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*Full Length Research*

# Exploring the impact of turkey age on gut morphological parameters and nutrient utilisation

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**ABSTRACT:** This study was carried out to determine the influence of turkey age on gut morphology and nutrient utilisation. Data on the gut morphology and nutrient utilisation were collected at the different age intervals of turkeys' growth: the starter (day-old - 8 weeks), grower (9 - 16 weeks), and finisher (17 - 24 weeks) phases. Nicholas turkeys were fed commercial turkey diets *ad libitum* for the eight weeks of each phase: starter, grower, and finisher. The energy utilisation parameters such as the efficiency of metabolisable energy used for energy retention (KRE), the efficiency of metabolisable energy used for lipid retention ($KRE_F$), the efficiency of metabolisable energy used for protein retention ($KRE_P$) were determined. Results indicate a significant increase in NEP and REP over time, underscoring the increasing energy demands associated with growth, protein and fat deposition. Results of the gut morphological parameters, encompassing villus and crypt dimensions, muscular wall thickness, epithelial characteristics, goblet cell sizes, and the ratio of villus height to crypt depth. There was significant increase ($p<0.05$) in villus height, crypt depth, and epithelial thickness over the age intervals, suggesting an increase in the absorptive surface area and barrier function of the gut. The ratio of villus height to crypt depth further indicates a maturation of the gastrointestinal tract. This study highlights the pivotal role of gut development in shaping the nutrient utilisation of turkeys.

**Keywords:** Gut morphology, nutrient utilisation, turkey age.

## INTRODUCTION

The turkey industry plays a vital role in the agricultural sector and has been the subject of extensive research and production efforts. The poultry industry continually seeks ways to enhance the efficiency of turkey production while ensuring the health and welfare of the birds (Hafez and Shehata, 2021). Among the multifaceted factors influencing turkey performance, age has emerged as a pivotal parameter affecting gut morphology and nutrient utilisation. Although poultry are classified as monogastric creatures, their digestive system differs from that of pigs and humans. In particular, the gastrointestinal tract (GIT) of poultry is noticeably shorter and lighter in comparison to these species. However, when compared to other types of livestock, the GIT of poultry is relatively lengthier (measured in cm/kg body weight) and more substantial (measured in g/kg body weight) (Jha and Mishra, 2021).

The age of turkeys at any given stage of production is more than just a chronological marker; it is intricately tied to the development of their digestive system and metabolic capacities. As such, understanding how the age of turkeys influences gut morphological parameters and nutrient utilisation is a critical aspect of optimising their growth, feed efficiency, and overall well-being. Nutrient use and gastrointestinal tract development are tightly linked (Choct, 2008). For a feed material to be a helpful source of energy, it must be digested and absorbed (Singh *et al*., 2021). According to Dibaji *et al*. (2014), the gut accounts for 20% of energy utilisation, indicating that factors that negatively impact the gut can affect nutrient intake and animal output (Kallam and Sejian, 2021).

It is crucial to assess the absorption of nutrients from all of the feed elements in the diet since various substances' efficiency of nutrient utilisation varies (NRC, 2012). Assessing the absorptive surface area for nutrient intake can be facilitated by examining gut morphology measurements (Geyra *et al*., 2001). The utilisation of net energy in chickens is influenced by various factors, including growth requirements, gut maintenance, and the overall surface area of the gut. Determining the surface area involves considering gross morphological features such as the length and cross-sectional area of the duodenal, jejunal, and ileal segments, as well as finer morphological features like the surface area and height of villi in each segment (Pourabedin and Zhao, 2015). The development of nutrient utilisation and the gastrointestinal tract are closely intertwined, underscoring the importance of comprehending the morphological and functional changes that occur during gut development to optimise nutrient utilisation in poultry. This research, therefore, explores the multifaceted relationship between turkey age, gut morphology, and nutrient utilisation.

## MATERIALS AND METHODS

### Experimental diets and management of birds

The experiment was carried out at the Poultry Unit, Teaching and Research Farm, University of Ibadan, located in Oyo State, Southwestern Nigeria. A total of 180-day-old Nicholas turkey poults were purchased from a reputable hatchery in Ibadan. The birds were tagged, individually weighed (50 – 55 g average weight), mass brooded on a deep litter floor and randomly allotted to five replicates. The birds were fed with standard turkey starter, grower, and finisher diets (Table 1) for 8 weeks of each phase.

### Gut Morphological parameters measurement

Prior to slaughter at various ages 56, 112, and 168 days, feed was withdrawn for 24 hours, and two birds were selected per replicate. The birds were slaughtered, and the gastrointestinal tracts were removed for gut morphological parameters. Samples were taken from the junction between Merkel's diverticulum and ileo-caeco-colonic junction and placed into sample bottles containing 10% formalin solution. The samples were transported to the laboratory for further analysis.

The specimens underwent a preservation process, involving exposure to a mixture of acetic acid and ethanol (in a ratio of 25:75, v/v) for 24 hours. Subsequently, rehydration occurred in an ethanol-water bath (50:50, v/v), followed by immersion in distilled water. The Feulgen reaction was then employed for staining: this entailed subjecting the samples to hydrolysis in 1 Normal hydrochloric acid (1N HCL) at a temperature of 60°C for 6 minutes. The samples underwent three rounds of rinsing with distilled water and were subsequently stained with Schiff reagent for 30 minutes. Following staining, additional rinsing with distilled water was carried out, and the samples were conserved in acetic acid-water solution (in a ratio of 45:55, v/v) at a temperature of 4°C until further examination.

For the analysis of intestinal villi alongside their crypts, an individual separation of these structures was performed using a dissecting microscope, employing the technique outlined by Goodlad *et al*. (1991). Measurements of villi length and width were conducted utilising an optical microscope equipped with a camera, following the methodology described by Hampson (1986). The following parameters were assessed and recorded: Epithelial cells, villus height, villus width, crypt depth, crypt width, muscular wall thickness, and goblet cells.

### Net energy and energy utilisation determination

80 male turkeys each were utilised at the starter, grower, and finisher phases respectively to determine net energy and energy utilisation. Each experimental phase lasted for eight weeks, and the following procedures were carried out:

1. Before the metabolic trial, the poults were divided into two groups with similar average body weight (50 – 55 g; starter, 588 – 590 g; grower, 1994 -1998 g; finisher) as follows;
   Group 1 = Initial slaughter group (10 birds)
   Group 2 = Final slaughter group (70 birds).
2. The initial slaughter group was slaughtered on day 1. The birds were chopped, coarsely powdered, and fully combined. Next, a subsample of roughly 200 g each (wet weight) was extracted from each poult, finely processed, and freeze-dried following the procedure of Olukosi *et al*. (2008). Following the drying process, the subsample was ground once more to get it ready for chemical analysis.
3. The final slaughter group of 70 turkeys were distributed randomly among five different replicates in a completely randomised design on day 1. Each replicate comprised 14 turkeys. Data on the birds' body weight and feed consumption were taken weekly.
4. On day 55, the feed was withdrawn for 24 hours.
5. On day 56, two birds per replicate were slaughtered using cervical dislocation, and their entire carcasses were promptly frozen and processed. The two poults from the same cage were processed. The whole poult was chopped, coarsely powdered, and fully combined. Next, two subsamples of roughly 200 g each (wet weight) were extracted, finely processed, and freeze-dried following the procedure of Olukosi *et al*. (2008). Following the drying process, the two subsamples

**Table 1.** Gross composition of standard commercial turkey diets.

| Ingredients composition | Starter (0 – 8 weeks) | Grower (9 – 16 weeks) | Finisher (17 -24 weeks) |
|---|---|---|---|
| Maize | 436.62 | 441.71 | 606.93 |
| Soybean meal | 446.05 | 418.25 | 274.85 |
| Fish meal | 20.00 | 10.00 | 10.00 |
| Soya oil | 39.41 | 70.52 | 65.48 |
| Dicalcium phosphate | 35.42 | 31.56 | 21.77 |
| Premix | 2.50 | 2.50 | 2.50 |
| Limestone | 7.07 | 11.05 | 7.34 |
| Methionine | 3.75 | 3.48 | 2.20 |
| Lysine | 4.18 | 4.11 | 3.01 |
| Salt | 2.23 | 2.61 | 2.95 |
| Sodium Bicarbonate | 1.69 | 1.79 | 1.35 |
| Phytase | 0.10 | 0.10 | 0.10 |
| Toxin Binder | 1.00 | 1.00 | 1.00 |
| Total | 1000 | 1000 | 1000 |
| Calculated values | | | |
| Crude protein (g/kg) | 260.00 | 240.52 | 185.00 |
| Metabolisable energy (kcal/kg) | 2900.00 | 3100.000 | 3250.00 |
| Crude fibre (g/kg) | 37.52 | 35.82 | 30.88 |
| Calcium (g/kg) | 13.30 | 13.30 | 9.30 |
| Phosphorus (g/kg) | 9.89 | 8.88 | |

Provided per kilogram of diet: vitamin A, 12,500 IU; vitamin D3, 3,500 IU; vitamin E (DL-α-tocopheryl acetate), 20 IU; vitamin K3, 3 mg; thiamine hydrochloride, 0.01 mg; riboflavin, 8.00 mg; pyridoxine hydrochloride, 4.5 mg; vitamin B12, 0.02 mg; nicotinic acid, 34 mg; calcium pantothenate 12 mg; folic acid, 0.5 mg; biotin, 0.2 mg; Fe, 80 mg; Cu, 8 mg; Zn, 80 mg; Mn, 80 mg; I, 0.7 mg; Se 0.3 mg.

were mixed and ground once more to get them ready for chemical analysis.

## Chemical analysis and calculations for net energy determination

The ground carcass samples were analysed for Gross Energy (GE), diethyl ether extractable fat (EE) and Nitrogen (N).

Net energy of production (NEP) was calculated as follows:

Net Energy (NE) = Final GE of carcass (KJ) - Initial GE of carcass (KJ)

Initial GE content of carcass (KJ) = Carcass GE (KJ/g) x BW of birds (g)

Final GE content of carcass (KJ) = Carcass GE (KJ/g) x BW of birds (g)

Heat Production (HP), which consists of the heat increment of feeding and fasting heat production was calculated as the difference between NEP and ME intake (MEI):

HP (KJ) = MEI – NEp

Where: ME intake (MEI) was calculated using the following formula:

MEI (KJ) = ME (KJ/g) x FI (g)

Energy retained as fat (REF) and as protein (REP) was calculated as follows:

REF (KJ) = [Final TBFC (g) – Initial TBFC (g)] x 38.2KJ/g

REP (KJ) = [Final TBPC (g) – Initial TBPC (g)] x 23.6KJ/g

Where TBFC = Total body fat content

TBPC = Total body protein content

The values 38.2 and 23.6 kJ $g^{-1}$ are energy values per gram of fat and protein respectively, as derived by Larbier and Leclercq (1992).

## Data collection

Data on the birds' body weight and feed consumption were taken weekly.

**Table 2.** Gut morphological parameters of turkeys at different ages.

| Parameters | 8 weeks | 16 weeks | 24 weeks | SEM | p-value |
|---|---|---|---|---|---|
| Villus height (µm) | 370.67 | $499.16^{b}$ | $969.22^{a}$ | 181.92 | 0.000 |
| Villus width (µm) | $84.42^{b}$ | $105.39^{a}$ | $85.77^{b}$ | 6.78 | 0.026 |
| Crypt depth (µm) | $64.67^{c}$ | $81.25^{b}$ | $124.34^{a}$ | 17.78 | 0.001 |
| Crypt width (µm) | 42.04 | 56.92 | 61.40 | 5.85 | 0.231 |
| Muscular wall thickness (µm) | $174.11^{b}$ | $183.27^{b}$ | $203.27^{a}$ | 8.61 | 0.009 |
| Epithelial (µm) | $13.40^{c}$ | $19.71^{b}$ | $37.25^{a}$ | 7.14 | 0.000 |
| Goblet cells (µm) | $110.82^{a}$ | $80.52^{b}$ | $70.52^{c}$ | 12.12 | 0.000 |
| Villus height: Crypt depth | $4.04^{a}$ | $5.38^{b}$ | $7.64^{a}$ | 1.05 | 0.761 |

$^{a.b.c}$ Means with different superscripts on the same row are significantly different (p<0.05).

**Table 3.** Energy utilisation of turkeys at different ages.

| Parameters | 8 weeks | 16 weeks | 24 weeks | SEM | p-value |
|---|---|---|---|---|---|
| $NE_P$ (KJ) | 6.26 | 11.36 | 12.19 | 2.02 | 0.012 |
| $RE_F$ (KJ) | 54.43 | $162.75^{a}$ | $154.57^{a}$ | 38.33 | 0.008 |
| $RE_P$ (KJ) | $129.64^{c}$ | $480.78^{b}$ | $594.59^{a}$ | 139.93 | 0.041 |
| TRE (KJ) | $173.53^{c}$ | $643.53^{b}$ | $749.17^{a}$ | 176.92 | 0.047 |
| KRE (%) | $27.81^{b}$ | $34.76^{a}$ | $34.43^{a}$ | 2.26 | 0.002 |
| $KRE_F$ (%) | 7.03 | 8.79 | 7.10 | 0.58 | 0.193 |
| $KRE_P$ (%) | $20.78^{b}$ | $25.97^{ab}$ | $27.33^{a}$ | 1.20 | 0.061 |

$^{a.b.c}$ Means with different superscripts on the same row are significantly different (p<0.05). **Key:** $NE_P$ = Net energy of production, KRE = Efficiency of ME used for energy retention, $RE_F$ = Retained energy as fat, $KRE_F$ = Efficiency of ME used for lipid retention, $RE_P$ = Retained energy as protein, $KRE_P$ = Efficiency of ME used for protein retention, TRE = Total Retained energy.

## Statistical analysis

Data obtained were analysed using ANOVA of SAS (SAS, 2012). The significant level of p = 0.05 was used. The treatment was compared using Duncan's Multiple Range Test (Duncan, 1955).

## RESULTS

Results of gut morphological parameters of turkeys at different stages of growth were presented in Table 2. There was a significant increase (p<0.05) in villus height from 8 weeks (370.67 µm) to 24 weeks (969.22 µm), indicating an enhancement in the absorptive surface area of the small intestine. This is likely associated with increased nutrient absorption capacity as turkeys mature. The crypt depth increased (p<0.05) significantly from 8 weeks (64.67 µm) to 24 weeks (124.34 µm). A deeper crypt may be associated with increased cell turnover and nutrient absorption capacity, contributing to the overall gut health status. The crypt width remains relatively stable, indicating that the width of the crypts did not undergo significant changes during the specified age intervals. The muscular wall thickness shows a gradual increase, which may be linked to the maturation and strengthening of the muscular layers of the gut as turkeys age. Also, there were significant increase (p<0.05) in epithelial thickness from 8 weeks (13.40 µm) to 24 weeks (37.25 µm), suggesting an increase in the number of cells comprising the gut lining, potentially contributing to improved barrier function and nutrient absorption. The goblet cell size decreases over the age intervals, indicating potential changes in mucin secretion and mucus composition. The villus height/crypt depth ratio increased (p<0.05) progressively from 8 weeks (4.04 µm) to 24 weeks (7.64 µm), indicating a more pronounced difference between the absorptive surface area (villi) and the area for cell turnover and regeneration (crypts).

Table 3 shows the energy utilisation of turkeys at different ages. Net Energy for Production ($NE_P$) significantly increased (p<0.05) from 8 weeks (6.26 KJ) to 24 weeks (12.19 KJ), indicating a rise in the energy available for productive processes. The retained energy as fat ($RE_F$) also significantly increased from 8 weeks (54.43 KJ) to 16 weeks (162.75 KJ) but remained relatively stable at 24 weeks (154.57 KJ), suggesting that fat deposition increased at week 16 but slightly declined at week 24. $RE_P$ also showed a substantial increase from 8 weeks (129.64 KJ) to 24 weeks (594.59 KJ), highlighting the increasing energy demand for protein deposition as turkeys age increased. Total Retained Energy (TRE) shows a consistent upward trend, reflecting the cumulative energy utilised for both maintenance and reproduction.

The efficiency of ME used for energy retention (KRE) displays a gradual improvement from 8 weeks (27.81%) to 24 weeks (34.43%), indicating enhanced utilisation of feed for overall processes. The efficiency of ME used for lipid retention ($KRE_F$) remains relatively stable, suggesting that the efficiency of feed utilisation for fat deposition remains consistent across the age groups. The efficiency of ME used for protein retention ($KRE_P$) increased significantly as turkey age increased indicating an increased efficiency for protein deposition.

## DISCUSSION

Studies have demonstrated the influence of bird age on nutrient utilisation (Batal and Parsons, 2002; An and Kong, 2022). Upon examining the impact of age on energy utilisation parameters, it was observed that NE increased with the advancement of turkey age, which coincided with the enhanced efficiency of energy utilisation as turkey age increased. This outcome could be attributed to the incomplete development of the turkeys' gastrointestinal tract (GIT) at 8 weeks, as bird age has been proven to significantly affect the digestion and absorption of energy-yielding nutrients (Khalil *et al*., 2022). The investigation conducted by Yaghobfar (2016) on the utilisation of apparent metabolisable energy corrected for zero nitrogen retention (AMEn) and true metabolisable energy corrected for zero nitrogen retention (TMEn) for net energy in broiler diets revealed a decline in protein and energy efficiencies between the starter and finisher phases, contradicting the findings of this study where protein and energy efficiencies increased with turkey age. Comparing the gut parameters to turkey age also gave insights into the results obtained for the energy utilisation parameters. Most of the existing research focused on the development of the gastrointestinal tract (GIT) in poultry has primarily centred around broilers (Iji *et al*., 2001; Barua *et al*., 2021). However, it is important to note that these findings may not be directly applicable to turkeys. Broilers are rapid-growth birds that have been genetically optimised to reach market weight within a short period of 35-56 days, which implies that their GIT development occurs at a faster rate compared to turkeys. The observed improvement in nutrient utilisation with increasing turkey age in this study might be attributed to the corresponding increase in gut parameters. As turkey age increases, gut parameters such as surface area expand, allowing for enhanced nutrient absorption and consequently leading to improved energy utilisation efficiency. The results obtained in this study corroborate previous research indicating that certain feeds may be utilised more effectively by older birds compared to younger ones (Plavnik *et al*., 1997).

The observed age-dependent morphological changes in the gastrointestinal tract underscore the significance of understanding and considering the evolving physiological needs of turkeys for optimising their overall nutritional strategies.

## Conclusion

This comprehensive analysis provides valuable insights into the age-dependent changes occurring in the turkey gut, shedding light on the intricate balance between morphological adaptations and energy utilisation efficiency. These findings contribute essential insights for the poultry industry, emphasising the critical interplay between gut morphology and nutrient utilisation in enhancing the growth, health, and productivity of turkeys across different stages of development.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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