STUDY ON BLOOD, IMMUNITY AND DIGESTIVE TRACT HISTOLOGY OF BROILER CHICKENS IN RELATION TO FEED FORM AND FEEDING PROGRAM

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SUMMARY

This experiment was conducted on 270-day-old broiler chicks using a 2×3 factorial arrangement. Two protein levels as feeding programs were compared, each program was combined with three feed forms (mash, crumble, and pellet), resulting in six treatment groups. Each treatment included three replicates of 15 birds, providing balanced experimental units. Data was analyzed using ANOVA, and differences among means were considered significant at $p< 0.05$. The study was designed to evaluate immune organ development, blood biochemistry, skeletal traits, and intestinal histomorphology. Results showed that feed form had a stronger influence than feeding program. Bursa percentage was significantly higher in birds fed pellets or crumbles, while spleen remained unaffected. The feeding program had minimal effects on blood parameters, except that reduced crude protein (CP) (program 2) increased cholesterol and alanine amino transferase activity. Feed form altered serum proteins, with mash-fed birds showing higher total protein and globulin, whereas crumble feeding lowered aspartate amino transferase, suggesting reduced hepatic stress. Bone traits were strongly affected by feed form as pelleted diets enhanced tibia length, width, and volume, while the combination of program 2 with pellets produced the greatest tibia breaking strength, reflecting a synergistic benefit. Intestinal morphology revealed that higher CP improved villus height, pellets promoted taller villi but reduced villus width/crypt depth ratio, mash increased goblet cell numbers, and crumbles enhanced crypt depth but lowered goblet cells. In conclusion, feed form was more decisive than feeding program in shaping immunity, metabolism, skeletal integrity, and gut morphology. Pellets and crumbles optimized growth and bone strength, while mash favored humoral and mucosal defense. Pelleting is recommended for performance, with limited mash inclusion to sustain gut health.

Keywords: *immunity, histology, feed form, feeding program, broilers.*

INTRODUCTION

Poultry production has become one of the fastest-growing agricultural sectors worldwide, providing an affordable and efficient source of animal protein to meet the nutritional demands of the increasing global population. Among poultry species, broiler chickens play a pivotal role due to their rapid growth rate, efficient feed conversion, and high meat yield (Havenstein *et al.,* 2003and Zuidhof *et al.,* 2014). However, the optimization of broiler productivity is highly dependent on dietary strategies and feeding management systems, which continue to be refined to maximize performance, health, and product quality under commercial conditions.

Nutrition is one of the primary determinants of broiler growth, feed efficiency, and carcass quality. Crude protein (CP) levels and dietary energy have historically been central to broiler diet formulation, as they directly affect nutrient utilization, carcass yield, and production economics (NRC, 1994; Leeson and Summers, 2001). More recently, however, the poultry industry has shifted toward precision feeding, in which diets are tailored to specific growth phases to improve nutrient efficiency, reduce feed costs, and mitigate nitrogen excretion and environmental pollution (Chrystal *et al.,* 2020 and Belloir *et al.,* 2017). Phase feeding programs allow for the adjustment of protein and amino acid levels according to the age and growth potential of broilers, thereby optimizing performance while supporting sustainable poultry production (Corzo *et al.,* 2005 and Kidd *et al.,* 2021).

In addition to nutrient composition, feed form plays an equally crucial role in poultry performance. Traditionally, broiler diets have been offered in mash form; however, advances in feed technology have made pellets and crumbles the preferred forms in intensive production systems (Cutlip *et al.,* 2008 and

Amerah *et al.,* 2007). Pelleting improves feed intake, digestibility, and nutrient utilization by reducing feed wastage and increasing nutrient density, whereas crumbles are particularly beneficial during the starter phase by improving early feed intake and gut development (Abdollahi *et al.,* 2013 and Amerah *et al.,* 2011). Several studies have reported significant improvements in growth rate, feed conversion ratio, and carcass yield in birds fed pelleted or crumbled diets compared with mash diets (Nir *et al.,* 1995; Abdollahi *et al.,* 2013 and Lemme *et al.,* 2020).

Beyond growth performance, feed form and feeding program can influence intestinal morphology, nutrient absorption, bone strength, and immune organ development, all of which are critical indicators of overall bird health and welfare (Khusro *et al.,* 2016 and Liu *et al.,* 2020). For example, pelleted diets are associated with increased villus height and improved gut integrity, while high-protein diets during early growth stages may enhance muscle development but also impose additional metabolic stress (Macari and Maiorka, 2017). Understanding these physiological responses is essential for designing optimal feeding strategies.

Despite extensive research on broiler nutrition, the interaction between feeding programs and feed forms remains insufficiently understood, particularly under modern high-performance genetics. The combined effects of dietary protein levels across different growth phases and feed form presentation may have synergistic or antagonistic impacts on growth performance, carcass characteristics, and internal physiological parameters. Moreover, in the context of increasing consumer demand for efficiency, meat quality, and animal welfare, there is a growing need to re-evaluate these factors to provide evidence-based feeding recommendations for commercial production systems.

Therefore, the present study was designed to investigate the effects of different feeding programs (varying crude protein levels across growth phases) and feed forms (mash, crumble, and pellet) on broiler chickens. The research specifically focused on evaluating intestinal histomorphology, bone quality, and immune organ development. The outcomes are expected to provide a comprehensive understanding of how feeding strategies can be optimized to balance productivity, animal welfare, and environmental sustainability.

MATERIALS AND METHODS

The study was conducted at the Poultry Nutrition Farm and the Poultry Feed Quality Control Laboratory, Department of Poultry Production, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Qalyobia, Egypt, to evaluate the effects of feed form, feeding program, and their interaction on broiler blood, immunity and digestive tract histology up to 35 days of age.

Experimental birds and management:

A total of 270 one-day-old Indian River broiler chicks were obtained from a commercial hatchery and randomly assigned to six treatments in a 2 × 3 factorial arrangement (two feeding programs × three feed forms). Each treatment consisted of three replicates with 15 chicks per replicate as chicks were weighed and housed in cage batteries.

All birds were reared under uniform environmental and managerial conditions. Temperature was maintained at 33 ± 1 °C during the first week and gradually reduced by 2-3 °C per week until reaching 24°C. Continuous lighting was provided during the first 3 days, followed by 23 h light: 1 h dark throughout the experimental period. Feed and water were offered *ad libitum*, and stainless-steel feeders and nipple drinkers were used to ensure hygiene. Excreta were removed daily, and pens were disinfected regularly to minimize microbial load.

A routine vaccination program was followed. Chicks were vaccinated against Newcastle disease at day 7, infectious bursal disease (Gumboro) at day 14, and Newcastle disease (LaSota strain) at days 18 and 28 via drinking water. Vaccines were procured from the Veterinary Serum and Vaccine Research Institute (Cairo, Egypt) and administered according to the manufacturer's recommendations.

Experimental design and diets:

Two feeding programs were tested:

- P1: 23.0, 21.0, 19.0% CP in starter, grower, and finisher phases, respectively.
- P2: 21.0, 19.0, 17.0% CP in starter, grower, and finisher phases, respectively.

Each program was offered in three forms:

- F1: Mash

- F2: Crumble
- F3: Pellet

This factorial arrangement (2 feed programs × 3 feed forms) resulted in six treatments: T1 (P1F1), T2 (P1F2), T3 (P1F3), T4 (P2F1), T5 (P2F2), and T6 (P2F3). Each treatment consisted of three replicates, with 15 chicks per replicate (total 270 birds). Diets were formulated to meet or exceed NRC (1994) nutrient requirements. Feed ingredient composition and calculated nutrient content are presented in Table 1, and all diets included a commercial vitamin–mineral premix, emulsifier, and enzyme blend (phytase, xylanase).

Table (1): Feed Ingredients composition and calculated nutrient content of experimental diets.

Ingredients	Starter (P1) 23%	Starter (P2) Grower (P1) 21%	Finisher (P1) Grower (P2) 19%	Finisher (P2) 17%
Yellow Corn	54.269	62.345	66.879	71.057
Soybean Meal (46% CP)	32.283	24.484	17.228	14.281
Corn Gluten Meal (60% CP)	4.020	5.000	4.925	2.666
Full Fat Soybeans	3.500	5.000	8.000	9.000
Calcium Carbonate	1.372	1.372	1.297	1.246
Mono-Calcium Phosphate	0.788	0.614	0.566	0.596
Salt (NaCl)	0.275	0.248	0.207	0.221
Sodium Bicarbonate	0.061	0.100	0.161	0.143
L- Lysine	0.231	0.248	0.187	0.147
DL- Methionine	0.218	0.184	0.169	0.239
L-Threonine	0.063	0.035	0.011	0.034
Choline Chloride	0.100	0.050	0.050	0.050
Phytase	0.010	0.010	0.010	0.010
Xylanase Enzymes	0.010	0.010	0.010	0.010
Mineral-Vitamin Premix*	0.300	0.300	0.300	0.300
**Additives	2.500	2.500	2.500	2.500
Total	100.000	100.000	100.000	100.000
Nutrient Content				
Crude Protein (CP) %	23.000	21.000	19.000	17.000
ME (Kcal/Kg)	2950	3040	3125	3150
Crude Fat %	3.326	3.697	4.285	4.519
Crude Fiber %	3.192	3.020	2.916	2.896
Calcium %	0.950	0.900	0.850	0.830
Available P %	0.450	0.400	0.380	0.380
Methionine %	0.581	0.530	0.491	0.522
Methionine + Cystine %	0.988	0.914	0.850	0.850
Lysine %	1.350	1.200	1.025	0.920
Threonine %	0.960	0.850	0.750	0.700
Sodium %	0.180	0.180	0.180	0.180
Chloride %	0.250	0.237	0.200	0.200
Price (EGP/ Ton)	18157	18034	17968	17385

**Vitamins-Minerals mixture (per kg diet): Vit. A 12000 IU, Vit. D3 5000 IU, Vit. E 10 mg, Vit. K3 2 mg, Vit. B1 1mg, Vit. B2 5 mg, Vit. B6 1.5 mg, Vit. B12 10 µg, Biotin 50 µg, Pantothenic acid 10 mg, Niacin 30 mg, Folic acid 1 mg, Mn 60 mg, Zn 50 mg, Fe 30 mg, Cu 10 mg, I 1 mg, Se 0.1 mg, Co 0.1 mg. #Contains Mycotoxin binder, Anti-clostridia, and Anti-coccidia additives. **Additives: Emulsifier & Phytase & Xylanase Enzymes.*

Lymphoid organ measurements:

At 35 days of age, immediately after slaughter and evisceration, the thymus and bursa of Fabricius were carefully excised from two birds per replicate. The thymus was identified as a series of paired lobes located along each side of the trachea and jugular vein in the neck region, and all visible lobes were removed. The bursa of Fabricius, situated dorsal to the cloaca, was dissected free from surrounding connective tissue. Both organs were gently blotted with absorbent paper to remove surface moisture and weighed individually using a digital analytical balance (±0.001 g accuracy). This method allowed standardization of organ size relative to bird size, enabling comparison across treatments. Organ weights were expressed as a percentage of live body weight according to the formula:

Relative organ weight (%) = [Organ weight (g)/ Live body weight (g)] ×100

Blood biochemistry:

At 35 days of age, two blood samples per replicate were collected from the brachial vein after 12 h feed withdrawal. Samples for antioxidant assays were centrifuged immediately at 2,000 rpm for 10 min, and the separated serum was stored at −20 °C until analysis. Samples for biochemical assays were allowed to clot at room temperature and centrifuged 30 min post-collection at 3,000 rpm for 10 min. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined using an automated biochemical analyzer (7600; Hitachi, Japan) with commercial reagent kits (Nanjing Jiancheng Bioengineering Institute, China). Total protein and albumin concentrations were determined colorimetrically using the Biuret and bromocresol green methods, respectively (Dumas *et al.,* 1971). Globulin(G) concentration was calculated as the difference between total protein and albumin (A), and the albumin: globulin (A/G) ratio was obtained by division. Total cholesterol was determined enzymatically using the cholesterol oxidase–peroxidase method (Henry, 1974). Creatinine was measured by the colorimetric Jaffe reaction (Henry, 1974), and uric acid concentration was determined by the uricase–peroxidase method (Caraway, 1955). All colorimetric assays were performed using a UV–visible spectrophotometer (Shimadzu UV-1800, Japan).

Tibia characteristics:

At 35 days of age, two birds per replicate were slaughtered for tibia evaluation. The right tibiae were carefully excised, cleaned of adhering soft tissue, and stored at −20 °C until analysis. Prior to assessment, bones were thawed at room temperature. Tibia length, proximal width, and midshaft width were measured using a precision digital micrometer. Tibia weight and volume (ml displacement method) were also recorded. Bone breaking strength was determined using an Instron Universal Testing Machine (maximum load 50 kg). In addition, tibia weight relative to live body weight (tibia % from LBW) was calculated. Physical bone density was estimated following the procedure of Watkins and Southern (1992), while the tibia Seedor index (TSI; bone weight/length) was calculated according to Seedor et al. (1991).

Intestinal morphology:

At 35 days of age, two birds per replicate were slaughtered for intestinal histomorphological assessment. A 2 cm segment of the mid-jejunum was excised, gently flushed with physiological saline, and immediately fixed in 10% neutral-buffered formalin for 48 h. Thereafter, tissues were dehydrated in a graded series of ethanol, cleared in xylene, embedded in paraffin, and sectioned at 5 μm thickness. The prepared sections were stained with hematoxylin and eosin (H&E) for histological evaluation following the method described by Iji et al. (2001).

Morphometric measurements were performed using a light microscope (×40 magnification) equipped with an image analysis system. Villus height (from the tip of the villus to the villus–crypt junction), villus width (measured at the mid-point), and crypt depth (from the villus–crypt junction to the base of the crypt) were determined on at least 10 well-oriented villi per sample, according to the procedures outlined by Awad et al. (2009). Goblet cells were counted along a standardized 100 μm section of the villus epithelium (Pluske *et al.,* 1996). In addition, the villus width/crypt depth (VW/CD) ratio was calculated as an index of mucosal structural efficiency, reflecting the balance between absorptive surface area and regenerative crypt activity (Awad *et al.,* 2009).

Statistical analysis:

Data was analyzed by two-way ANOVA using the GLM procedure of SAS (2004). Treatment means were separated by Duncan's multiple range test (Duncan, 1955) at $p < 0.05$. The statistical model was:

$$Yijk=\mu+S_i+P_j+ (S\times P)_{ij}+Y_{eijk}$$

Where: Si = feed form, Pj = feeding program, and eijk = random error.

RESULTS AND DISCUSSION

Lymphoid organs:

In the current study as presented in Table (2), feed form significantly affected the relative weight of the bursa of Fabricius, whereas neither feed program nor the interaction between program and form had any influence. The results indicate that broilers fed crumble and pellet diets exhibited greater bursal percentages (0.23–0.25%) compared with mash-fed birds (0.10%), while spleen percentage remained unaffected across all treatments. These findings suggest that the physical form of the diet, rather than moderate differences in CP content, plays a more important role in modulating lymphoid organ development.

The bursa of Fabricius is a primary lymphoid organ that is crucial for B-cell differentiation and humoral immunity. Its relative size is often used as a proxy for immunological development in broilers (Tahir *et al.,* 2024). The observed enlargement in birds fed crumble or pellet diets may be explained by improved nutrient availability and uniform feed intake associated with processed feed forms, which can enhance the allocation of nutrients to rapidly developing immune tissues (Hosseini *et al.,* 2017). Similar trends have been observed in other studies where improved diet form or fermentation-based feed strategies enhanced bursal indices or immune responses (Zhu *et al.,* 2023).

Table (2): Effects of feed program and feed forms on lymphoid organs.

Items	Spleen %	Bursa %
Feed Program		
Program 1 (P1)*	0.65	0.21
Program 2 (P2)**	0.69	0.18
Feed Form		
Mash (F1)	0.68	0.10b
Crumble (F2)	0.67	0.23a
Pellets (F3)	0.65	0.25a
Interaction		
T1 (P1F1)	0.64	0.13
T2 (P1F2)	0.72	0.07
T3 (P1F3)	0.66	0.23
T4 (P2F1)	0.68	0.23
T5 (P2F2)	0.64	0.25
T6 (P2F3)	0.67	0.24
Significance		
Feed Program	NS	NS
Feed Form	NS	**
Interaction	NS	NS

*a, b: The values' means different letters are significantly different (P<0.05). *P1: 23.0, 21.0, 19.0% CP in starter, grower, and finisher phases, respectively. *P2: 21.0, 19.0, 17.0% CP in starter, grower, and finisher phases, respectively.*

In contrast, the spleen percentage was not affected by either feed program or form. This result is consistent with previous research, which generally reports that spleen weight remains stable unless the birds experience a marked immunological challenge or stress (Kithama *et al.,* 2023). The lack of differences in spleen size in this study suggests that the immune system was not activated by infection or stress, and that the observed bursal differences were more likely linked to nutrient delivery efficiency rather than pathological changes.

The absence of a feed program effect on either bursa or spleen indices is noteworthy. Within the CP ranges tested, birds maintained comparable immune organ weights, suggesting that if amino acid balance is preserved, moderate CP adjustments do not compromise lymphoid organ development. This agrees with recent findings that moderate reductions in CP, especially when protease supplementation or amino acid balancing is provided, do not negatively affect immune organ development (Kamely *et al.,* 2020 and Qiu *et al.,* 2023).

From a practical perspective, these findings highlight that feed physical quality is a more critical determinant of lymphoid organ development than feeding program adjustments under standard nutritional conditions. Crumble and pellet feeding not only provide well-documented advantages in growth performance and feed efficiency (El-Faham *et al.,* 2024), but, may also contribute to enhanced bursal development and potentially stronger humoral immunity. However, since lymphoid organ indices are only proxy indicators, future research should integrate functional measures such as vaccine antibody

titers or disease challenge responses to validate whether the observed increase in bursa percentage indeed translates into superior immune competence (Attia *et al.,* 2020).

Blood biochemical parameters:

As shown in Table (3), serum total protein, albumin, globulin, creatinine, and uric acid were not significantly influenced by the CP program. This indicates that moderate changes in CP level across feeding phases did not compromise protein metabolism or renal function. Similar results were reported by Qiu et al. (2023) and Kamely et al. (2020), who found that when essential amino acid balance is maintained, reductions in dietary CP do not markedly affect serum protein fractions or uric acid excretion in broilers.

Interestingly, total cholesterol was significantly elevated in Program 2 (87.33 mg/dL) compared with Program 1 (72.13 mg/dL). Lowering CP has been associated with altered lipid metabolism and increased fat deposition, leading to higher serum cholesterol levels (Abudabos *et al.,* 2017). In parallel, AST (aspartate aminotransferase) and ALT (alanine aminotransferase) responded differently: Program 1 showed higher AST (200.71 IU) but lower ALT (14.48 IU), whereas Program 2 yielded lower AST (184.50 IU) but higher ALT (16.97 IU). Since these enzymes are important liver function markers, the elevation in ALT in Program 2 may reflect a slightly higher hepatic metabolic load under reduced CP diets. However, values remained within the normal physiological range for broilers, suggesting no pathological liver impairment (Abdelqader *et al.,* 2020).

Table (3): Effects of feed program and feed forms on blood parameters.

Items	Total protein mg/dL	Albumin mg/dL	Globulin mg/dL	Total cholesterol mg/dL	AST* IU/L	ALT** IU/L	Creatinine mg/dL	Uric acid mg/dL
Feed Program								
Program 1	3.23	1.21	2.02	72.13 b	200.71 a	14.48b	0.415	10.816
Program 2	3.16	1.18	1.98	87.33a	184.50 b	16.97a	0.413	11.058
Feed Form								
Mash (F1)	3.29a	1.21a	2.08a	80.06	203.29a	16.22a	0.412	10.225
Crumble (F2)	3.19ab	1.14b	2.05a	76.79	172.44b	16.21a	0.413	10.209
Pellets (F3)	3.09b	1.23a	1.86b	82.34	202.08a	14.75b	0.417	10.754
Interaction								
T1 (P1F1)	3.43	1.17	2.25	76.21	218.15a	16.02ab	0.411	11.438
T2 (P1F2)	3.16	1.25	1.91	83.92	188.43b	16.43ab	0.412	10.729
T3 (P1F3)	3.16	1.23	1.93	62.75	181.04b	14.12b	0.412	10.344
T4 (P2F1)	3.22	1.04	2.18	90.82	163.84c	18.31a	0.414	11.288
T5 (P2F2)	3.10	1.22	1.88	77.44	202.93a	13.32b	0.421	10.198
T6 (P2F3)	3.09	1.24	1.85	87.23	201.23a	16.19ab	0.414	10.262
Significance								
Feed Program	NS	NS	NS	*	*	**	NS	NS
Feed Form	*	*	*	NS	*	*	NS	NS
Interaction	NS	NS	NS	NS	*	*	NS	NS

*a, b: The values' means different letters are significantly different (p <0.05). *AST: aspartate aminotransferase. **ALT: alanine aminotransferase. ***P1: 23.0, 21.0, 19.0% CP in starter, grower, and finisher phases, respectively. ****P2: 21.0, 19.0, 17.0% CP in starter, grower, and finisher phases, respectively*

Feed form significantly influenced serum protein fractions. Mash-fed broilers exhibited higher total protein (3.29 mg/dL), and globulin (2.08 mg/dL), while pellet-fed birds showed the lowest values (3.09, and 1.86 mg/dL, respectively). The higher serum proteins in mash-fed birds may be linked to slower feed passage rate and more stable absorption of amino acids, whereas pelleting enhances nutrient digestibility but favors tissue deposition rather than circulating protein pools (Amerah *et al.,* 2007). The relatively higher globulin values in mash and crumble groups suggest improved humoral immune potential, consistent with previous findings that dietary structure influences immunoglobulin synthesis and immune status (Attia *et al.,* 2020).

With respect to liver enzymes, crumble-fed birds showed significantly lower AST (172.44 IU) compared with mash (203.29 IU) and pellets (202.08 IU), indicating potentially lower hepatic stress. Conversely, ALT was lowest in pellet-fed birds (14.75 IU) compared with mash and crumble (16.2 IU).

This agrees with observations that feed processing methods alter nutrient metabolism and may modulate enzyme leakage from hepatocytes (Choct, 2009).

Creatinine and uric acid did not differ among feed programs or forms. This indicates that neither protein level nor feed physical form imposed detrimental effects on kidney function or nitrogen excretion. Such stability in renal biomarkers under different dietary regimens has also been documented by Kithama et al. (2023).

Collectively, these results suggest that while feeding program adjustments within practical ranges do not markedly alter serum protein or renal indices, they may affect lipid metabolism and liver enzyme activities. Feed form, on the other hand, plays a more pronounced role in shaping blood protein profiles and liver health markers. Mash feeding favored higher serum protein fractions, whereas crumble and pellet feeding improved enzyme stability, reflecting subtle trade-offs between nutrient utilization for growth and circulating biochemical indices.

Tibia bone development:

Bone quality is a key indicator of skeletal health and overall broiler performance, and it is strongly influenced by nutrition and feed form (Rath *et al.,* 2000 and Williams *et al.,* 2004). The results presented in Table (4) show that tibia weight, tibia percentage relative to live body weight, and tibia volume were not significantly ($p > 0.05$) affected by the feeding program. However, tibia length was significantly higher in broilers fed under program 2 compared to program 1 (84.31 vs. 80.01 mm, respectively), indicating improved skeletal growth when dietary crude protein was supplied at a relatively lower but more balanced level (Angel, 2007).

Table (4): Effects of feed program and feed forms on tibia bone measurements.

Items	Tibia weight (g)	Tibia (%) - LBW	Tibia length (mm)	Tibia proximal width (mm)	Tibia middle width (mm)	Tibia volume (ml)	Tibia Breaking Strength (N)
Feed Program							
Program 1 (P1)*	133.75	6.50	80.01b	7.99	6.76	7.39	130
Program 2	133.80	6.45	84.31a	7.86	6.73	7.34	135
Feed Form							
Mash (F1)	134.00	6.32b	83.10ab	7.75b	6.57c	7.14b	128
Crumble (F2)	134.07	6.78a	77.89b	7.77b	6.79b	7.07b	133
Pellets (F3)	133.25	6.32b	85.49a	8.26a	6.87a	7.89a	135
Interaction							
T1 (P1F1)	133.77	6.50	82.00	8.07	6.75	7.37	125b
T2 (P1F2)	134.23	6.15	84.20	7.44	6.39	6.91	129b
T3 (P1F3)	133.9	6.73	72.67	7.67	6.87	7.24	131ab
T4 (P2F1)	134.24	6.83	83.11	7.87	6.72	6.90	127b
T5 (P2F2)	133.57	6.27	85.37	8.24	6.67	7.57	135a
T6 (P2F3)	132.94	6.37	85.62	8.27	7.07	8.20	138a
Significance							
Feed Program	NS	NS	*	NS	NS	NS	NS
Feed Form	NS	*	**	*	*	**	NS
Interaction	NS	NS	NS	NS	NS	NS	*

*a, b: The values' means different letters are significantly different (p <0.05). *P1: 23.0, 21.0, 19.0% CP in starter, grower, and finisher phases, respectively. **P2: 21.0, 19.0, 17.0% CP in starter, grower, and finisher phases, respectively*

Feed form had a marked effect on bone characteristics. Birds fed pelleted diets recorded the highest tibia length (85.49 mm), proximal width (8.26 mm), middle width (6.87 mm), and volume (7.89 ml), with significant differences compared to mash or crumble forms. This finding agrees with previous reports that pelleted diets enhance nutrient digestibility and promote better bone mineralization and growth compared to mash diets (Amerah *et al.,* 2007 and Abdollahi *et al.,* 2013). On the other hand, tibia percentage relative to body weight was higher in crumble-fed birds (6.78%), which could suggest an improvement in skeletal proportion relative to body size during early growth phases (Nir *et al.,* 1995).

Tibia breaking strength (N) was not significantly influenced by either feed program or feed form alone; however, a significant interaction effect ($p < 0.05$) was observed. Birds in treatment T6 (program 2 ×

pellets) showed higher tibia breaking strength (138 N), significantly greater than treatments T1, T2, and T4. This result indicates that the combination of an optimized protein feeding program and pelleted feed form synergistically improves bone mechanical properties, supporting stronger skeletal development (Onyango *et al.,* 2003 and Shim *et al.,* 2012).

Overall, these results confirm that feed form plays a more decisive role than protein level in shaping bone morphology and strength in broilers, while the interaction between diet composition and feed processing can provide additional benefits for skeletal integrity.

Intestinal morphology:

The results of intestinal histomorphology (Table 5 & Figure 1) showed that feed program had a significant effect only on villus height (VH), where broilers in program 1 recorded higher villus height (986.82 µm) compared to program 2 (938.59 µm). This suggests that diets with relatively higher crude protein levels supported better intestinal villus development, which is directly associated with enhanced absorptive surface area and improved nutrient utilization (Awad *et al.,* 2009 and Rehman *et al.,* 2020). The lack of significant effect of feed program on villus width, crypt depth, and goblet cell numbers indicates that protein level mainly influences mucosal elongation rather than structural remodeling of crypts.

Table (5): Effects of feed program and feed forms on intestine histomorphology.

Items	Villus height	Villus Width (VW)	Crypt Depth (CD)	VW / CD Ratio	Goblet Cells Number
Feed Program					
Program 1 (P1)*	986.82a	91.33	201.15	0.454	5.29
Program 2 (P2)**	938.59b	84.15	203.03	0.414	6.30
Feed Form					
Mash (F1)	961.35ab	92.06a	193.13b	0.477a	6.35a
Crumble (F2)	950.97b	94.60a	206.85a	0.457a	4.93b
Pellets (F3)	975.79a	76.55b	206.28a	0.371b	6.11a
Interaction					
T1 (P1F1)	922.2	91.71	197.32	0.465	5.52
T2 (P1F2)	990.5	92.41	188.93	0.489	7.19
T3 (P1F3)	964.04	102.51	205.46	0.499	4.57
T4 (P2F1)	987.89	118.69	208.24	0.570	5.28
T5 (P2F2)	1004.2	79.75	200.65	0.397	5.78
T6 (P2F3)	947.37	73.34	211.91	0.346	6.44
Significance					
Feed Program	**	NS	NS	NS	NS
Feed Form	*	*	*	*	**
Interaction	NS	NS	NS	NS	NS

*a, b: The values' means different letters are significantly different (p <0.05). *P1: 23.0, 21.0, 19.0% CP in starter, grower, and finisher phases, respectively. **P2: 21.0, 19.0, 17.0% CP in starter, grower, and finisher phases, respectively.*

Regarding feed form, significant effects were observed on villus height, villus width, crypt depth, villus width/crypt depth ratio, and goblet cell number. Birds fed pellets had higher villus height (975.79 µm) compared with crumble (950.97 µm), while mash-fed birds showed intermediate values (961.35 µm). This agrees with previous reports that pelleted diets improve feed efficiency and gut morphology due to reduced feed sorting, better nutrient density per volume, and improved digestibility (Svihus, 2011; Amerah *et al.,* 2007).

Interestingly, villus width was greater in birds fed mash and crumble compared to pellets, while crypt depth was deeper in crumble and pellet-fed groups relative to mash. A deeper crypt generally reflects higher cell proliferation activity, which may be a compensatory response to higher villus turnover in crumble and pellet forms (Caspary, 1992 and Montagne *et al.,* 2003). The villus width/crypt depth ratio, an indicator of gut health, was significantly reduced in pellet-fed birds (0.371), suggesting that despite taller villi, the balance between villus function and crypt renewal may be stressed under pelleted diets.

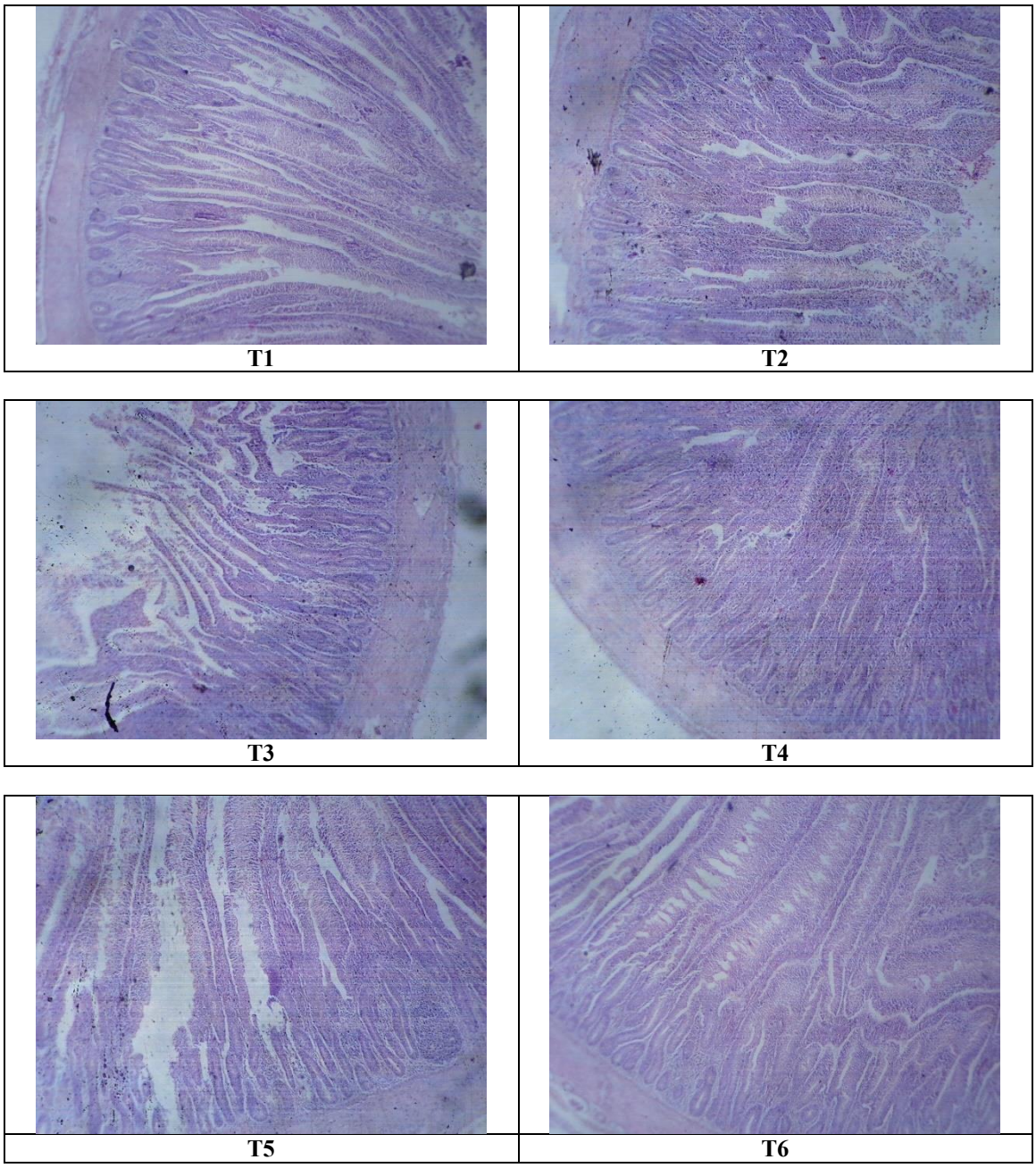

Figure (1): Photomicrographs of intestinal histomorphology of birds of different dietary groups.

In terms of goblet cell numbers, which are essential for mucus secretion and intestinal protection, mash (6.35) and pellet-fed groups (6.11) had higher counts than crumble (4.93). A higher number of goblet cells enhance mucosal defense mechanisms, thereby potentially protecting birds from enteric pathogens (Smirnov *et al.,* 2004 and Uni *et al.,* 2003).

The interaction between feed program and feed form showed no significant effects on all measured histomorphological traits, suggesting that the main effects of protein level and feed form were independent. However, the photomicrographs (Figure 1) confirm numerical variations among treatments (T1–T6) that correspond to differences in villus architecture, crypt morphology, and goblet cell density, reinforcing the statistical findings.

Overall, these results highlight that pelleted diets improve villus height but may reduce villus width/crypt depth ratio, while mash diets support higher goblet cell counts, both contributing differently to gut health. Crumble diets, although intermediate in villus height, induced deeper crypts and lower

goblet cell counts, which may compromise mucosal defense. These findings are in line with recent evidence that diet form not only influences performance but also profoundly affects intestinal health and morphology in broilers (González-Alvarado *et al.,* 2008 and Mateos *et al.,* 2012).

CONCLUSION

In conclusion, feed form influenced immunity, bone strength, and gut morphology more than feed program. Pellets and crumbles enhanced growth traits, while mash supported serum proteins and gut defense. Practically, pelleting is recommended for performance, with mash inclusion to sustain gut health.

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دراسة مقاييس الدم، المناعة، والتركيب النسيجي للجهاز الهضمي في دجاج التسمين وعلاقتها بشكل العلف وبرنامج التغذية

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أُجريت هذا الدراسة على 270 طائر تسمين عمر يوم واحد، تم توزيعهم عشوائياً بتصميم عاملي 2 × 3. حيث تمت مقارنة برنامجين غذائيين، تم توزيع كل برنامج خلال ثلاثة أشكال من العلف (ناعم، مفتت، ومصبعات)، بحيث تم التوزيع في ست مجموعات تجريبية. شملت كل مجموعة ثلاث مكررات، بكل منها 15 طائر. تم تحليل البيانات باستخدام تحليل التباين ANOVA، واعتُبرت الفروقات بين المتوسطات ذات دلالة إحصائية عند $p< 0.05$، وقد تناولت الدراسة تقييم تطور الأعضاء المناعية، بعض مقاييس الدم، صفات عظم الساق، وصفات التركيب النسيجي للأمعاء. أظهرت النتائج أن شكل العلف كان له تأثير أقوى من برنامج التغذية. فقد كانت نسبة البرسا (Bursa) أعلى بشكل ملحوظ في الطيور التي تغذت على المصبعات أو المفتتات، بينما لم تتأثر نسبة الطحال. وكان لبرنامج التغذية تأثير محدود على مقاييس الدم، باستثناء زيادة في تركيز الكوليسترول ونشاط إنزيم ALT عند تقليل .CP (برنامج غذائي 2) أما شكل العلف فقد غير مستويات البروتينات في السيرم، حيث أظهرت الطيور المغذاة على العلف الناعم ارتفاع في البروتين الكلي والجلوبيولين في حين قلل العلف المفتت من تركيز إنزيم AST، مما يشير إلى انخفاض الإجهاد الواقع على الكبد. تأثرت صفات عظم الساق بصورة كبيرة بشكل العلف؛ فقد حسنت المصبعات من طول وعرض وحجم عظم الساق، بينما أنتج الجمع بين البرنامج الغذائي 2 والمصبعات أقوى مقاومة للكسر، مما يعكس فائدة تجمعية للبرنامج الغذائي مع صورة وشكل العلف المقدم للطيور. وأوضحت نتائج فحص التركيب النسيجي للأمعاء أن البروتين المرتفع (برنامج غذائي 1) حسّن ارتفاع الخملات المعوية، بينما عززت المصبعات طول الخملات لكنها قللت نسبة villus width/crypt depth، وزاد العلف الناعم من عدد الخلايا الكأسية، بينما عزز العلف المفتت crypt depth مع خفض عدد خلايا الكأسية. يمكن الإستنتاج بأن لشكل العلف تأثير أكبر من برنامج التغذية في تحديد القدرة المناعية، والتمثيل الغذائي، وسلامة الهيكل العظمي، وبنية الأمعاء. فقد حسنت التغذية على العلف المصبع والعلف المفتت من النمو وقوة العظام، بينما دعم العلف الناعم القدرة المناعية الخلوية والمخاطية للأمعاء. لذلك، يُنصح باستخدام العلف المصبع لتحسين الأداء الإنتاجي مع دمج كمية محدودة من العلف الناعم للحفاظ على صحة الأمعاء وتطور القناة الهضمية.