

EFFECTS OF KOMBUCHA PELLICLE SUPPLEMENTATION ON BROILER MORTALITY AND INTERNAL ORGANS OVER 35 DAYS

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Abstract. This study investigated the effects of Sabah tea kombucha pellicle supplementation on mortality rate, physical and behavioural condition, and internal organ development in broiler chickens over 35 days of feeding trial. Broilers were divided to four groups: a basal diet control and three treatment groups supplemented with 0.05% (T1), 0.1% (T2), or 0.2% (T3) kombucha pellicle. The control group exhibited the highest mortality rate (7.69%), while T1 recorded 100% survival and T2 and T3 showed lower mortality (2.56% and 5.13%, respectively) than the control. Control birds also displayed poorer physical and behavioural indicators, including damp litter, watery excreta, ruffled feathers, panting, high water intake, and reduced activity. These signs are consistent with heat stress and gut disturbance, whereas treatment groups exhibited healthier plumage, normal fecal consistency, and no panting. Carcass assessment revealed no significant differences in relative liver weight or small intestine length across groups, indicating the absence of hepatotoxicity or intestinal morphological damage. A dose-related increase in caeca length was observed, suggesting enhanced fermentative activity at higher pellicle inclusion. Collectively, these findings suggest that kombucha pellicle supplementation, at inclusion levels up to 0.2%, is safe for broiler consumption and may improve survivability, general health, and gut function without adverse effects on internal organs.

Keywords: *kombucha pellicle, feed supplementation, broiler chickens, mortality rate, safety assessment*

Introduction

The broiler chicken industry remains one of the most significant players in food supply globally valued for its short production cycle, efficient feed conversion, and relatively low production cost compared to other livestock (Pesti and Choct, 2023). However, the industry frequently associated with elevated stress levels, poor gut health, and increased susceptibility to pathogenic infections, which can contribute to higher mortality rates. Generally, antibiotic growth promoters (AGPs) have been widely used to mitigate these issues by improving growth performance and reducing disease incidence, however, growing concerns over antibiotic resistance and residue accumulation in poultry products have led to increasing restrictions on AGP use in many countries (Zhu et al., 2021). This has led to the search for safe, natural, and cost-effective alternatives capable of supporting poultry health and performance without the drawbacks associated with synthetic antibiotics. Among the alternatives being explored, fermented functional foods and beverages have gained a lot of interest from researchers due to their rich content of bioactive compounds, organic acids, and probiotic microorganisms (Valero-Cases et al., 2020). During fermentation of kombucha, a beverage produced through the fermentation of sweetened tea by a symbiotic culture of bacteria and yeast (SCOBY), a cellulose-based biofilm known as the kombucha pellicle was produced. This pellicle contains polyphenols, organic acids (such as acetic and gluconic acid), and residual probiotic microorganisms from the fermentation process

(Ojo and de Smidt, 2023). While the liquid component of kombucha has been studied for its antimicrobial, antioxidant, and gut-modulating properties (Hartono et al., 2026), the pellicle itself, despite being a byproduct rich in similar bioactive compounds, remains comparatively underexplored as a functional feed supplement.

While kombucha and tea polyphenols individually show promising benefits, only few studies have specifically explored kombucha pellicle as compared to the liquid, as a feed supplement in broiler chickens (Salehi et al., 2021; Afsharmanesh and Sadaghi, 2013; Murugesan et al., 2005). Limited data exists on how the supplementation may influence broiler mortality rates and the physiological condition of internal organs, which serve as critical indicators of overall chicken health and feed safety (Basit et al., 2020). Given that 35 to 42 days represents a standard rearing period commonly used to assess broiler growth performance and marketability, evaluating the effects of this byproduct of kombucha as feed supplementation across this duration provides a practical and industry-relevant timeframe for assessment (Wang et al., 2025; Bortoluzzi et al., 2013). Therefore, this study aims to investigate the effect of kombucha pellicle supplementation on the mortality rate and internal organ condition of broiler chickens over 35 days of rearing period. The findings of this study are expected to contribute to the growing body of knowledge on natural feed additives and provide insight into the viability of kombucha pellicle as a sustainable, locally sourced alternative to conventional growth promoters in poultry production.

Materials and Methods

Preparation of kombucha pellicle

The pellicle was prepared following the same method from our previous study (Nasir et al., 2024). Sabah tea (*Camellia sinensis*), obtained from a local market in Kota Kinabalu, Sabah, Malaysia, was used as the fermentation substrate. Briefly, black tea (10 g) was added to 1 L of sterile water containing 10% (w/v) sucrose and boiled for 15 minutes. Once brewed, the tea was transferred into a sterile container and allowed to cool to room temperature prior to inoculation with 10% (v/v) SCOBY starter culture. The container was then covered with a sterile cloth and secured with a rubber band to prevent contamination. Fermentation was carried out in the dark at room temperature (23–27 °C) for 30 days to allow sufficient pellicle formation. Following fermentation, the pellicles were aseptically separated and pooled into a sterile flask containing fresh sugared black tea to ensure homogeneity across batches, prior to storage at 4 °C for up to one month until further use.

Feed preparation

The kombucha pellicle was weighed, cut into smaller pieces, and thoroughly blended into the commercial chicken feed to ensure even distribution at concentrations of 0.05%, 0.1%, and 0.2% for the T1, T2, and T3 treatment groups, respectively. As the pellicle is inherently moist while the feed is dry, the supplemented feed was freshly prepared daily to minimize the risk of contamination.

Animal study

The animal study was conducted at the Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Sandakan Campus, following the ethical guidelines provided by the

Animal Ethics Committee, University Malaysia Sabah, with reference number AEC0010/2022. The experimental diets and procedures were conducted following previous studies by Ooi et al. (2018) and Olnood et al. (2015) with slight modifications. A total of 150 one-day-old broiler chickens (Cobb500) were purchased from Tshun Wah Trading, Sandakan, Sabah, Malaysia. The birds were acclimatized for 24 hours and fed the starter and grower basal diet (BD) (*Table 1*) free of antibiotics from Gold Coin Feedmills Sdn. Bhd., Malaysia, with water provided ad libitum. Following acclimatization, the birds were randomly divided into four groups: BD (control, C), BD supplemented with 0.05% kombucha pellicle (T1), BD supplemented with 0.1% kombucha pellicle (T2), and BD supplemented with 0.2% kombucha pellicle (T3) (*Table 2*). For the first 14 days, the birds were fed a starter basal diet supplemented with the pellicle, followed by a grower basal diet similarly supplemented with kombucha pellicle from day 15 onward.

Table 1. *Ingredients and guaranteed analysis of the basal diet fed to broiler chickens.*

Basal diet	Starter (Day 1-14)	Grower (Day 15-35)
Ingredients ^a	Corn, soybean meal, other grains and grain by-products, vegetable oil, dicalcium phosphate, amino acids, vitamins, approved antimicrobials and additives	Corn, soybean meal, other grains and grain by-products, vegetable oil, dicalcium phosphate, amino acids, vitamins, approved antimicrobials and additives
Nutrient composition		
Crude protein, min	21.0	19.0
Crude fibre, max	5.0	5.0
Crude fat, min	5.0	5.0
Moisture, max	13.0	13.0
Ash, max	8.0	8.0
Calcium, min	0.8	0.8
Phosphorus, min	0.6	0.6

Note: ^aIngredient composition as declared by the manufacturer (Gold Coin Feedmills Sdn. Bhd., Malaysia); exact inclusion percentages are proprietary and not disclosed on the product packaging.

Table 2. *The experimental diet given to the broiler chickens throughout the study.*

Group	Experimental diets
C	Basal diet (BD)
T1	BD with 0.05% kombucha pellicle (w/w)
T2	BD with 0.1% kombucha pellicle (w/w)
T3	BD with 0.2% kombucha pellicle (w/w)

Sample collection

After 35 days of feeding trials, the broiler chickens were slaughtered at the Post-harvest Processing Facility, Universiti Malaysia Sabah, Sandakan Campus, where the breast and thigh muscles of broiler chickens were collected and weighed, while the small intestine and caeca were harvested, and their lengths were measured.

Statistical analysis

Data were analyzed using GraphPad Prism 9.5.1 for Windows (GraphPad Software Inc., USA) and are presented as mean $\pm$ standard deviation. Differences among groups were evaluated using one-way ANOVA followed by Tukey's multiple comparison test, with a p-value of less than 0.05 considered statistically significant.

Results and Discussion

Safety consumption assessment

The mortality rate of broiler chickens is shown in *Table 3*. The control group recorded the highest mortality rate (7.69%), followed by the T3 (5.13%) and T2 (2.56%) groups after 35 days of feeding trials, while the T1 group maintained a 100% survival rate throughout the study. This pattern suggests no dose-dependent toxicity was induced by increasing pellicle inclusion, since the highest inclusion level (T3) did not produce the highest mortality. The mortality data also indicate that pellicle supplementation, at all tested inclusion levels, did not increase mortality relative to the basal-diet control. Notably, the mortality rates observed across all groups in this study were lower than the average mortality rate of broiler production in Sabah reported by Md Isa et al. (2019), which was 8.7% per cycle, indicating that the broiler chickens in this study were in good health.

Table 3. Mortality rate of broiler chickens.

Group	Number of deaths					Total number of chieckens	Mortality rate (%)
	W1	W2	W3	W4	W5		
Control	0	0	0	1	2	39	7.69
T1	0	0	0	0	0	33	0
T2	0	0	0	1	0	39	2.56
T3	0	1	1	0	0	39	5.13

Note: Control: Basal diet (BD); T1: BD + 0.05% pellicle; T2: BD + 0.1% pellicle; T3: BD + 0.2% pellicle; W1=Week 1 until W5=Week 5.

Based on the data, it was observed that the mortality in the control group was concentrated in the later growth phase (week 4 to 5), a period during which broilers remain within the recognized risk window for metabolic stress, rapid weight gain, and increased heat production. These factors are known to predispose fast-growing birds to cardiac arrhythmias and sudden death syndrome, which typically peaks between weeks 3 and 4 but can continue to affect birds through to market age (Sosnówka-Czajka and Skomorucha, 2022). In contrast, mortality in T3 occurred earlier (week 2 to 3), coinciding with the period when broiler chicks require an adaptation window to fermented feed products, marked by reduced feed intake driven by the higher organic acid content and lower palatability typical of such ingredients during the starter and grower phases (Katu et al., 2025; Sugiharto and Ranjitkar, 2019). This is consistent with reports that fermented feed inclusion can transiently affect performance in the early phases without carrying over into later stages of growth (Zhou et al., 2023), supporting the interpretation that the earlier mortality in T3 reflects a transient physiological adjustment to the higher inclusion rate of the kombucha pellicle by product rather than a cumulative toxic effect, particularly given that no further deaths occurred beyond week 3.

In addition, T2 recorded a single mortality in Week 4, coinciding with the same late-phase mortality window observed in the control. Since mortality in broilers is known to be strongly influenced by non-identifiable environmental and flock-level factors rather than a single specific cause (Richter et al., 2024; Van Limbergen et al., 2020), and given the isolated nature of this event, it is more plausible that this mortality reflects general flock-related or environmental conditions rather than the dietary treatment itself. Broiler mortality rates in well managed commercial flocks over 35 days of production cycle typically range between 3 to 5% (Slegers et al., 2024; Hasnul et al., 2015).

Benchmarked against this range, the control mortality rate (7.69%) appears elevated, whereas T1 (0%) and T2 (2.56%) fall below or within the expected range, and T3 (5.13%) lies at the upper end of normal variation. These findings further support the safety of pellicle supplementation for broiler consumption within the tested inclusion range (0.05 - 0.2%) and suggest a potential improvement in survivability in broiler chickens. The physical and behavioural observations of broiler chickens further reveal a clear pattern distinguishing the control group from all pellicles supplemented groups as depicted in *Table 4*. The control group exhibited persistently damp litter and watery excreta with mucus, both classic indicators of digestive disturbance, subclinical enteric issues, or excessive water intake associated with gut dysbiosis. In contrast, T1 and T3 showed partially dry litter with normal to slightly watery droppings, while T2 achieved the driest litter condition and fully normal fecal consistency. This gradient suggests that pellicle supplementation, even at the lowest inclusion level (0.05%), exerted a beneficial effect on gut health and moisture regulation, potentially through modulation of the gut microbiota or improved nutrient digestibility or improved (Salehi et al., 2021; Iqbal et al., 2020), both of which are commonly attributed to kombucha derived polyphenols and organic acids.

Table 4. *Physical and behavioural observations of broiler chickens.*

Parameters	Control	T1	T2	T3
Litter condition	Often damp	Partial dry	Dry	Partial dry
Fecal dropping	Watery excreta with mucus	Normal	Normal	Slightly watery
Feather condition	Slightly ruffled and dirty	Smooth and clean	Smooth and clean	Smooth but slightly dirty
Panting	Often	Never	Never	Never
Drinking	High water intake	Regular water intake	Regular water intake	Regular water intake
Activity level	Less active	Always active	Slight reduction in activity in a few birds	Always active
Alertness	Mostly alert	Always alert	Always alert	Always alert

Note: Control: Basal diet (BD); T1: BD + 0.05% pellicle; T2: BD + 0.1% pellicle; T3: BD + 0.2% pellicle.

Figure 1a shows the one-day old birds in comparison with the 35 day old birds. Throughout the 35 days observation period, the control group exhibited ruffled, dirty feathers, whereas all treatment groups (T1, T2, and, to a slightly lesser extent, T3) displayed smooth, cleaner plumage. Since feather condition is widely used as an external indicator of general health, nutritional adequacy, and welfare status in poultry (Saraiva et al., 2016), this improvement in treatment groups further supports a positive effect of pellicle supplementation on overall bird condition, independent of dose. Panting, a well-established behavioural indicator of heat stress in poultry (Olayiwola and Adedokun, 2025), was frequently observed in the control group but was never recorded in the T1, T2, or T3 groups. This is a particularly meaningful finding, as heat stress in broilers is strongly associated with reduced feed efficiency, immune suppression, and increased mortality risk, especially during the later growth phase (Apalowo et al., 2024; Brugaletta et al., 2022). The complete absence of panting across all treatment groups suggests that pellicle supplementation may confer a protective effect against heat stress, which could plausibly account for the lower late-phase mortality observed in T1 and T2 relative to the control, as discussed previously. Consistent with this, the control group also demonstrated high water intake, which, alongside its watery excreta and panting behaviour, indicating a compensatory response

to heat stress or mild dehydration inducing gut disturbance (Liu et al., 2025). Interestingly, birds in the treatment groups showed regular water intake, aligning with the absence of panting and improved fecal consistency. The control group was also notably less active than the treatment groups, and this sign is often associated with lethargy, subclinical illness, or heat stress (Kim et al., 2025; Brugaletta et al., 2022). The alertness scores mirrored this activity pattern, reinforcing the overall trend that pellicle supplemented birds exhibited better general health and vitality compared to control birds (*Figure 1b*).



Figure 1. One-day old birds (A) and 35-days old birds (B).

Carcass assessment

Figure 2 showed the effects of kombucha pellicle supplementation on liver weights and small intestine and caeca lengths of broiler chickens. The relative liver weights were numerically comparable across the control (32.69 ± 2.14 g) and T1 (32.67 ± 0.35 g) groups, with a modest, dose-related increase observed in T2 (33.50 ± 7.47 g) and T3 (34.50 ± 2.65 g). However, none of the treatment groups differ significantly from the control, indicating that the supplementation, even at the highest inclusion level (0.2%), did not induce hepatomegaly or overt hepatic stress. On the other hand, the small intestine length was similar between the control (203.07 ± 5.05 cm) and T1 (203.02 ± 7.68 cm) groups, while T2 (189.50 ± 16.49 cm) and T3 (201.98 ± 18.66 cm) showed slightly shorter mean lengths. Since intestinal length is often used as an indirect indicator of gut adaptation or nutrient absorption capacity (Gorenz et al., 2024), in which the numerically shorter intestines in T2 could suggest a mild adaptive response to pellicle inclusion. Nevertheless, as no statistically significant differences were detected among groups, this suggests that pellicle supplementation, even at the highest inclusion level (0.2%), does not adversely affect small intestinal morphology or growth in broiler chickens. In addition, caeca length showed a slight upward trend with increasing pellicle inclusion with control (13.30 ± 1.49 cm), T1 (13.89 ± 0.49 cm), T2 (13.80 ± 1.04 cm), and T3 (15.18 ± 0.88 cm). This pattern is the most consistent dose-related trend, with T3 showing the longest caeca and the tightest variability (± 0.88) among treatment

groups. Since the caeca are a key site for microbial fermentation and short-chain fatty acid production in poultry, this trend could tentatively suggest enhanced fermentative activity or microbial adaptation associated with higher pellicle inclusion (0.2%), which may align with the polyphenol and prebiotic-like properties often attributed to kombucha derived products (Park and Manna, 2025; Uțoiu et al., 2018).

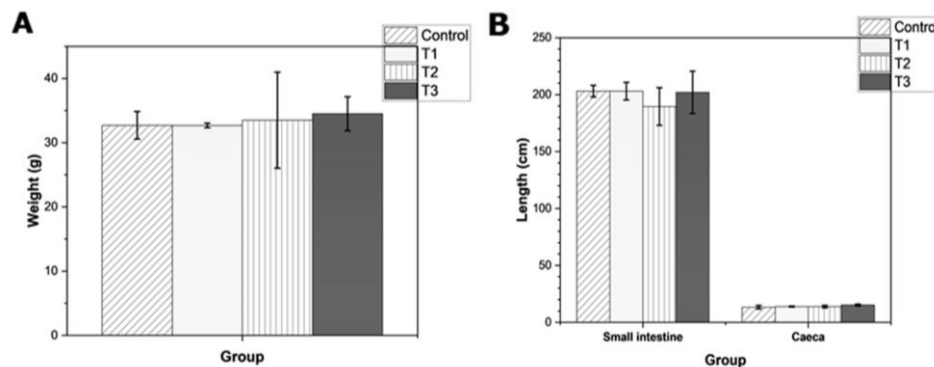


Figure 2. Effects of Sabah Tea Kombucha Pellicle Supplementation on Liver Weights (A) and Small Intestine and Caeca Lengths (B) of Broiler Chickens.

Note: The values are presented as mean $\pm$ standard deviation. * Means is considered statistically significant at $P < 0.05$; Control: Basal diet (BD); T1: BD + 0.05% pellicle; T2: BD + 0.1% pellicle; T3: BD + 0.2% pellicle.

Conclusion

Overall, dietary inclusion of kombucha pellicle, up to 0.2%, did not adversely affect liver mass, gastrointestinal tract morphology, or organ development in broiler chickens, as evidenced by the absence of statistically significant differences ($P < 0.05$) across these parameters. The mild, non-significant upward trends in relative liver weight and caeca length with increasing inclusion level may reflect early, dose-dependent physiological adaptations, such as mild hepatic metabolic activity or enhanced hindgut fermentation rather than any adverse effect, though this warrants further investigation in a larger cohort. These findings are further supported by consistent improvements in physical and behavioural indicators across all treatment groups, including better litter condition, fecal consistency, feather quality, reduced heat-stress-related panting, and higher activity and alertness levels relative to the control. Taken together with the carcass, organ, and mortality data, including lower mortality rates in T1 and T2 relative to control, these results collectively indicate that kombucha pellicle supplementation enhanced growth performance without inducing organ-level toxicity. Rather than merely demonstrating an absence of harm, the data suggest that pellicle supplementation may confer measurable welfare and health benefits, supporting its safety and potential value as a functional feed additive for broiler chickens under the conditions of this study.

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Conflict of interest

The authors have declared that no conflict of interest exists. The animal study was conducted according to the guidelines and approval by the Animal Ethics Committee, Universiti Malaysia Sabah (UMS) (AEC 0010/2022).

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