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# Effect of different ratios of male-to-female in broilers on performance and nutrients digestibility

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## Abstract

Male and female broiler chickens differ in performance and this will cause unwanted experimental errors in research. For this reason, single-sex or mixed-sex broilers are used in most studies. This study aimed to assess the performance differences between groups of chickens with varying male/female ratios to determine how sex ratio can affect performance criteria. Birds (N = 550) were separated by sex and placed in 11 groups (pens) according to the male/female ratios, including group 1 (10 males + 0 female), group 2 (9 males + 1 female), group 3 (8 males + 2 females), group 4 (7 males + 3 females), group 5 (6 males + 4 females), group 6 (5 males + 5 females), group 7 (4 males + 6 females), group 8 (3 males + 7 females), group 9 (2 males + 8 females), group 10 (1 male + 9 females), and group 11 (0 male + 10 females). The results showed that male broiler chickens had higher feed intake and body weight gain than female broiler chickens, but the feed conversion ratio was not affected by gender. The digestibility of phosphorus, bone strength, bone density, bone calcium and phosphorus, pH, and redness and water holding capacity of meat were higher in male broilers. The dripping loss was higher in female broilers. This study showed that male and female broiler chickens differed in most of the parameters examined in the research, and the use of separate breeding affected the research results.



## Introduction

In the poultry industry, male chickens have better growth performance than female chickens in a similar genetic background and diet. It has been discovered that sex differences in broiler chickens begin in the early embryonic stages in terms of weight and muscle growth,[1] and differences in growth rate, feed intake (FI), and feed efficiency are also observed between males and females during the breeding period.[2] Gender effects on performance can be attributed to a variety of factors, including chromosomal differences, sex hormones, gut bacteria community, and so on.[3] Male broilers are used in the majority of feeding trials, which can help to minimize fluctuation in results and guarantee a more consistent response.[4] However, the use of only male birds may introduce a potential bias in determining the nutritional requirements of male broilers, as males make up only half of the birds used in the industry. If single-sex birds are to be used, practical methods of sexing are also needed.

Recently, it has become increasingly difficult to obtain single-sex birds for use in experiments, because it is not always possible to determine the sex of feathers under certain conditions. The Ross 308 continues to produce sexable birds in certain regions, but shortly, producing sexable broilers will become more challenging due to the genetic alterations in broiler breeders. Feather sex determination is sometimes substituted with genital sex determination, which is fast and precise. However, it requires trained and experienced personnel, and most hatcheries are reluctant to allow these personnel to routinely segregate small numbers of birds for researchers due to the biosecurity risks. This is because it takes skilled and experienced individuals.[5] Genital sexing also involves handling the birds, and the resulting stress can increase early chick mortality by up to 1.00%.[6] Other techniques for sexing eggs include estrogen radioimmunoassay,[6] genetic engineering breeding,[7] and near-infrared fluorescence and spectroscopy.[7] Due to their technological complexity, these procedures cannot be used on a wide scale, such as in

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nutritional research. Furthermore, genetic modifications have rendered broiler strains that are typically utilized in research non-sexable.[8]

Thus, mixed-sex broilers are required for research purposes. Still, not much study has been done to find out whether the breeding strategy (mixed or single) produces the least amount of variance across repetitions and the best uniformity inside each experimental unit. On the other hand, early research results suggested that single-sex breeding had no optimum or positive impacts on body weight gain (BWG).[9] However, some research findings and reports have suggested that the single-sex approach also yields positive outcomes.[10] Recently, Da Costa *et al*.,[11] have reported that male broilers benefit from mixed-sex rearing in terms of BWG, while female broilers perform better in single-sex rearing. More research should be done to determine how separate and mixed-sex parentings affect feeding study outcomes. Finding out the physiological and nutritional variations between male and female broilers will be helpful in obtaining more data for modern breeds of the bird. So far, no study has investigated the effect of different male/female ratios on the performance of broiler chickens to show how the performance will change with the change of gender composition, and the studies that have been done in this field have only compared male and female sexes. The results of this study can help the researchers to determine which parameters suffer from experimental error if different ratios of males to females are used. Also, the results of this research can be used to estimate the effect of gender on performance by intelligent computing methods in future studies. This study aimed to assess the performance differences between groups of chickens with varying male/female ratios to determine how sex ratio can affect performance criteria. Furthermore, it examined the differences between male and female chickens concerning digestibility, bone structure, and meat quality.

## Materials and Methods

**Birds, treatments, and management.** In this study, 550 one-day-old mixed-sex Ross 308 broiler chickens were obtained from a commercial hatchery. Upon arrival, all birds were weighed, vent sexed (genital sex determination), and allocated to floor pens according to the treatments. The whole experiment lasted for 42 days. All the chicks (275 males and 275 females) with a body weight (BW) mean of 49.66 ± 0.28 g were randomly divided into 11 groups with five replicates *per* group and 10 birds *per* pen. Birds (N = 550) were separated by sex and placed in 11 groups (pens) according to the male/female ratios as follows: Group 1 (10 males + 0 female), group 2 (9 males + 1 female), group 3 (8 males + 2 females), group 4 (7 males + 3 females), group 5 (6 males + 4 females), group 6 (5 males + 5 females), group 7 (4 males + 6 females), group 8 (3 males + 7 females), group 9 (2 males + 8 females), group 10 (1 male + 9 females), and group 11 (0 male + 10 females). All groups were fed the same diets during starter (1 - 10 days), grower (11 - 24 days), and finisher (25 - 42 days) periods (Table 1). The birds had free access to feed and water. The lighting, relative humidity, and temperature were maintained following Ross 308 guidelines.[12] The birds received veterinary care, and vaccination programs were implemented following husbandry practices for broiler chickens. Care and management of chicks were performed according to the commercial guidelines and approved by the Urmia University Animal Ethic Committee, Urmia, Iran (IR-UU-AEC-3/76).

**Performance.** The FI and live BW were recorded at 1, 10, 24, and 42 days of age. Further, BWG and feed conversion ratio (FCR) values were calculated. Mortality was also recorded daily for each group individually.

**Digestibility assay.** To measure the digestibility from day 17 to day 21, 5.00 g $kg^{-1}$ of titanium dioxide was added to the diets as an indigestible indicator to determine the digestibility of nutrients.[13] Excreta samples were collected four times a day on days 18, 19, 20, and 21. The collection pans were lined with new waxed papers to accumulate excreta and immediately stored at – 20.00 ˚C.[14] Duplicate analyses were performed on the diets and excreta samples for all chemical assays. Diets and dried excreta were ground (< 0.75 mm) using a mill grinder (ZM 100; Retsch GmbH, Haan, Germany). Diet and excreta samples were ashed in a muffle furnace at 600˚C for 16 hr for subsequent titanium, calcium, and phosphorus analyses. After wet-ash digestion with sulphuric acid and hydrogen peroxide,[15] titanium concentrations in experimental diets and excreta samples were analyzed by spectrophotometry (Spectronic 21D; Milton Roy, New York, USA) at an absorption of 410 nm, as described by Short *et al*.[16] Ash residues of the diet and excreta samples were digested with nitric and perchloric acids (Merck, Darmstadt, Germany), after which calcium concentration was measured by flame atomic absorption spectroscopy (FAAS) using Spectra AA 220FS (Varian, Belrose, Australia). Similarly, upon digestion with perchloric acid and nitric oxide, acid molybdate (Merck) and Fiske-SubbaRow reducer solution (Merck), were added to form a phospho-molybdenum complex. The color intensity, which is proportional to the phosphorus concentration, was determined by a spectrophotometric reading of absorbance at a wavelength of 630 nm. Apparent nutrient utilization was calculated by the index method, following the equation described by Kong and Adeola:[17]

$$\text{Apparent nutrient utilization (\%)} = [1 - (Ti / To) \times (Xo / Xi)] \times 100$$

where, apparent nutrient utilization is the apparent total tract retention of Ash, calcium, and phosphorus expressed in percentage, *Ti* represents the concentration of titanium

(g $kg^{-1}$ dry matter) in experimental diets, To represents the concentration of titanium (g $kg^{-1}$ dry matter) in excreta output, and Xi and Xo are the concentration of nutrients in experimental diets and excreta output, respectively.

**Bone parameters.** To investigate the effects of gender on weight, length, diameter, and strength of tibia, 20 birds (10 males and 10 females) were euthanized at the age of 42 days. The tibia of the left leg of the chickens was transferred to the freezer after removing all the surrounding tissues. Bone weight was measured with a digital scale (K-E-B-602, Kerona, Shanghai, China), and bone length and diameter were measured with a digital caliper (Bakingwin, Shenzhen, China). Tibia density was determined by a portable X-ray system (Ultra 124 HF-ATX; Veterinary Solutions, Queensland, Australia). The strength of the tibia of the left leg against pressure was measured in newtons *per* meter.[18] Strength test was performed by a Santam device (DBBP-500; Santam Engineering Co., Tehran, Iran) at 60.00 mm *per* min. The ultimate tibia-breaking force was directly obtained from the load-deformation curve recorded by a computerized monitor.[19] After the strength test, the tibia was transferred to an electric furnace and placed at a temperature of 600 ºC for 16 hr.[20] After leaving the furnace and cooling, the remaining ash was weighed, and the ash percentage of each sample was calculated separately. Tibia calcium was determined using FAAS by Spectra AA 50B system (Varian Inc., Palo Alto, USA). Tibia phosphorus was also measured using a spectrophotometer (6300; Jenway, Staffordshire, UK).[20]

**Meat quality.** Sampling was done from breast tissue to check the meat quality at the age of 42 days. To measure water holding capacity (WHC), first, 4.00 g of breast samples were centrifuged at 1,500 rpm for 4 min after being placed in a filter paper. After centrifugation, the samples were placed in an oven at 70.00 ˚C for 24 hr.[21] To measure dripping loss, 5.00 g of breast meat was weighed and placed in a linen cloth. Then, the desired sample was placed in a plastic bag and kept at 4.00 ˚C for 24 hr, after which the meat sample was weighed again.[22] To measure cooking loss, 5.00 g of breast meat with a thickness of 1.00 $cm^3$ was weighed and kept for 24 hr at a temperature of 4.00 ˚C , then, placed in a hot water bath at a temperature of 85.00 ˚C for 10 min, and finally cleaned with a linen

**Table 1.** Components and chemical compositions of the diets used in the starter, growth, and finisher periods of the experiment.

| *Diet ingredients (%)* | Starter (1 - 10 days) | Grower (11 - 24 days) | Finisher (25 - 42 days) |
|---|---|---|---|
| **Corn** | 55.97 | 59.97 | 66.35 |
| **Soybean meal 44.00%** | 37.58 | 34.16 | 28.43 |
| **Soybean oil** | 1.60 | 1.57 | 1.53 |
| **Dicalcium phosphate** | 2.47 | 2.09 | 1.65 |
| **Calcium carbonate** | 0.68 | 0.57 | 0.46 |
| **L-lysine HCl** | 0.31 | 0.29 | 0.27 |
| **DL-methionine** | 0.35 | 0.31 | 0.28 |
| **L-threonine** | 0.14 | 0.13 | 0.11 |
| **Sodium bicarbonate** | 0.10 | 0.12 | 0.15 |
| **Vitamin and mineral-premix*** | 0.50 | 0.50 | 0.50 |
| **Salt** | 0.30 | 0.29 | 0.27 |
| **Sum** | 100 | 100 | 100 |
| ***Nutrients (%)*** | | | |
| **AMEn (Kcal $Kg^{-1}$)** | 2,796 | 2,843 | 2,914 |
| **Crude protein** | 21.62 | 20.37 | 18.33 |
| **Ether extract** | 4.03 | 4.08 | 4.15 |
| **Crude fiber** | 3.29 | 3.21 | 3.10 |
| **Linoleic acid** | 2.20 | 2.27 | 2.35 |
| **Calcium** | 0.89 | 0.77 | 0.61 |
| **Available phosphorus** | 0.47 | 0.40 | 0.33 |
| **Sodium** | 0.17 | 0.17 | 0.17 |
| **Digestible methionine** | 0.63 | 0.58 | 0.53 |
| **Digestible methionine + cysteine** | 0.94 | 0.88 | 0.80 |
| **Digestible lysine** | 1.24 | 1.14 | 1.01 |
| **Digestible threonine** | 0.82 | 0.75 | 0.67 |
| **Digestible arginine** | 1.23 | 1.15 | 1.01 |
| **Digestible valine** | 0.86 | 0.80 | 0.72 |
| **Digestible isoleucine** | 0.82 | 0.75 | 0.67 |
| **DCAB (mEq $kg^{-1}$)** | 231.76 | 217.69 | 199.31 |

AMEn: Apparent metabolizable energy corrected for nitrogen; DCAB: Dietary cation-anion balance.

* Provides the following *per* kg of diet: 4.13 mg retinol, 60.00 µg chole-calciferol, 30.00 mg Dl-α-tocopherol, 3.00 mg menadione, 2.20 mg thiamine, 8.00 mg riboflavin, 5.00 mg pyridoxine, 11.00 µg cyanocobalamin, 1.50 mg folic acid, 150µg biotin, 25.00 mg calcium pantotenate, 65.00 mg nicotinic acid, 60.00 mg manganese sulfate, 40.00 mg zinc oxide, 0.33 mg potassium iodate, 80.00 mg ferrous sulfate, 8.00 mg copper sulfate, 0.15 mg sodium selenite, and 150 mg ethoxyquin.

cloth and reweighed.[22] The pH of the meat was measured by a digital pH meter (D55122; ProLab, Mainz, Germany) after homogenizing the meat in distilled water.[23] Meat color is determined based on the International Commission on Illumination system with three parameters, including lightness, redness, and yellowness, which was measured by a chronometer (CR-400; Minolta, Osaka, Japan). This device considers the lightness of poultry meat color as light, pale, normal, and dark.[24]

**Statistical analysis.** The obtained data were tested for normality using the PROC UNIVARIATE of SAS Software (version 9.2; SAS Institute, Cary, USA), and whenever needed, the percentage data were normalized by ArcSin√x transformation. The data were analyzed using the PROC GLM of SAS. The difference among means was determined using Duncan's test, and $p$ values < 0.05 were considered statistically significant. The statistical model of the current study was as follows:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

where, $Y_{ij}$, $\mu$, $T_i$, and $\varepsilon_{ij}$ represent the observation, mean of observations, treatment effect, and experimental error of each observation, respectively.

## Results

**Broiler performance.** The results related to the effect of different ratios of male and female broiler chickens on performance are shown in Table 2. The FI was affected by the sex of the birds, and single-sex male groups or those with 1, 2, and 3 female chicks had more FI than the single-sex female group (group 11; $p$ < 0.05). The BWG in the total period was affected by the sex of broilers. The average BWG of birds in the group with 9 males (group 2) was significantly higher than the groups with 0, 1, 2, 3, and 4 males during the total period ($p$ < 0.05). The FCR was not affected by the different ratios of male and female broiler chickens in the experimental groups ($p$ > 0.05).

**Nutrient digestibility.** Table 3 shows the results of the effect of different ratios of male-to-female in broilers on ash, phosphorus, and calcium digestibility. The digestibility of phosphorus was affected by the gender of broiler chickens, and in groups group 1, group 3, and group 4 (more male broilers) was higher than group 5, group 6, group 8, group 10, and group 11 (more female broilers). The digestibility of ash and calcium was not significantly affected by different ratios of male-to-female in broilers.

**Bone characteristics.** The effect of broiler gender on tibia bone indices is reported in Table 4. Bone weight, strength, density, length, ash concentration, calcium, and phosphorus were influenced by gender. The weight, strength, density, length, ash, calcium, and phosphorus were significantly greater in male birds than females ($p$ < 0.05). As shown in Figure 1, the density and length of the tibia bone in male broiler chickens were higher than those of female broiler chickens.

**Meat quality.** The effect of gender on the meat quality of broilers is indicated in Table 5. The results showed that the pH, WHC, dripping loss, and color were affected by gender. Male broilers had higher pH, WHC, and redness than female birds ($p$ < 0.05). But the dripping loss was higher in female broilers. Cooking loss, lightness, and yellowness were not affected by gender ($p$ > 0.05).

**Table 2.** The effect of different gender ratios of broiler chickens on performance parameters (1 - 42 days).

| Groups | FI (g *per* day) | BWG (g *per* day) | FCR |
|---|---|---|---|
| **$G_1$** | 101.20$^{a}$ | 58.81$^{ab}$ | 1.72 |
| **$G_2$** | 101.59$^{a}$ | 61.20$^{a}$ | 1.66 |
| **$G_3$** | 100.47$^{a}$ | 59.89$^{ab}$ | 1.68 |
| **$G_4$** | 99.09$^{a}$ | 60.42$^{ab}$ | 1.64 |
| **$G_5$** | 98.47$^{ab}$ | 57.85$^{ab}$ | 1.70 |
| **$G_6$** | 99.79$^{ab}$ | 58.02$^{ab}$ | 1.72 |
| **$G_7$** | 97.99$^{ab}$ | 56.19$^{bc}$ | 1.74 |
| **$G_8$** | 97.81$^{ab}$ | 56.28$^{bc}$ | 1.73 |
| **$G_9$** | 97.73$^{ab}$ | 57.30$^{bc}$ | 1.70 |
| **$G_{10}$** | 97.33$^{ab}$ | 55.62$^{c}$ | 1.75 |
| **$G_{11}$** | 95.10$^{b}$ | 56.02$^{bc}$ | 1.69 |
| **SEM** | 1.17 | 0.95 | 0.03 |
| ***p*-value** | 0.019 | 0.006 | 0.44 |

FI: Feed intake; BWG: Body weight gain; FCR: Feed conversion ratio; SEM: Standard error of means; $G_1$: 10 males + 0 females; $G_2$: 9 males + 1 females; $G_3$: 8 males + 2 females; $G_4$: 7 males + 3 females; $G_5$: 6 males + 4 females; $G_6$: 5 males + 5 females; $G_7$: 4 males + 6 females; $G_8$: 3 males + 7 females; $G_9$: 2 males + 8 females; $G_{10}$: 1 males + 9 females; $G_{11}$: 0 males + 10 females.
$^{abc}$ Means in the same column with different superscripts differ significantly ($p$ < 0.05).

**Table 3.** The effect of different gender ratios of broiler chickens on nutrient digestibility at the age of 17 - 21 days.

| Groups | Ash (%) | Calcium (%) | Phosphorus (%) |
|---|---|---|---|
| **$G_1$** | 58.92 | 44.98 | 44.06$^{a}$ |
| **$G_2$** | 60.50 | 44.24 | 40.11$^{ab}$ |
| **$G_3$** | 59.39 | 43.26 | 43.56$^{a}$ |
| **$G_4$** | 56.66 | 44.84 | 43.19$^{a}$ |
| **$G_5$** | 57.68 | 43.99 | 37.75$^{b}$ |
| **$G_6$** | 59.29 | 44.04 | 37.66$^{b}$ |
| **$G_7$** | 59.13 | 44.38 | 40.06$^{ab}$ |
| **$G_8$** | 56.90 | 44.22 | 37.85$^{b}$ |
| **$G_9$** | 57.49 | 44.02 | 40.10$^{ab}$ |
| **$G_{10}$** | 57.80 | 44.19 | 35.81$^{bc}$ |
| **$G_{11}$** | 55.78 | 42.98 | 33.29$^{c}$ |
| **SEM** | 4.86 | 1.22 | 1.13 |
| ***p*-value** | 0.14 | 0.33 | 0.001 |

SEM: Standard error of means; $G_1$: 10 males + 0 females; $G_2$: 9 males + 1 females; $G_3$: 8 males + 2 females; $G_4$: 7 males + 3 females; $G_5$: 6 males + 4 females; $G_6$: 5 males + 5 females; $G_7$: 4 males + 6 females; $G_8$: 3 males + 7 females; $G_9$: 2 males + 8 females; $G_{10}$: 1 males + 9 females; $G_{11}$: 0 males + 10 females.
$^{abc}$ Means in the same column with different superscripts differ significantly ($p$ < 0.05).

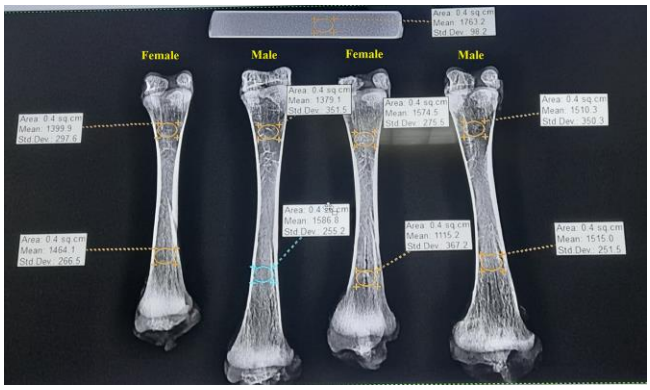


**Fig. 1.** Bone density in male and female broilers.

**Table 4.** The effect of gender on tibia bone characteristics of broiler chickens at the age of 42 days.

| Parameters | Male | Female | SEM | *p*-value |
|---|---|---|---|---|
| **Weight (g)** | 23.69[a] | 19.09[b] | 0.31 | 0.02 |
| **Strength (N)** | 296.80[a] | 240.04[b] | 20.28 | 0.01 |
| **Density (g *per* cm²)** | 1.84[a] | 1.46[b] | 0.03 | 0.001 |
| **Length (mm)** | 118.40[a] | 103.20[b] | 0.15 | 0.04 |
| **Minimum diameter (mm)** | 9.20 | 8.03 | 0.48 | 0.07 |
| **Ash (%)** | 38.40[a] | 35.47[b] | 1.20 | 0.001 |
| **Calcium (%)** | 12.48[a] | 10.13[b] | 0.26 | 0.01 |
| **Phosphorus (%)** | 4.24[a] | 3.47[b] | 0.09 | 0.03 |

SEM: Standard error of means.
[ab] Means in the same row with different superscripts differ significantly ($p < 0.05$).

**Table 5.** The effect of gender on meat quality of broiler chickens at the age of 42 days.

| Parameters | Male | Female | SEM | *p*-value |
|---|---|---|---|---|
| **pH** | 6.23[a] | 5.81[b] | 0.11 | 0.04 |
| **Water holding capacity (%)** | 80.62[a] | 72.94[b] | 2.08 | 0.01 |
| **Cooking loss (%)** | 27.34 | 28.66 | 1.17 | 0.45 |
| **Dripping loss (%)** | 10.78[b] | 14.50[a] | 0.84 | 0.007 |
| **Color parameters** | | | | |
| **Lightness value** | 40.37 | 42.71 | 1.64 | 0.39 |
| **Redness value** | 5.34[a] | 4.79[b] | 0.13 | 0.02 |
| **Yellowness value** | 4.81 | 4.95 | 0.15 | 0.27 |

SEM: Standard error of means.
[ab] Means in the same row with different superscripts differ significantly ($p < 0.05$).

## Discussion

Many factors can influence broiler chicken growth performance, including breed, age, sex, and environmental factors, such as diet, stocking density, and broiler chicken housing, as well as the combination of these factors.[25] To conduct robust research, any undesirable variance must be eliminated or accounted. Differences in growth performance between male and female broilers are often not considered when mixed-sex broilers are used in experiments. Much research has been published that clearly reveals that male and female broilers differ in terms of BWG, FI, and FCR. The current study found that male broiler chickens had a higher FI and BWG than single-sex female chickens. López *et al*.,[25] have investigated the effect of sex on broiler performance and discovered that male broilers weigh more than females at 42 days of age. The heavier weight of male broilers at 42 days of age has been reported by some other researchers.[11,26] Furthermore, female broilers have been shown to have lower FI than male broilers.[27] In agreement with the findings of the current study, Madilindi *et al*.[26] have reported that the gender of broiler chickens does not affect the FCR at all stages of rearing, and male and female broiler chickens have the same efficiency at all ages. These findings contradict prior research, found that males used food more efficiently than females.[27] This could be attributed to environmental or genetic differences among the birds utilized in the tests. These inconsistent findings may explain why FCR is not an appropriate measure for comparison. If both sexes have a similar FCR, it does not necessarily imply that they are using feed of the same efficiency. Correcting FCR for BW and comparing using this figure will be more accurate. In truth, FCR uncorrected for BW is used as a reference parameter and is not an exact measurement; yet, producers utilize FCR as a calculation to calculate the economic impact of poultry farming. Madilindi *et al*.,[26] have ascribed male birds' larger weights to physiological differences between sexes in BWG and FI. Also, a positive genetic correlation between these traits is known.[28] However, Zerehdaran *et al*.,[29] have argued that the difference between males and females in one feature cannot be explained by a single cause. Greater competition for food, aggressive male behavior, social dominance, variances in nutritional needs, hormonal effects on development, and obesity are all contributing factors. A recent study by Da Costa *et al*.,[11] showed that single-sex female birds weighed more than mixed-sex females. Da Costa *et al*.,[11] concluded that rearing females in a mixed-sex environment negatively affected their performance, while male broilers benefited from mixed-sex rearing. These differences may be related to the feeder space and competition. When female broilers are reared mixed, competition for feeding space with males may lead to lower FI and lighter weight in females. Conversely, males reared in mixed-sex conditions can easily drive smaller birds, such as females, away from feeders, leading to increased FI and faster growth in males. However, in single-sex male conditions, the competition for feeding space among males will be higher and will result in a slower growth rate compared to the male birds raised in mixed-sex conditions. This issue was also observed in the present study and BWG of the whole period was higher in group 2, group 3, and group 4 than group 1. In contrast, England *et al*.,[30] have reported that as birds become heavier, they tend to move less around the feeder. This means that even in mixed-sex groups, there is plenty of time for all birds to access the feeder, and the males will not dominate the females.

In the present study, the digestibility of crude ash and calcium was not affected by gender. However, in male birds, the digestibility of phosphorus was higher than that of females. The results of Singh *et al*.,[31] showed that male broiler chickens had a higher digestibility coefficient for crude ash and calcium compared to the female broiler chickens. In the study of Muñoz *et al*.,[32] gender did not have a significant effect on the digestibility of diet. Moreover, Ten Doeschate *et al*.,[33] have reported that female broiler chickens have a higher phosphorus digestibility coefficient than males. These results are inconsistent, which may be due to the effect of FI on nutrient digestibility. The higher digestibility of phosphorus in male broilers may be related to the gut microbiota. Bacteria found at high levels in male broilers have been reported to have the ability to break down indigestible fiber in the digestive tract.[34] Therefore, probably the improvement in fiber digestibility has reduced the binding of phosphorus phytate with soluble fibers, ultimately resulting in higher phosphorus digestibility of male broiler chickens. These results are in line with the results of Ziaei *et al*.,[35] reported higher apparent digestibility of phosphorus in male chickens compared to the female broiler chickens.

In this study, tibia bone was used to investigate the effect of gender on bone parameters. Other researchers who examined this bone showed higher values of mechanical and geometric parameters and mineralization than pelvic bones.[36] Accordingly, it has been proven that the tibia bone has more mechanical resistance and is more suitable for research than the femur bone.[37] The present study showed that the tibia bone in male broilers had more weight, length, strength, ash, calcium, and phosphorus than females. These results agree with most of the studies.[38,39] Greater weight, size, composition, and strength of tibia bone in male broiler chickens indicate more mineral retention in the bone, which may explain the higher digestibility of phosphorus in male broiler chickens compared to the females.[35] Rath *et al*.,[40] showed that testosterone implants led to increased bone strength in male broilers. Therefore, the observed changes in bone quality may be due in part to hormonal differences. On the other hand, a healthy and high-quality skeleton may improve locomotion and thus, increase water and FI, which may contribute to better performance.[41] Therefore, another reason for higher BWG and FI in male broilers can be related to the bone quality.

It has been found that pH is an important indicator for meat quality, because the decrease in pH after slaughter may be due to the denaturation of proteins,ultimately leading to a decrease in WHC and a lighter color of the meat. Reducing the tissue's ability to maintain and store water causes the nutritional value of meat to be lost, which depends on the degree of tissue denaturation.[42] In agreement with our results, it has been reported that female broilers have lower meat pH than males.[43] The lower pH observed in female broilers may result in higher droplet losses in females than males. Sex-related differences in pH and subsequent effects on drop loss and meat color may be the result of differences in muscle glycogen content or different regulatory patterns of post-mortem metabolism (*e.g.*, phospholipase $A_2$ activity).[44] The amount of breast WHC in male broilers was significantly higher than that of female broilers. Similarly, breast WHC was significantly affected by gender.[43] Also, differences in breast meat WHC values have been attributed to gender differences in meat water retention, which can be explained by variations in composition and quality.[45] Color is considered as an important quality indicator and can be influenced by various factors, including gender.[46] Redness of breast meat was significantly higher in male broilers than females. In this regard, researchers have reported that male meat has higher redness values than female meat.[47] Fletcher has reported that meat color is the first characteristic of meat quality seen by consumers and is the determining factor in whether buy or not to buy a particular product.[42] A higher redness score of the breast is attributed to higher myoglobin, heme pigment, cytochrome C, and degree of vascularization.[48]

This study highlighted that groups of chickens treated with the same nutrition and conditions produced variations in performance, nutrient digestibility, bone quality, and meat quality due to the differing male/female ratios. When conducting practical single-sex or mixed-sex experiments, it is crucial to be ensured about an accurately known sex ratio (*e.g.*, exact 100:0, 50:50, or any other known male/female ratios) to avoid bias in data and results. Our findings suggest that adjustments are necessary when generalizing results from single-sex or mixed-sex experiments to practical applications, ensuring accurate and reliable outcomes. This study could be the first step in making these necessary adjustments.

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**Conflict of interest**

There is not conflict of interest with any person or institute/organization regarding this manuscript.

**References**

1. Henry MH, Burke WH. Sexual dimorphism in broiler chick embryos and embryonic muscle development in late incubation. Poult Sci 1998; 77(5): 728-736.

2. May JD, Lott BD. Relating weight gain and feed: gain of male and female broilers to rearing temperature. Poult Sci 2001; 80(5): 581-584.
3. England A, Gharib-Naseri K, Kheravii SK, et al. Influence of sex and rearing method on performance and flock uniformity in broilers-implications for research settings. Anim Nutr 2023; 12: 276-283.
4. Gous RM, Moran ET Jr, Stilborn HR, et al. Evaluation of the parameters needed to describe the overall growth, the chemical growth, and the growth of feathers and breast muscles of broilers. Poult Sci 1999; 78(6): 812-821.
5. Otsuka M, Miyashita O, Shibata M, et al. A novel method for sexing day-old chicks using endoscope system. Poult Sci 2016; 95(11): 2685-2689.
6. Phelps P, Bhutada A, Bryan S, et al. Automated identification of male layer chicks prior to hatch. Worlds Poult Sci J 2003; 59(1): 33-38.
7. Doran TJ, Morris KR, Wise TG, et al. Sex selection in layer chickens. Anim Prod Sci 2017; 58(3): 476-480.
8. England AD, Kheravii SK, Musigwa S, et al. Sexing chickens (*Gallus gallus domesticus*) with high-resolution melting analysis using feather crude DNA. Poult Sci 2021; 100(3):100924. doi: 10.1016/j.psj.2020.12.022.
9. Lamoreux WF, Proudfoot FG. Effects on body weight and feed conversion of broiler chickens from three commercial crosses reared with the sexes separated or intermingled. Can J Anim Sci 1969; 49(1): 23-28.
10. Albuquerque R, Marchetti LK, Fagundes AC, et al. Effect of different population densities and sex on performance and uniformity in broilers (2006) [Portuguese]. Braz J Vet Res Anim Sci 2006; 43(5): 581-587.
11. Da Costa MJ, Colson G, Frost TJ, et al. Straight-run *vs.* sex separate rearing for two broiler genetic lines Part 2: economic analysis and processing advantages. Poult Sci 2017; 96(7): 2127-2136.
12. Aviagen. Ross 308 parent stock: nutrition specifications. Available at: https://aviagen.com/ assets/Tech_Center /Ross_PS/Ross308-EuropeanParentStock-Nutrition Specifications-2021-EN.pdf. Accessed: 18 September, 2025.
13. Osunbami OT, Walk CL, Adeola O. Digestible calcium equivalency of phytase and nutrient utilization of broiler chickens fed graded levels of limestone or phytase during the starter phase. Poult Sci 2024; 103(2): 103360. doi: 10.1016/j.psj.2023.103360.
14. Haetinger VS, Adeola O. Comparison of different protein sources on the phosphorus digestibility of soybean meal for broiler chickens determined using the regression method. Poult Sci 2024; 103(2): 103327. doi: 10.1016/j.psj.2023.103327.
15. Fenton TW, Fenton M. An improved procedure for the determination of chromic oxide in feed and feces. Can J Anim Sci 1979; 59(3): 631-634.
16. Short FJ, Gorton P, Wiseman J, et al. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim Feed Sci Technol 1996; 59(4): 215-221.
17. Kong C, Adeola O. Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. Asian-Australas J Anim Sci 2014; 27(7): 917-925.
18. Pedersen IJ, Tahamtani FM, Forkman B, et al. Effects of environmental enrichment on health and bone characteristics of fast growing broiler chickens. Poult Sci 2020; 99(4): 1946-1955.
19. Liu SB, Liao XD, Lu L, et al. Dietary non-phytate phosphorus requirement of broilers fed a conventional corn-soybean meal diet from 1 to 21 d of age. Poult Sci 2017; 96(1): 151-159.
20. AOAC. Official methods of analysis. 18th ed. Washington DC, USA: Association of Official Analytical Chemists, 2006.
21. Dai SF, Gao F, Xu XL, et al. Effects of dietary glutamine and gamma-aminobutyric acid on meat colour, pH, composition, and water-holding characteristic in broilers under cyclic heat stress. Br Poult Sci 2012; 53(4): 471-481.
22. Christensen LB. Drip loss sampling in porcine m. longissimus dorsi. Meat Sci 2003; 63(4): 469-477.
23. Aziz Mesgari Z, Daneshyar M, Aghazadeh A. Effects of betaine on performance, some blood indices, antioxidant status and internal organ weights of broiler chickens under heat stress [Persian]. J Vet Res 2017; 72(3): 297-304.
24. Qiao M, Fletcher DL, Northcutt JK, et al. The relationship between raw broiler breast meat color and composition. Poult Sci 2002; 81(3): 422-427.
25. López KP, Schilling MW, Corzo A. Broiler genetic strain and sex effects on meat characteristics. Poult Sci 2011; 90(5): 1105-1111.
26. Madilindi MA, Mokobane A, Letwaba PB, et al. Effects of sex and stocking density on the performance of broiler chickens in a sub-tropical environment. S Afr J Anim Sci 2018; 48(3): 459-468.
27. Goo D, Kim JH, Choi HS, et al. Effect of stocking density and sex on growth performance, meat quality, and intestinal barrier function in broiler chickens. Poult Sci 2019; 98(3): 1153-1160.
28. Aggrey SE, Karnuah AB, Sebastian B, et al. Genetic properties of feed efficiency parameters in meat-type chickens. Genet Sel Evol 2010; 42(1): 25. doi: 10.1186/1297-9686-42-25.
29. Zerehdaran S, Vereijken AL, van Arendonk JA, et al. Effect of age and housing system on genetic parameters for broiler carcass traits. Poult Sci 2005; 84(6): 833-838.
30. England AD, Gharib-Naseri K, Kheravii SK, et al. Rearing broilers as mixed or single-sex: relevance to

performance, coefficient of variation, and flock uniformity. Poult Sci 2022; 101(12): 102176. doi: 10.1016/j.psj.2022.102176.
31. Singh V, Singh U, Sethi AP, et al. Gender effect on growth performance and nutrient digestibility in (IBL-80) broilers. Haryana Vet 2020; 59(1): 6-9.
32. Muñoz JA, Suckeveris D, Demuner LF, et al. Effects of nutritional levels on performance, carcass characteristics and nutrient digestibility of sexed broilers. Rev Bras Cienc Avic 2018; 20(1): 53-62.
33. Ten Doeschate RA, Scheele CW, Schreurs VV, et al. Digestibility studies in broiler chickens: influence of genotype, age, sex and method of determination. Br Poult Sci 1993; 34(1): 131-146.
34. Lee KC, Kil DY, Sul WJ. Cecal microbiome divergence of broiler chickens by sex and body weight. J Microbiol 2017; 55(12): 939-945.
35. Ziaei N, Guy JH, Edwards SA, et al. Effect of gender on factors affecting excreta dry matter content of broiler chickens. J Appl Poult Res 2007; 16(2): 226-233.
36. Tatara MR, Sierant-Rozmiej N, Krupski W, et al. Quantitative computed tomography for the assessment of mineralization of the femur and tibia in turkeys. Med Weter 2005; 61(2): 225-228.
37. Charuta A. Evaluation of densitometric and geometric parameters of the femur in 14-month-old ostriches depending on sex with the use of computed tomography. J Vet Res 2013; 57(2): 287-291.
38. Alkhtib A, Sanni CO, Burton E, et al. What is "normal"? Morphology and mineralization of tibias from healthy, on farm broilers. J Appl Poult Res 2021; 30(3): 100190. doi: 10.1016/j.japr.2021.100190.
39. Müsse J, Louton H, Spindler B, et al. Sexual dimorphism in bone quality and performance of conventional broilers at different growth phases. Agriculture 2022; 12(8): 1109. doi: 10.3390/agriculture12081109.
40. Rath NC, Huff WE, Balog JM, et al. Effect of gonadal steroids on bone and other physiological parameters of male broiler chickens. Poult Sci 1996; 75(4): 556-562.
41. Torres TR, Ludke M do CM M, Ludke JV, et al. Performance of broilers fed during 21 days on mash or pellet diets containing whole or ground pearl millet grain. Rev Bras Cienc Avic 2013; 15(4): 371-378.
42. Fletcher DL. Poultry meat quality. Worlds Poult Sci J 2002; 58(2): 131-145.
43. Hailemariam A, Esatu W, Abegaz S, et al. Effect of genotype and sex on breast meat quality characteristics of different chickens. J Agric Food Res 2022; 10: 100423. doi: 10.1016/j.jafr.2022.100423.
44. Schneider BL, Renema RA, Betti M, et al. Effect of holding temperature, shackling, sex, and age on broiler breast meat quality. Poult Sci 2012; 91(2): 468-477.
45. Barbut S, Leishman EM. Quality and processability of modern poultry meat. Animals (Basel) 2022; 12(20): 2766. doi: 10.3390/ani12202766.
46. Jaturasitha S, Srikanchai T, Kreuzer M, et al. Differences in carcass and meat characteristics between chicken indigenous to northern Thailand (Black-boned and Thai native) and imported extensive breeds (Bresse and Rhode Island Red). Poult Sci 2008; 87(1): 160-169.
47. Gálvez F, Domínguez R, Pateiro M, et al. Effect of gender on breast and thigh turkey meat quality. Br Poult Sci 2018; 59(4): 408-415.
48. Özbek M, Petek M, Ardıçlı S. Physical quality characteristics of breast and leg meat of slow-and fast-growing broilers raised in different housing systems. Arch Anim Breed 2020; 63(2): 337-344.