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# The Effect ff Feed Acidification on Protein, Fat, and Cholesterol Content of Meat Broiler Fed Two Different Commercial Diets

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**Abstrak**

Penelitian ini bertujuan untuk menganalisis pengaruh perbedaan jenis pakan komersial, tingkat pengasaman, dan interaksinya terhadap bobot karkas, pH daging dada, kadar protein, lemak, dan kolesterol ayam pedaging. Sebanyak 160 ekor ayam pedaging (umur 21 hari, campuran jantan dan betina) dibagi secara acak ke dalam 8 kelompok dengan 4 kali ulangan per kelompok, dan 5 ekor ayam per ulangan. Metode penelitian menggunakan Rancangan Acak Lengkap dengan pola faktorial 2x4 yang melibatkan 2 jenis pakan komersial (pakan A dan pakan B), dan 3 level (L) pengasaman (0%; 0,5%; 1%; 1,5%). Karkas serta protein, lemak, dan kolesterol daging dada dianalisis pada hari ke-35. Hasil penelitian menunjukkan bahwa dengan peningkatan level pengasaman, maka kadar lemak dan kolesterol menurun secara nyata (P<0,05), mencapai nilai terendah pada level pengasaman 1 dan 1,5%. Berat karkas dan kandungan protein daging dada tidak dipengaruhi oleh jenis pakan dan tingkat pengasaman. Pakan serta interaksi antara pakan dan tingkat pengasaman tidak memberikan pengaruh yang signifikan terhadap semua parameter yang diukur. Kesimpulan, kandungan lemak dan kolesterol menurun ketika tingkat pengasaman berada di antara 1 dan 1,5% terlepas dari jenis pakan komersial.

## 1. INTRODUCTION

Broiler meat without antibiotics is increasingly sought after due to consumer concerns about antibiotic resistance and a preference for what's perceived as a healthier product. A source of acidifier can be simply applied as an alternative to antibiotics. Studies have demonstrated that acidifiers can enhance growth performance, particularly during the finisher phase (Šamudovská *et al.*, 2018; Lückstädt & Akyürek, 2004).

Lime juice is one of the natural acidifiers that contains bioactive compounds and citric acid that improve nutrient utilization (Imam *et al.*, 2018) and enhance the nutritional profile of poultry meat. The primary organic acids found in lime juice are citric acid and ascorbic acid. Citric acid in lime juice can alter intestinal pH and morphology of the gastrointestinal tract which may contribute to improved health, performance, and feed efficiency (Ao, *et al.*, 2009). Acidification also enhances protein content and reduces fat in breast meat, while decreasing lipid oxidation and improving antioxidant activity.

The optimal level of acidifier supplementation varied among studies, with effective ranges from 0.7% to 3% of the diet (Abdelrazek *et al.*, 2016). Frasiska *et al.*, (2022) indicated that excessive use of acidic substances like lime juice could lead to a decrease in pH levels in meat, which might affect protein stability and functionality. Therefore, careful consideration of its concentration is necessary to avoid potential adverse effects on protein integrity.

Research on the impacts of acidifiers on poultry has so far mostly concentrated on intestinal organ development, performance, and microbiota using synthetic acidifiers; however, little is known about the effects of natural acidifiers on meat's protein fat, and cholesterol content. Additionally, the feed given, together with specific feed additives or supplements, can also affect the amount of protein, fat, and cholesterol in chicken meat. Studies have shown that different feed types or additives can affect the quality of fat in chicken meat, potentially influencing the overall nutritional profile. Each feed company has different ration formulations and sources of feed ingredients, so there are differences in growth responses in chickens. Commercial diets with different brands is expected to improve the physical quality of meat. Therefore, the objective of this study is to investigate the effect of different diets supplemented with different levels of natural acidifier, and their interaction on carcass weight, pH of breast meat, protein, fat, and cholesterol content of broiler chickens

## 2. MATERIALS AND METHODS

One hundred and sixty birds of day-old-chicks broiler with an average initial body weight of 44±1.2 g were used as experimental animals. The birds were given two different commercial diets (Diet A and Diet B) in pelleted form. Diets were given ad libitum and free access to drinking water. The nutrition composition of diet A (DA) is presented in Table 1, and the nutrition composition of diet B (DB) can be seen in Table 2.

### 2.1. Source of The Acidifier

The blended acidifier used in the current experiment was lime fruits as natural acidifiers. The clean lime fruits were split into two transverse portions to create a natural source of acidifier. After that, the juice from the lime fruits was physically squeezed out. To produce usable juice, the liquid extracted from lime fruits was filtered to exclude any potential seeds or other particles. To ensure the birds could finish the

experimental meal by the treatment level, lime juice as an acidifier was combined with a portion of the feed and served in the morning. The treatment levels (L) of acidifiers are: 0% (without lime juice); 0,5%; 1%; and 1,5%.

Tabel 1. Composition and nutrient levels of diet A (%, as-fed basis) for starter and finisher.

| Nutrition | DA (for starter) | DA (for finisher) |
|---|---|---|
| Moisture (%) | Max 13.00 | Max 14.00 |
| Crude protein (%) | 21,00-23,00 | 19,00 |
| Crude fat (%) | 5.00 | 5.00 |
| Crude fiber (%) | 5.00 | 6.00 |
| Ash (%) | 7.00 | 8.00 |
| Ca (%) | 0,90 | 0,80 |
| P (%) | 0,60 | 0,45 |

Table 2. Composition and nutrient levels of the diet B (%, as-fed basis) for starter and finisher.

| Nutrition | DB (for starter) | DB (for finisher) |
|---|---|---|
| Moisture (%) | Max 14% | Max 14.00 |
| Crude protein (%) | 20.00 | 19.00 |
| Crude fat (%) | 5.00 | 5.00 |
| Crude fiber (%) | 5.00 | 6.00 |
| Ash (%) | 8.00 | 8.00 |
| Ca (%) | 0,50-1,10 | 0,80-1,10 |
| P (%) | 0,50 | 0,45 |

### 2.2. Analytical Procedures

At day 35, five birds per pen were slaughtered at a processing plant. The carcasses were weighed, and a 100g sample of breast meat from each treatment was analysed in the laboratory for the content of protein, lipid, and cholesterol in each treatment. After a 24-hour slaughter, breast pH was measured. A probe was inserted directly into the breast meat muscle using a pH meter (Testo 205, Germany) to measure pH.

### 2.3. Cholesterol

Total cholesterol was extracted from lyophilized meat (dry matter) following saponification with saturated methanolic KOH. The total cholesterol content of each meat sample was determined in duplicate using the standard approach. Meat's (dry matter) total lipids were extracted and measured using the Soxhlet method (Association of Official Analytical Chemists, 1980). The AOAC's (1980) procedures were followed while analyzing the feed for dry matter, ether extract, crude protein, and dietary fiber.

### 2.4. Experimental Design and Statistical Analysis

Research methods using a Completely Randomized Design with a 2x4 factorial pattern to establish the effect of 2 types of commercial diet (Diet A and Diet B) and the level of lime juice (0%, 0,5%, 1%, 1,5% per kg diet). There are 8 combinations of treatments, each treatment was repeated 3x, and in each repetition, there were 5 chickens. To assess the impact of treatments, analysis of variance was used. In cases where there was no significant two-way interaction, the primary effects were explored. When two-way interactions were significant, the simple effects were examined. Tukey's HSD was used to separate means when there were significant interactions with a 5% probability for P-value.

## 3. RESULTS AND DISCUSSION

Data concerning the carcass weight, protein, fat content, pH of breast meat, and cholesterol of breast meat are presented in Table 3.

### 3.1. The Effect of Treatment on Carcass

Results in Table 3 showed that there were no significant interactions (P>0.05) between diets and levels of acidifiers on carcass weight. Different diets and different levels of acidifier had no significant effect (P>0.05) on carcass weight.

The results of this investigation are consistent with those of Gapsiso & Shua (2021), who found no significant differences in the carcass weight of broilers given three distinct commercial diets. The amount of protein in the ration is one of the elements that influences the proportion of carcass (Bansal *et al.*, 2011). Because diet A (DA) and diet B (DB) in the finisher phase had the same protein content (19%), their effects on the percentage of carcass are identical. This study demonstrated that the nutrient composition of the diets used satisfied the nutritional needs of all birds, despite differences in brand, and may contain various mixing ingredients that result in variations in quality. Diets are designed to supply the precise amount of nourishment required for good performance. To achieve the ideal carcass weight, having the right standard

nutrition for poultry meals is crucial. The study's carcass weight is between the normal range of 1.1 to 1.2 kg at 35 days.

The levels of acidifier up to 1,5% in the diet had no significant effect on carcass weight. That could be because of insufficient acidifier supplementation to improve feed efficiency. The result of this research showed that the levels of feed acidification up to 1.5% were not able to increase the absorption of nutrients, especially protein to build carcass yields, and to increase enzyme pepsin activity and protein digestibility. In contrast with the finding of (Brzoska *et al.,* 2013) who reported that feed acidification improved carcass yield by reducing pH in the bird's digestive tract, and increased mineral absorption and digestibility of protein by enhancing pepsin activity.

Table 3. The effect of treatment on the variables measured.

| Diet (D) | Acidifier level (%) | carcass weight (g) | Protein (%) | Lipid (%) | Cholesterol (mg/100g) | Breast pH | Dry matters (%) |
|---|---|---|---|---|---|---|---|
| DA | 0 | 1205 | 28.05 | 7.11 | 42.6 | 5.8 | 27.4 |
| | 0.5 | 1298 | 27.91 | 7.45 | 40.5 | 6.0 | 25.3 |
| | 1 | 1209 | 28.10 | 7.60 | 36.4 | 5.9 | 25.1 |
| | 1.5 | 1198 | 27.66 | 6.35 | 36.2 | 6.1 | 24.7 |
| DB | 0 | 1214 | 26.12 | 11.51 | 43.6 | 5.7 | 27.1 |
| | 0.5 | 1210 | 28.33 | 9.11 | 42.3 | 5.7 | 28.4 |
| | 1 | 1245 | 21.90 | 9.99 | 35.1 | 6.0 | 26.3 |
| | 1.5 | 1285 | 25.40 | 6.24 | 34.8 | 6.2 | 25.2 |
| SEM | | 11.24 | 7.03 | 1.27 | 2.04 | 1.45 | 1.47 |
| Main effect | | | | | | | |
| Diet (D) | DA | 1227,5 | 27.9 | 7.1[b] | 38.9 | 6.0 | 25.6 |
| | DB | 1238,5 | 25.4 | 9.2[a] | 39.0 | 5.9 | 26.8 |
| P-value | | 0.278 | 0.126 | 0.003 | 0.207 | 0.04 | 0.171 |
| Level (L) | 0 | 1209.5 | 27.1 | 9.3[a] | 43.1[a] | 5.8 | 27.3 |
| | 0.5 | 1254.0 | 28.1 | 8.3[a] | 41.4[a] | 5.9 | 26.9 |
| | 1 | 1227.0 | 25.0 | 8.8[a] | 35.8[b] | 6.0 | 25.7 |
| | 1.5 | 1241.5 | 26.5 | 6.3[b] | 35.5[b] | 6.2 | 25.0 |
| P-value | | 0.325 | 0.207 | 0.003 | 0.002 | 0.34 | 0.17 |
| Interaction effect | | | | | | | |
| D x L | P-value | 0.278 | 0.302 | 0.115 | 0.345 | 0.43 | 0.114 |

### 3.2. The Effect of Treatment on Cholesterol

As shown in Table 3, different diets had no significant effect (P>0.05) on the cholesterol content. However, different levels of lime juice had a significant effect (P<0.05) on the cholesterol. The current study showed that lime juice at the levels 1% and 1,5% gave lower cholesterol content than the other treatments. Results in Table 3 also showed there were no significant interactions (P>0.05) between different feeds and the levels of acidifier on breast meat cholesterol.

According to the current experiment, birds fed DA and DB did not exhibit significant (P>0.05) differences in meat cholesterol. In contrast, broilers that were given 1.5% lime juice had noticeably (P<0.05) reduced cholesterol levels, implying that the feed acidity may have been the primary factor influencing the cholesterol levels of the meat in these trials. The data support the earlier findings by Waghmare *et al.* (2025), who found that supplemented organic acid blends (acidapure powder 1 kg/MT feed) in broiler feeds significantly decreased the serum cholesterol in broiler chickens. The reduction of cholesterol is ascribed to the suppression of coenzyme reductase activity, a crucial regulatory enzyme implicated in the production of cholesterol (Lee *et al.*, 2004).

The fat content of broilers today is far larger than it was in the past (Wang *et al.*, 2006). It is essential to assess the cholesterol content of meat. In this current study shown that meat cholesterol concentration is associated with total fat content of the tissue. The amount of intramuscular fat determines the cholesterol percentage; that is, meat with a high intramuscular fat content has a higher cholesterol content (Alfaia *et al.*, 2007).

### 3.3. The Effect of Treatment on Protein and Fat Content

Table 3 shows that different diets had no significant (P>0.05) effect on protein content of meat broilers, but diets had a significant effect on fat content. Table 3 also shows that different levels of acidifier had a significant effect on fat content. It can be observed that with an increase in acidifier level, the total lipid significantly decreases. However, the interaction between sources of diets and the levels of acidifiers had no significant impact (P > 0.05) on the protein and fat content of broiler chicken breast meat.

The total lipid content of breast meat from birds exposed to DA was substantially lower than that of DB. This could be due to different protein levels in starter broiler diets. DA had higher protein content (21-23%) than DB (20%), which can lead to increased fat deposition in the body, including the breast meat. As Rodriguez *et al.* (2016) stated that higher protein diets generally result in decreased fat in breast meat. The protein sources and types of protein could be different between DA and DB. Different feed protein sources can impact fat deposition (Milczarek & Osek, 2019).

The birds fed 1,5% lime juice had a lower (P<0.05) fat proportion in breast meat than those without lime juice. The results of this study agree with Hasanuddin (2013) who found that the fat content of chicken breast can be reduced by providing 1,2% lime juice in feed (20,7ml/100g feed) because the citric acid in lime juice extract can reduce the activity of enzymes that digest fat, so that fat absorption in the intestines is reduced. The acidic conditions created in the digestive tract can also reduce the synthesis of fat in the blood, such as cholesterol.

Meanwhile, there was no difference (P>0.05) in the proportions of protein across the treatments. This is because chicken breast is a part of the chicken body that has a protein content, and is the main component in the structure of the breast meat of chickens. Lime juice can help smooth digestion and increase the absorption of other nutrients, but it does not directly affect the protein content of chicken meat. So, giving lime juice in chicken feed is more functional to improve the quality of feed in general, not to change or increase the protein content of chicken breast.

### 3.4. The Effect of Treatment on pH Breast

As shown in Table 3 that different diets had no significant (P>0.05) effect on pH of breast meat broilers, but different levels of acidifier had a significant effect on pH of breast meat. It can be seen that the pH of breast meat considerably rises as the acidifier level increases. However, the interaction between sources of diets and the levels of acidifiers had no significant impact (P > 0.05) on pH of breast meat.

The experiment's findings demonstrated that adding acidifiers (1%), and (1,5%) raised the breast meat's pH at 24 hours. This is in line with (Lee *et al.,* 2003), who found that supplementing with acidifier raised the pH of broiler thigh meat. The increase in antioxidant activity in the thigh meat of the birds fed the acidifiers may be the reason why supplementing with acidifiers prevented the drop in muscle pH. Previous studies have indicated that antioxidant activity in meat can be affected by the acidifier supplementation (Wang *et al.,* 2018). Furthermore, the gut microbiota and its metabolites may be impacted by acidifier supplements, which could mitigate the decline in muscle pH. Lime juice acidifier was successful in creating an acidic environment in the gastrointestinal tract. Acidifiers have been shown in trials to lower feed pH and enhance gut health, but their effects on meat pH are less consistent and may vary depending on the kind and dosage of acidifier.

## 4. CONCLUSION

In conclusion, the inclusion of 1-1.5% lime juice in broiler chicken diets, regardless of diet A or diet B, appears to have beneficial effects in lowering the fat and cholesterol content of the breast meat of broiler chickens.

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