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To cite this article: Ivan Muhumuza , Isaac Kasoro , Brian Asimwe & Donald Rugira Kugonza (2026) Fertility duration in indigenous hens following controlled mating, and growth performance of their offspring, Journal of Applied Animal Research, 54:1, 2649467, DOI: [10.1080/09712119.2026.2649467](https://doi.org/10.1080/09712119.2026.2649467)

To link to this article: <https://doi.org/10.1080/09712119.2026.2649467>



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Published online: 26 Mar 2026.



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# Fertility duration in indigenous hens following controlled mating, and growth performance of their offspring

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## ABSTRACT

In Uganda, indigenous chickens constitute 70% of the poultry population, but their reproductive efficiency is affected by insufficient fertility data. This study evaluated the fertility duration in indigenous hens after mating and compared the growth performance of their offspring with that of Broiler and Kuroiler chickens. Thirty-six indigenous hens from three districts were assigned to four pens of eight hens each. Twelve cocks were introduced in batches for a 2-week mating period following a 3-week hen isolation phase. Eggs were collected weekly over 15 weeks, incubated, and candled on day 13 to assess fertility. Chicks hatched from these eggs were monitored for growth and compared with Broiler and Kuroiler chicks for 14 weeks. The average egg fertility post-mating was 75%, with fertile eggs laid approximately 87.3 h after mating, while fertility declined to 49.6%, 3 weeks post-cock removal, with effective fertility lasting approximately 7.12 days. Live body weight was highest in broilers and lowest in indigenous chickens at all ages until the broilers left the study at five weeks. Among indigenous and Kuroiler chickens, cocks were significantly heavier ( $P < 0.05$ ) than hens at all ages. To enhance fertility and optimize egg production in smallholder systems, weekly mating of indigenous hens is recommended.

## ARTICLE HISTORY

Received 1 December 2025  
Accepted 18 March 2026


## KEYWORDS

Indigenous hens; fertility duration; offspring; growth; breeding

## 1. Introduction

The poultry industry is a vital pillar of food security, with chicken meat and eggs dominating African markets. In Uganda, indigenous chickens (IC) account for approximately 69.9% of the national flock (Ministry of Agriculture and Animal Industry for Fishing Uganda Burden Statistics Kampala UBOS 2021), but supply a small proportion of the eggs on the market (Kyarisiima et al. 2004; Beyihayo et al. 2022). These birds are economically and culturally important, especially for rural households (Nampijja et al., 2025), with the current IC stock contributing an estimated 9.8 billion Ugandan Shillings to the economy. Despite their resilience, indigenous chickens have low productivity and reproductive performance, largely due to poor breeding strategies (Assan 2015) and poor management (Mujiyambere et al. 2021; Desta and Wakeyo 2024; Ochieng et al. 2013; Ngogo et al. 2023). To improve the IC, egg fertilization is indispensable for the production of next-generation offspring (Ichikawa et al. 2016). Fertility is a major indicator of poultry reproductive performance (Wen et al. 2022), as it affects hatchability (Hristakieva et al. 2022) and the supply of day-old chicks (Ogbu and Oguike 2019). To achieve fertilization, sperm must encounter the oocytes at the right time and at the right place (Sasanami et al. 2013; Sasanami et al. 2015). Since chickens lack an oestrus cycle for the synchronization of copulation and ovulation (Kheawkanha et al. 2024), they rely on the Sperm Storage Tubules (SSTs), which allow hens to store sperm for up to 2–15 weeks after mating (Sasanami et al. 2013; Sasanami et al. 2015; Khillare et al. 2018).

Ordinarily, rural farmers unintentionally utilize the knowledge of this reproductive biology through temporarily borrowing cocks of desired physical characteristics (Kayitesi et al. 2014), such as (i) naked-neck, (ii) feathered shanks, (iii) frizzled feathers, and production traits such as (iv) chicken size and (v) the number

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of eggs. However, since egg production is known to occur hours or days after mating, fertility declines over time following cock removal, likely due to the depletion of stored viable sperm cells (Bui et al. 2018). Consequently, farmers must periodically reintroduce a cock to maintain egg fertility in their flock.

To date, the relationship between time and fertile egg production in Ugandan indigenous chicken ecotypes remains unclear. This research gap leads to the collection of infertile eggs for incubation, resulting in poor hatchability and wasted resources. Understanding this temporal relationship is key in planning hand mating breeding programs, as it determines the optimum time for cock introduction and removal without affecting egg fertility. Additionally, knowledge of the onset and fertility period of IC under natural mating would provide guidance for improving their reproduction. Therefore, this study aimed to determine the onset of fertility and the fertility period of laying indigenous hens after cock removal, and comparatively evaluate the growth performance of indigenous chickens and two exotic chicken strains reared under a deep litter intensive system to help farmers make informed breeding decisions.

## 2. Materials and methods

### 2.1. Study location

The study was conducted at the poultry research facility of the Makerere University Agricultural Research Institute Kabanyoro (MUARIK) in Wakiso district, Central Uganda. The research station is located 14 km north of Kampala Capital City at 0°27'60"N, 32°36'24"E, at an altitudinal range of 1250–1320 m above sea level. The area lies within the Lake Victoria basin that receives a bimodal rainfall pattern with an annual average precipitation of approximately 1150 mm. This rainfall is distributed between two wet seasons, one running from March to June, and the other from August to November, with a mean annual temperature ranging from 18–26 °C (Sserumaga et al. 2015).

### 2.2. Birds and their husbandry

**Fertility trial:** Fertile eggs were initially collected from three districts of Uganda (Kabale, Kabarole and Kiryandongo), hatched at a commercial hatchery, brooded for one month and then reared for 13 weeks. The first selection of breeding hens and cocks was performed at 13 weeks of age, with birds that were below 1 kg in weight being dropped. The second selection was for females and was performed after 18 weeks by considering clutch size. The selected birds became the parent stock, and their offspring, which went through the same selection process, produced the second generation.

In this study, 12 indigenous cocks and 36 indigenous hens were selected and reared for four months for use in breeding. These breeding cocks and hens were obtained at 40 weeks of age and kept under a deep litter system. All the male birds used were vigorous and healthy, and all the hens had already started laying eggs before the start of the experiment. The hens were housed in identical floor pens within a room measuring 8 × 10 m and 3 m high at the front and sloping to 2.5 m at the back. The front wall was constructed to a height of 1 m in solid bricks and mortar, with the remaining 2 m covered by wire mesh to provide ventilation. Ten pens were used in total: four for hens, three for cocks and three for chicks. Each pen measured 2 × 2.5 m and had 2 m-high walls. For the cock pens, the lower half of each wall was solid, while the pens for hens had full solid sides to prevent visual interaction between birds in adjacent pens. Each pen allocated to hens was fitted with a laying box containing at least ten covered nests designed to trap the hen after egg laying, allowing accurate identification of each egg in terms of the parent hen and cock. Feeders, drinkers and perches were provided in all pens. The floors were covered with a 5 cm layer of wood shavings as litter. All birds were fed a restricted breeder ratio of approximately 120 g per bird per day, following standard breeder management recommendations, and clean water was provided.

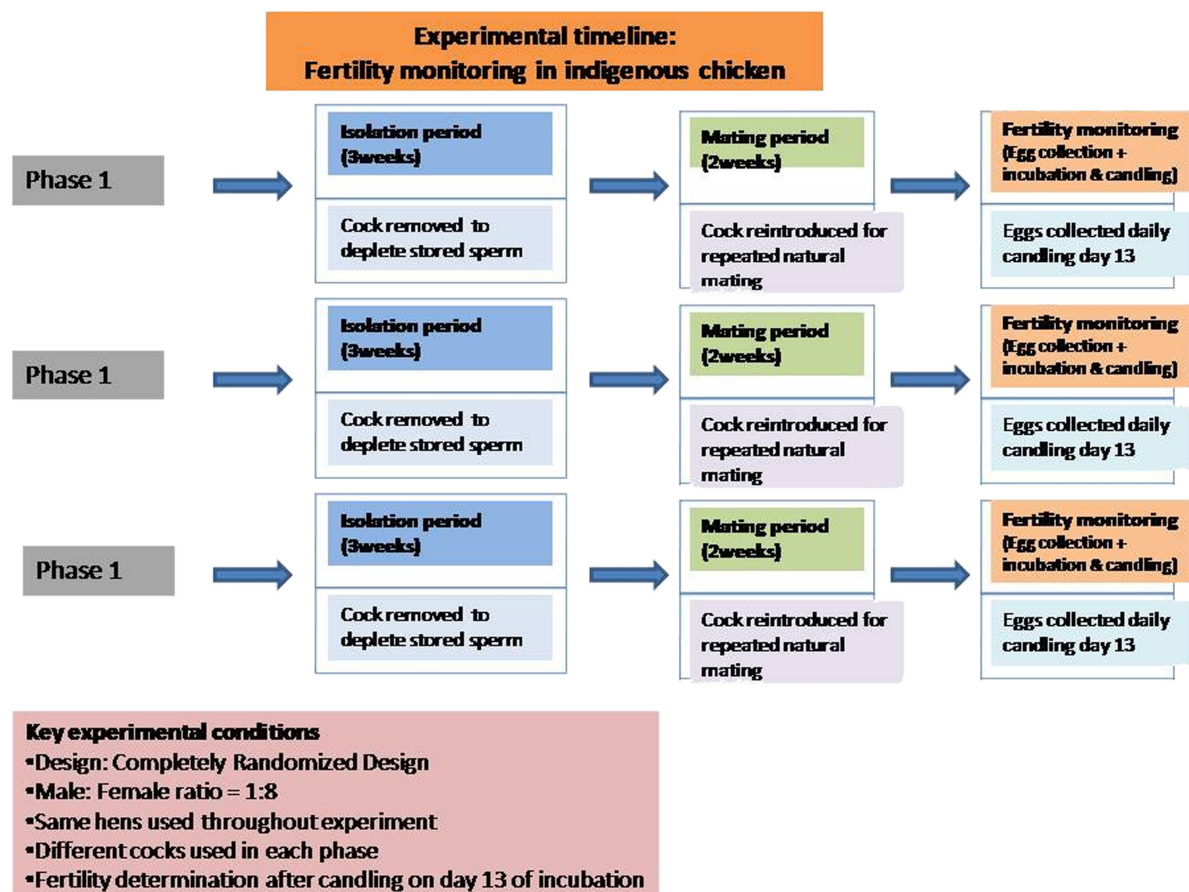
**Growth performance trial:** A sample of 50 IC chicks used for this study was randomly obtained from a batch of 135 chicks hatched from the pure IC flock. A batch of 50-day-old Kuroiler chicks was purchased from a local commercial hatchery, and a sample of 50 broiler chicks was randomly selected from a batch of 200 chicks from the Department of Animal Science facility. All chicks were marked with

leg bands and wing tags, brooded artificially using a charcoal stove, provided with commercial chick pellets until 2nd week of age, and thereafter subjected to a well-mixed ration. Drinking water was supplied to all experimental birds *ad libitum*. Vaccinations against Marek's disease, Newcastle disease, Gumboro, infectious bronchitis, Fowl pox and Fowl typhoid were conducted following veterinary vaccination schedules.

### 2.3. Experimental design and data collection

**Fertility of indigenous chickens:** A completely randomized design was used in this experiment, consisting of three sequential phases, each following the same procedure. Each phase starts with the removal of the cock from a flock for a period of 3 weeks to allow depletion of previously stored sperm (Sasanami et al. 2015; Bui et al. 2018), and then reintroduced for a 2-week mating period to provide sufficient time for natural mating to occur repeatedly and for sperm to be deposited and stored in the sperm storage tubules (Sasanami et al. 2015). The ratio of one male to eight females was used per pen. The eggs were collected, identified and recorded daily. The incubation was done weekly, and candling was performed on the 13th day of incubation to detect fertility. Infertile eggs were then isolated but kept in the incubator until hatching for further analysis of their internal content to differentiate embryonic mortality from infertility. Fertility was defined as the proportion of the total number of eggs set in each laying period (that is to say, from the time the cock was withdrawn) that had an embryo.

A schematic timeline for the fertility experiment.



This design led to repeated observations of fertility onset and decline in the same flock under consistent management.

**Growth performance of the chicken strains:** The same completely randomized design was used for this study as well. The Indigenous, Kuroiler and Broiler chicks after leg banding were divided into three experimental units (replicates). Live body weight was recorded on day 0 and every 2 weeks for a period of 16 weeks for indigenous and Kuroiler chickens using a sensitive weighing scale (Salter Ltd., U.K.). The weight was increased to the market weight for the Broiler strain. The feed intake was measured daily for the entire study period.

## 2.4. Data analysis

**Fertility trial:** The data were entered into a Microsoft Excel (2016) spreadsheet and subjected to one-way analysis of variance (ANOVA) using SPSS ver. 16. Pens were treated as replicates, while weeks or days served as treatment factors. The sources of variation between the weeks, within-week laying and the hypothesis for the differences in fertility among the 5 weeks were tested using the F-statistic = 5.14 at alpha = 0.05. The percentage of the average fertile eggs per day was taken, and 60% was considered the minimum acceptable percentage, following the recommendation of (2014).

**Growth performance trial:** The data obtained on the body weight and feed intake were used to compute the average daily gain (ADG), feed intake (FI) and feed conversion ratio (FCR). The generated data were then subjected to two-way analysis of variance (ANOVA) using SPSS Ver. 16, with chicken strain and sex as fixed effects.

## 3. Results

### 3.1. Evaluation of egg fertility as affected by the duration of cocks in hen flocks

The time taken for the onset of laying fertile eggs ranged from 48 to 160 h with an average of  $87.33 \pm 4.3$  h (Table 1). The duration of fertility ranged from 2 to 19 days and was an average of  $7.12 \pm 5.5$  days.

A typical pattern of laying fertilized and unfertilized eggs following cock removal from the flock after mating is shown in Figure 1. From day one to day 17, fertility decreased gradually and then rapidly increased until day 21 post cock removal. On average, egg fertility post cock removal was 49.59%, with a maximum of 71.43% in the first 3 days. Similarly, the regression trend indicated that fertility remained high immediately after cock removal, and gradually decreased as the stored sperm were depleted.

### 3.2. Growth performance of indigenous, Kuroiler and broiler chickens

Broiler chickens showed a significantly higher body weight and average daily gain compared to other strains within the first five weeks, while the indigenous birds presented the lowest values (Figures 2 and 3). The feed consumption and feed efficiency were significantly higher ( $P = 0.000$ ) in broilers and lowest in indigenous chickens (Figures 4 and 5). Over a 14-week period of feeding, the body weight significantly differed ( $P = 0.001$ ) between indigenous chickens from the three selected districts. On average, all the birds gained 1.5 kg of body weight (Table 2), with cocks gaining significantly more body weight than hens did for the entire 16 weeks; however, all categories of cocks gained approximately 2 kg of body weight over the study period (Table 3).

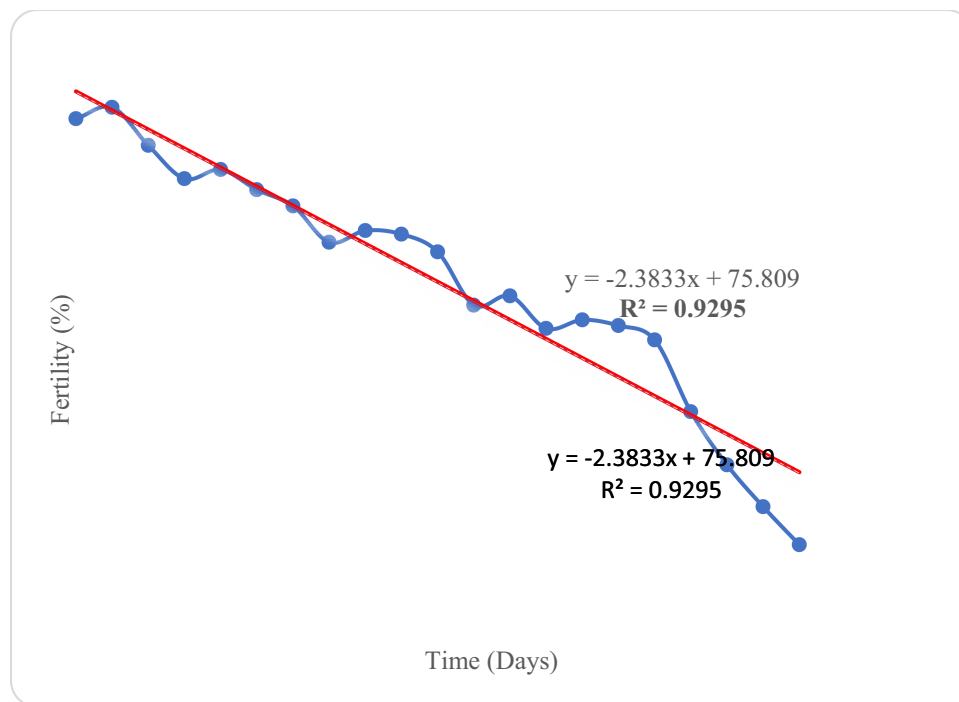
**Table 1.** Time (hours) after mating to the onset of laying fertile eggs, and average duration (days) of hen fertility after cock removal.

Test run	n	Av. time for the onset of fertile eggs after mating	Av. duration of fertility after cock removal	F-value
Replicate1	319	$93.40 \pm 7.052$	$8.27 \pm 5.550$	6.49
Replicate2	303	$82.02 \pm 6.850$	$6.92 \pm 5.805$	8.33
Replicate3	138	$88.56 \pm 5.550$	$6.18 \pm 4.997$	6.49
Mean (with cock)	342	$87.33 \pm 4.305$	-	81.87
Mean (without cock)	419	-	$7.12 \pm 5.473$	20.42

Mean (with cock) represents the mean onset time (in hours) of fertile eggs after cock introduction.

Mean (without cock) represent mean fertility duration (days) after cock removal.

n-represents number of eggs collected and incubated.



**Figure 1.** Relationship between the percentage fertility of indigenous hen eggs and time after cock removal following natural mating.

## 4. Discussion

### 4.1. Fertility of eggs as influenced by the duration of cocks in a hen flock

Understanding the time of the onset of fertile egg production following cock introduction, as well as the duration of fertility after cock removal, is crucial for optimizing poultry breeding strategies. In this study, the onset of fertile egg production under pen mating ranged from 48 to 160 h, with a mean of  $87.33 \pm 4.3$  h following cock introduction. These findings are comparable to previous studies, such as Nicolaidis (2014), which reported a range of 24–384 h with a 104.7-h mean. Additionally, they align with the contemporary maximum values of 240–336 h (Dameraw, 2018). The reported differences among the studies could be attributed to differences in breeds, laying intensity, environmental conditions, and levels of bird selection. Although group size has been previously reported to influence fertility (Zheng et al. 2015), this study standardized the male-to-female ratio across pens. Therefore, based on the observed onset of fertile egg production time, fertile eggs in the IC could be expected after approximately 3 days following cock introduction.

From this study, the average fertility duration after cock removal was  $7.12 \pm 5.5$  days, which was slightly higher than the 6.5 days reported following artificial insemination (Asmarawati et al. 2019). However, the observed fertility duration was slightly lower than 8.7 days reported for pen mating systems (Nicolaidis 2014). These discrepancies are likely due to differences in breed, strain and age factors previously identified as influencing fertility performance (Wen et al. 2022). Additionally, natural mating systems are known to result in higher sperm deposition compared to Artificial Insemination which could explain the high fertility duration observed (Kheawkanha et al. 2024).

Furthermore, the observed gradual decline in fertility after cock removal reflects the depletion of sperm stored in the sperm storage tubules (SSTs). Several biological processes have been shown to contribute to this decline, including (i) the progressive release of sperm from SSTs, (ii) partial sequestration of sperm by albumen during egg formation, (iii) physical obstruction by the egg mass in the magnum, (iv) diffusion of sperm into the body cavity near the infundibulum and (v) loss of sperm that fail to reach the site of fertilization (Bakst 2011; Chai et al. 2024a, 2024b). The fertility trend observed in this study, where hens laid a sequence of fertile and subsequently infertile

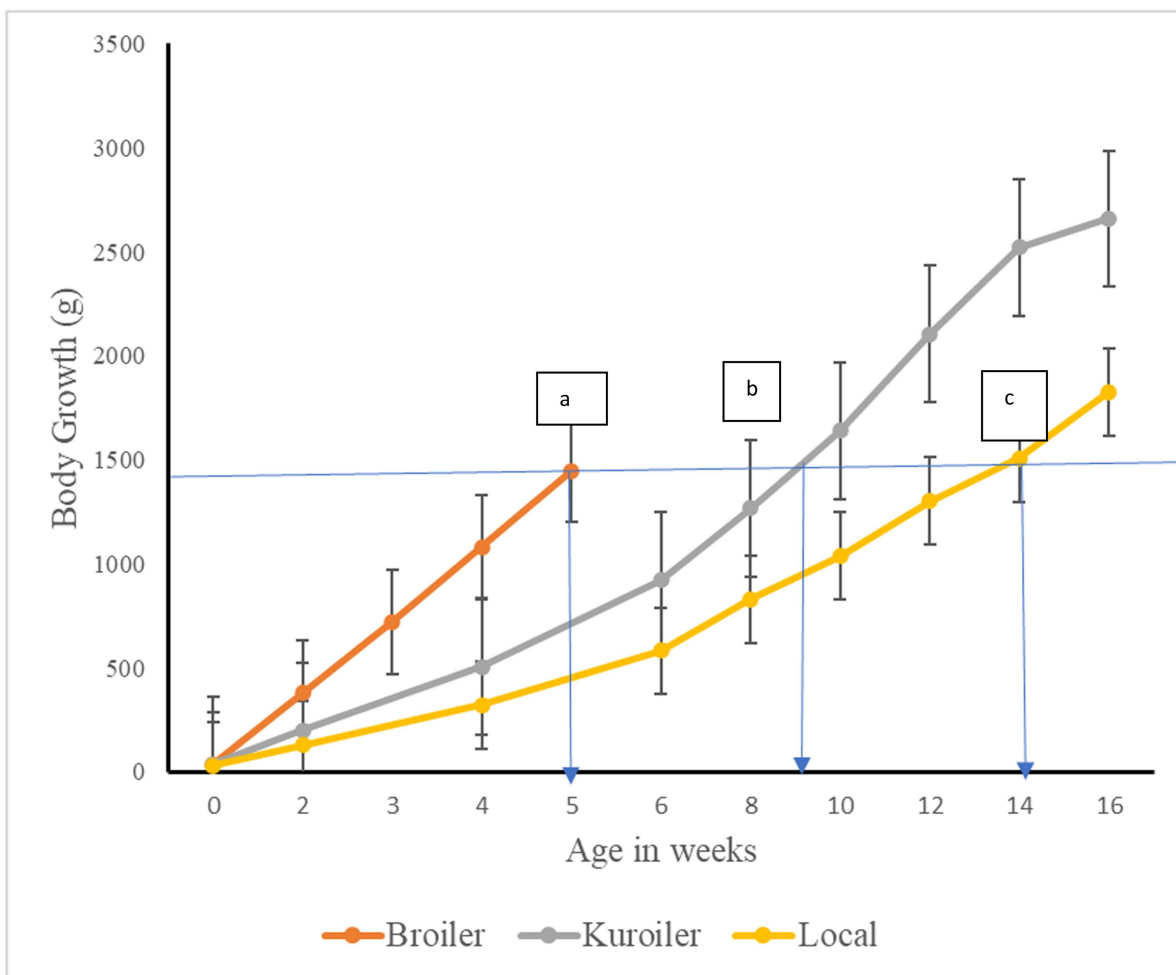


Figure 2. Growth curves of the three chicken strains studied (a, b, c show means with significant differences).

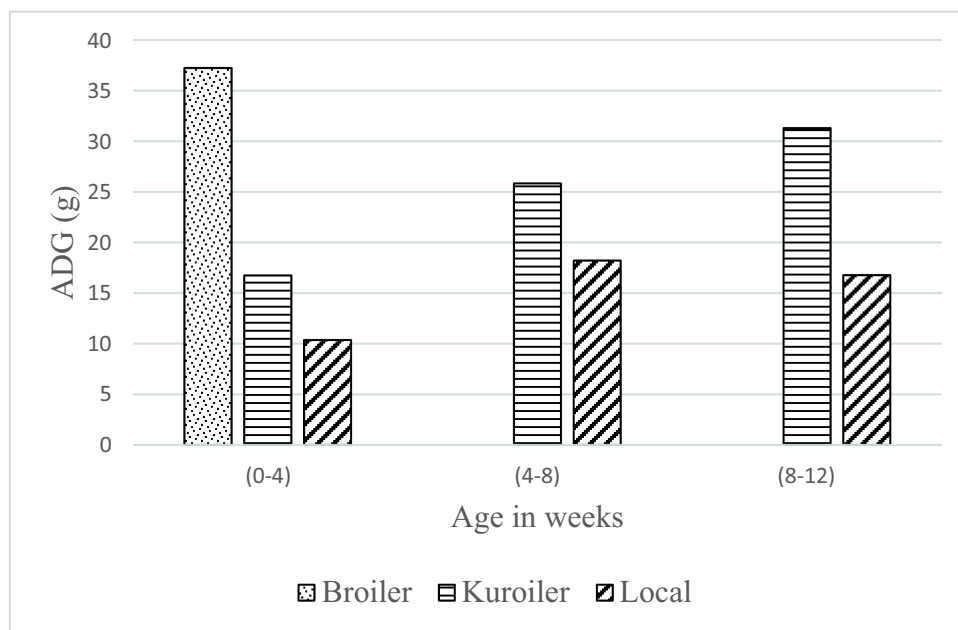
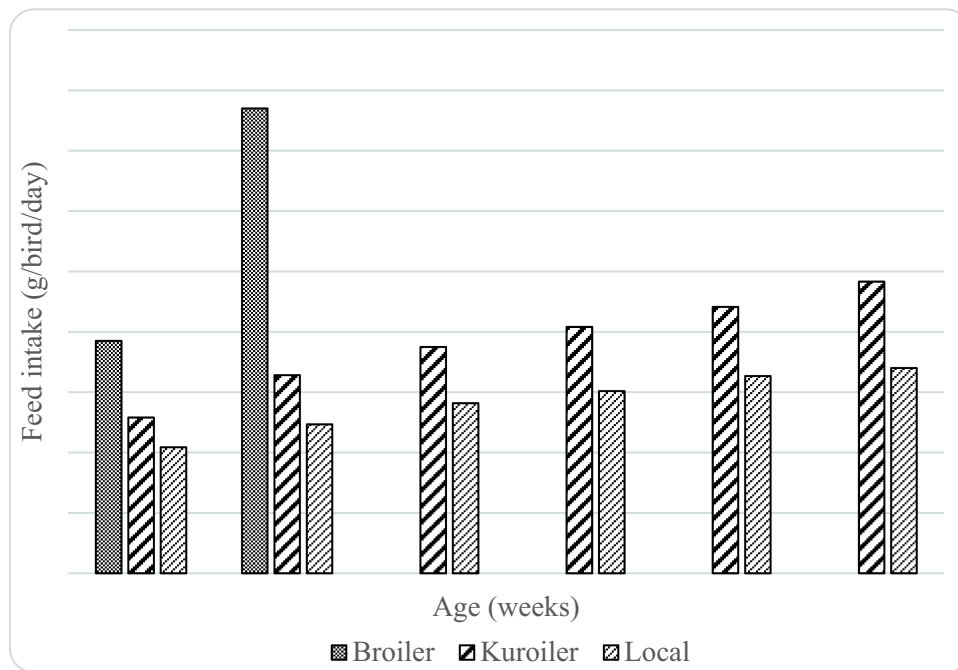
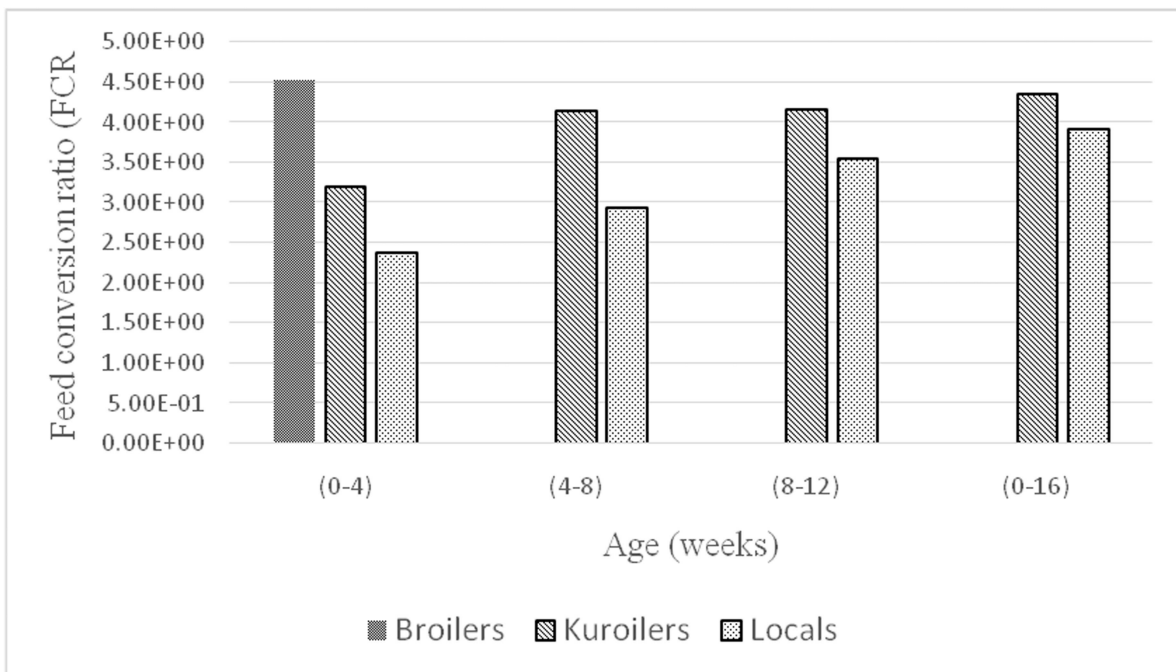


Figure 3. Average daily gain of the three chicken strains studied.



**Figure 4.** Feed consumption per bird from the different chicken strains.



**Figure 5.** Feed conversion ratio of the different chicken strains.

eggs, reflects the gradual depletion of stored sperm. Occasional fertile eggs following several infertile eggs suggest asynchronous depletion among hens, where fertility in some birds ceases abruptly, while in others, it declines gradually. This progressive decline in fertility has been widely reported (Kheawkanha et al. 2024; Brady et al. 2024) and attributed to the cessation of fertility in a large proportion of hens after male removal.

**Table 2.** Mean body weight (g) of indigenous chickens originating from Kiryandongo, Kibaale and Kabarole.

Trait	Kiryandongo (n = 16)		Kibaale (n = 16)		Kabarole (n = 19)		Overall (n = 27)		F-value
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
BW0	29.62 <sup>b</sup>	±0.894	28.12 <sup>b</sup>	±0.700	32.7 <sup>a</sup>	±0.954	32.74 <sup>a</sup>	±0.902	39.818
BW2	138.12 <sup>b</sup>	±9.600	103.12 <sup>c</sup>	±3.257	140.05 <sup>a</sup>	±9.125	130.56 <sup>b</sup>	±3.129	39.0409
BW4	345.25 <sup>a</sup>	±36.247	232.12 <sup>c</sup>	±9.769	337.05 <sup>b</sup>	±27.469	348.67 <sup>a</sup>	±12.658	32.754
BW6	658.44 <sup>a</sup>	±70.699	429.62 <sup>d</sup>	±18.920	641.79 <sup>b</sup>	±51.494	583.7 <sup>c</sup>	±17.896	31.281
BW8	847.12 <sup>b</sup>	±87.934	651.06 <sup>c</sup>	±27.842	974.58 <sup>a</sup>	±73.570	827.85 <sup>b</sup>	±29.420	27.553
BW10	1212.25 <sup>a</sup>	±139.53	761.06 <sup>c</sup>	±38.366	1114.11 <sup>b</sup>	±87.318	1042.67 <sup>b</sup>	±41.726	26.451
BW12	1392.00 <sup>b</sup>	±143.712	1005.56 <sup>b</sup>	±46.997	1462.47 <sup>a</sup>	±107.478	1308.04 <sup>c</sup>	±51.998	34.113
BW14	1534.69 <sup>b</sup>	±144.448	1231.81 <sup>c</sup>	±59.555	1711.21 <sup>a</sup>	±121.445	1512.59 <sup>b</sup>	±59.342	28.54

<sup>a, b, c, d</sup>Means within a row with different superscript letters are significantly different ( $P < 0.05$ ).

SEM: Standard Error of the mean, BW: Body weight (g/bird) from week (0–14).

Overall is the mixture of offspring from the three districts.

**Table 3.** Effect of sex on the body weight (g) of indigenous chickens.

Body weight trait (g/bird)	Hens (n = 107)		Cocks (n = 87)		F-value	P-value
	Mean	SEM	Mean	SEM		
week.0	34.37 <sup>a</sup>	±0.515	35.94 <sup>a</sup>	±0.534	4.324	<b>0.039</b>
week.2	199.92	±9.984	224.62	±11.100	2.712	0.101
week.4	519.13 <sup>b</sup>	±29.170	609.19 <sup>a</sup>	±35.71	3.882	0.05
week.6	692.19 <sup>b</sup>	±26.120	810.63 <sup>a</sup>	±36.39	7.38	<b>0.007</b>
week.8	974.63 <sup>b</sup>	±35.277	1114.59 <sup>a</sup>	±48.074	5.756	<b>0.018</b>
week.10	1218.42 <sup>b</sup>	±48.156	1460.43 <sup>a</sup>	±67.294	9.038	<b>0.003</b>
week.12	1550.63 <sup>b</sup>	±60.910	1855.55 <sup>a</sup>	±78.161	9.707	<b>0.002</b>
week.14	1570.14 <sup>b</sup>	±60.233	2004.14 <sup>a</sup>	±114.235	13.636	<b>0.000</b>
week.16	2204.67	±80.553	2467.72	±159.813	2.16	0.151

<sup>a, b</sup>Means within a row with different superscript letters are significantly different ( $P < 0.05$ ).

SEM: Standard Error of the mean, BW: Body weight.

#### 4.2. Growth performance of indigenous, Kuroiler and broiler chickens

Marked differences in body weight were observed among the three chicken genetic groups, with exotic strains exhibiting superior growth performance compared to indigenous chickens across all ages (Figure 2). This pattern is consistent with previous studies comparing Kuroiler and indigenous chickens raised under scavenging systems in Uganda (Sharma et al. 2015) and under tropical conditions in Nigeria (Okoli et al. 2021). Broilers presented the highest mean body weight, significantly surpassing Kuroiler and indigenous chickens ( $P < 0.001$ ) at all ages. This superior growth performance could be attributed to intensive genetic selection for rapid growth and improved feed efficiency in commercial broiler strains (Binda et al. 2012; Biazen et al. 2021). Kuroilers, on the other hand, exhibited intermediate performance and outperformed indigenous chickens. This could be due to the heterotic effect of their synthetic genetic composition derived from multiple crossbreeding programs of Rhode Island Red, broiler, and/or Leghorn lines, which improved growth and production potential (Okoli et al. 2021).

In this study, the mean body weight at 16 weeks for Kuroiler and Indigenous chickens were 2.6 and 1.8 kg, respectively, which were higher than the 1.6 kg for Kuroiler chickens and the 1 kg for Indigenous chickens reported by Sharma et al. (2015). These observed differences could be attributed to variations in management conditions, specifically feeding and housing systems. While Sharma et al. (2015) evaluated the growth performance of birds under the free-range systems, a deep-litter intensive system was used in this study, which could have enhanced growth performance. Additionally, the feed intake and feed conversion ratio differed significantly ( $P < 0.000$ ) among the genetic groups (Figures 4 and 5). Broilers consumed more feed but converted it more efficiently into body mass, which is consistent with previous findings (Binda et al. 2012).

As expected, differences in body weights were also observed among indigenous chickens from the three districts included in this study. This variation reflects the genetic diversity among the IC ecotypes and their adaptations to diverse production environments. Similar variations among IC ecotypes have been reported in other tropical regions (Tadelle et al. 2003; Bekele and Abebe 2010; Mwakilembe et al. 2013). In addition, both planned and unplanned crossbreeding with exotic breeds may also contribute to the genetic variability observed among and within the IC ecotypes (Beyihayo et al. 2022).

Overall, the relatively higher body weights observed in the present study compared with previous reports may be attributed to improved management practices, particularly housing and feeding regimes associated with intensive production systems (Magala and Kugonza 2012). These findings highlight the importance of management conditions in enhancing the growth performance of both improved and IC genotypes.

#### 4.3. Limitations

While we sampled three major districts, the performance may vary in different agro-ecological zones like the semi-arid Northern Uganda and West-Nile.

### 5. Conclusions

The findings from this study demonstrated that the onset of fertile egg production in indigenous hens starts at approximately 3.5 days after cock introduction. Following cock removal, fertility persisted for an average of  $7.12 \pm 5.47$  days, despite some hens maintaining fertility for up to 19 days, indicating that the indigenous hens are capable of storing viable sperm for an extended period. Therefore, this information could be used by smallholder farmers in managing mating intervals and optimizing fertility in their breeding flocks. As predicted, Broiler birds exhibited the highest growth rates, with Indigenous birds showing the slowest growth rate. Despite their slow growth rates, the indigenous chickens achieved an average body weight of approximately 1.5 kg at 14 weeks of age. This finding demonstrates their potential for enhanced productivity when provided with better management conditions. Generally, these results highlight the importance of both reproductive management and improved production systems in enhancing the productivity of indigenous chicken ecotypes.

The average duration for the onset of fertile eggs after mating was 3.5 days after introducing the cock. After cock removal, the average duration of fertility was  $7.12 \pm 5.47$  days, although considerable variation was observed among hens, with some maintaining fertility for up to 19 days. These findings indicate that indigenous hens are capable of storing viable sperm for a limited period that supports continued egg fertilization after mating. In the growth trial, the broiler chickens exhibited the highest growth rate, followed by the Kuroiler chickens, while indigenous chickens showed comparatively slower growth. Male indigenous chickens consistently presented higher body weights than females. Despite their slower growth rate, ICs gained an average body weight of 1.5 kg within 14 weeks, with the males gaining more body weight than the females under improved management conditions.

### 6. Recommendations

1. The length of time taken for the onset of fertile eggs suggests that hatchable eggs should be collected at least 3 days after introducing the cock.
2. The gradual increase in the number of infertile eggs laid by fertile hens prior to the cessation of fertility suggests that mating once per week may help maintain higher fertility levels in smallholder flocks.
3. Further study based on the reproductive biological of indigenous chickens, particularly sperm storage dynamics in the sperm storage tubules and the effects of controlled or stud mating systems on fertility and hatchability.
4. Since all the IC birds gained an average body weight of 1.5 kg within 14 weeks, a robust selective breeding and improved management practices should be encouraged to enhance their growth performance and productivity.

### Author contributions

CRediT: **Ivan Muhumuza**: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing; **Isaac Kasoro**: Investigation, Resources, Validation, Writing – original draft, Writing – review & editing; **Brian Asimwe**: Formal analysis, Investigation, Resources, Validation, Writing – original draft; **Donald Rugira Kugonza**: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Funding

This research was financially supported by the Native Chicken Project (AURG II-2-115-2018) of Makerere University, financed by the African Union Commission.

## Ethical statement

This research study was conducted after approval of the Department of Animal and Range Sciences Research and Ethics Committee, School of Agricultural Sciences, College of Agricultural and Environmental Sciences of Makerere University.

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