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Screening of Fermentation Conditions for Liver-Protecting Tablets Residues and Their Effect on the Slaughtering Performance of Brolier Chickens

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Abstract **Original Research Article**

In order to optimise the solid-state fermentation process of liver tablet dregs and evaluate its feed application value, Bacillus subtilis Bu412 was used as the strain. This was achieved by optimising the water content, temperature, time and inoculum amount by one-way test, and by detecting the changes of saponins and other active ingredients. One hundred and twenty 7-day-old white-feathered broilers were selected and randomly divided into the control group and 1%, 3% and 5% fermented dregs group to determine the slaughtering performance and HE staining to observe the liver and kidney morphology. The results demonstrated that the optimal process was identified as a water content of 60%, a temperature of 30°C, a duration of 72 hours, and an inoculum of 20%. The study revealed a significant increase in polysaccharides and polyphenols ($P<0.05$) and a decrease in saponins and flavonoids following fermentation. While no significant differences ($P>0.05$) were observed in the slaughter rate indices of the feeding group, they did exhibit higher values compared to the control group. Furthermore, the morphology of liver and kidney tissues was found to be normal. This finding indicates that the optimised fermentation process enhances the content of polysaccharides and polyphenols in the dregs, which is safe and has the potential to be valuable as a feed additive for broiler chickens. Furthermore, it provides a foundation for the effective utilisation of Chinese medicine dregs and the research and development of alternative-resistant feeds.

Keywords: liver tablet dregs; fermentation conditions; one-way experiment; feed additive; slaughtering performance.

1. INTRODUCTION

As the core product of Heilongjiang Sunflower Pharmaceutical Co., Ltd, liver protection tablets are formulated with over ten Chinese medicinal plants, including Chai Hu, Yin Chen, and Panlangen, which are commonly used in clinical hepatoprotective drugs. Notably, the dregs from these medicinal plants contain 15% to 20% residual active ingredients, such as polysaccharides, flavonoids, and saponin-like substances [1-2]. However, due to the high crude fibre content and dense lignified structure, the dregs are difficult to use as feedstuffs directly, and are mostly disposed of in landfills or incinerated, resulting in waste of resources and environmental pollution problems [3]. In recent years, there has been considerable interest in the utilisation of herbal dregs as a green resource. Among the various methods of doing so, solid-state fermentation technology has emerged as a particularly effective approach due to its ability to adapt to a wide range of substrates, its low energy requirements, and the diversity of metabolites it produces. Research has demonstrated that the concurrent fermentation of Astragalus dregs by Bacillus subtilis and Aspergillus niger results in the degradation of 42.3% of cellulose, accompanied by the generation of immunologically active polysaccharides [4]. The polysaccharide content of the optimised solid-state fermentation product of Panax quinquefolium dregs has been shown to reach 28%.17 mg/g and shows significant antioxidant activity [5], which corroborates the potentiation potential of solid-state fermentation on the functional constituents of the drug dregs.

In the context of livestock production, the fermented dregs have demonstrated efficacy in enhancing the health and performance of livestock and poultry. The incorporation of 1.5% fermented dregs has been shown to significantly regulate serum lipid metabolism indexes [6]. Compounded fermented dregs have been observed to reduce the incidence of piglet diarrhea and optimise the structure of the intestinal flora [7]. Furthermore, the fermentation process has been found to degrade antinutritional factors and enhance the

palatability of feeds.

1. MATERIALS

1.1 Test Animal

One hundred and twenty 1-day-old healthy white-feathered broilers, with a weight range of 35-40 g, were procured from a standardized farm in Daqing City, Heilongjiang Province. These birds underwent a 7-day acclimatization period prior to the commencement of the experiment, with the objective of ensuring that their health status and feeding behaviour were within the normal parameters.

1.2 Samples and reagents

Liver tablet dregs (Sunflower Pharmaceutical Group Co., Ltd.); Bacillus subtilis Bu412 Wuhan University Chinese Typical Cultures Preservation Center); LB agar, MH agar, MH broth, peptone (Qingdao HaiBo Biotechnology Co., Ltd.); Folinol (Shanghai YuanYe Biotechnology Co., Ltd.); dextrose, anhydrous ethanol, and sodium carbonate (Tianjin YungDa Chemical Reagent Co., Ltd.).

1.3 Main Instruments

CNC Ultrasonic Cleaner (Kunshan Ultrasonic Instrument Co., Ltd.); UV-Vis Spectrophotometer (Shanghai Yuananalytical Instrument Co., Ltd.); Constant Temperature Cultivation Oscillator (Tianjin OuNuo Instrumentation Co., Ltd.); Electronic Balance (Shanghai Tianmei Balance Instrument Co., Ltd.); Full-Temperature Oscillator (Harbin Donglian Electronic Technology Development Co., Ltd.); Electric Heating Blast Drying Oven (Shanghai Boxun Industry Co., Ltd. Medical Treatment) Ltd. Medical Equipment Factory).

2 METHODS

2.1 Screening of fermentation conditions

In accordance with the one-way experimental method, the effects of four factors – namely, fermentation time, inoculum amount, water content and fermentation temperature – on the solid-state fermentation effect of liver-protecting tablet dregs were investigated separately, with the initial conditions (fermentation temperature of 35°C, water content of 70%, fermentation time of 72 h and inoculum amount of 10%) established as the baseline. The preserved Bacillus subtilis was transferred to a LB plate for activation, and after purification, it was transferred to liquid LB medium and incubated at 30°C, 170 rpm-min-1 for 24 h, with the number of bacterial liquid ≥108 CFU-mL-1. Peptone and glucose with a mass volume fraction of 4 g/mL were added into the sterile water, and then stirred and mixed uniformly, and then loaded into the sealed fermentation bag with a one-way respiratory valve, and placed in the incubator at 35℃ for continuous fermentation. The fermentation process was conducted in an incubator at 35°C for a period of 72 hours, with three replicates being performed for each sample. Following the fermentation process, the residue from the hepatoprotective tablets was subjected to drying at 70°C, crushing, and sieving through a 60-mesh sieve. The fermentation products were then diluted and spread on LB agar medium, and the number of colonies (CFU/g) was enumerated after 24 h incubation at 30℃, using the plate counting method.

2.2 Detection of bioactive substances before and after fermentation

Saponins, flavonoids, polysaccharides and polyphenols were determined by a combination of ultrasound-assisted extraction and spectrophotometry, with the samples collected before and after the fermentation process of the dregs of liver protection tablets. The total flavonoid content was determined by the sodium nitrite-aluminum nitrate colorimetric method (510 nm) following hot reflux extraction with ethanol, with rutin serving as the control; polysaccharides were extracted by ultrasonic extraction and alcohol precipitation, and the glucose content was subsequently detected by the phenol-sulfuric acid method (490 nm); The analysis of total polyphenols was conducted using the Folinol method (760 nm) after extraction by ultrasonication, with chlorogenic acid serving as the reference standard. The determination of total saponins was performed using Chaihu saponins as the control, followed by analysis by the vanillin-glacial acetic acid method (540 nm) after hot reflux extraction with 75% ethanol. The analysis of total saponins was conducted using the vanillin-glacial acetic acid method (540 nm) after heat reflux extraction. The contents of all indicators were calculated by standard curve, and three parallel samples were set in each group.

2.3 ASSESSMENT OF BROILER INDICATORS

2.3.1 Experimental design for broiler chickens

The experiment was designed as a one-way randomised grouping, and 120 white-feathered broilers at 7 days of age were selected and divided into four experimental groups according to the principle of consistency of mean body weight (180.32 ± 0.27 g), with three replicates in each experimental group and 10 broilers in each replicate. The control group was fed a basal diet, while the experimental group was given a diet containing 1%, 3% and 5% of fermented liver tablet dregs, in place of an equal amount of maize diet (see Table 1: Experimental grouping of white-feathered broilers).

Table 1 Experimental grouping of white feather broilers

Process	Experimental diet
Control subjects	Basic diet
Fermentation 1% group	Basic diet + Fermentation 1% group
Fermentation 3% group	Basic diet + Fermentation 1% group
Fermentation 5% group	Basic diet + Fermentation 1% group

2.3.2 Observation of liver and kidney morphology

Following the conclusion of the experimental trial, the broilers were subjected to a 12-hour fast and subsequently weighed. Subsequently, the broilers were euthanised by cervical dislocation, and the livers and kidneys were expeditiously extracted and thoroughly rinsed with saline to eliminate blood and extraneous substances from the surface. Subsequently, a portion of the liver and kidney tissues were harvested and preserved in 10% neutral formalin for a minimum of 24 hours. Following this, the tissues were routinely paraffin-embedded, sectioned (5 μm thickness), and stained with hematoxylin-eosin (HE). The morphological and structural changes of the liver and kidney tissues were then observed under a light microscope.

2.3.3 Measurement of slaughtering performance

At the conclusion of the experiment, at 21 days of age, six broilers each with similar body weight and health status were selected from each group for slaughtering. The large and small leg muscles and pectoral muscles were separated for weighing and calculation. The formula is as follows:

$$\text{Slaughtering rate}(\%) = \text{Carcass weight（g） / Live weight（g） } \times 100\%$$
$$\text{Semi} - \text{clean carcass rate}(\%) = \text{Semi} - \text{clean carcass weight（g） / Lean carcass rate }(\%) \times 100\%$$
$$\text{Full clean carcass rate}(\%) = \text{ Full clean carcass weight（g） / Butchery weight（g） } \times 100\%$$
$$\text{Pectoral rate}(\%) = \text{Pectoral weight（g） / Full net chamber weight（g） } \times 100\%$$
$$\text{Leg rate}(\%) = \text{Full leg weight（g） / Live weight（g） } \times 100\%$$
$$\text{Leg muscle rate}(\%) = \text{Leg muscle weight（g） / Live weight（g） } \times 100\%$$
$$\text{Wing rate}(\%) = \text{Wing rate（g） / Live weight（g） } \times 100\%$$

2.3.4 Statistical analysis

The experimental data were organised using Excel 2019 and SPSS 28.0 for one-way ANOVA, with multiple comparisons conducted using Duncan's method. The results were expressed as "mean ± standard deviation", with $P < 0.05$ denoting significant differences and $P > 0.05$ denoting non-significant differences.

3. RESULTS

3.1 Morphological characteristics of fermented and unfermented drug residues

As illustrated in Figure 1, a comparison of the morphological characteristics of fermented and unfermented dregs reveals notable distinctions. The dregs of Bacillus subtilis fermented tablets exhibited a black-green hue, while those in the control group displayed a yellow colouration. Furthermore, the fermented dregs of liver protection tablets exhibited a more compact structure, reduced flocculent content, and a pronounced olfactory profile.

Figure 1 Morphological characteristics of fermented and unfermented drug residues

3.2 RESULTS OF ONE-WAY EXPERIMENTS

3.2.1 Effect of water content on solid fermentation of dregs of liver protection tablets

As shown in Fig. 2A, the Bacillus subtilis population showed a significant decreasing trend as the moisture content increased from 60% to 90%, reaching a peak of 2.36×10^9 CFU/g at a moisture content of 60%. Therefore, the optimum moisture content for inoculation was determined to be 60%.

3.2.2 Effect of fermentation time on solid fermentation of dregs of liver protection tablets

As shown in Fig. 2B, the Bacillus subtilis count showed a tendency of increasing and then decreasing with the increase of fermentation time. The bacterial count reached a peak of 5.49×10^8 CFU/g at the time of fermentation up to 72 h. Therefore, the optimal fermentation time was determined to be 72 h. The optimal fermentation time was determined to be 72 h. The optimal fermentation time was determined to be 72 h.

3.2.3 Effect of inoculum amount on the solid fermentation of dregs of liver protection tablets

As shown in Fig. 2C, the live bacterial count of Bacillus subtilis showed a continuous upward trend as the inoculum amount was incremented from 5% to 20%. The peak count of 1.65×10^9 CFU/g was reached when the inoculum was 20%. therefore, the optimum inoculum was determined to be 20%.

3.2.4 Effect of fermentation temperature on solid fermentation of dregs of liver protection tablets

As shown in Fig. 2D, when the fermentation temperature was increased from 25°C to 30°C, the viable count of Bacillus subtilis showed a significant increase, reaching a peak value of 3.55×10^9 CFU/g at 30°C. Therefore, the optimal fermentation temperature was determined to be 30°C.

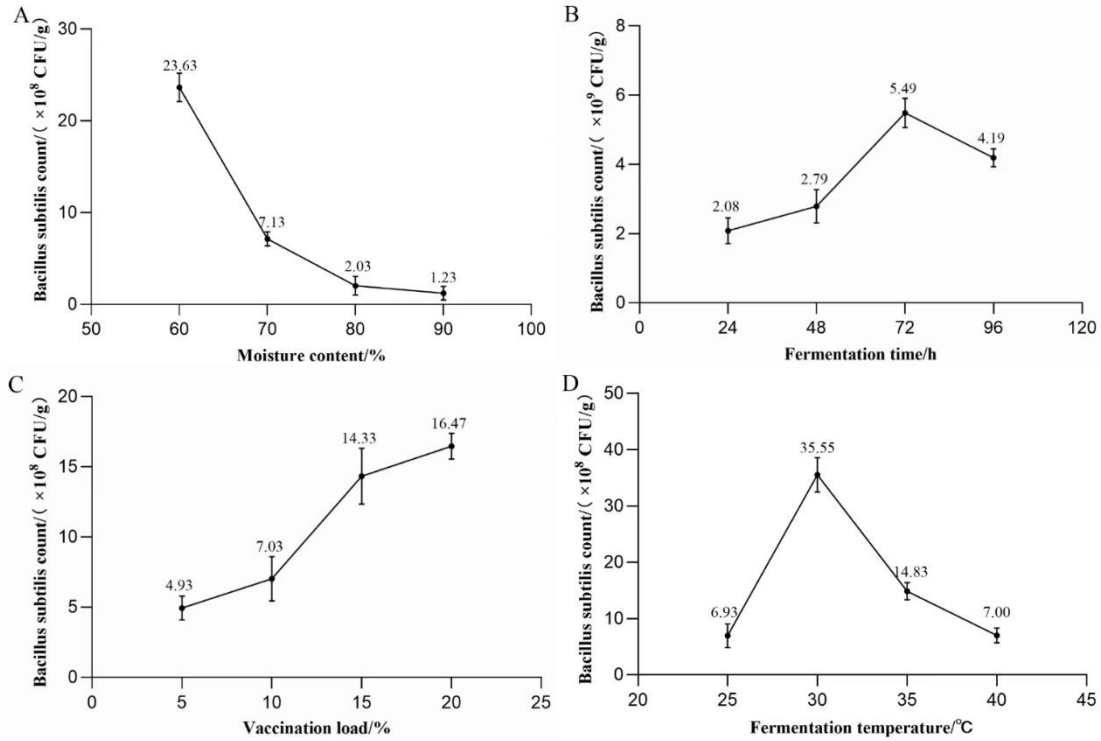

Figure 2. One-factor experiment on the fermentation of dregs of liver protection tablets

Note: Figure A: Effect of water content on solid fermentation of liver protectant tablet dregs, Figure B: Effect of fermentation time on solid fermentation of liver protectant tablet dregs, Figure C: Effect of inoculum amount on solid fermentation of liver protectant tablet dregs, Figure D: Effect of fermentation temperature on solid fermentation of liver protectant tablet dregs.

3.3 Changes of bioactive substances before and after fermentation of pharmaceutical dregs

In the context of a 60% water content, a fermentation temperature of 30℃, a fermentation time of 72 h, and an inoculum amount of 20%, a significant increase in the polysaccharide and polyphenol contents of the dregs of liver protection tablets was observed ($P < 0.05$). Concurrently, a significant decrease in saponins and flavonoids was noted ($P < 0.05$) post-fermentation, as illustrated in Table 2. The decline in saponins and flavonoids in the dregs of the tablets during fermentation can be ascribed to the decomposition or transformation of constituent components, resulting from specific chemical reactions during the fermentation process. Conversely, an increase in the content of polysaccharides and polyphenols was observed, which is likely attributable to the fermentation process, as it has been demonstrated that this process can promote the extraction of these original compounds.

Table 2: Comparison of bioactive substances per milligram of fermented dregs versus dregs

Bioactive substance	Liver Tablet Dregs（mg）	Fermented Liver Tablet Dregs（mg）
Saponin	0.31±0.09 a	0.26±0.04 b
Flavonoid	1.66±0.14 a	1.52±0.05 b
Polysaccharide	3.12±0.13 b	3.41±0.03 a
Polyphenol	2.18±0.17 b	4.25±0.14 a

Note: Peer data labeled with different lowercase letters on the shoulder indicate significant differences ($P < 0.05$), while the same or no letter indicates non-significant differences ($P > 0.05$). The following table is the same.

3.4 EFFECT OF FERMENTED MEDICINAL DREGS ON LIVER AND KIDNEY TISSUES OF BROILERS

In order to further evaluate the safety of the fermented dregs, a morphological analysis was performed on liver and kidney tissue sections of broilers. The addition of 1%, 3% and 5% fermented liver tablet dregs did not cause significant pathological changes in the morphological characteristics of liver and kidney tissues of white feather broilers (see Fig. 3 Liver and kidney tissue sections). As demonstrated in Fig. 3, compared with the control group, the hepatocytes of the experimental group exhibited well-arranged structures, clear hepatic blood sinusoids, and no signs of cell swelling, necrosis, or inflammatory infiltration. In the kidney tissue section, the structure of renal tubules and glomeruli was clear and intact, and the distribution of renal mesenchyme was normal, and no characteristic pathological changes were triggered by the change of dosage of drug residue. The results indicated that the dregs of fermented liver protection tablets did not produce significant hepatorenal toxicity in white feather broilers.

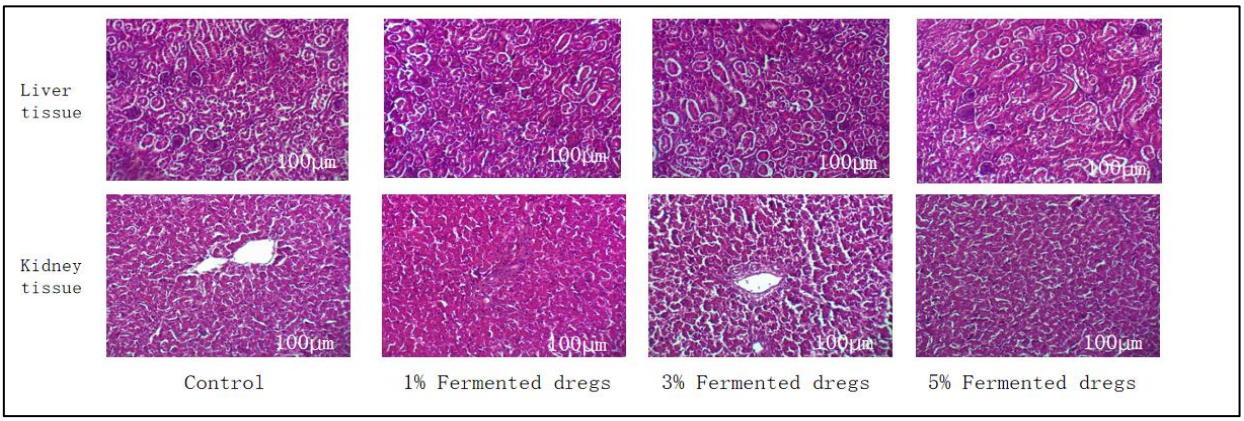

Fig. 3 Liver and kidney tissue sections

3.5 EFFECT OF FERMENTED DRUG RESIDUES ON THE SLAUGHTERING PERFORMANCE OF BROILER CHICKENS

The effects of fermented liver tablet dregs on the slaughtering performance of white-feathered broiler chickens are shown in Table 3. In the 21-day feeding test, no significant differences (P>0.05) were observed between the groups with the exception of live weight. However, the experimental groups demonstrated varying degrees of enhancement in slaughtering performance indicators in comparison with the control group. The 5% fermented dregs group exhibited the most significant enhancements, with an increase of 10.7% in slaughter weight, 8.89% in half-clearance weight, 3.63% in full-clearance weight, 7.87% in pectoral weight, 6.79% in full leg weight, 10.9% in leg muscle weight, and 17.6% in full wing weight compared with the control group. The 3% fermented dregs group demonstrated a 14.0% increase in pectoral weight compared with the control group. Conversely, the enhancement effect was observed to be less pronounced in the indexes of 1% slaughter rate, semi-clearance and pectoral muscle rate. The results indicated that the addition of fermented liver protection tablets dregs to the feed could improve the slaughtering performance of broilers.

Table 3: Effect of fermented liver tablet dregs on slaughtering performance of white feather broilers

Project	Control subjects	1% Fermented dregs group	3% Fermented dregs group	5% Fermented dregs group
Deadweight (g)	686.07±36.95 b	718.45±78.77 b	764.4±63.96 a	774.72±37.52 a
Butcher's weight (g)	663.4±37.13 a	687.35±76.72 a	716.1±61.04 a	744.31±47.46 a
Slaughtering rate (%)	0.97±0.04 a	0.96±0.02 a	0.94±0.01 a	0.95±0.01 a
Half-naked chamber (g)	611.45±32.09 a	634.22±81.06 a	642.3±49.65 a	671.11±49.22 a
Half-clearance rate (%)	0.89±0.03 a	0.88±0.02 a	0.84±0.01 a	0.86±0.04 a
Complementary weight (g)	557.85±32.00 a	578.18±64.01 a	578.16±58.13 a	578.11±59.07 a
Full bore rate (%)	0.81±0.03 a	0.80±0.01 a	0.76±0.04 a	0.77±0.02 a
Pectoral weight (g)	117.2±12.59 a	120.45±16.79 a	136.12±17.48 a	127.81±19.57 a
Pectoral muscle rate (%)	0.21±0.01 a	0.21±0.02 a	0.24±0.02 a	0.22±0.05 a
Full-legged weight (g)	169.37±14.69 a	174.82±24.46 a	175.38±21.49 a	181.7±16.45 a
Leg ratio (%)	0.30±0.01 a	0.30±0.01 a	0.30±0.01 a	0.32±0.04 a
Hamstring muscle weight (g)	80.48±10.79 a	87.42±10.28 a	83.94±26.88 a	90.3±13.64 a
Hamstring muscle rate (%)	0.14±0.01 a	0.15±0.01 a	0.14±0.03 a	0.16±0.03 a
Total wing weight (g)	51.02±8.79 a	54.98±9.43 a	55.88±9.15 a	62.11±5.38 a
Wing rate (%)	0.09±0.02 a	0.09±0.01 a	0.10±0.01 a	0.11±0.02 a

4. DISCUSSION

In this study, an optimisation of the solid-state fermentation process of liver protection tablets dregs was conducted, resulting in a significant increase in the content of polysaccharides and polyphenols, and a concomitant decrease in the content of saponins and flavonoids. These findings were achieved under the synergistic effect of water content (60%), temperature (30°C), time (72 h) and inoculum (20%). This outcome may be associated with the metabolic characteristics of Bacillus subtilis Bu412 [9]. The secreted enzymes of this organism have been observed to facilitate the decomposition of certain macromolecules (e.g., saponins and flavonoids) present within the dregs, while concurrently promoting the release or biotransformation of polysaccharides and polyphenols [10]. The increased content of polysaccharides and polyphenols, which are natural antioxidant actives, can improve the nutritional value of the dregs and lay the foundation for subsequent feed applications [11]. In the feeding test, although the addition of fermented dregs did not significantly improve the slaughtering performance of white-feathered broilers, the indexes of each experimental group were superior to those of the control group. Furthermore, the histomorphometric analysis of liver and kidney tissues did not reveal any pathological damage, which indicated that fermented dregs had potential for optimisation in improving the production performance of broilers, and that they were safe. The results of the study provide a reference for the secondary development and utilisation of Chinese medicine dregs and the research and development of alternative-resistant feed additives.

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