs), I-CMHs (DG+I-CMHs). Values (mean $\pm$ S.D.) in a column with different lowercase letters indicate significant differences at $P < 0.05$ level by Duncan.

Treatments	Slaughter Weight (g)	Semi-Eviscerated Weight (g)	Eviscerated Weight (g)	Wing Weight (g)	Leg Weight (g)	Leg Muscle Weight (g)	Breast Muscle Weight (g)	Dressing Percentage	Half-Eviscerated Dressing Percentage	Fully Eviscerated Dressing Percentage	Wing Ratio (%)	Leg Muscle Ratio (%)	Breast Muscle Ratio (%)	Lean Meat Ratio (%)
CK	420.3 $\pm$ 131.4d	345.8 $\pm$ 14c	267.5 $\pm$ 98.0c	36.8 $\pm$ 12.7d	75.0 $\pm$ 25.4c	44.3 $\pm$ 21.6e	42.0 $\pm$ 21.2b	87.3 $\pm$ 4.2a	71.6 $\pm$ 5.8de	54.8 $\pm$ 4.6df	13.8 $\pm$ 1.0bc	28.8 $\pm$ 4.7c	13.6 $\pm$ 6.6a	26.7 $\pm$ 9.8a
DG	634.5 $\pm$ 99.2bc	523.9 $\pm$ 8.2b	428.4 $\pm$ 78.0b	50.0 $\pm$ 10.6b	119.0 $\pm$ 21.7ab	68.6 $\pm$ 13.1b	47.8 $\pm$ 12.8b	89.1 $\pm$ 1.7a	73.5 $\pm$ 3.7cde	60 $\pm$ 3.1bc	11.6 $\pm$ 0.7bc	27.8 $\pm$ 1.5c	11.0 $\pm$ 1.4a	27.1 $\pm$ 2.1a

D-CMHs	514.2± 89.0cd	446.5±8 9.0bc	347.7 ±78.1 bc	46.8± 6.4bc d	94.8± 24.7b c	52.8± 14.6d e	34.3 ±22. 8b	89.7 ±2.7 a	77.6±3 .7ab	60.2± 5.1bc	13.8 ±2.2 bc	27. 2±1 .9c	9.3± 4.6a	24.7± 6.3a
A-CMHs	397.6± 43.1d	320.4±3 5.3c	245.4 ±31.1 c	39.2± 4.5d	81.2± 12.0c	42.0± 7.6e	25.2 ±11. 2b	86.9 ±3.4 a	70.0±2 .0e	53.6± 2.4ef	16.0 ±1.2 a	33. 1±2 .0b	9.9± 3.4a	27.0± 4.3a
T-CMHs	500.6± 84.7cd	420.3±7 7.0bc	329.8 ±70bc	46.0± 8.1cd	93.1± 17.3b c	59.0± 15.9c de	42.6 ±17. 2b	88.3 ±2.2 a	74.0±3 .1bcd	57.8± 3.9bc de	14.1 ±1.5 b	28. 4±1 .4c	12.6 ±2.6 a	30.3± 3.1a
I-CMHs	445.2± 61.8d	375.2±6 6.2c	266.0 ±51.8 c	40.4± 7.0d	95.6± 17.1b c	51.0± 13.2d e	32.0 ±12. 6b	87.7 ±2.8 a	73.7±5 .5cde	52.1± 4.8f	15.3 ±0.6 ab	36. 2±3 .8a	11.7 ±2.7 a	30.7± 4.0a
DG+D-CMHs	754.1± 126.5a b	685.9±1 17.9a	548.4 ±99.8 a	62.0± 12.4a b	146.1 ±36.2 a	82.4± 21.5a b	50.0 ±15. 3b	89.1 ±0.6 a	81.0±1 .0a	64.7± 4.2a	11.5 ±2.0 d	26. 7±4 .5c	9.2± 2.3a	24.3± 5.3a
DG+A-CMHs	619.7± 128.7b c	514.5±1 11.5b	413.8 ±99.6 b	56.0± 10.9a bc	133.8 ±33a	76.5± 23.7a bc	44.3 ±16. 3b	88.5 ±1.7 a	73.4±1 .1cde	58.8± 2.0bc d	13.7 ±0.9 bc	32. 3±0 .8b	10.6 ±2.5 a	28.9± 2.8a
DG+T-CMHs	820.5± 160.1a	691.0±1 42.4a	565.0 ±121. 9a	69.4± 18.4a	151.6 ±35.9 a	97.0± 20.9a	73.6 ±25. 2a	88.9 ±0.9 a	74.8±1 .9bcd	61.1± 2.0ab	12.2 ±0.9 dc	26. 8±1 .2c	12.8 ±1.7 a	30.0± 2.4a
DG+I-CMHs	632.4± 154.4b c	539.9±1 41.8b	399.5 ±121. 3b	60.5± 17.1a bc	130.0 ±33.9 a	73.8± 22.0b cd	47.6 ±22. 0b	89.3 ±1.5 a	76.0±1 .2bc	55.8± 2.7cd ef	15.2 ±0.8 ab	32. 8±1 .8b	11.5 ±1.7 a	30.0± 2.0a
<i>F</i>	9.448*	9.593*	9.610*	5.078*	6.067*	6.000*	3.406*	0.980	6.708	7.418	9.435*	10.311*	1.708	2.039
<i>P</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	0.467	<0.001	<0.001	<0.001	<0.001	0.109	0.052

Table 3 Variation analysis of distillery grains and chinese medicine herbs addition on broiler chicken on body weight after feed treatment (BW), average daily gain (ADG) and carcass quality (SW: Slaughter Weight; SEW: Semi-Eviscerated Weight; EW: Eviscerated Weight; WW: Wing Weight; LW: Leg Weight; LMW: Leg Muscle Weight; BMW: Breast Muscle Weight; DP: Dressing Percentage; HDP: Half-Eviscerated Dressing Percentage; FEDP: Fully Eviscerated Dressing Percentage; WR: Wing Ratio; LMR: Leg Muscle Ratio; BMR: Breast Muscle Ratio; LMR: Lean Meat Ratio). Sources of Variation are the Distillery Grains (DG), Digestive-Promoting Chinese Medicines Herbs (D-CMHs), Antibacterial & Anti-inflammatory Chinese Medicines Herbs (A-CMHs), Typhoid and Miscellaneous Disease-Fighting Chinese Medicines Herbs (T-CMHs), Immune-Boosting Chinese Medicines Herbs (I-CMHs). DFT mean Days after feed treatment. * and NS indicate significant and no differences at $P < 0.05$ level, respectively.

Parameters	DF T	Sources of Variation											
		DG versus D-CMHs			DG versus A-CMHs			DG versus T-CMHs			DG versus I-CMHs		
		DG	D-CMHs	Interaction	DG	A-CMHs	Interaction	DG	T-CMHs	Interaction	DG	I-CMHs	Interaction
BW	35	*	*	NS	*	NS	NS	*	NS	NS	*	NS	NS
ADG	10	NS	NS	NS	*	NS	NS	*	NS	*	*	NS	NS
	15	*	NS	NS	*	NS	NS	*	NS	NS	*	NS	NS
	20	*	NS	NS	*	NS	NS	*	NS	NS	*	*	NS
	25	*	NS	NS	NS	*	NS	*	*	NS	*	*	NS
	30	*	*	NS	*	NS	NS	*	*	NS	*	NS	NS
	35	NS	*	NS	NS	NS	NS	NS	*	*	NS	NS	NS
	Average	*	*	NS	*	NS	NS	*	*	*	*	NS	NS

	ge												
SW	----	*	*	NS	*	NS	NS	*	*	NS	*	NS	NS
SEW	----	*	*	NS	*	NS	NS	*	*	NS	*	NS	NS
EW	----	*	*	NS	*	NS	NS	*	*	NS	*	NS	NS
WW	----	*	*	NS	*	NS	NS	*	*	NS	*	NS	NS
LW	----	*	NS	NS	*	NS	NS	*	*	NS	*	NS	NS
LM W	----	*	NS	NS	*	NS	NS	*	*	NS	*	NS	NS
BM W	----	NS	NS	NS	NS	NS	NS	*	NS	NS	*	NS	NS
DP	----	NS	NS	NS	NS	NS	NS	*	*	NS	*	NS	NS
HED P	----	*	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
FED P	----	*	*	NS	*	NS	NS	*	NS	NS	NS	NS	NS
WR	----	*	NS	NS	*	*	NS	*	NS	NS	NS	NS	NS
LM R	----	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS
BM R	----	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
LM R	----	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

3. Discussion

Our findings highlight the potent capability of sauce-flavored baijiu distiller's grains to markedly enhance the average weight gain in broiler chickens, alongside improvements in slaughtering quality. The effects of Chinese medicinal herbs (CMHs) supplementation, however, were contingent on the specific type of herb used. Notably, the inclusion of digestive enhancers and fever-reducing herbs proved effective in boosting both production performance and slaughtering quality, exhibiting an additive effect when combined with distiller's grains. Moreover, fever-reducing herbs in conjunction with distiller's grains displayed a synergistic interaction, characterized by mutually reinforcing effects. This discovery offers innovative formulation strategies for the utilization of distiller's grains in feed, holding considerable value in regions with thriving baijiu industries, particularly in Southwest China. Simultaneously, it provides fresh theoretical foundations for CMH agriculture, specifically regarding their incorporation into animal feeds. Given the substantially larger consumption of CMHs in feed compared to medicinal purposes, our research catalyzes the

exploitation and development of CMH resources. Moreover, the integrated use of distiller's grains and CMHs advances greener poultry farming practices, reduces reliance on veterinary drugs, diminishes the environmental burden posed by distiller's grains in baijiu production areas, and consequently fosters regional agricultural sustainability.

As a byproduct of the distilling industry, distiller's grains from baijiu production are recognized for their rich protein, energy, and micronutrient content, garnering attention for their application in the feed sector, particularly in broiler chicken farming (Jang et al., 2022; He et al., 2023). Extensive studies have explored the effects and mechanisms of distiller's grains derived from major feedstocks such as corn (Abudabos et al., 2017), sorghum (Mohammad et al., 2013), wheat (Bolarinwa and Adeola, 2012), and rice (Om et al., 2019)) on poultry feeding. Evidence supports that utilizing distiller's grains as alternative or supplementary feed ingredients can significantly enhance broiler growth performance (Abudabos et al., 2017; Jessica et al., 2020), improve meat quality encompassing carcass traits, color, tenderness, and water retention (Om et al., 2018; Shim et al., 2018), increase lean meat ratio,

improve bone density without raising fat content (Castro et al., 2019), largely attributed to their capacity to promote cecal microbiota in broilers (Abudabos et al., 2017; Jazi et al., 2017; Kim et al., 2018), reduce oxidative stress, and mitigate intestinal damage by alleviating inflammation (Barekatin et al., 2013), thus improving nutrient digestibility, notably amino acids (Kim et al., 2018). Furthermore, yeast derivatives in distiller's grains can bolster the immune response and enhance resistance to pathogens in broilers (Alizadeh et al., 2016; Alizadeh et al., 2016). In our study, supplementing baijiu distiller's grains significantly enhanced broiler growth and slaughtering quality. Diverging from previous studies, our results revealed a striking 390% increase in weight gain compared to the control group, markedly exceeding findings reported elsewhere (Kim et al., 2018; Jessica et al., 2020). This heightened gain can be attributed to the challenging conditions in our open-air feeding environment, characterized by harsh nighttime temperatures and high humidity (no data), which induced negative weight gains in the control group. Distiller's grains may have mitigated these adverse effects, preventing significant weight loss. Additionally, the superior quality of sauce-flavored baijiu distiller's grains, produced from sorghum and wheat through multiple rounds of high-temperature fermentation, contributed to the exceptional weight gain due to their higher crude protein, crude fat, soluble sugar, free amino acid, and antioxidant content (Yang et al., 2023). It is important to note, however, that while high-protein distiller's grains enhance nutritional value, they might also increase nutrient excretion, necessitating a balance between environmental concerns and economic viability (Applegate et al., 2009). Indeed, excessively high inclusion rates can negatively impact growth performance (Damasceno et al., 2020). Thus, further investigation is warranted to determine the optimal feeding level of sauce-flavored baijiu distiller's grains in broiler chicken farming.

In the quest for sustainable and efficient modern livestock production, the application of CMHs in animal feed has emerged as a promising avenue, albeit with limited exploration into the differentiated effects of various CMHs categories. Grounded in traditional Chinese medicine principles, our study selected Digestive-Promoting, Antibacterial & Anti-inflammatory,

Typhoid & Miscellaneous Disease-Fighting, and Immunity-boosting CMHs for feeding trials, revealing positive impacts on broiler growth performance and health, particularly from Digestive-Promoting and Typhoid & Miscellaneous Disease-Fighting CMHs. Digestive-Promoting CMHs, featuring ingredients like tangerine peel (*Citrus reticulata*) and hawthorn (*Crataegus pinnatifida*), have been long-standing staples in traditional Chinese medicine for promoting digestion. Modern research underscores the dual regulatory effect of tangerine peel on gastrointestinal motility, its beneficial modulation of gut microbiota, and protective action against oxidative stress and inflammation through bioactive components such as naringin (Shi et al., 2024). Terpenoids, flavonoids, and alkaloids in tangerine peel are key bioactive constituents with broad pharmacological activities, including anticancer, anti-inflammatory, immunomodulation, hepatoprotective, cardiovascular benefits, antioxidation, antimicrobial, and neuroprotective effects (Zhang et al., 2024). Similarly, anthocyanins in hawthorn exert notable beneficial effects on gut microbiota and colonic phenolic acids (Han et al., 2023), supporting the observed improvement in broiler performance following the administration of digestive enhancers. By enhancing digestive tract physiology, these herbs directly facilitate nutrient absorption and indirectly bolster overall health, fostering an environment conducive to rapid growth. Typhoid & Miscellaneous Disease-Fighting CMHs like dandelion (*Taraxacum mongolicum*) and platycodon (*Platycodon grandiflorus*), traditionally used in traditional Chinese medicine for respiratory ailments, contain phenolics and flavonoids that demonstrate potent antimicrobial activity against *Streptococcus pneumoniae* and *Haemophilus influenzae*, responsible for respiratory infections, while also modulating the immune system, notably augmenting Th1 responses (Sun et al., 2018). Platycodon's saponins inhibit key inflammatory mediators and cytokines, alleviating airway inflammation and reducing cough reflex sensitivity (Lee et al., 2020). These pharmacological actions align with the observed reduction in mortality and enhanced disease resistance in our study. Notably, antimicrobial and anti-inflammatory, as well as immunostimulatory CMHs, did not significantly affect broiler

performance in our study, highlighting the complexity of CMH effects. This could be due to the absence of specific pathogen challenges or the robust innate defenses of the broilers. Whether these CMHs should be excluded as feed additives in practical applications requires further investigation through large-scale feeding trials.

An important highlight of our study is the elucidation of the synergistic effects between fever-reducing herbs and distiller's grains supplementation. In concert, these additives not only enhanced broiler growth performance but also significantly improved slaughtering quality, achieving dual goals of meat quality enhancement and productivity boost. This discovery of complementary mechanisms opens new avenues for the formulation of composite feeds, suggesting that judicious selection and combination of herbs can synergize with distiller's grains to elevate feed nutritional value and formulate novel eco-friendly and efficient feedstuffs. Certainly, the underlying nutritional interplay between distiller's grains and fever-reducing herbs warrants deeper investigation. Our data indicate that while standalone distiller's grains positively impact weight gain in the early stages (up to 20 days), their efficacy wanes or becomes negligible thereafter, suggesting the presence of mycotoxins and antinutritional factors (ANFs) in distiller's grains (Zhang *et al.*, 2009), which limit their long-term inclusion. However, in conjunction with herbs, the diminishing effects of late-stage distiller's grains are notably mitigated, hinting at the potential of active compounds in Typhoid and Miscellaneous Disease-Fighting CMHs to neutralize or inhibit ANFs in distiller's grains, or alleviate their toxic effects. Extensive research has explored strategies to mitigate ANFs in distiller's grains and enhance feed utilization, such as physical drying (Dzuman *et al.*, 2016), preservative addition (Patience *et al.*, 2014), and fungal fermentation (Xiao Suna, 2021). Our study contributes to this discourse from the perspective of formula optimization and herbs agriculture, offering novel insights into ANF inhibition. Beyond ANF mitigation, the interplay between distiller's grains and Typhoid and Miscellaneous Disease-Fighting CMHs may stem from other mechanisms, such as the enhanced gut environment facilitated by distiller's grains promoting better absorption and utilization of herbal bioactives, or herbs bolstering host

immunity and pathogen suppression, further optimizing gut health and facilitating the assimilation of beneficial nutrients from distiller's grains. Beyond the synergy between distiller's grains and Typhoid & Miscellaneous Disease-Fighting CMHs, we also observed additive effects with Digestive-Promoting CMHs, while Antibacterial & Anti-inflammatory CMHs and Immunity-boosting CMHs did not influence broiler performance under distiller's grains supplementation. This suggests that although these herbs do not potentiate the feed efficacy of distiller's grains, there is no antagonism between them, alleviating concerns about herb inclusion in distiller's grains-based feeds. However, the cost-effectiveness of adding herbs must be considered in relation to their contribution to poultry production enhancement, duration of use, and other factors, warranting future research attention. Furthermore, employing advanced methodologies such as molecular biology and metabolomics to unravel the intricate synergistic regulation of gut microbiota, metabolic pathways, and immune systems by composite feeds containing herbs and distiller's grains is imperative for a comprehensive understanding of their effects on broiler chickens.

4. Conclusion

Our study substantiates the potent ability of distiller's grains to accelerate growth rates and enhance carcass characteristics in broiler chickens, with the effects of Chinese medicinal herbs (CMHs) contingent upon their functional classifications. Digestive-Promoting CMHs and Typhoid & Miscellaneous Disease-Fighting CMHs were found to significantly bolster production performance and slaughtering quality, whereas Antibacterial & Anti-inflammatory CMHs and Immunity-boosting CMHs had no discernible impact. Notably, a synergistic effect was observed between distiller's grains and Typhoid & Miscellaneous Disease-Fighting CMHs, while an additive effect was noted with Digestive-Promoting CMHs. Importantly, CMH supplementation did not compromise the beneficial nutritional effects of distiller's grains, regardless of the herb category. This finding underscores the potential of tailored combinations of distiller's grains and CMHs to optimize avian nutrition, fostering synergistic and sustainable advancements in poultry farming, the baijiu

industry, and CMH agriculture. Future investigations should delve into the nutritional interactions between distiller's grains and CMHs, particularly focusing on their modulation of gut microbiota and stimulation of immune responses. Moreover, refining herbal formulations and optimizing dosages could unlock the full potential of this integrative approach, paving the way for more efficient and environmentally friendly livestock production systems.

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Statement of Animal Rights

Statement on Ethical Approval and Animal Welfare

The research involving animal subjects reported in this manuscript has been conducted in strict compliance with international ethical standards and guidelines for the care and use of laboratory animals. Specifically, our study adhered to the principles outlined in the 'Guiding Principles in the Care and Use of Animals'.

Minimization of Animal Suffering

Throughout the study, every effort was made to minimize pain, distress, and discomfort to the animals. We utilized appropriate anesthetic, analgesic, and euthanasia techniques as detailed in the methods section. Moreover, we strictly followed the [3Rs principle] (Replacement, Reduction, Refinement) to limit the number of animals used while maintaining the scientific validity of our findings.

Animal Welfare Measures

Poultry were meticulously maintained under conditions optimized to ensure their health and welfare, encompassing secure shelter that safeguarded them against environmental adversities such as rainfall. They were provided with continuous access to a nutritious diet and fresh water *ad libitum*, facilitating their physiological needs without restriction. Professional veterinary oversight was a routine part of their care regimen, complemented by diligent daily monitoring of each animal's health status to promptly address any potential health concerns.

Ethical Approval

All experimental protocols and procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Moutai Institute. This ensures that our research meets the standards of animal welfare and ethical consideration.

Conflict of Interest Statement:

The authors declare no conflict of interest.

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Authors' contributions

Kaixian Wu, Mo Chen and Hongli Yang contributed to the study conception and design. Material preparation, data collection were performed by Kaixian Wu, Haiyan Zhang, Guang Zeng. Analysis were performed by Mo Chen, Changbing Pan, Xinhong Liu and Hongli Yang. The first draft of the manuscript was written by Mo Chen and Kaixian Wu. Manuscript was reviewed and edited by Haiyan Zhang, Xiaoming Liu, Guang Zeng and Hongli Yang. All authors commented on previous versions of the manuscript and approved the final manuscript.

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