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RESEARCH ARTICLE

Effects of Probiotics and Chinese Herbal Additives on Body Weight, Antioxidant Status and Fecal Microbiota in Xizang Chickens

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A B S T R A C T

The Xizang chicken is an important indigenous poultry breed in high-altitude regions; however, its growth is often constrained by environmental stress under intensive cage-rearing conditions. This study evaluated the effects of dietary probiotics, Chinese herbal additives, and their combination on body weight, serum antioxidant and biochemical indices, and gut microbiota composition in Xizang chickens. In this 42-day experiment, 420 one-day-old chicks were randomly assigned to seven treatment groups, including control, probiotic, herbal, and combination treatments. Our findings demonstrated that all the dietary treatments had a significant beneficial effect on body weight, as evidenced by the increase in body weight at 28 and 42 days; the low-dose combination group (MIX1) showed the most significant growth-promoting effect in the early growth period (7-21 days). In addition, the antioxidant analysis revealed that the high dosage of the herbal group (ZY2) significantly increased glutathione (GSH) levels, whereas the low dosage of the probiotic group (YSJ1) significantly elevated the levels of superoxide dismutase (SOD) and total antioxidant capacity (T-AOC). Moreover, microbiota profiling demonstrated a significantly different gut microbiota composition, such as an increase in *Limosilactobacillus* in the combination group and a decrease in the abundance of *Enterococcus* in the herbal treatment. These results suggest that probiotics and Chinese herbal additives, especially in combination and at low doses, have beneficial effects on body weight in Xizang chickens, possibly via improved antioxidant status and specific modulation of gut microbiota.

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INTRODUCTION

The Xizang chicken is an indigenous poultry breed native to the Qinghai-Tibet Plateau (Zhou *et al.*, 2016). It is well adapted to severe climates and high altitudes, thus constituting a valuable genetic resource for alpine regions (Zhang *et al.*, 2019). Nonetheless, in intensive rearing environments, these chickens remain vulnerable to environmental stress and inflammatory issues. This usually leads to decreased growth performance and reduced production efficiency, which restricts their large-scale commercialization (Niu *et al.*, 2022; Stefanetti *et al.*, 2023). Thus, the development of safe and effective natural feed additives is necessary, especially in antibiotic-free production systems.

Probiotics are popular as a type of functional feed additives used in the nutrition of poultry and are also considered to have a very promising future as an alternative to antibiotics (Abd El-Hack *et al.*, 2020; Yaqoob *et al.*, 2021). Their mechanism of action mainly includes the modulation of gut microbiota in the process of promoting beneficial bacteria and suppressing pathogens (Ahmed *et al.*, 2019; Ma *et al.*, 2023). This helps in the enhancement of intestinal health, nutrient absorption, and immunity (Bogusławska-Tryk *et al.*, 2021). Also, probiotics may increase the antioxidant capacity, decrease inflammatory response, and ultimately improve the growth and health of poultry (Larsberg *et al.*, 2024; Cai *et al.*, 2024).

Chinese herbal additives represent another category of natural feed supplements (Phillips *et al.*, 2023; Wang *et al.*,

2024). They contain bioactive constituents, such as polysaccharides and flavonoids, which exhibit antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory properties. Additionally, the supplementation of Chinese herbal additives has been found to enhance immune responses in poultry (Song *et al.*, 2022; Liu *et al.*, 2024), improve intestinal development (Gui *et al.*, 2023), and modulate intestinal microbiota composition, respectively, thereby contributing to better poultry performance (Huang *et al.*, 2021; Peng *et al.*, 2023; Zou *et al.*, 2024).

Because probiotics mainly regulate gut microecology, whereas Chinese herbal additives exert multi-target physiological effects, their combined use may induce complementary benefits. The combination of these additives has become a topic of concern as a sustainable approach to enhancing poultry production with a reduction in the use of antibiotics (Wang *et al.*, 2019; Liang *et al.*, 2021). However, a majority of previous studies have been done on commercial broilers, and little evidence has been found on indigenous breeds such as Xizang chickens. Notably, there is no systematic assessment of graded supplementation and the cumulative effects of these supplements on body weight, antioxidant regulation, and gut microbiota of Xizang chickens under intensive rearing systems. Therefore, the aim of the current study was to examine the impact of various levels of dietary probiotics, Chinese herbal additives, and their combination on body weight, antioxidant capacity, and gut microbial composition in Xizang chickens.

MATERIALS AND METHODS

Experimental Design and Animals: All animal experimentation was conducted under the approval of the Animal Protection Committee of the Xizang Academy of Agriculture and Animal Husbandry Sciences (Approval No. XKS2024062). In this experiment, 420 healthy Xizang chicks were used. Birds were randomly distributed to seven dietary treatments with five replicates (n=12). The control group (CON) was fed the basal diet only, whereas the other groups were fed the basal diet supplemented with graded levels of probiotics, Chinese herbal additives, or their combination. The probiotic supplement was a combination of *Lactiplantibacillus* P*lantarum* and *Bacillus licheniformis*, with a total viable count of 1.0×10^9 CFU/g. The herbal additive was a mixture of Astragalus, Licorice, Hawthorn, and Eucommia (Table 1).

Table 1: Experimental grouping of Xizang chickens

No.	Experimental Group	Dosage (g/kg)	Abbreviation
1	Control (basal diet)	No additive	CON
2	Basal Diet + Probiotics	30	YSJ1
3	Basal Diet + Probiotics	60	YSJ2
4	Basal Diet + Chinese herbal additives	30	ZY1
5	Basal Diet + Chinese herbal additives	60	ZY2
6	Basal Diet + Probiotics (50%) + Chinese herbal additives (50%)	30	MIX1
7	Basal Diet + Probiotics (50%) + Chinese herbal additives (50%)	60	MIX2

The experiment was conducted at Snow Region Chicken Breeding Base in Lhasa, Tibet Autonomous Region, and lasted 42 days. The experimental house was cleaned and disinfected before the trial started. Before placement, all chicks were immunized as per the routine vaccination program. Feed and water were provided ad libitum throughout the experiment. The basal diet ingredient composition and nutrient levels are shown in Table 2.

Table 2: Composition and nutrient levels of the basal diet

Feed Ingredients	Content (%)	Nutrient Level	%
Corn	64	Metabolizable energy (MJ/kg)	12.00
Wheat bran	6.4	Crude protein (%)	19.00
Soybean meal	14.00	Crude fiber (%)	3.50
Fish meal	9.00	Calcium (%)	1.24
Alfalfa meal	3.50	Phosphorus (%)	1.00
Bone meal	2.00	Lysine (%)	0.99
Salt	0.10	Methionine (%)	0.29
Chick premix	1.00		
Total	100		

Body weight Determination: The body weight of each bird was measured weekly at a predetermined time in the absence of feed after 4 h of feed deprivation. Statistical analysis was conducted using the mean value of the cages, with each replicate cage serving as the experimental unit (with n=5 for each treatment).

Sample Collection: On the last day of the experiment, one bird was randomly selected from each replicate cage for sample collection, resulting in five birds per treatment. Fresh feces were stored in sterile cryotubes at -80 °C until the microbiota analysis. Blood samples were collected from the wing vein, centrifuged, and serum was separated and stored at -20 °C for biochemical and antioxidant analyses.

Serum Biochemical Analysis: An automatic biochemical analyzer was used to measure serum levels of glucose (GLU), urea (UREA), creatinine (CREA), triglycerides (TG), total cholesterol (CHO), high-density lipoprotein (HDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (γ-GT), and the electrolytes Na^+, K^+, and Cl^-.

The assay of serum total antioxidant capacity (T-AOC), superoxide dismutase (SOD), glutathione (GSH), and malondialdehyde (MDA) was performed using commercial diagnostic kits (Shanghai Beyotime Biotechnology Co., Ltd., China) according to the instructions provided by the manufacturer.

Analysis of Microbial Community using 16S rRNA Gene Sequencing: The microbial composition in fecal samples was described using high-throughput 16S rRNA sequencing. Five fecal samples from each treatment group were used for sequencing, resulting in a total of 35 samples. Total DNA was extracted using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA), and DNA integrity was assessed by agarose gel electrophoresis. The V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified with barcoded primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Xu *et al.*, 2025). Amplicons were purified, quantified, and pooled for library construction, followed by paired-end sequencing on an Illumina platform. A total of 2,955,415 raw reads were generated from 35 fecal samples. After quality filtering, 2,583,270 effective reads were retained for downstream

analysis, with an average of 73,807.71 effective reads per sample. Raw reads were assembled, quality controlled, and clustered into operational taxonomic units (OTUs) at 97% sequence similarity. Representative sequences were assigned taxonomic classification using the SILVA database. After normalization, the resulting dataset was used for analyses of alpha diversity, beta diversity, and differences in community structure (Liu *et al.*, 2026).

Statistical Analysis: Data were first organized in Microsoft Excel 2019 and then processed statistically with SPSS 25.0. Body weight data were analyzed using the replicate cage as the experimental unit, with five replicate values per treatment. For serum biochemical and antioxidant analyses, one bird was sampled from each replicate cage, resulting in five biological replicates per treatment. Before the analysis, the normality and homogeneity of the variances were evaluated. Data satisfying these assumptions were compared among groups by one-way ANOVA, followed by Duncan's multiple range test for multiple comparisons. Results are expressed as mean ± SEM, and differences were considered significant at $P<0.05$. Microbial diversity indices and relative abundance data were evaluated with non-parametric methods, and differences among multiple groups were tested using the Kruskal-Wallis test; pairwise comparisons were performed when the overall test was significant. Beta-diversity differences were examined by PERMANOVA.

RESULTS

Body weight: Initial body weight did not differ among groups ($P>0.05$). During the early growth phase (7-21 days), supplemented groups generally exhibited improved body weight compared to the CON group, with the low-dose composite group (MIX1) showing the most pronounced effect ($P<0.05$). At day 14, MIX1 remained heavier than CON, YSJ1, YSJ2, ZY2, and MIX2 ($P<0.05$), while ZY1 also exceeded CON and YSJ1 ($P<0.05$). By day 21, both MIX1 and MIX2 had higher body weight than CON and ZY1, and ZY2 was also higher than CON ($P<0.05$). During the later growth stage (28–42 days), all supplemented groups showed greater body weight than the control ($P<0.05$), whereas no significant differences were detected among the supplemented treatments ($P>0.05$) (Table 3).

Serum Antioxidant Capacity: Serum antioxidant indices are summarized in Table 4. The ZY2 group showed higher GSH than CON, YSJ1, YSJ2, and MIX1 ($P<0.05$), whereas ZY1 also exceeded YSJ1 and MIX1 ($P<0.05$). In addition, YSJ1 had greater SOD activity than CON, YSJ2, ZY1, ZY2, and MIX1, and greater T-AOC than all other groups ($P<0.05$), while the other comparisons were not significant ($P>0.05$). Conversely, dietary treatments had no significant effect on levels of MDA ($P>0.05$), indicating that the status of lipid peroxidation did not change across groups. This could imply that the oxidative stress levels were not excessive in the experimental conditions, or that the levels of MDA were already low in the control conditions, hence limiting the magnitude of the effect of supplementation that could be detected.

Serum Biochemical Indices: Serum biochemical indices are summarized in Table 5. For liver function, ALT levels were higher in YSJ1 and some other groups compared with ZY1 ($P<0.05$). For nitrogen metabolism, YSJ1 showed higher UREA and CREA than most other groups ($P<0.05$), whereas YSJ2 also exceeded ZY1 for both indices ($P<0.05$). GLU and CHO differed among treatments ($P<0.05$), with ZY1 showing relatively lower values than several other groups. Regarding electrolyte balance, K^+ levels were lower in ZY1 than in all other groups, whereas Cl^- levels were lower in ZY1 than in CON, YSJ1, YSJ2, and ZY2 ($P<0.05$). No other significant differences were detected among groups ($P>0.05$).

Sequencing depth and fecal microbial beta diversity: The rarefaction curves of fecal samples from all groups (CON, YSJ1, YSJ2, ZY1, ZY2, MIX1, and MIX2) reached a plateau (Figure 1), indicating that the sequencing depth was sufficient to cover the majority of bacterial diversity in the samples.

Principal coordinate analysis (PCoA) based on Bray-Curtis distances revealed clear separation of fecal microbial communities among different treatment groups ($R^2=0.56$, $P=0.001$; Figure 2), indicating that probiotics, Chinese herbal additives, and their composite supplementation significantly altered the intestinal microbiota of Xizang chickens. Cluster patterns showed that the microbial communities in the composite groups (MIX1 and MIX2) differed most markedly from the control, while single additive groups (YSJ1, YSJ2, ZY1, ZY2) also displayed varying degrees of community modulation.

Table 3: Effects of different additives on body weight of Xizang chickens

Age / body weight (g)	CON	YSJ1	YSJ2	ZY1	ZY2	MIX1	MIX2	*P*-value
Initial body weight	25.98±0.43	27.36±0.77	27.26±0.89	25.71±0.39	25.61±0.52	26.09±0.67	26.43±0.57	0.280
Body weight at 7 days	31.27±0.47b	32.68±0.57ab	30.97±0.68b	32.49±0.68ab	32.79±0.58ab	33.91±0.99a	32.10±0.62ab	0.030
Body weight at 14 days	52.51±1.10a	51.85±1.59a	52.81±1.66ab	58.63±2.25bc	55.75±1.93ab	61.67±3.02c	55.96±1.82ab	0.003
Body weight at 21 days	73.95±1.96a	81.60±3.34abc	83.12±2.93abc	77.60±3.58ab	84.56±3.28bc	87.24±3.44c	89.80±2.81c	0.002
Body weight at 28 days	104.06±2.65a	125.20±5.92b	123.87±4.46b	119.08±4.99b	123.93±4.30b	119.97±5.18b	128.79±5.16b	0.002
Body weight at 42 days	185.10±4.14a	225.64±7.79b	211.00±6.15b	217.89±8.59b	215.94±9.20b	210.68±8.77b	232.94±8.25b	<0.001

Note: Different lowercase letters within a row indicate significant differences among groups ($P < 0.05$). Values sharing the same lowercase letter are not significantly different ($P>0.05$)

Table 4: Effects of different additives on serum antioxidant indices of Xizang chickens

Index/group	CON	YSJ1	YSJ2	ZY1	ZY2	MIX1	MIX2	*P*-value
GSH (μmol/L)	1.69±0.28ab	1.30±0.27a	1.75±0.42ab	2.82±0.59bc	3.11±0.56c	1.40±0.30a	2.50±0.26abc	0.023
MDA (mmol/ml)	2.58 ±0.11	34.37±4.51	3.12 ± 0.18	2.50 ± 0.19	2.85 ± 0.22	2.90 ± 0.21	2.68 ± 0.29	0.248
SOD (units)	1.30±0.06a	1.65±0.09b	1.12±0.08a	1.15±0.11a	1.19±0.14a	1.09±0.08a	1.41±0.16ab	0.016
T-AOC (U/mll)	2.84 ± 0.12	4.94 ± 0.25b	3.42 ± 0.20a	2.75 ± 0.21a	3.14 ± 0.24a	3.19 ± 0.23a	3.20 ± 0.22a	<0.001

Note: Different lowercase letters within a row indicate significant differences among groups ($P < 0.05$). Values sharing the same lowercase letter are not significantly different ($P>0.05$)

Table 5: Effects of different additives on serum biochemical indices of Xizang chickens

Index/group	CON	YSJ1	YSJ2	ZY1	ZY2	MIX1	MIX2	*P*-value
ALT (U/L)	4.32±0.39bc	4.48±0.63c	4.03±0.12bc	2.07±0.47a	3.36±0.62abc	4.02±0.52bc	2.92±0.39ab	0.012
AST(U/L)	235.79±6.95	236.73±30.26	220.04±31.16	137.44±14.89	250.84±19.82	193.49±39.27	173.56±35.69	0.172
γ-GT (U/L)	24.97±1.92	21.81±2.45	21.11±4.31	7.97±3.20	18.88±5.69	18.23±5.78	13.44±4.04	0.131
UREA (mmol/L)	0.29±0.07ab	0.49±0.03c	0.37±0.05bc	0.15±0.03a	0.27±0.07ab	0.20±0.06ab	0.29±0.07ab	0.009
CREA (μmol/L)	23.07±1.20bc	30.96±1.93c	23.28±4.23bc	9.87±1.03a	16.23±3.11ab	16.34±3.16ab	15.48±2.40ab	<0.01
GLU (mmol/L)	15.32±0.33c	14.96±1.03c	12.08±1.86bc	5.50±0.88a	9.04±1.97ab	10.30±1.85abc	11.24±2.53bc	0.006
TG (mmol/L)	0.92±0.22	0.92±0.12	0.77±0.15	0.28±0.05	0.48±0.10	0.59±0.20	0.67±0.19	0.078
CHO (mmol/L)	2.85±0.27bc	3.68±0.26c	2.74±0.41bc	1.41±0.27a	2.44±0.55ab	2.17±0.35ab	2.17±0.39ab	0.013
HDL (mmol/L)	1.98±0.22	2.71±0.26	1.95±0.39	1.11±0.24	1.93±0.50	1.60±0.31	1.62±0.34	0.097
Na^+ (mmol/L)	147.52±1.11	148.24±1.05	133.53±15.25	79.30±12.42	123.15±24.73	122.51±16.48	120.36±16.57	0.060
K^+ (mmol/L)	4.91±0.10c	4.22±0.23bc	3.72±0.44bc	2.20±0.23a	3.58±0.61b	3.53±0.42b	3.84±0.45bc	0.005
Cl^- (mmol/L)	90.60±0.68b	93.31±1.77b	82.71±9.08b	49.17±6.38a	77.14±14.69b	72.89±9.84ab	73.32±10.03ab	0.038

Note: Different lowercase letters within a row indicate significant differences among groups ($P < 0.05$). Values sharing the same lowercase letter are not significantly different (P>0.05)

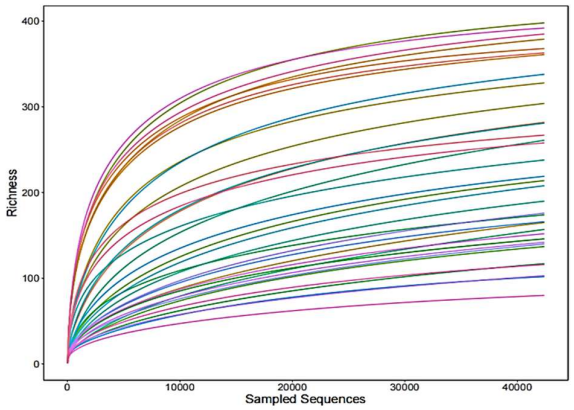

Fig. 1: Rarefaction curves of fecal bacterial communities in Xizang chickens under different additive treatments.

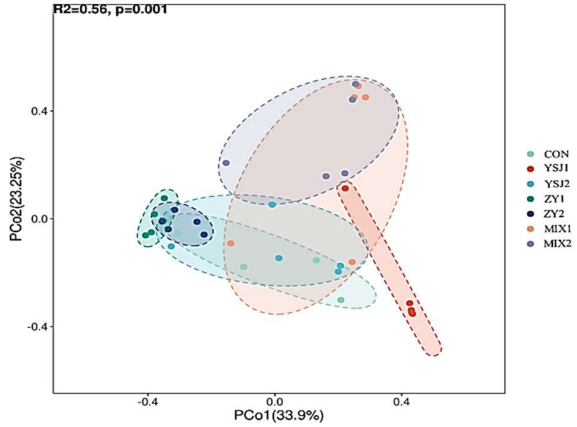

Fig. 2: Principal coordinate analysis (PCoA) of fecal bacterial communities in Xizang chickens under different additive treatments.

Fecal microbial alpha diversity: Fecal microbial alpha diversity is shown in Figure 3. For species richness, the Observed index was lower in ZY1 than in YSJ2 and ZY2 (P<0.05), whereas YSJ2 and ZY2 were both higher than MIX2 (P<0.05). Similarly, the Ace index was higher in YSJ1, YSJ2, and ZY2 than in MIX2 (P<0.05), and YSJ2 also exceeded ZY1 (P<0.05). For diversity and evenness, the Shannon, Simpson, and Pielou indices were all lower in ZY1 than in CON, YSJ1, YSJ2, ZY2, and MIX2 (P<0.05). In addition, the Simpson index in ZY2 was lower than that in CON and YSJ1 (P<0.05). No significant differences were detected for the remaining alpha-diversity comparisons (P>0.05).

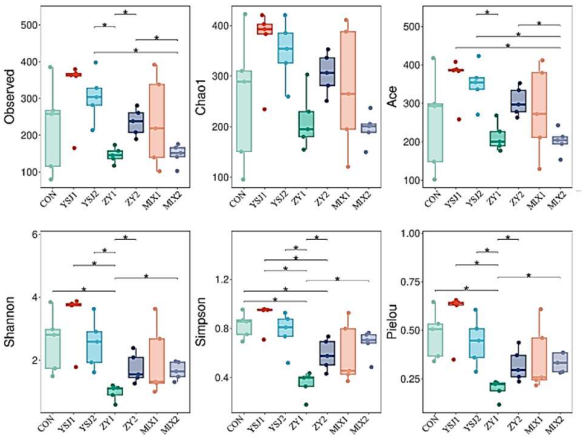

Fig. 3: Effects of different additives on fecal microbial alpha diversity in Xizang chickens.

Fecal Microbiota Composition: Phylum-level composition is shown in Figure 4 and Table 6. A total of nine bacterial phyla were identified, with Firmicutes and Proteobacteria as the dominant phyla, together accounting for about 85% of the total relative abundance. Six phyla showed mean relative abundances above 1%, including Firmicutes, Proteobacteria, Bacteroidota, Fusobacteriota, Actinobacteriota, and Synergistota. Significant differences in phylum-level composition were detected among treatments. Specifically, Firmicutes was lower in YSJ1 and YSJ2 than in ZY1 (P<0.05), whereas Proteobacteria was lower in ZY1 than in YSJ2 (P<0.05). In addition, Actinobacteriota was higher in YSJ2 and MIX1 than in CON (P<0.05), and Campylobacterota was higher in YSJ2 than in ZY1 and MIX2 (P<0.05). No significant differences were found for the remaining phyla (P>0.05).

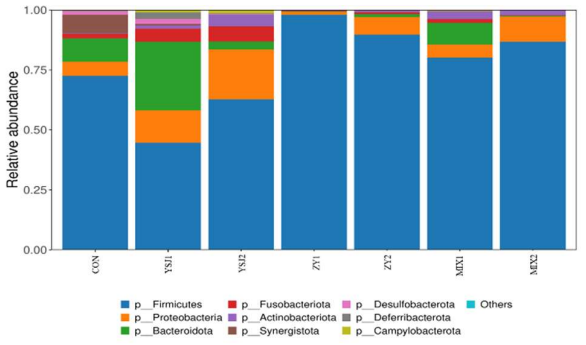

Fig. 4: Effects of different additives on the relative abundance of fecal bacterial phyla in Xizang chickens.

Genus-level composition is shown in Figure 5 and Table 7. A total of 15 dominant genera were compared, with *Romboutsia*, *Lactobacillus, Veillonella,* and *Ligilactobacillus* as the predominant genera, together accounting for about 60% of the total relative abundance. Significant differences in genus-level composition were detected among treatments. Specifically, *Romboutsia* was higher in ZY1 than in YSJ1, MIX1, and MIX2 (P<0.05), whereas *Romboutsia* was lower in YSJ1 than in ZY2 (P<0.05). In addition, *Veillonella* was higher in MIX2 than in ZY1 (P<0.05), and *Ligilactobacillus* was higher in MIX2 and CON than in MIX1 (P<0.05). *Enterococcus* was higher in CON than in ZY1 (P<0.05), whereas *Escherichia-Shigella* was higher in YSJ1 and YSJ2 than in CON (P<0.05). Moreover, *Limosilactobacillus* was higher in MIX1 than in CON (P<0.05), *Faecalibacterium* was higher in YSJ1 and YSJ2 than in ZY1 (P<0.05), and *Corynebacterium* was higher in YSJ2 than in CON (P<0.05). No significant differences were found for the remaining genera (P>0.05).

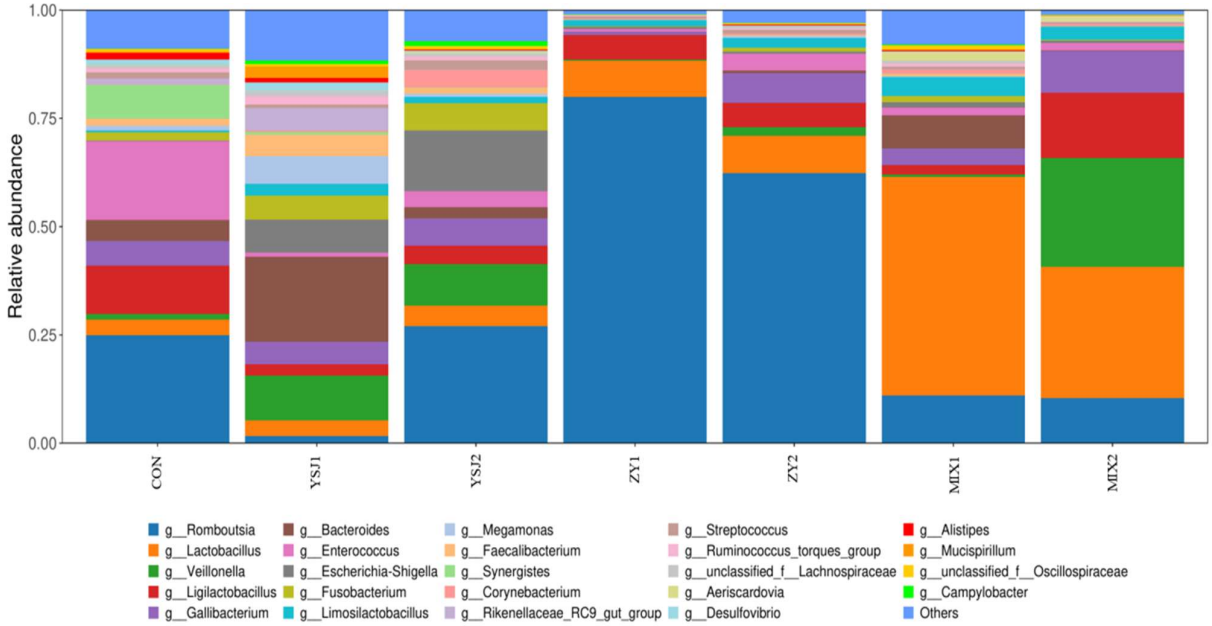

Fig. 5: Effects of different additives on the relative abundance of fecal bacterial genera in Xizang chickens.

Table 6: Effects of different additives on the relative abundance of fecal bacterial phyla in Xizang chickens

	CON	YSJ1	YSJ2	ZY1	ZY2	MIX1	MIX2	SEM	*P*-value
Firmicutes	72.47ab	44.67b	62.71b	97.93a	89.68ab	80.19ab	86.81ab	6.85	< 0.001
Proteobacteria	6.03ab	13.54ab	20.78a	1.28b	7.43ab	5.45ab	10.55ab	2.41	0.004
Bacteroidota	9.71	28.55	3.38	0.21	1.12	9.07	0.20	3.83	0.058
Fusobacteriota	1.87	5.51	6.32	0.17	0.90	1.43	0.21	0.95	0.096
Actinobacteriota	0.19c	1.30abc	4.82a	0.29bc	0.56abc	3.16ab	2.09abc	0.65	0.001
Synergistota	7.79	0.58	0.05	0.01	0.03	0.06	0.01	1.10	0.08
Desulfobacterota	1.46	2.16	0.31	0.05	0.14	0.14	0.02	0.32	0.108
Deferribacterota	0.26	2.68	0.22	0.01	0.02	0.10	0.03	0.37	0.057
Campylobacterota	0.13abc	0.87ab	1.27a	0.03c	0.08abc	0.28abc	0.06bc	0.18	0.002

Note: Different lowercase letters within a row indicate significant differences among groups (P<0.05). Values sharing the same lowercase letter are not significantly different (P>0.05)

Table 7: Effects of different additives on the relative abundance of fecal bacterial genera in Xizang chickens

	CON	YSJ1	YSJ2	ZY1	ZY2	MIX1	MIX2	SEM	*P*-value
Romboutsia	24.94abc	1.64c	27.06abc	79.93a	62.32ab	11.05bc	10.40bc	11.03	< 0.001
Lactobacillus	3.61	3.66	4.68	8.36	8.64	50.42	30.34	6.79	0.095
Veillonella	1.29ab	10.33ab	9.60ab	0.29b	2.02ab	0.54ab	25.13a	3.42	0.023
Ligilactobacillus	11.17a	2.59ab	4.24ab	5.63ab	5.57ab	2.16b	15.02a	1.79	0.004
Gallibacterium	5.63	5.17	6.34	0.68	6.87	3.84	9.65	1.05	0.130
Bacteroides	4.87ab	19.62a	2.60ab	0.10b	0.63ab	7.67ab	0.15b	2.64	0.031
Enterococcus	18.21a	1.01ab	3.65ab	0.58b	3.87ab	1.80ab	1.69ab	2.35	0.003
Escherichia-Shigella	0.18b	7.60a	14.01a	0.56ab	0.47ab	1.25ab	0.57ab	2.01	< 0.001
Fusobacterium	1.87	5.51	6.32	0.17	0.90	1.43	0.21	0.95	0.096
Limosilactobacillus	0.40b	2.72ab	1.46ab	1.35ab	2.28ab	4.38a	2.95ab	0.49	0.010
Megamonas	1.09	6.39	0.74	0.12	0.47	0.19	0.08	0.86	0.089
Faecalibacterium	1.69ab	5.01a	1.37a	0.03b	0.19ab	0.50ab	0.09ab	0.67	0.016
Synergistes	7.79	0.58	0.05	0.01	0.03	0.06	0.01	1.10	0.080
Corynebacterium	0.07b	0.37ab	4.12a	0.24ab	0.35ab	0.96ab	0.52ab	0.54	0.008
Rikenellaceae_RC9	1.38	5.20	0.01	0.00	0.00	0.04	0.00	0.73	0.103

Note: Different lowercase letters within a row indicate significant differences among groups (*P* < 0.05). Values sharing the same lowercase letter are not significantly different (P>0.05)

Correlation Analysis: Spearman correlations between serum antioxidant parameters, biochemical indices, and the relative abundances of the top 15 fecal bacterial genera are shown in Figure 6. *Escherichia, Megamonas, Fusobacterium, Romboutsia, Bacteroides,* and *Faecalibacterium* were positively correlated with serum GSH and T-AOC (R>0.5, P<0.05). In addition, *Fusobacterium, Romboutsia, Bacteroides*, and *Faecalibacterium* were positively correlated with CREA and UREA (R>0.5, P<0.05). By contrast, *Synergistes* was negatively correlated with MDA, T-AOC, CREA, UREA, and GLU (R < -0.5, P<0.05). These findings indicate close associations between fecal microbial composition and serum antioxidant as well as biochemical traits in Xizang chickens.

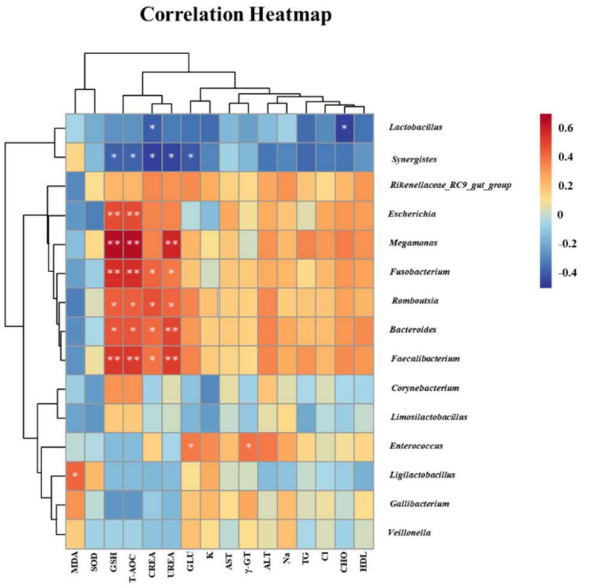

Fig. 6: Spearman correlation analysis between fecal bacterial genera and blood antioxidant and biochemical indices in Xizang chickens.

DISCUSSION

The present study demonstrates that dietary supplementation with probiotics, Chinese herbal additives, and their combination enhanced the body weight of Xizang chickens to different extents. Notably, the composite additive exhibited a more pronounced effect during early growth, suggesting a potential complementary effect, whereas individual probiotic or herbal supplementation primarily influenced antioxidant regulation and metabolic homeostasis. The observed improvements are probably mediated by the interaction between antioxidant regulation and gut microbiota modulation, which together influence nutrient utilization and physiological stability under intensive rearing conditions.

The superior early body weight response observed in the composite group suggests a potential complementary effect between probiotics and herbal additives, while herbal components support metabolic and immune functions. We found significantly higher body weight in all additive-treated groups in comparison with the CON group, which further supports the growth-promoting effects of individual and combined supplementation. Previous research has confirmed that *Lactobacillus* and *Bacillus* species have the capacity to improve body weight gain in poultry by enhancing the composition of the gut microorganisms and nutrient utilization (Al-Fataftah and Abdelqader, 2014; Incharoen *et al.*, 2019). Similarly, herbal ingredients known as Astragalus, Glycyrrhiza, and Crataegus have been reported to benefit the health and productive performance of animals through various regulatory pathways (Jiang *et al.*, 2020; Song *et al.*, 2023; Ahmadipour *et al.*, 2024). In addition, previous studies indicate that the overall regulatory effects of concomitant probiotics and herbs are generally better than those of individual supplementation (Liang *et al.*, 2021). Our findings are in line with these observations. Notably, the low-dose composite group (MIX1) showed higher growth-promoting effects between 7 and 21 days compared to the high-dose composite group (MIX2). This finding suggests that higher supplementation levels do not necessarily produce stronger responses and that appropriate inclusion levels should be further evaluated.

Oxidative stress is commonly linked to high-altitude conditions and could negatively impact the growth and health of Xizang chickens (Wasti *et al.*, 2020; Chen *et al.*, 2021; Niu *et al.*, 2022). The present study revealed that dietary supplementation can significantly raise some serum antioxidant parameters, suggesting that the growth-promoting effects of the additives might be related to the improved antioxidant capacity. Specifically, high-dose herbal supplementation (ZY2) had a significant effect on serum GSH levels. GSH is an important non-enzymatic antioxidant that is involved in cellular redox homeostasis, and higher GSH levels generally reflect better antioxidant capacity (Sies, 1999; Fraternale *et al.*, 2009). In comparison, the group with a low dose of probiotic (YSJ1) exhibited increased superoxide dismutase (SOD) activity and total antioxidant capacity (T-AOC). SOD is typically employed to indicate potential antioxidant activity, and T-AOC is commonly used to reflect the overall antioxidant potential at the organismal level. These results suggest that the action of probiotics might be primarily to enhance enzymatic antioxidant activity and overall redox status, while Chinese herbal additives may be more to enhance non-enzymatic antioxidant defenses. The observed differences in the effects of the treatments on antioxidant indicators suggest that the probiotics and herbs can regulate the oxidative balance through different pathways. This compensatory effect implies that probiotics and herbal additives can regulate various components of the antioxidant system that may work together to enhance cellular stability and metabolic efficiency. The fact that no dramatic changes occurred in MDA further suggests that these additives might be acting in preventing an oxidative imbalance instead of reversing severe oxidative imbalance of lipids. Earlier research has also documented that probiotics and Chinese herb additives improve the body weight gain of poultry through an enhancement in the activities of antioxidant enzymes and the prevention of oxidative damage (Derakhshan *et al.*, 2023; Song *et al.*, 2023; Liu *et al.*, 2023). Therefore, our results further confirm the notion that enhanced antioxidant capacity may be the most critical mechanism for promoting the growth of Xizang chickens under high-altitude conditions, although whether antioxidant regulation is the common factor is yet to be explored at the molecular level.

In addition to the antioxidant defense, modulation of the gut microbiota can also help to increase the growth-promoting effects of the additives. Fecal microbial profiles showed that the fecal microbiota significantly changed after the supplementation, indicating that there could be some regulatory impacts on the intestinal functionality and host development. At the phylum level, the abundance of *Actinobacteriota* was greater in the probiotic and composite groups, suggesting that probiotics may help to enrich the abundance of this bacterial group. *Actinobacteriota* members are involved in the breakdown of complex carbohydrates and production of antimicrobial metabolites, and hence play an important role in intestinal homeostasis in poultry (Fathima *et al.*, 2024). *Enterococcus* abundance at the genus level was more abundant in the CON group. Since *Enterococcus* serves as an opportunistic pathogen in the avian gut, a high abundance of this genus may predispose to enterococcal disease and have a detrimental impact on growth and health (Jung *et al.*, 2018). Specifically, the low-dose herbal supplementation (ZY1) decreased *Enterococcus* abundance, indicating that these additives could reduce the potential pathogen existence and help in maintaining the gut microbial balance. Moreover, the low-dose composite group (MIX1) showed the increased Limosilactobacillus abundance, indicating that combined supplementation could be used more effectively to enrich the beneficial lactic acid bacteria that can inhibit the harmful bacteria through the production of lactic acid, organic acids, and other metabolites, which support host health (Sánchez-Maldonado *et al.*, 2011; Lehri *et al.*, 2017; Racines *et al.*, 2023). The changes in these microbes offer a functional explanation of the observed growth responses. The increase in beneficial taxa like *Limosilactobacillus* can help to enrich nutrient digestion and energy conversion and decrease opportunistic pathogens like *Enterococcus*, thereby improving the body weight gain. Correlation analyses revealed that the abundances of *Escherichia*, *Megamonas*, *Fusobacterium*, *Romboutsia*, *Bacteroides*, and *Faecalibacterium* were positively correlated with GSH and T-AOC levels, indicating a relationship between the change in gut microbial abundances and the host antioxidant status. It has also been reported in previous studies that the high-altitude-adapted lines of chicken harbor distinct gut bacteriomes related to environmental adaptation (Huang *et al.*, 2021; Peng *et al.*, 2023; Bhagat *et al.*, 2025). Collectively, these findings can imply that additive-induced modulation of the gut microbiota may also contribute to enhanced physiological homeostasis and body weight of Xizang chicken.

Overall, the findings suggest that combined supplementation with low doses is the most effective in promoting the early growth of Xizang chickens. This phenomenon appears to be the result of concerted changes in the antioxidant capacity and the composition of gut microbiota rather than the effect of one dominant pathway. These findings are, however, largely based on associative evidence, and causal relationships between microbial changes and body weight are yet to be discovered. Moreover, the analysis of fecal microbiota might not be sufficient to reflect intestinal functional dynamics. Thus, more research that incorporates metabolomics, intestinal barrier functionality, and molecular studies is needed to further understand the underlying mechanisms and further evaluate additive strategies.

Conclusions: This research indicates that dietary supplementation with probiotics, Chinese herbal additives, and their combination enhanced the body weight of the Xizang chickens and modulated serum antioxidant status and fecal microbiota. The low-dose composite treatment (MIX1) showed a relatively greater early body weight response. Moreover, probiotic and herbal additives demonstrated different impacts on the regulation of antioxidants and metabolic reactions. These results indicated that the use of targeted natural feed additives can support the growth and physiological stability of Xizang chickens. Further research is also needed to refine supplementation plans and to explain the underlying mechanisms.

Authors contribution: LX and JF contributed to conceptualization and methodology. LX, JF, HW, DP, LZ, and YZ contributed to providing reagents, materials, and analysis tools. LX contributed to writing and preparing the original draft. LX and JF contributed to review and editing. JF contributed to visualization and supervision. All authors reviewed and approved the final manuscript.

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Ethics approval: All animal experimentation was conducted under the approval of the Animal Protection Committee of the Xizang Academy of Agriculture and Animal Husbandry Sciences (Approval No. XKS2024062). All Xizang chickens used in this study were sourced from local farms, and we obtained written consent from the farm manager prior to the experiment.

Data Availability Statement: The raw sequencing data have been deposited in the NCBI Sequence Read Archive under accession number PRJNA1465701.

Competing interests: The authors declare no conflict of interest.

REFERENCES

Abd El-Hack ME, El-Saadony MT, Shafi ME, *et al.*, 2020. Probiotics in poultry feed: a comprehensive review. Journal of Animal Physiology and Animal Nutrition104(6): 1835-1850.

Ahmed Z, Vohra MS, Khan MN, *et al.*, 2019. Antimicrobial role of lactobacillus species as potential probiotics against enteropathogenic bacteria in chickens. Journal of Infection in Developing Countries 13(2): 130-136.

Ahmadipour B, Kalantar M, Abaszadeh S, *et al.*, 2024. Antioxidant and antihyperlipidemic effects of Hawthorn Extract (Crataegus oxyacantha) in broiler chickens. Veterinary Medicine and Science 10(3): e1414.

Al-Fataftah AR and Abdelqader A, 2014. Effects of dietary bacillus subtilis on heat-stressed broilers performance, intestinal morphology and microflora composition. Animal Feed Science and Technology 198: 279-285.

Bhagat NR, Maan NS, Bhanja SK, *et al.*, 2025. Gut bacteriome dynamics in high altitude-adapted chicken lines: A key to future poultry therapeutics. Scientific Reports 15: 96178.

Bogusławska-Tryk M, Ziółkowska E, Sławińska A, *et al*., 2021. Modulation of intestinal histology by probiotics, prebiotics and synbiotics delivered in ovo in distinct chicken genotypes. Animals (Basel) 11(11): 3293.

Cai Y, Xiao C, Tian B, *et al*., 2024. Dietary probiotic based on a dual-strain bacillus subtilis improves immunity, intestinal health, and growth performance of broiler chickens. Journal of Animal Science 102: skae183.

Chen S, Yong Y and Ju X, 2021. Effect of heat stress on growth and production performance of livestock and poultry: Mechanisms of prevention. Journal of Thermal Biology 99: 103019.

Derakhshan M, Ghasemian SO and Gholami-Ahangaran M, 2023. The effects of probiotic and phytase on growth performance, biochemical parameters and antioxidant capacity in broiler chickens. Veterinary Medicine and Science 9(2): 860-866.

Fathima S, Al Hakeem WG, Shanmugasundaram R, *et al*., 2024. The effect of supplemental arginine on the gut microbial homeostasis of broilers during sub-clinical necrotic enteritis challenge. Frontiers in Physiology 15: 1463420.

Fraternale A, Paoletti MF, Casabianca A, *et al*., 2009. GSH and analogs in antiviral therapy. Molecular Aspects of Medicine 30(1-2): 99-110.

Gui J, Azad MAK, Lin W, *et al*., 2023. Dietary supplementation with chinese herb ultrafine powder improves intestinal morphology and physical barrier function by altering jejunal microbiota in laying hens. Frontiers in Microbiology 14: 1185806.

Huang P, Wang P, Xu J, *et al*., 2021. Fermented traditional chinese medicine alters the intestinal microbiota composition of broiler chickens. Research in Veterinary Science 135: 8-14.

Incharoen T, Charoensook R, Onoda S, *et al*., 2019. The effects of heat-killed *Lactobacillus Plantarum L-137* supplementation on growth performance, intestinal morphology, and immune-related gene expression in broiler chickens. Animal Feed Science and Technology 257: 114272.

Jiang M, Zhao S, Yang S, *et al*., 2020. An "Essential Herbal Medicine"—Licorice: A review of phytochemicals and its effects in combination preparations. Journal of Ethnopharmacology 249: 112439.

Jung A, Chen LR, Suyemoto MM, *et al*., 2018. A review of Enterococcus cecorum infection in poultry. Avian Diseases 62(3): 261-271.

Larsberg F, Sprechert M, Hesse D, *et al*., 2024. In vitro assessment of the immunomodulatory effects of probiotic *Bacillus strains* on chicken PBMCs. Frontiers in Immunology 15: 1415009.

Lehri B, Seddon AM and Karlyshev AV, 2017. *Lactobacillus fermentum 3872* as a potential tool for combatting *Campylobacter jejuni* infections. Virulence 8(8): 1753-1760.

Liang W, Li H, Zhou H, *et al*., 2021. Effects of Taraxacum and Astragalus extracts combined with probiotic *Bacillus subtilis* and *Lactobacillus* on *Escherichia coli*–infected broiler chickens. Poultry Science 100(4): 101007.

Liu B, Ma R, Yang Q, *et al*., 2023. Effects of traditional chinese herbal feed additive on production performance, egg quality, antioxidant capacity, immunity and intestinal health of laying hens. Animals (Basel) 13(15): 2510.

Liu M, Chen R, Wang T, *et al*., 2024. Dietary chinese herbal mixture supplementation improves production performance by regulating reproductive hormones, antioxidant capacity, immunity, and intestinal health of broiler breeders. Poultry Science 103(1): 103201.

Liu Y, Dong Y, Safdar M, *et al*., 2026. Lactobacillus johnsonii DY2 isolated from yaks alleviated acute escherichia coli infection via modulating inflammatory responses, antioxidant capacity, and gut microbiota. Veterinary Sciences 13(2):132.

Ma K, Chen W, Lin XQ, *et al*., 2023. Culturing the chicken intestinal microbiota and potential application as probiotics development. International Journal of Molecular Sciences 24(3): 3045.

Niu X, Ding Y, Chen S, *et al*., 2022. Effect of immune stress on growth performance and immune functions of livestock: mechanisms and prevention. Animals (Basel) 12(7): 909.

Peng Y, Zhang M, Zheng Q, *et al*., 2023. Effects of anti-inflammatory and digestive-promoting chinese herbs on intestinal microbiota in broiler chickens. Italian Journal of Animal Science 22(1): 1467-1478.

Phillips CJC, Hosseintabar-Ghasemabad B, Gorlov IF, *et al*., 2023. Immunomodulatory effects of natural feed additives for meat chickens. Life (Basel) 13(6): 1287.

Racines MP, Mosquera M, Erazo DA, *et al*., 2023. an overview of the use and applications of *Limosilactobacillus fermentum* in broiler chickens. Microorganisms 11(8): 1944.

Sánchez-Maldonado AF, Schieber A and Gänzle MG, 2011. Structure-function relationships of the antibacterial activity of phenolic acids and their metabolism by lactic acid bacteria. Journal of Applied Microbiology 111(5): 1176-1184.

Sies H, 1999. Glutathione and its role in cellular functions. Free Radical Biology and Medicine 27(9-10): 916-921.

Song B, Li P, Yan S, *et al*., 2022. Effects of dietary astragalus polysaccharide supplementation on the Th17/Treg balance and the gut microbiota of broiler chickens challenged with necrotic enteritis. Frontiers in Immunology 13: 781934.

Song W, Zou Z, Chen X, *et al*., 2023. Effects of traditional chinese herbal feed supplement on growth performance, immunity, antioxidant levels, and intestinal health in chickens: A study on ningdu yellow chickens. Poultry Science102(10): 102986.

Stefanetti V, Cartoni Mancinelli A, Pascucci L, *et al*., 2023. Effect of rearing systems on immune status, stress parameters, intestinal morphology, and mortality in conventional and local chicken breeds. Poultry Science 102(12): 103110.

Wang J, Deng L, Chen M, *et al*., 2024. Phytogenic feed additives as natural antibiotic alternatives in animal health and production: a review of the literature of the last decade. Animal Nutrition 17: 244-264.

Wang R, Cai W, Wang X, *et al*., 2019. Progress in chinese medicine-probiotics compound microecological preparations for livestock and poultry. Chinese Journal of Biotechnology 35(6): 972-987.

Wasti S, Sah N and Mishra B, 2020. Impact of heat stress on poultry health and performances, and potential mitigation strategies. Animals (Basel) 10(8): 1266.

Xu C, He Q, Zhu Z, *et al*., 2025. Propolis improves intestinal barrier function against Cryptosporidium parvum via NLRP6 inflammasome. mBio 16(11): e0231725.

Yaqoob MU, Wang G and Wang M, 2021. An updated review on probiotics as an alternative of antibiotics in poultry. Frontiers in Veterinary 8: 769348.

Zhang Y, Gou W, Zhang Y, *et al*., 2019. Insights into hypoxic adaptation in tibetan chicken embryos from comparative proteomics. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics 31: 100602.

Zhou X, Jiang X, Yang C, *et al*., 2016. Cecal microbiota of tibetan chickens from five geographic regions determined by 16S rRNA Sequencing. Microbiology Open 5(5): 753-762.

Zou C, Xing X, Li S, *et al*., 2024. Effects of a combined chinese herbal medicine on growth performance, intestinal barrier function, immune response, and cecal microflora in broilers infected with Salmonella enteritidis. Animals (Basel) 14(18): 2670.