

APPLE CIDER VINEGAR SUPPLEMENTATION ENHANCES GROWTH PERFORMANCE, FEED EFFICIENCY, AND PHYSIOLOGICAL RESPONSES IN JAPANESE QUAILS

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Japanese quail, feed intake, feed conversion ratio, carcass characteristics, serum biochemistry, intestinal morphology, gut health, organic acid, poultry nutrition

Article History

Received: 16 May 2026

Accepted: 12 June 2026

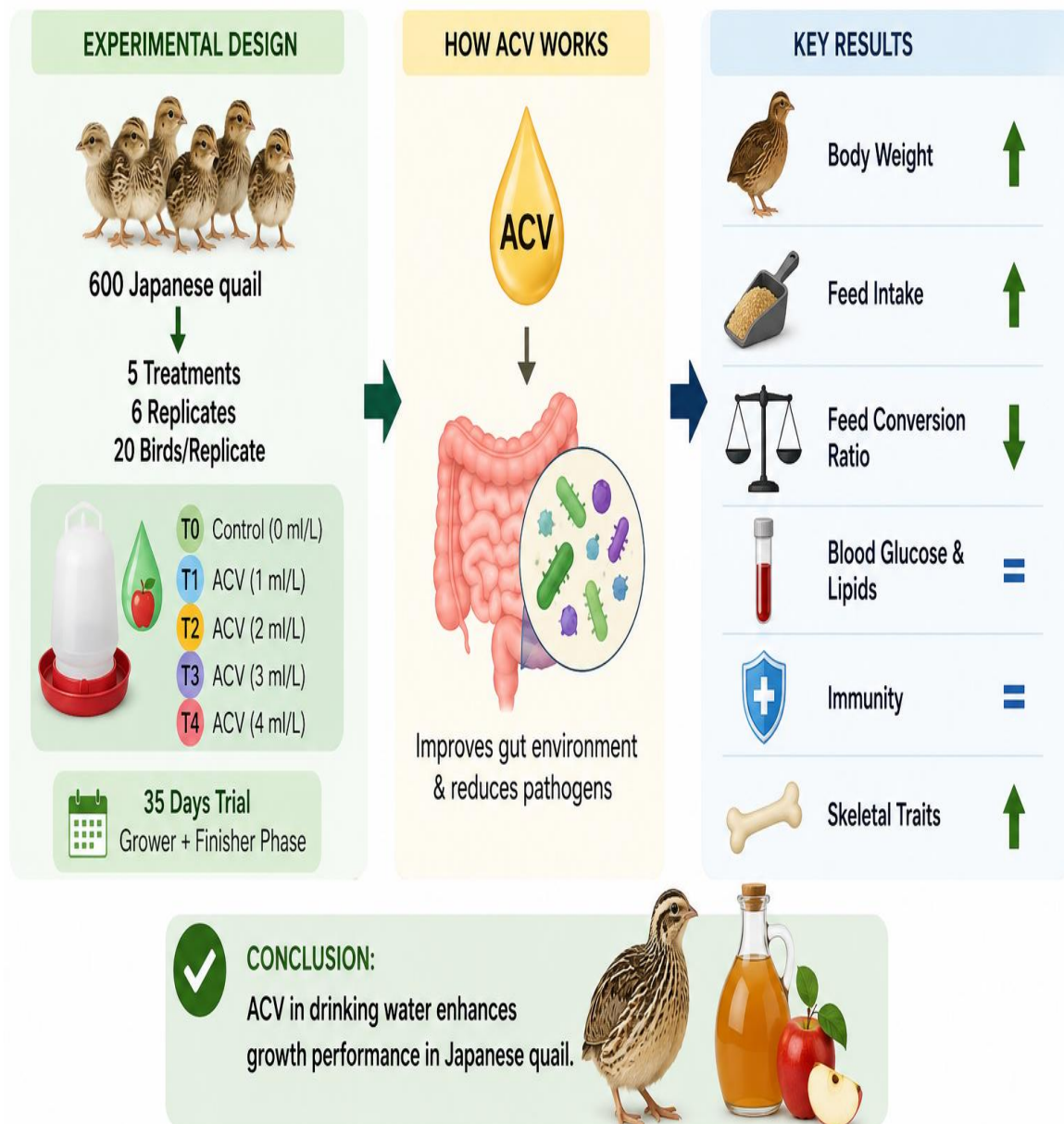
Published: 17 August 2026

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Muhammad Arif**Abstract**

Vinegar, known as acetic acid, is a short-chain fatty acid used to improve poultry growth and digestive health due to its antibacterial properties and ability to provide energy to epithelial cells. Many food products include acetic acid, which prevents the growth of pathogenic germs in the feed, hence preserving the amount of beneficial bacteria in the gastrointestinal system. The use of antibiotic growth promoters in poultry may result in antibiotic resistance and the accumulation of toxic residues in different chicken products, endangering poultry and human health. Apple cider vinegar is a phytochemical chemical that has sparked widespread interest due to its capacity to enhance performance. This study represents how drinking water enriched with ACV affects the growth performance of Japanese quail species. For this reason, 600 quails were randomly assigned to five experimental groups, each with six replicates and 20 chicks, using a completely randomized design (CRD). The control group had a basal diet and fresh drinking water, whereas Groups A, B, C, and D were given ACV in their drinking water at concentrations of 1, 2, 3, and 4 ml per liter, respectively. All groups adhered to the identical eating plan. The trial lasted 35 days and consisted of two phases: grower and finisher. All data were subjected to analysis of variance (ANOVA) using the general linear model procedures of Statistics 8.1. Where the main effects were significant, Tukey's test was employed to separate the means. Dietary inclusion of ACV had a substantial effect ($p < 0.05$) on the body weight of quails. There were substantial differences in initial body weight across treatment groups ($p < 0.05$). There were substantial changes in total feed consumption across treatment groups ($p < 0.05$). Dietary ACV substantially altered the feed conversion ratio ($p < 0.05$). Blood glucose levels and lipid concentrations remained steady between treatments, with no significant change. There was no significant effect ($p > 0.05$) on immunological markers. Significant influences on skeletal characteristics ($p < 0.05$). Finally, ACV boosted the performance of Japanese quail.

Apple Cider Vinegar (ACV) in Drinking Water Enhances Performance of Japanese Quail



INTRODUCTION

One of the agricultural subsectors with the quickest growth is poultry farming, which contributes to the world's population with a variety of products. Common producers of eggs and meat are broiler chickens, industrial layers, turkeys, ducks and quails. These are high-yielding birds that have undergone genetic enrichment to grow larger and produce more eggs. The purpose of these strains was to provide the population of the world with the required

proteins in their diets (Hussain 2, 2018; Ghayas *et al.*, 2020). Moreover, improvements in feed formulation, health management, and housing systems have greatly enhanced production efficiency and bird performance. Poultry farming also serves as a cost-effective source of high-quality animal protein, making it essential for food security in developing countries (FAO, 2021).

The current intensive poultry production has achieved impressive levels of efficient and

economical production of high-quality, safe poultry meat, eggs and other products thereof. The most organized animal husbandry sector is poultry, which is worth rupeeing one lakh crore. Large-scale systems are nearly the only ones to find quail and ostriches (FAO, 2019).

Recent advances in genetics, nutrition, and disease management have considerably increased poultry output and profitability. Furthermore, the implementation of new technologies and biosecurity controls has resulted in enhanced animal health and product quality. The industry also plays an important role in food security, job creation, and agricultural economic growth.

The integration of quail farming into chicken production systems is a critical step toward diversifying sources of high-quality animal protein. Quails play an important role in supplying the growing demand for eggs and meat because of their high growth rate, early maturity, and effective feed conversion. The Japanese quail (*Coturnix coturnix japonica*) is a migratory, ground dwelling game bird. The bird was a product of a wild habitat as any other domesticated animal, and it was domesticated in Japan in 1595. Quail meat and eggs have become popular since 1910.

Quail has 45 species across the world. Nevertheless, there are only two quail species in Pakistan, the black-breasted jungle or rain quail (*Coturnix coromandelica*), inhabiting the jungle and the brown colored Japanese quail raised as food and utilized commercially. Quails in Pakistan were introduced in 1974 at Central Avian Research Institute (CARI), Izat-nagar, in the development project of United International Institute and Pakistan Council of Agricultural Research (ICAR), in joint research (Arya *et al.*, 2018). Japanese quails are also called Bater in Pakistan and have a fast expansion rate, high rate of egg production, early sexual maturity, short generation period, less floor space, less feed and genetic resistance to most diseases (Shanaway, 1994).

The rearing of quail has led to a reduction in protein deficiency among the Pakistani population owing to the availability of 33 eggs and 770 gm. of meat per person per annum (Arya *et al.*, 2018). Japanese quail possesses some special traits of toughness and the ability to

adjust to diversified agroclimatic conditions (Chitrambigai *et al.*, 2015). Quail meat has a peculiar taste and attributes; this has led to an increase in the interest of consumers in different regions of the world (Genchev *et al.*, 2008).

Generally, Japanese quail production can be increased by expanding its natural capacity and providing advantageous conditions of administration. Two of the most significant economic aspects in any genetic enhancement strategy are weight gain and egg production for a variety of reasons and the connection between their increase to additional meat-related attributes and the comparative simplicity of their quantification. Public and private organizations focused on the growth of quail farming as a way of producing quail meat at a competitive price; however, the efforts were inadequate to overcome the hurdles. The commercial quail farming sector in the country was seemingly experiencing a major setback due to the low live body weight and meat production (Jatoi, 2012). The nutritional value and taste of quail meat are major factors that have led to the rise in the demand for the product among consumers. The excellence and features of the meat are impacted by several factors, such as the dietary pattern and age of the birds at the time of slaughter. Quail meat production is very economically profitable (Genchev & Mihaylova, 2008).

The rapid growth of the poultry sector is widely seen in many developing countries, primarily driven by the rising consumer demand for poultry meat and eggs (Aziz-Mousavi *et al.*, 2012; Shamlo *et al.*, 2014). Following the 2006 ban by the European Union (EU) on the use of antibiotics as growth promoters on poultry meat and eggs, considerable research efforts have been directed toward identifying safe and effective alternative feed additives for livestock production. This shift has accelerated the development of natural, sustainable, and residue-free growth-promoting strategies in poultry nutrition.

The popular poultry industry is actively researching and flourishing unique feed additives to increase feed efficiency and chicken health. To address concerns about antibiotic residues in meat and the danger of antimicrobial resistance, alternatives such as organic acids and salts, probiotics, prebiotics, and exogenous

enzymes have been developed. These additives not only promote intestinal health and nutritional utilization but also help to create safer and more sustainable poultry production systems.

The business related to poultry is regularly looking for new feed additives to increase feed efficiency and improve the health of chickens. Among these possibilities, organic acids such as acetic acid have received much attention as excellent feed additions in animal production. These chemicals promote the growth of the intestinal epithelium, which improves gut integrity (Langhout and Sus, 2005). Furthermore, consuming different types of organic acids and their salts can reduce the multiplication of harmful microbes while preserving a healthy balance of beneficial gut flora. They also reduce gastrointestinal pH, increasing nutrient solubility, digestion, and absorption efficiency (Vogt *et al.*, 1981; Patten and Waldroup, 1988; Skinner *et al.*, 1991). These factors are associated with the source, quantity of organic acids used, location, environmental status, and diet ingredients (Gama *et al.*, 2000).

Gut health is one of the primary elements that degrades bird performance, the economy of poultry farming, and thus the profitability of a specific organization (Samik *et al.*, 2007). The gut microflora has a substantial impact on gut health, host nutrition, and growth performance because it regulates the utilization and development of the host's gut system (Barrow, 1992). Many forms of pathogens (bacteria, etc.) such as *E. coli*, different species of *Salmonella*, *Clostridium*, and others pose a constant threat to broilers; thus, the trend of adding subtherapeutic doses of antibiotics to feed as additives to gain protection against certain intestinal pathogens and as growth promoters is growing by the day, despite the prohibition on their use (Dibner and Richards, 2002).

Many forms of organic acids are divided into two types: simple monocarboxylic acids (such as acetic acid) and hydroxylated carboxylic acids (lactic, malic, tartaric, and citric acids). In their undissociated state, these acids can pass through the semipermeable membranes of bacterial cells, enter the cytoplasm, and lower the intracellular pH, disrupting critical enzyme processes such as

decarboxylases and catalases. This process disrupts glycolysis, active transport systems, and cellular signalling pathways.

As a result, the use of organic acids in broiler feed is becoming more widely recognized as a viable and safer alternative to subtherapeutic antibiotic use, especially in light of growing concerns about antimicrobial resistance and food and environmental safety. It has also been observed that organic acids can protect young chicks by competitive exclusion (La Ragione and Woodward, 2003) and increase nutrient intake, growth, and feed ratio (Denli *et al.*, 2003). They can also stimulate pancreatic juice secretion and modify gut shape in terms of villus height, increasing the nutritional absorption area (Dibner and Buttin, 2002). In contrast, it has been claimed that organic acids can influence food palatability and hunger in broiler chickens (Cave, 1982).

Acetic acid is widely used as an anticoccidial agent in the chicken business. It has been reported to be useful in the treatment of avian coccidiosis by poultry farmers. This trait leads to higher bioavailability of nutrients and, as a result, improved bird production performance, which contributes to a rise in business profitability (Rao *et al.*, 2011).

Materials and Methods

Experimental Site

The present experiment was designed and performed at the Department of Animal Sciences Research and Development farm at the College of Agriculture, University of Sargodha, Sargodha, Pakistan. All study approaches were utilized to assess the performance of Japanese quail (*Coturnix coturnix japonica*) given varying quantities of acetic acid (ACV). The study's findings were assessed in terms of growth, feed intake, feed conversion ratio, blood biochemical parameters, carcass characteristics, gastrointestinal parameters, nutrient digestibility, and quail production economics. The current study presents extensive data on various techniques, methodologies, and materials used during the course of the current examination. Research was conducted according to the institutional guidelines for the proper care and experimentation of experimental animals.

Experimental Birds and Housing

This study on quail involves an experimental investigation including 600-day-old Japanese quail (*Coturnix coturnix japonica*) chicks. The research was carried out from May 10th to June 14th, 2025. Day-old chicks were purchased from Hatchery in Lahore. After arrival, the chicks were weighed and randomly assigned to experimental groups. The birds were housed in cages with consistent management and environmental conditions. Throughout the trial, sufficient lighting, ventilation, and hygiene were maintained and monitored. All of the birds in the experimental network were vaccinated and grown in accordance with standard quail rearing practices. The quails were brooded and grown in standardized conditions. To decrease stress and illness spread, clean and sanitized equipment was used.

Biosecurity Measures

The research trial was conducted under strict hygienic conditions, and complete biosecurity

measures were followed. Foot dips with 1% potassium permanganate solution were arranged at the entrance of the experimental shed. No visitors were allowed until completion of this project.

Experimental Design and Treatments

The experiment was carried out using a completely randomized design (CRD). The birds were randomly divided into five treatment groups, each of which had six replicates and 20 birds per replication.

- **Control (T₀):** Basal diet + f drinking water (without ACV)
- **Treatment (T₁):** Basal diet + Apple Cider Vinegar at 1 ml/L of drinking water
- **Treatment (T₂):** Basal diet + Apple Cider Vinegar at 2 ml/L of drinking water
- **Treatment (T₃):** Basal diet + Apple Cider Vinegar at 3 ml/L drinking water
- **Treatment (T₄):** Basal diet + apple cider vinegar at 4 ml/L of drinking water

Table 1: Details of dietary treatments with respect to the number of birds and replications

Sr. No.	Treatment group	No. of birds/pen/replication	No. of replication	Total no. of birds
1.	T ₀ - Standard broiler quail diet	20	6	120
2.	T ₁ 0.1% Apple Cider vinegar	20	6	120
3.	T ₂ 0.2% Apple Cider vinegar	20	6	120
4.	T ₃ 0.3% Apple Cider vinegar	20	6	120
5.	T ₄ 0.4% Apple Cider vinegar	20	6	120
	Total		30	600

All birds in the experimental groups were given the **same** basal diet. Feeding and watering management was produced to equalize or surpass the nutritional requirements of Japanese quail, with the only difference between treatments being the amount of Apple Cider Vinegar added to the drinking water. Fresh, clean drinking water was given continuously, and ACV was mixed routinely in drinking water as per assigned treatment levels to verify and maintain consistency and freshness. Chemical composition of feed ingredients (% DM basis).

Field Data Collection and Medication

Daily feed intake was calculated for each replicate by subtracting the leftover feed from

the total feed supplied. Weekly feed consumption was determined accordingly. On the arrival day, all quail chicks were given proper heat and jaggery water as a source of energy to lessen the stress caused by transportation.

Experimental Analysis of Different Parameters

On the first day of the experiment, all the chicks were weighed randomly and then on a weekly basis up to five weeks of age using an electronic weighing scale. The average body weight gain/week was calculated from the difference in body weight gained at the time of weighing and the start of the concerned period for each bird in replicates of groups. The feed consumption of quail from each pen was recorded on a weekly

basis, and the average feed intake was calculated in g/chick/week by dividing the total amount of feed consumed by the number of chicks in the particular pen. These steps must be followed to determine the villus length, height, and crypt depth in broiler chickens and quails.

Histological and Anatomical Analysis

Samples of the birds' intestinal tissue, usually from the ileum, jejunum, and duodenum, were taken. Hematoxylin and eosin (H&E) staining, embedding in paraffin, and fixation of the samples in formalin. Measure the following using a light microscope and a calibrated ocular micrometer: The distance between the villus tip and the crypt-villus junction. Villus height and villus length are sometimes used interchangeably, from the villus-crypt junction to the crypt's base.

Feed conversion ratio (FCR)

The feed conversion ratio was determined on a weekly and overall basis using the following formula:

$$FCR = \frac{\text{Feed intake (g)}}{\text{gained body weight (g)}}$$

Carcass Characteristics

At the end of the trial (day 35), two birds from each replicate were randomly selected for carcass assessment, as described by Fernandes *et al.* (2013). Carcass yield was calculated using the eviscerated carcass weight (without the head, feet, and abdominal fat) represented as a percentage of live body weight. Prior to slaughter, the birds were fasted for 8-10 hours before being weighed and slaughtered in accordance with halal standards. This method guarantees reliable assessment of carcass features by reducing gut fill variance and preserving consistency in processing methods.

The following carcass parameters were recorded:

- Live body weight
- Carcass weight
- Dressing percentage
- Giblet weights (liver, heart, and gizzard)

The dressing percentage was estimated using the formula:

$$\text{Dressing \%} = \left(\frac{\text{Carcass weight}}{\text{Live body weight before slaughter}} \right) \times 100$$

Blood Biochemical Profiles

At the conclusion of the experiment, blood was drawn from the blood of selected birds from each experimental group. The serum was separated by centrifugation at 3000 RPM for 10 minutes and stored at -20°C until additional research was completed. Using biochemical kits with the aid of a biochemical autoanalyzer, biochemical parameters included the estimation of total protein, albumin, globulin, cholesterol and triglycerides by following m

Serum Total Protein (g/dL)

Using a conventional commercial diagnostic kit and an AGD autoanalyzer, the serum total protein was calculated. To do this, 0.01 ml of sample was added to a clean, dry micro test tube containing 1 ml of total protein reagent, and the tubes were carefully mixed. Next, the reaction mixture was incubated for five minutes at room temperature. Following incubation, the autoanalyzer was used to detect the absorbance (A) at 546 nm. The reagent kit's instructions were followed to calculate the serum total protein concentration.

$$\text{Total protein } \left(\frac{g}{dl} \right) =$$

$$\frac{A_{\text{sample}}}{A_{\text{standard}}} \left(\text{concentration of standard} \left(\frac{g}{dL} \right) \right)$$

Serum Albumin (g/dL)

An AGD autoanalyzer and a common commercial diagnostic kit were used to quantify serum albumin. A 0.01 ml blood sample was added to 1.0 ml of albumin reagent in a dry, clean small test tube and carefully mixed for this analysis. Next, the reaction mixture was incubated for five minutes at room temperature. Following incubation, the autoanalyzer was used to measure the absorbance (A) at 630 nm. The following formula was used to determine the serum albumin concentration in accordance with the guidelines included with the reagent kit:

$$\begin{aligned} \text{Albumin } \left(\frac{g}{dL} \right) &= \frac{A_{\text{sample}}}{A_{\text{standard}}} \\ &\times \text{concentration of standard} \end{aligned}$$

Globulin in serum (g/dL)

The method below was used to compute serum globulin: serum total protein - serum albumin. Albumin was subtracted from total protein.

$$\text{Serum globulin} = \text{Serum total protein} - \text{serum albumin}$$

Serum Cholesterol (g/dL)

Using a conventional commercial diagnostic kit and an AGD autoanalyzer, serum cholesterol was calculated. To do this, 1.0 ml of cholesterol reagent and 0.01 ml of blood sample were placed in a clean, dry small test tube and carefully mixed. Next, the reaction mixture was incubated for five minutes at room temperature. Following incubation, the autoanalyzer was used to detect the absorbance (A) at 505 nm. The reagent kit's instructions were followed to determine the serum cholesterol concentration.

$$\text{Cholesterol conc.} \left(\frac{\text{mg}}{\text{dL}} \right) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{conc. of standard}$$

Serum Triglyceride (mg/dL)

Serum triglyceride levels were measured using an AGD autoanalyzer and a commercial diagnostic kit. Briefly, 1.0 mL of triglyceride reagent was poured into a clean, dry test tube, followed by 0.01 mL of serum sample. The mixture was carefully combined and incubated at room temperature for 5 minutes. Following incubation, the absorbance at 505 nm was measured using an autoanalyzer, and triglyceride concentrations were estimated in accordance with the manufacturer's instructions. This standardized approach guarantees the reliable and precise determination of blood triglyceride levels, which are essential indicators of lipid metabolism and the general physiological state.

$$\text{Triglyceride conc.} \left(\frac{\text{mg}}{\text{dL}} \right) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{conc. of standard}$$

Gut Parameters

At the end of the experiment, two birds from each replication (eight birds per treatment) were chosen at random and slaughtered to determine intestinal parameters such as weight, ileal pH, length, thickness, and total microbial count. Following slaughter, the carcasses were meticulously opened, and the entire digestive tract was aseptically removed for further examination. This approach ensured accurate evaluation of gut parameters while minimizing contamination, resulting in valid data on intestinal health and microbial activity.

Gut weight

A computerized weighing balance was used to measure the gut weight following aseptic evacuation of the intestine.

Gut pH

A digital pH meter was used to measure the pH after 10 g of intestinal material from the ileum was aseptically collected in 90 ml of sterilized physiological saline (1:10 dilution) (Al-Natour and Alshawabkeh, 2005).

Gut Length

Using a measuring tape and aseptic circumstances, the length of the gut was measured.

Gut Thickness

A digital Vernier calliper was used to measure the thickness of the gut while aseptic conditions were maintained.

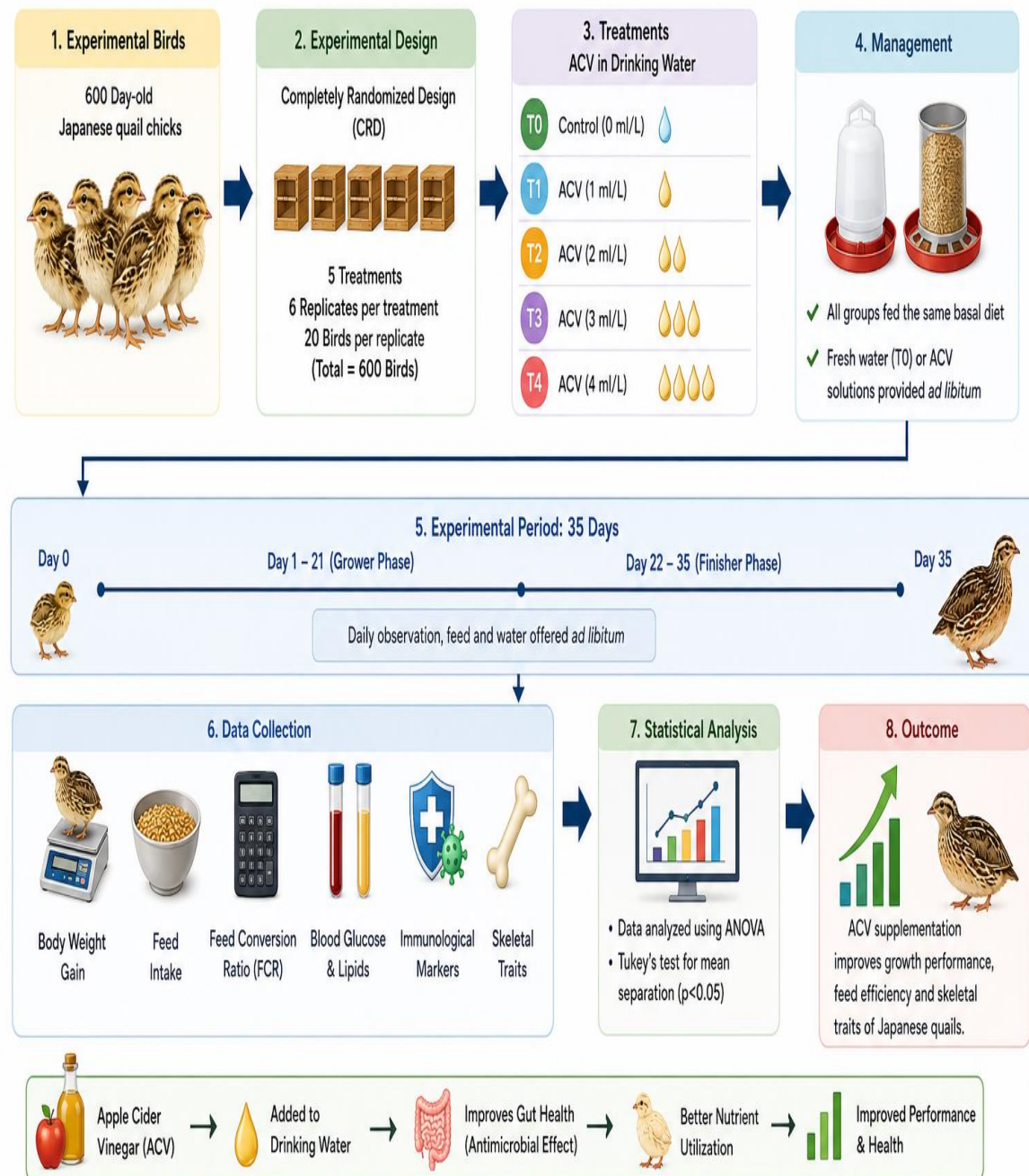
Total Microbial count (*E. coli* and *Salmonella*)

Cecal contents were collected aseptically from the birds, packed in sterile plastic bags, and transferred to the laboratory via cold chain. For microbiological analysis, 1 g of each sample was homogenized in sterile saline at a 1:9 (w/v) dilution and then diluted tenfold. To isolate *Salmonella* and *E. coli*, 0.1 mL of each dilution was plated in duplicate on selective media such as *Salmonella*-Shigella agar and Eosin Methylene Blue (EMB) agar. Plates were incubated at 37 °C for 22 ± 2 hours under aerobic (*E. coli*, *Salmonella*) and anaerobic (*Clostridium*) conditions. Following incubation, the distinctive colonies were identified and counted. The microbial load was quantified as log₁₀ colony-forming units per gram of ileal digesta (CFU/g). In addition, samples were qualitatively tested for *Salmonella* and *E. coli*. This approach provides a thorough study of intestinal microbial populations, allowing for reliable identification of both benign and harmful microorganisms.

Statistical Analysis

All collected data (weekly live body weight, weekly weight increase, feed consumption, feed conversion ratio, etc.) were subjected to an analysis of variance (ANOVA) using appropriate statistical software in a completely randomized design. Treatment means were compared to identify significant differences between groups. Significant differences between treatment means were isolated using Duncan's Multiple Range test and judged significant when the P value was less than 0.05.

Methodology: Effect of Apple Cider Vinegar (ACV) in Drinking Water on Growth Performance of Japanese Quails



Methodology for the analysis of apple cider vinegar supplementation enhances growth performance, feed efficiency, and physiological responses in Japanese quails
Results and Discussion

The present research work “Effects of Apple Cider Vinegar on the Growth Performance of Japanese Quails” was carried out, and performance metrics such as growth, feed intake, feed conversion ratio, blood biochemicals,

carcass features and quail production economics were used to assess the study's impact.

Table 2: Average weekly live body weight (g) of quail for different dietary treatments

Week/Treatment	W ₁	W ₂	W ₃	W ₄	W ₅
T ₀	34.85 ^b	70.69 ^d	130.87 ^d	180.14 ^d	231.14 ^d
T ₁	34.14 ^c	71.69 ^c	131.87 ^c	181.14 ^c	232.14 ^c
T ₂	35.14 ^b	71.69 ^c	131.87 ^c	181.14 ^c	232.14 ^c
T ₃	34.14 ^c	72.69 ^b	132.87 ^b	182.14 ^b	233.14 ^b
T ₄	38.14 ^a	74.69 ^a	134.87 ^a	184.14 ^a	235.14 ^a
SEM	0.1603	0.1415	0.1822	0.1948	0.1607

T₀ indicates the control group, while T₁, T₂, T₃ and T₄ indicate the effect of apple cider vinegar supplementation at different dietary levels. SEM = standard error of the mean. Means sharing different superscripts (a, b, c) within the same column differ significantly ($P < 0.05$).

Effects of Apple Cider Vinegar on Body Weight Gain

Table 2 shows that adding ACV to the diet had a substantial ($p < 0.05$) influence on the body weight of quails. The highest value was observed in T₄ at various weeks of age, such as W₁-W₅. The average live body weights for weeks 1, 2, 3, 4, and 5 were 38.24, 74.69, 134.87, 184.14, and 235.14 grams, respectively ($p < 0.05$). Quails fed T₄ had the highest final live body weight (235.14, $p < 0.05$), followed by those fed T₃. The birds fed T₀ or Control diets had the lowest end body weight. During different weeks of age, all treatment groups exhibited nearly identical responses.

This suggests that birds fed an ACV-rich diet continued to grow at a faster rate. Broiler chicks have also been shown to gain more weight when supplemented with ACV. The T₄ group's final body weight of 235.14 g falls well within the healthy range for mature Japanese quails, which typically ranges from 100 to 130 g for males and 130 to 160 g for females in normal-sized strains or higher in particular meat strains (Sezer, 2007). The consistently lower body weight of the control group (T₀) illustrates the favourable benefits of ACV and its potential as a feed additive to promote growth performance. This finding is consistent with past research indicating lower performance control groups

without ACV or equivalent feed additives (Ibrahim *et al.*, 2023).

Effects of Apple Cider Vinegar on FCR and TFC

The results showed significant effects of ACV inclusion on the initial body weight, final body weight, total feed consumption, and feed conversion ratio of Japanese quails ($p < 0.05$). Initial body weight showed significant differences among treatment groups ($p < 0.05$). Quails in the T₀ group (12.36 g) had a significantly higher IBW compared to T₁ and T₂ (both 11.36 g), while T₃ and T₄ (both 10.36 g) exhibited the lowest IBW. Total feed consumption also displayed significant differences between treatment groups ($p < 0.05$). The T₀ group consumed the highest amount of feed (606.31 g), which was statistically similar to T₁ (605.31 g) but significantly higher than T₃ (602.31 g) and T₄ (599.31 g). The lowest TFC was recorded in the T₄ group (599.31 g).

The feed conversion ratio was significantly influenced by dietary ACV ($p < 0.05$). The T₄ group demonstrated the most efficient FCR (2.666), which was significantly lower than all other groups. Progressively poorer FCR values were observed from T₃ (2.703) to T₁ and T₂ (both 2.741 and 2.735, respectively), with the control group T₀ showing the least efficient FCR (2.771). The observed significant differences in initial body weight among treatment groups, with T₀ having a higher initial weight than ACV-supplemented groups (T₁-T₄), is noteworthy. While not directly indicative of ACV's growth-promoting effects, this suggests a preexisting variation in the experimental units, which was accounted for in the statistical analysis (Figure 1).

The T4 group consistently achieved the highest body weight (235.14 g) at the end of the experimental period, significantly outperforming the control group (T0, 231.14 g) and other ACV inclusion levels. This outcome is consistent with previous research demonstrating that ACV and its active component acetic acid can act as a natural growth promoter in poultry (Jahantigh *et al.*, 2021; Tasharofi *et al.*, 2017). Similar positive influences on body weight in Japanese quails have been reported, where certain dietary strategies, including the use of feed additives, led to increased growth (Al-

Hassani *et al.*, 2023). The enhancement in FCR by ACV is widely attributed to its role as an acidifier, which optimizes the gastrointestinal environment. By lowering gut pH, ACV suppresses the growth of pathogenic bacteria, improves the activity of digestive enzymes, and thereby enhances the digestibility and absorption of nutrients (Alagawany *et al.*, 2021; Ibrahim *et al.*, 2023). These results align with other studies where ACV supplementation led to improved feed efficiency in broilers (Al-Jubori, 2023).

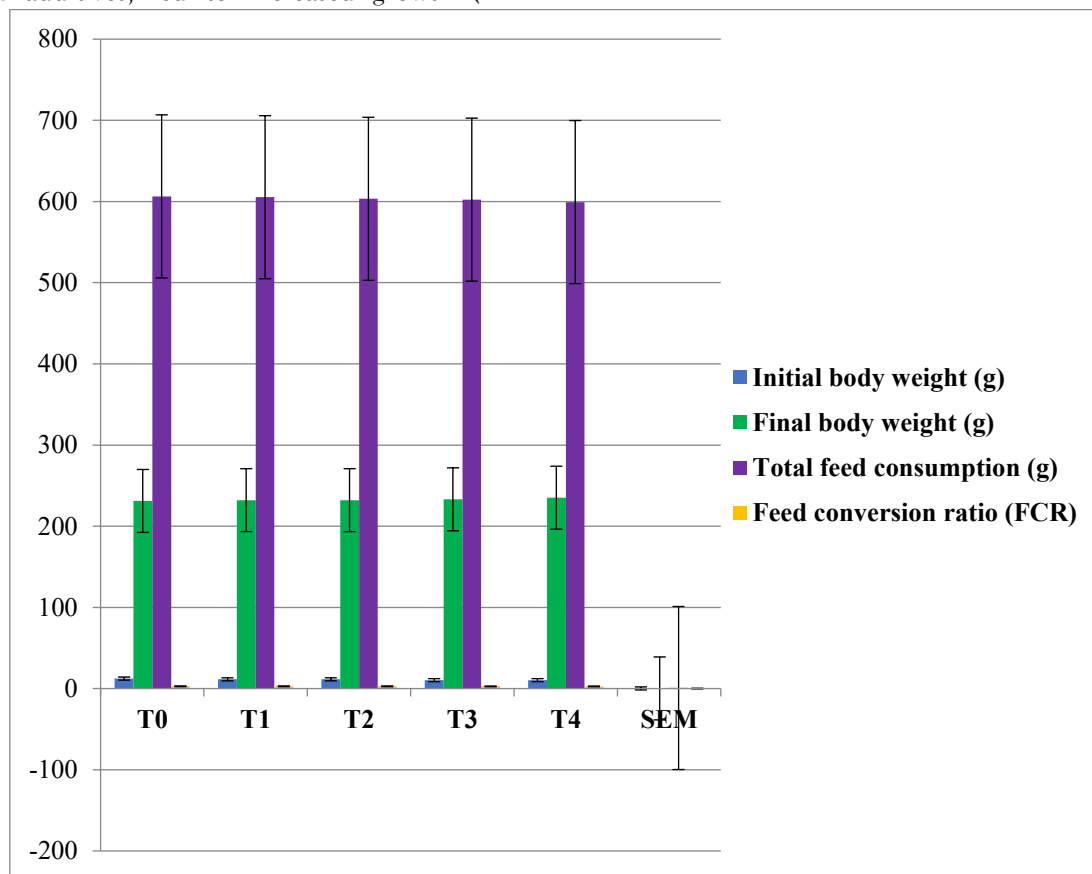


Figure 1: Initial and final body weight (g) and FCR analysis

Blood Parameters

The phosphorus concentrations ranged from 4.906a to 5.144a, with no significant differences ($p>0.05$) observed among the control T0 and ACV-supplemented groups T1, T2, T3, and T4, which were 4.906, 5.118, 5.140, and 5.024, respectively. Similarly, calcium levels showed no significant variations across treatments, with values ranging from 11.05 to 11.12. The T0 group exhibited 11.11, while ACV groups T1, T2, T3, and T4 showed 11.12, 11.05, 11.06, and 11.12, respectively. The results showed that red blood cell (RBC) counts were similar across treatments ($3.655\text{--}3.722 \times 10^6/\mu\text{L}$), and no significant ($p>0.05$) difference was observed, with hemoglobin levels ranging from 10.94 to 11.20 g/dL (Figure 2). The lowest Hb value was in T3, and the highest was in T0, but differences were small and within normal physiological ranges for broilers (Maxwell & Robertson, 1998).

These results indicate that hematological integrity was preserved across treatments. This stability is consistent with other studies evaluating dietary supplements or feed additives,

which generally report negligible impacts on RBC counts and hemoglobin unless interventions are extreme or cause metabolic stress (Abdel-Magid *et al.*, 2018; Younis *et al.*, 2024). Blood glucose levels were stable across treatments and showed no significant change ($p>0.05$), ranging from 95.88 to 96.63 mg/dL. The minimal variation suggests that the treatments did not affect glucose homeostasis in the birds. This aligns with reports that moderate dietary interventions, including feed additives or micronutrient supplementation, typically do not alter basal glucose concentrations in healthy broilers (Rath *et al.*, 2016; Oliveira *et al.*, 2019). Serum low-density lipoprotein (LDL) values showed a slight decreasing trend across treatments (20.43 in T0 to 19.60 mg/dL in T4) but no significant changes ($p>0.05$), whereas high-density lipoprotein (HDL) remained largely unchanged, and nonsignificant ($p>0.05$) results were observed (109.55–110.62 mg/dL). The reduction in LDL, although small, may suggest a modest improvement in lipid metabolism with incremental treatments.

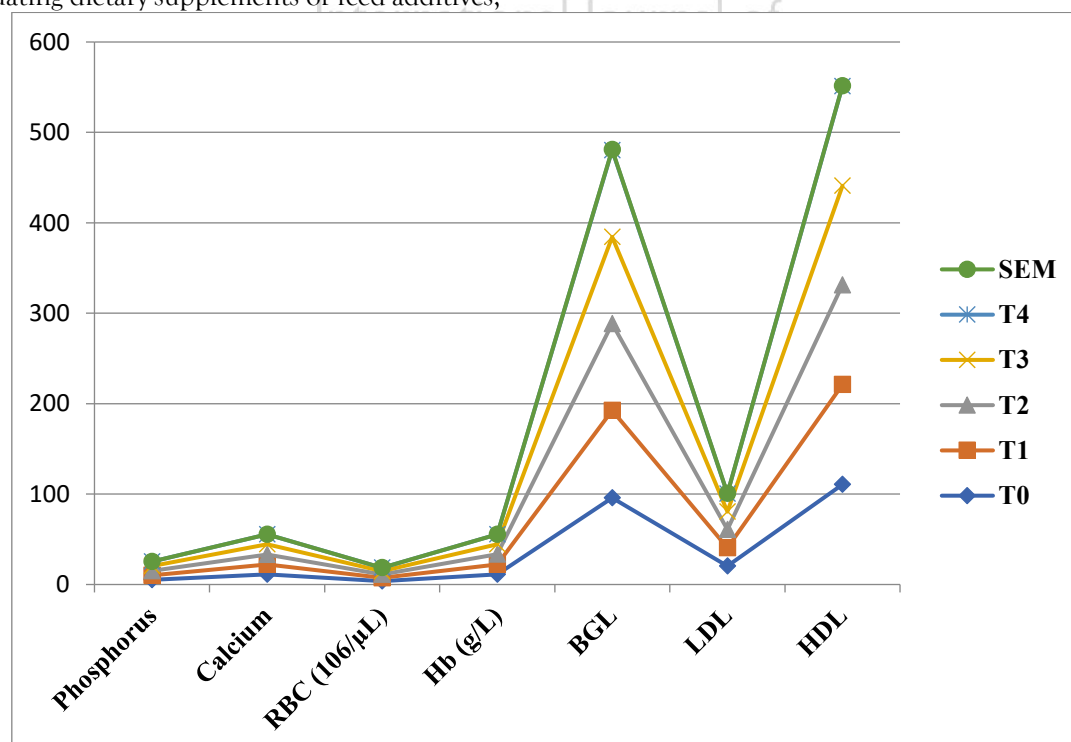


Figure 2: Effects of Apple Cider Vinegar on Blood Parameters of Japanese Quails
Effects of Apple Cider Vinegar on Hematology and Biochemistry

Past research in broilers shows that some feed supplements, such as probiotics, enzymes, and

phytochemicals, have the ability to reduce LDL and maintain HDL, which is an indication of better cardiovascular health and lipid metabolism (Attia *et al.*, 2017; Younis *et al.*,

2024). Nevertheless, there are no reported significant differences in the levels of cholesterol, albumin, total protein, triglycerides, HDL, and LDL after the administration of ACV in other broiler studies (Jahantigh *et al.*, 2021). The findings indicated that the triglyceride and cholesterol concentrations in serum were astonishingly constant during the study, and there was no significant difference ($p>0.05$). The average triglyceride was between 107.53 and 108.61 mg/dl, and cholesterol was between 132.29 and 133.77 mg/dl. The insignificant difference in the present study indicates that the amount of ACV supplementation or physiological disparities in Japanese quails might not have been high enough to cause any observable systemic response in lipid metabolism.

The hematological parameters were also stable, and no significant difference was found among the results ($p>0.05$). Packed cell volume (PCV) did not differ significantly and was 39.7, which does not imply any alterations in red cell mass or hydration. This eliminates the possibility of dehydration, hemorrhage or hemolysis. On the same note, there was no significant difference in the overall number of white blood cells (WBCs), although there was minimal nonsignificant variation ($p>0.05$), as all means were found to lie within the range of 3.395 to $3.468 \times 10^3/\mu\text{L}$. The percentage of lymphocytes, which is one of the indicators of adaptive immunity, was also stable (88.6% to 89.7%), which also confirms the finding of immune system quiescence (Table 3).

The outcome indicated no significant ($p>0.05$) response in total protein and net total protein. For example, total protein had a minimum of 2.2883 g/dL at T3 and a maximum of 2.7192 g/dL at T1. These small changes do not follow a

regular pattern and, therefore, can probably be attributed to normal physiological variability and not the direct impact of the experimental treatment. The general stability, however, indicates that liver synthetic functioning and nutritional condition were not impaired negatively.

Statistically, the inclusion of dietary apple cider vinegar (ACV) did not show any significant effects ($p>0.05$) on the percentage of monocytes, eosinophils, aspartate aminotransferase (AST), alanine aminotransferase (ALT) or alkaline phosphatase (ALP) in Japanese quails in all treatment groups. The proportional numbers of both monocytes (2.03% to 2.61) and eosinophils (2.02% to 2.60) in this study had only slight, statistically insignificant ($p>0.05$) changes across the treatment groups.

This is a noteworthy finding since there is no substantial variation. This indicates that systemic inflammatory or immune-stimulatory responses were not elicited by the experimental treatments. Research performed on some probiotics or plant extracts has frequently found that there are major changes in leukocyte populations. Awaad *et al.* (2020) noted that dietary supplementation with a multistrain probiotic had a significant impact on the percentage of lymphocytes and reduction in the heterophil-lymphocyte ratio among broiler chickens. Conversely, the stability of our data is more consistent with the results where the tested material is demonstrated to be immunologically inactive or is given at the dose that is subclinical in its effect. These data are highly suggestive that the treatments were not stressors or pathogens, as would be anticipated to cause changes in leukocyte profiles (Davis, Maney, & Maerz, 2008).

Table 3: Effects of ACV on Hematological and Biochemical Parameters (Mean $\pm$ SEM)

Parameter	T0	T1	T2	T3	T4	SEM
Triglycerides	107.94 ^a	107.78 ^a	108.12 ^a	108.61 ^a	107.53 ^a	0.4376
Cholesterol	132.71 ^a	132.29 ^a	132.64 ^a	133.77 ^a	133.17 ^a	0.4261
White Blood Cells ($10^3/\mu\text{L}$)	3.4683 ^a	3.4583 ^a	3.4625 ^a	3.395 ^a	3.4242 ^a	0.0201
Total Protein	2.3625 ^a	2.7192 ^a	2.54 ^a	2.2883 ^a	2.7067 ^a	0.2315
Packed Cell Volume	39.758 ^a	39.808 ^a	39.758 ^a	39.758 ^a	39.542 ^a	0.2773

Net Total Protein	2.4592 ^a	2.7125 ^a	2.3875 ^a	2.6392 ^a	2.3092 ^a	0.1422
Lymphocyte %	89.681 ^a	88.764 ^a	89.599 ^a	88.597 ^a	89.262 ^a	0.2907

Effects of Apple Cider Vinegar on Liver Function

The findings of the present experiment show that there is no significant ($p>0.05$) difference and an unprecedented level of stability in the serum levels of AST, ALT, and ALP in all experimental groups (Table 4.5). The mean values for AST (ranging from 112.42 to 112.66 U/L), ALT (31.67 to 32.26 U/L), and ALP (1035.9 to 1036.5 U/L) remained virtually unchanged from the control group. The minimal deviations noticed are not beyond the normal physiological variation.

Parveen *et al.* (2020) noted that in broiler

chickens, serum levels of aflatoxin B1 increase significantly in serum of ALT, ALP, and AST, which are directly associated with histopathological evidence of liver necrosis and fatty degeneration. The lack of such a reaction in our experiment is a significant indication that the experimental treatments do not show hepatotoxicity at the experimental inclusion levels. The values observed are in agreement with baseline values mentioned in healthy subjects in a similar experimental setting, which also helps to conclude that liver functioning was not impaired (Awaad *et al.*, 2020).

Table 4: Effects of ACV on haematological and liver function parameters

Parameter	T0	T1	T2	T3	T4	SEM
Monocytes %	2.0333	2.35	2.2167	2.6083	2.2583	0.1518
Eosinophils %	2.0233	2.34	2.2067	2.5983	2.2483	0.1518
Aspartate Aminotransferase %	112.66	112.49	112.65	112.56	112.42	0.2764
Alanine Aminotransferase %	32.25	32.258	32.167	31.833	31.667	0.2999
Alkaline Phosphatase %	1036.5	1036.4	1036.2	1036.1	1035.9	0.3469

Effects of Apple Cider Vinegar on Carcass Characteristics

The most consistent and potentially economically relevant observation is the linear increase in Body Yield from T0 (60.188 g) to T4 (63.037 g). This indicates that the experimental treatment was successful in increasing overall growth and/or dressing percentage. This finding is consistent with previous research showing that nutritional measures such as optimizing dietary

protein and amino acid balance can greatly improve growth performance and carcass production (Musigwa *et al.*, 2024). For instance, studies on lysine and methionine supplementation have consistently shown improvements in body weight gain, feed efficiency, and breast meat yield (Tesseraud *et al.*, 1996; Corzo *et al.*, 2005; Siam *et al.*, 2017).

Table 5: Effects of Apple Cider Vinegar on Carcass characteristics of Japanese Quails

Parameter	T0	T1	T2	T3	T4	SEM
Breast Length(cm)	32.375	31.792	31.292	31.375	32.292	0.2829
Shank Length (inch.)	1.545	1.545	1.5442	1.5858	1.5858	0.0175
Shank Diameter (inch.)	1.9583	1.7917	1.825	1.7333	1.6333	0.0906

Keel Bone Length (inch.)	2.5325	2.6333	2.5958	2.5867	2.5717	0.0417
Drumstick Length (inch.)	2.1942	2.2017	2.2433	2.2642	2.1817	0.0356
Body Width (inch.)	1.4225	1.4692	1.4225	1.4517	1.4575	0.0303
Body Yield (g)	60.188	60.552	62.432	62.693	63.037	1.5691

Pectoral and Sternal Development: Breast and Keel Bone Length

In the present study, the Keel Bone Length showed only minor nonsignificant ($p>0.05$) fluctuations, peaking at T1 (2.6333 in.) before declining slightly in subsequent treatments. This finding appears to contradict studies that report a strong positive correlation between these traits (Ebong *et al.*, 2023). One possible explanation is that the treatment caused muscle hypertrophy (increase in muscle fibre size) rather than an extension of the skeletal frame. It is also possible that keel ossification, which can continue until 40-49 weeks of age in some breeds (Thøfner *et al.*, 2024), was not significantly influenced by ACV treatment within the timeframe of the experiment.

Breast length showed no significant differences among treatment groups ($p>0.05$). The values ranged from 31.292 cm (T2) to 32.375 cm (T0). The T0 group registered 32.375 cm, while ACV treatments ranged from 31.792 cm (T1) to 32.292 cm (T4). There were significant changes in shank length across treatment groups ($p<0.05$). The T3 and T4 groups both had the longest shanks at 1.5858 inches, which was much longer than T0 (1.545 in), T1 (1.545 in), and T2 (1.5442 in.). There were substantial variances in shank diameter ($p<0.05$). The T0 group had the largest shank diameter (1.9583 in.), which was much greater than that of the other groups. The diameters decreased gradually from T1 (1.7917 in.) to T4 (1.6333 in.), with T4 having the smallest diameter.

Keel bone length showed significant differences ($p<0.05$). The T1 group had the longest keel bone (2.6333 in.), well exceeding T0 (2.5325 in.), T2 (2.5958 in.), T3 (2.5867 in.), and T4 (2.5717 in.). The control group (T0) possessed the shortest keel bone. Drumstick length showed significant variations ($p<0.05$). The largest drumstick length was

found in T3 (2.2642 in), followed by T2 (2.2433 in) and T1 (2.2017 in). The T0 group measured 2.1942 inches, while T4 had the shortest drumstick length (2.1817 inches).

There were no significant differences in body width between the treatment groups (p values ranged from 1.4225 inches (T0 and T2) to 1.4692 inches (T1)). The width of the body, among other conformation characteristics, is a part of the total prediction of carcass yield in regression equations (Ebong *et al.*, 2023). The fact that body width was stabilized as body yield increased further confirms that muscle deposition and not an extension of skeletal frame was the main cause of the increase in yields. The findings show that there are differences in the effects of ACV on these parameters and that there are substantial improvements in some areas of carcass development and yield, which supports the usefulness of ACV as a useful feed additive in quail production (Figure 3).

Statistical analysis showed no significant ($p>0.05$) difference in the inclusion of dietary apple cider vinegar (ACV) on the weight of the spleen, bursa, ileum, pH, jejunum, pH, and cecum pH in Japanese quails among all treatment groups (T0-T4), as shown in Table 4.7. Comparisons of the treatment groups in all parameters were statistically nonsignificant. This observation is similar to a study on broilers in which dietary supplementation using 1-3 percent apple cider vinegar did not have a significant effect on the weight of the spleen. Nevertheless, studies have produced evidence that some organic acid formulations can raise the relative weight of the spleen, and this is deemed an improved immune mechanism (Attia *et al.*, 2013).

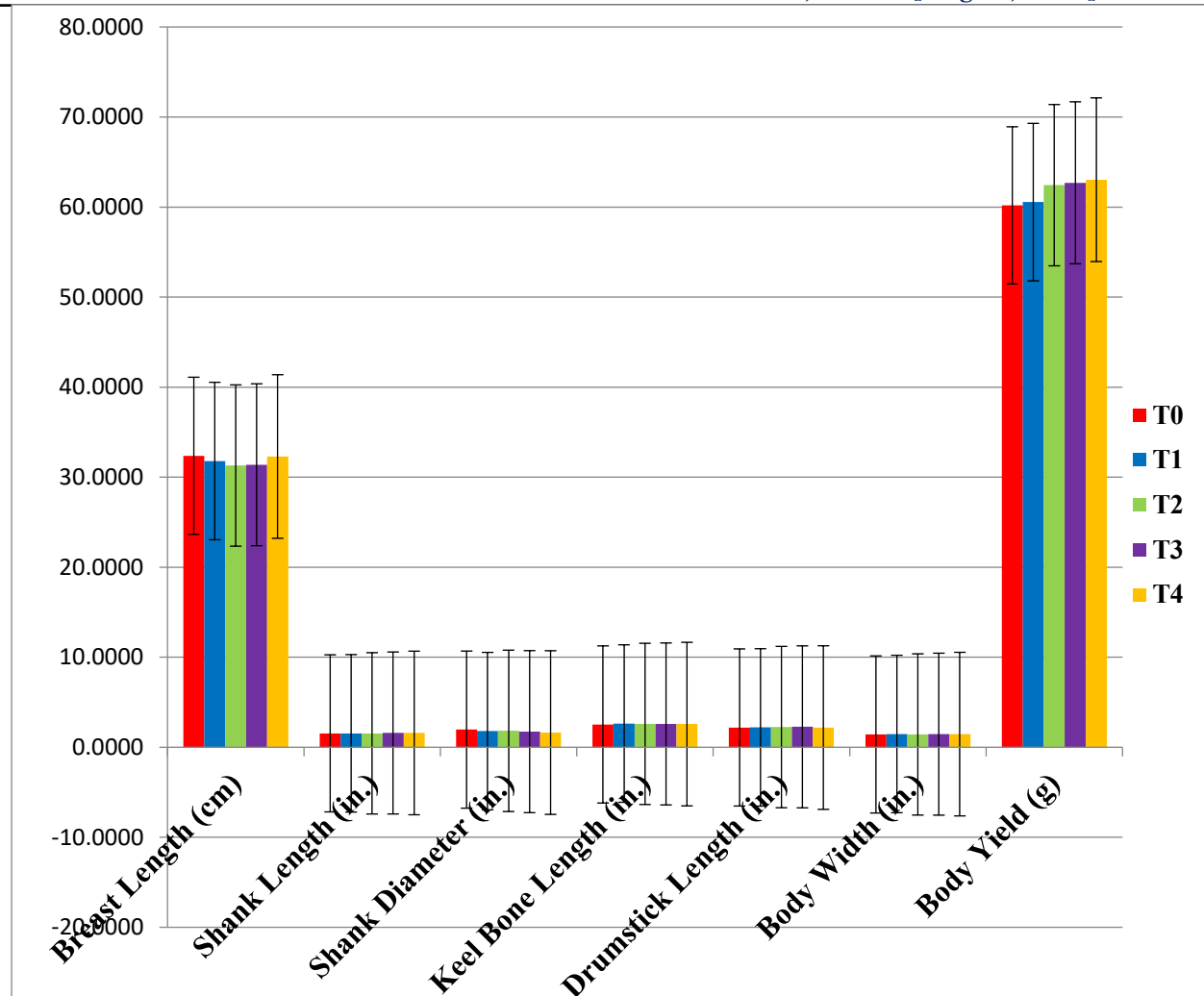


Figure 3: Analysis of pectoral and sternal Development: breast and keel bone length in Japanese Quails

Effects of Apple Cider Vinegar Supplementation on Immune Organ Weights and Gastrointestinal pH in Japanese Quails

The T2 and T4 groups expressed the greatest bursa weights. One of the major indicators of humoral immune capacity is the bursa of Fabricius, and an increment in relative weight is generally related to a stronger immune position. Although research on broilers indicated that there was no significant ($p > 0.05$) effect of ACV on the weight of the bursa, the trend in this case is that there is a possibility of a stimulatory effect on Japanese quails. This finding corresponds with other studies conducted on other immune-enhancing supplements. Another possible indirect cause of the increased growth of the bursa may be increased gut health. This indicates that ACV could be useful in enhancing humoral immunity in growing quails.

The findings indicated that there was no significant ($p > 0.05$) variance between the data. Spleen weights ranged from 0.1358 g (T2) to 0.1617 g (T1). The mean spleen weight of the control group (T0) was 0.1525 g. Other ACV-supplemented groups presented values of 0.1617 g (T1), 0.1358 g (T2), 0.1592 g (T3), and 0.1433 g (T4). These data statistically indicated no variation and were not significant ($p > 0.05$). Bursa weights varied from 0.1733 g (T0) to 0.2292 g (T4). The T0 group averaged 0.1733 g. ACV-supplemented groups showed mean weights of 0.2183 g (T1), 0.220 g (T2), 0.1958 g (T3), and 0.2292 g (T4).

Ileum pH values exhibited a narrow range from 6.1417 (T2) to 6.175 (T0 and T1). The ileum pH in the control group (T0) was 6.175. Similar values were found in T1 (6.175), T2 (6.1417), T3 (6.1667), and T4 (6.1583). No meaningful ($p > 0.05$)

differences were found. The jejunum pH values ranged from 5.8417 (T2) to 5.875 (T0 and T1). The pH of the jejunum in the T0 group was 5.875. The ACV-supplemented groups showed mean pH values of 5.875 (T1), 5.8417 (T2), 5.8667 (T3), and 5.8583 (T4). All the comparisons were not statistically significant ($p > 0.05$).

The pH of the cecum had close range values ranging between 6.7417 (T2) and 6.775 (T0 and T1). The control group (T0) had a cecum pH of 6.775. The ACV-treated groups had mean pH values of 6.775 (T1), 6.7417 (T2), 6.7667 (T3), and 6.7583 (T4). They did not find significant ($p = 0.05$) differences within any of the groups. The general observation is that the inclusion of ACV at all the experimental concentrations (T1-T4) did not affect statistically significant alterations in any of these parameters in comparison to the control group (T0).

The lack of major differences in the weight of the spleen indicates that ACV did not cause measurable immune stimulation or suppressive effects at the concentrations applied, which would have led to a change in the size of the spleen. This result is consistent with certain researchers in broiler chickens who found that dietary interventions such as the application of specific phytogens did not greatly influence spleen weight. As an illustration, one study ($p = 0.122$) found no significant effect of vinegar consumption on the weight of the spleen, whereas another reported a tendency of the weight of the spleen to rise, although not

significantly, in quails receiving protein-vitamin mineral supplements.

The studies have documented different effects, including a significantly lower relative weight of the spleen in positive control diet birds compared to ACV diet birds, indicating complex interactions that depend on the overall diet composition and individual ACV preparations. The existing findings indicate that ACV is homeostatic to the growth of the spleen and its functionality in Japanese quails under experimental conditions. The fact that there were no statistically significant differences in the pH of the ileum, jejunum, and cecum between the ACV-supplemented groups and the control group is one of the main results in terms of the direct local effects of ACV. ACV is generally regarded as an acidifier, and its beneficial effect is often explained by a reduction in the gastrointestinal pH, which can suppress the activity of pathogenic bacteria and increase the activity of digestive enzymes.

The present experiment examined how apple cider vinegar (ACV) supplementation influenced the intestinal histomorphology of Japanese quails, particularly the villus height (VH) and crypt depth (CD) at the duodenum, jejunum, and ileum. The findings, as summarized in Table 4.8, reveal that there was a slight difference in these parameters between the control (T0) and ACV-supplemented groups (T1, T2, T3, T4) on day 28, which means that ACV at the inclusion levels used did not cause a significant structural change in intestinal mucosa (Table 6).

Table 6: Study of different gut parameter measurements under different treatments

Parameter	T0	T1	T2	T3	T4	SEM
Spleen Weight (g)	0.1525	0.1617	0.1358	0.1592	0.1433	0.0185
Bursa Weight (g)	0.1733	0.2183	0.22	0.1958	0.2292	0.0221
Ileum pH	6.175	6.175	6.1417	6.1667	6.1583	0.0629
Jejunum pH	5.875	5.875	5.8417	5.8667	5.8583	0.0629
Cecum pH	6.775	6.775	6.7417	6.7667	6.7583	0.0629

Control group (basal diet); T1, T2, T3, T4: Basal diet supplemented with increasing levels of Apple Cider Vinegar. SEM: Standard Error of the Mean

Effect of Dietary Apple Cider Vinegar Levels on the Small Intestine

VH measurements were between 997.4 μm and 1001.3 μm , and CD measurements were between 124.75 μm and 125.42 μm in all duodenum treatment groups. The data were not

significantly different ($p > 0.05$). These results are in contrast to a number of studies that have found that ACV or other organic acids have a significant positive influence on the morphology of the intestines of poultry. To provide an example, Allahdo *et al.* (2018) found that not only the height of the villus but also the depth of the crypt of the small intestine of broilers increased significantly when vinegar was added to the drinking water. Likewise, Tasharofi *et al.* (2017) indicated that there was an improved intestinal morphology in broilers fed waste date vinegar. These are usually observed to be a result of a better nutrient-absorbing capacity because of an increased surface area, which affects the feed conversion ratio and overall growth performance. The lack of these alterations in the present research might indicate that the basal diet or environmental factors of the Japanese quails might not have provided enough intestinal stress to ACV to induce a significant morphological change.

Jejunum, which is one of the main areas of nutrient absorption, also showed steady levels of VH (797.42 μm to 801.33 μm) and CD (99.75 μm to 100.42 μm) across all dietary groups. The findings were not significant (p that is, greater than 0.05). This finding does not agree with the literature, which has shown that ACV can have a positive effect on the health of the gut, resulting in an increase in VH and an enhancement of the ratio of villus height to crypt depth. A higher VH:CD ratio is usually linked to a healthy intestine, which means that there is an improvement in the digestive and absorptive processes. The constant jejunal morphology in the current experiment is indicative of the fact that the level of ACV administered was not high enough to cause a significant response or that the jejunum of the quails was already optimized in the experimental conditions.

Similar to the duodenum and jejunum, the ileum of Japanese quails in this experiment did not have significant changes ($p > 0.05$) in VH

(718.42 μm to 722.33 μm) or CD (89.833 μm to 90.417 μm) with ACV supplementation. This is contrary to the reported positive effects of organic acids, such as ACV, which tend to increase the health of the intestinal epithelium by increasing villi length and crypt depth (or a better VH:CD ratio) in other poultry species. The absorption of the remaining nutrients and the regulation of the microbiota in the gut by the ileum suggest that morphological stability, which is already optimal, might not be significantly improved under certain conditions of a diet and environment with the aid of ACV supplementation (Figure 4).

The present results are in accordance with studies where no significant effect of ACV was observed on some of the production parameters or definite physiological responses in chickens. As an example, Jahantigh *et al.* (2021) reported that ACV did not affect broiler growth performance, and there are studies indicating that it also did not affect the weight of the lymphoid organs. This difference could be attributed to a number of factors. The doses of ACV (T1-T4) could have been low enough to cause no substantial improvement or, on the contrary, too high, and the extra action would not bring any more advantage without side effects. Another important factor that should not be overlooked is species variations in Japanese quails and broilers or other poultry because physiological reactions to dietary interventions are often diverse in terms of avian species. Moreover, the effectiveness of ACV could be affected by the composition of the basal diet and the general health condition of the birds. When the control diet already provides healthy gut health and morphology, the additional benefits of ACV may be less dramatic or even absent.

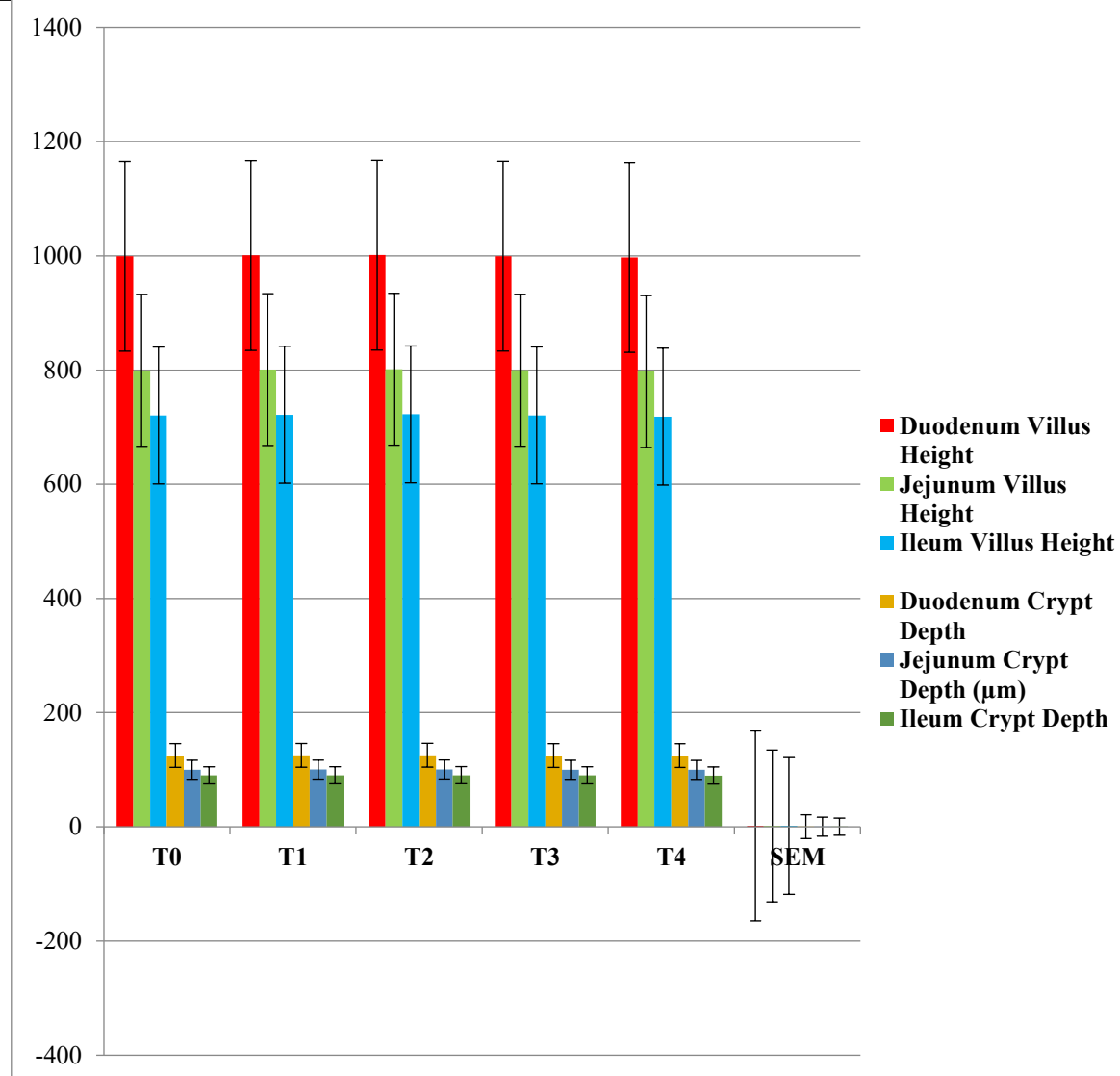


Figure 4: Effects of ACV on different morphological measurements of the intestine

Conclusion

The current study found that adding apple cider vinegar (ACV) to drinking water improved the growth performance of Japanese quail. Among the investigated treatments, 4 mL/L supplementation resulted in the greatest final body weight, increased feed conversion ratio, and decreased feed consumption, indicating greater feed utilization and production efficiency. ACV supplementation also resulted in a moderate increase in body yield and specific carcass features. However, the majority of hematological and serum biochemical indicators remained statistically unaltered, indicating that the measured ACV levels had no deleterious systemic effects. Similarly, supplementation had no significant effect on intestinal pH, villus

height, crypt depth, or splenic weight. ACV, particularly at 4 mL/L, has the potential to be a natural dietary water supplement that improves growth performance and feed efficiency in Japanese quails. Further studies under commercial farming conditions, with longer experimental periods and additional evaluation of gut microbiota and physiological responses, are required to confirm its practical applicability and establish an optimum supplementation level.

Declarations

Consent for publication

All subjects gave their “informed consent” for the publication of details within the text (“informed consent”) to be published in the above Journal and Article. Written “informed consent” was obtained from all authors for the publication of this manuscript.

Availability of data and materials

Data are provided within the manuscript or supplementary information files.

Competing Interest

All authors declare that there are no competing interests.

Conflict of interest

All the authors declare no conflicts of interest.

Funding

No funding

Acknowledgements

The authors extended their appreciation to all those contributed in the manuscript.

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