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Influence of Short-Term Starvation on Growth Performance and Biochemical Parameters of Indigenous Chicken in Kenya

*Abdi Maalim Adhan[1] https://orcid.org/0000-0003-3640-6283, Jemimah Simbauni[2] https://orcid.org/0000-0002-0985-725X & Syprine A. Otieno[3] https://orcid.org/0000-0001-9236-0166

[1]Department of Zoological Sciences, Kenyatta University

[2]Department of Zoological Sciences, Kenyatta University Email; simbauni.jemimah@ku.ac.ke

[3]Department of Zoological Sciences, Kenyatta University Email; otieno.syprine@ku.ac.ke

Corresponding Author's Email address: adhanmaalim10@gmail.com

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ABSTRACT

The demand for indigenous chicken is higher due to its better taste and nutritional value than broilers. However, production is low owing to many factors that include: a high mortality rate caused by the incidence of metabolic diseases, skeletal disorders, and other diseases. Short-term starvation followed by re-feeding has been shown to improve daily gain and reduce the occurrence of metabolic diseases in broiler chicken. This study was designed to determine the influence of short-term starvation on growth performance and biochemical parameters of the indigenous chicken in Kenya. A randomized experimental design consisting of three groups of indigenous chicken was adopted. The first group, the control, was fed on growers' chicken feed for 60 hours before they were sacrificed and dissected. The second group was starved for 60 hours, sacrificed, and dissected. The third group was starved for 60 hours and then given growers' chicken feed for five days, after which they were sacrificed and dissected to remove the gastrointestinal tract. For each group, weight in grams was taken after every 12 hours. Data were analyzed by one-way Analysis of Variance (ANOVA) at a 95% confidence level using SPSS version 26. Results on the size of organs showed that the volume of the stomach, pancreas, and small intestines was highest in group 3, followed by group 1, and lowest in group 2. The glucose, lipids, and cholesterol concentrations were highest in group 1, followed by group 3, and lowest in group 2. Protein was highest in Group 2, followed by Group I, and the lowest in Group 3, and the concentration of alkaline phosphatase in Group 3 was the highest, followed by Group 1, and the lowest in Group 2. Re-feeding of Group 3 resulted in an increase of 0.1925 g in females and 0.1197 g in males. It is concluded that short-term starvation and re-feeding increase the size of the stomach and pancreas and decrease glucose, lipid, and cholesterol concentration in the blood. Short-term starvation and re-feeding improve feed efficiency. The research recommends farmers rearing indigenous chicken to practice 60 hours of fasting due to the compensatory growth phenomenon.

Keywords: Short-term starvation, Re-feeding, Growth Performance, Biochemical parameters, indigenous chicken.

INTRODUCTION

The practice of keeping indigenous/native breeds of chicken is done in many developed and developing countries throughout the world (Padhi, 2016). In Kenya, it is estimated that 90 percent of people living in rural areas rear indigenous chicken, which plays an important role in rural economies (Kingori *et al.,* 2015). This chicken is kept to increase household income when sold and they provide a cheaper source of proteins since they require lower production costs (Atela *et al.,* 2016). It is estimated that among the poultry domesticated in Kenya, 77% are indigenous chicken (Okeno *et al.,* 2012). This indicates how valuable indigenous chicken are to the people of Kenya.

The demand for indigenous chicken has been increasing, and the trend is expected to continue (Gikunju *et al.,*

2010), The reason for the high demand is that their meat and eggs are more delicious and more nutritious than those of broilers (Afolayan *et al.,* 2016). Despite the high demand for indigenous chicken, their production is low (Atela *et al.,* 2016) due to the high mortality rate caused by infections such as Newcastle, incidences of metabolic disorders, and skeletal disorders (Ismail and Abdel-Wareth, 2011; Tumova *et al.,* 2019). A study conducted by Gonzales *et al.* (2010) revealed that Sudden Death Syndrome (SDS) and Ascites (AS) caused more than 50% of the deaths of a reared chicken. The average mortality of indigenous chicken in Kenya has been estimated to be 39.2% (Mutinda *et al.,* 2018). To reduce the mortality rate due to diseases, the Kenya Agricultural and Livestock Research Organization (KALRO) recommended an indigenous chicken vaccination program. Some of the vaccines are provided to treat the following diseases: Mareks, Newcastle, infectious bronchitis, Gumboro, Fowl pox, Fowl typhoid, and parasites (KALRO, 2016). Genetic selection research has been conducted, which has resulted in improved indigenous "Kienyeji" chicken which grows faster than the earlier indigenous chicken. However, these efforts have not led to an increase in indigenous chicken production as expected. Besides, the improved breeds are still not as delicious as the original "Kienyeji" chicken. This chicken also accumulates fat deposits around their breast region which are unhealthy for consumers.

One of the proposed remedies to this problem is feed restriction or subjecting chicken to short-term starvation. Feed restriction is based on a compensatory growth phenomenon that is known to achieve the same growth performance of chicken subjected to starvation as that given food throughout (Mahmoud *et al.*, 2019). This is because when an animal, whose growth has been retarded by dietary restriction, is given adequate nutrition, it grows faster than an animal of the same age with no restriction. Compensatory growth is sometimes termed catch-up growth (Dissanayake and David, 2017). Research on broiler chicken has shown that short-term starvation and feed restriction decrease body fat deposition, and feed intake (Omosebi *et al.*, 2014) and reduce the prevalence of metabolic disorders as well as leg problems (Mahmoud *et al.,* 2019: Butzen *et al.*, 2013). The strategy can be used to alter feeding management to decrease feed consumption to some extent and hence growth rate, alleviating the occurrence of metabolic disorders and improving feed efficiency.

Short-term starvation of chicken causes morphological and biochemical changes. Research conducted on Japanese quail revealed that starvation for 2.5 days causes a significant decrease in the mass of the body, liver, stomach, pancreas, small intestine, and kidney (Lone and Akhtar's, 1988; Ahmed *et al.*, 2016; Chediack *et al.,* (2022). Also, it was revealed that total protein level decreased, cholesterol increased while triglycerides remained unchanged among the starved Japanese quails. However, the reviewed studies were conducted in quails and house sparrows which though related to Indigenous chicken are of different species, therefore potential differences may arise when the same conditions are applied to Indigenous chickens. The current study therefore focused on the influence of short-term starvation on growth performance and selected biochemical parameters of the indigenous chicken in Kenya.

MATERIAL AND METHODS

Study Area

This study was carried out at the Department of Zoological Sciences in the main campus of Kenyatta University in Nairobi County, approximately 18 kilometers by road northeast of the Nairobi Central Business District.

Management of the Indigenous Chicken

A total of 24 males and females Indigenous chicken aged 16 weeks were bought from Ziwani Poultry Farm which is located in Thika Kiambu County, Kenya. The chicken were housed in a steel wire cage on a floor covered with wood shavings at a temperature of 25^{O}C and at a 14:10 photoperiod. They were all fed on Grower's pellets chicken feed and also provided with water *ad libitum.* They were allowed to acclimatize to the conditions of the experimental house for two weeks.

Study Design

This study adopted a randomized experimental research design. The chicken were divided into three groups, each group with 8 chicken, having 4 males and 4 females. The first group was the control group, which was fed on Grower's pellets chicken feed (*ad libitum-fed*) and also provided with water. The second group was starved for 60 hours but was provided with water. The third group of chicken was fasted for 60 hours and then given growers chicken feed and water for the next 5 days after short-term starvation.

Determination of the Size of Small Intestine, Liver, Pancreas, and Stomach

After 60 hours from the start of the experiment, Group 1 and Group 2 indigenous chicken were sacrificed and dissected. The gastrointestinal tract for each chicken from the stomach to the anus was removed from the body. The stomach, liver, pancreas, and small intestine were excised and each separated. The same was done to Group 3 after the initial 60 hours and five days of refeeding, which was

a total of $7^1/_2$ days. The size of the organ was determined by measuring its weight and volume of the organ. The weight of the pancreas, stomach, small intestine, and liver for each group was measured using a beam balance, while volume was measured by dipping the organ in a Eureka can containing water. The displaced water was collected in a measuring cylinder, and the volume was read and recorded.

Determination of Concentration of Biochemical Parameters

Collection of Blood Samples

Blood samples from each experimental indigenous chicken were collected. Samples of about 3 ml of blood were drawn from the branchial vein into a vacutainer blood collection tube using a butterfly needle. Then the blood collected was allowed to clot for one hour before being centrifuged at 3000 rpm for 15 minutes. Blood serum was then obtained and stored at -20°C until analysis.

Determination of Glucose Concentration

Glucose determination was done following the Glucose oxidase assay (GOD) procedure (Megazyme, 2020).

The Glucose oxidase (GOD) method was used, and it involved the following: GOD reagent was used together with a standard glucose concentration of 100mg/dl = 5.55 mmol/l (Multiply 100 mg/l with multiplication factor (0.0555) = 5.55 mmol/l). In the first step, 1 ml of GOD working reagent was placed in clean test tubes labeled Blank (B), Standard (S), and Test (T). The second step was placing 10 microliters of distilled water (0.01 ml) in the blank. The third step was adding 10 microliters (0.01ml) of glucose standard to the standard test tube (S). The fourth step was adding 10 microliters (0.01 ml) of samples to the test (samples):1.2.3.4.5. The fifth step was mixing thoroughly and starting the watch to incubate for 30 minutes the solution at room temperature for 25 °C. For the accuracy of the spectrophotometer, the researcher set the photometer at a wavelength of 546 nm using distilled water and then air. The sixth step was measuring the absorbance at 546 nm, starting with Blank, Standard, and Samples. Finally, the absorbance of each sample was recorded and determined using the following formula.

The concentration of samples =

$$\frac{\text{Absorbance of (T) test (sample)}}{\text{The absorbance of (S) standard}} \text{ X Concentration of standard}$$

The concentration of Standard is 100 mg/dl multiplied by a factor (0.0555) = 5.55 mmol/l

Determination of Lipid and Cholesterol Concentration

The cholesterol concentration was determined using the CHOD-PAP method (cholesterol oxidase phenol 4-aminoantipyrine peroxidase), which is common for the determination of cholesterol in serum or plasma (Atlas Medical, 2015). The lipid triglyceride was determined using the GPO-PAP method, which is commonly used for the in vitro quantitative determination of triglycerides in serum or serum (Pointe, 2016).

Preparation of working reagent

It involved measuring 60 ml L1 Buffer solution and 15 ml L2 substrate in a ratio of 60:15 (4:1). This was mixed in the ratio of 1 ml of the substrate and 4 ml of buffer solution. The procedure was as follows;

One milliliter of working reagent was added to a clean test tube labeled Blank (B), Standard (S), and Test (T), followed by adding 10 microliters (0.01 ml) of distilled water to the blank. This was followed by placing 10 microliters (0.01 ml) of standard reagent solution into a standard test tube (S). Next 10 microliters (0.01ml) of samples were added into each test tube, i.e., 1, 2, 3, 4, 5. This is followed by mixing thoroughly and incubating for 15 minutes at room temperature, 25°C. Finally, absorbance was measured at a wavelength of 546 nm starting with the blank, standard, and samples.

The concentration of samples =

$$\frac{\textit{Absorbance of T (Samples)}}{\textit{Absorbance of S (standard}} \text{ X } \textit{Concentration of standard}$$

NB: Concentration of cholesterol standard=5.18mmol/l (200mg/dl*0.0259 factor). Concentration of Triglyceride standard=2.36mmol/l (200mg/dl*0.0118factor).

Determination of Protein Concentration

Protein concentration was determined using the biuret test (Brava, 2016). The procedure was as follows

One ml of working reagent (A) was added to a clean test tube labeled Blank (B), Standard (S), and test (T). This was followed by adding 20 microliters of distilled water to the blank test tube (B). The next step was adding 20 microliters (0.02 ml) of protein standard reagent in a standard test tube (S). This was followed by adding 20 microliters (0.02 ml) of the samples to each test tube containing the samples and mixing thoroughly the solution, then starting the stopwatch and incubating the mixture for 15 minutes at a room temperature of 25°C. This was followed by measuring the absorbance at a **wavelength of 546 nm** starting with

Blank, Standard, and Samples. Finally, the absorbance of each sample was recorded. The formula below was used to determine the concentration of the samples.

The concentration of samples =

$$\frac{\text{Absorbance Test Sample}}{\text{Absorbance of Standard S}} \times \text{Concentration of standard}$$

The concentration of protein standard = 69.5g/l

Determination of Alkaline Phosphatase Concentration

The concentration of alkaline phosphatase was determined using the alkaline phosphatase pNPP Kinetic method, which is commonly used for the determination of Alkaline Phosphatase activity in serum (Atlas Medical, 2015). The procedure was as follows;

The working reagent was prepared by adding 1 tablet (substrate) to 3.2 ml of buffer solution and stirring well till the substrate dissolved. This was followed by setting the photometer at a **wavelength of 405 nm** using distilled water and then air. The next step was to measure 1 ml of working reagent into a clean test tube. This was followed by adding 20 microliters (0.02 ml) of the sample to each test tube containing the working reagent. The Next step was mixing the solution and starting the stopwatch. Then the absorbance was read after 30 seconds. The reading of the absorbance of test tubes containing the sample was repeated after 1 minute and 2 minutes and recorded. Finally, the mean absorbance change per minute (A/min) was calculated using the following formula.

ALP enzyme activity in u/l = A/min X 2754

Determination of Growth Performance

The parameters that were considered to measure growth performance are live body weight and body weight gain (Ismail & Abdel-Wareth, 2011). The weight of the indigenous chicken was taken twice at the start of the experiment. Also, after every 12-hour interval, each chicken's weight was taken until the end of the experiment. Weight gain was calculated by taking the difference between the final body weight and the previous body weight in each 12-hour interval.

Feed efficiency in this study was taken as weight gain in grams of each chicken per gram of feed taken by the chicken within the interval of weight gain (Zampiga, Calini, & Sirri, 2021). In each group of chicken, weight gain was calculated after 12-hour intervals. Also, the number of grams of feed eaten by the eight chicken after 12-hour intervals was recorded. For each group, the number of grams of feed eaten was averaged to get the feed eaten by one chicken. Feed efficiency was calculated as the total weight of feed consumed / total grams of weight gained.

Ethical consideration

When carrying out this research, ethical considerations were observed to ensure minimal suffering of the chicken so that there would be maximization of the benefits of the research while minimizing the potential harm. Key areas considered were: first, the freedom from discomfort, where suitable housing was provided for all three groups of indigenous chicken in this study. The second was freedom from pain, injuries, and diseases by ensuring prompt diagnosis and treatment of illnesses and minimization of pain during procedures. The third was freedom from fear and suffering, which was observed by maintaining husbandry conditions and treatment that do not cause physical suffering.

Data Analysis

The data on weight in grams and volume in cm^3 of the stomach, small intestines, liver, and pancreas, and concentration of the measured biochemical parameters from each group of experimental indigenous chicken were analyzed by one-way ANOVA at a 95% confidence level using SPSS version 26 for Windows. This was done to determine whether there was any significant difference in the parameters measured between the three groups of the indigenous chicken. Significant differences were taken at $p<0.05$.

RESULTS

Size of Body Organs

The analysis of variance showed no significant difference in mean volume between group 1, group 2, and group 3 of the Indigenous chicken for female liver (P value = 0.675), male stomach (P value = 0.054), and male pancreas (P value 0.734) (Table 1). The Post hoc test result further confirmed that they were statistically not significant since all the P values were above 0.05, therefore, the mean differences were due to sampling errors or by chance.

Furthermore, the result indicates that there was a statistically significant difference in mean volume between Group 1, group 2, and Group 3 of the Indigenous chicken for male liver (P value = 0.015), female stomach (P value = 0.004), female pancreas (P value = 0.025), and male small intestines (P value = 0.005) (Table 1).

Table 1: Summary of Descriptive and Analysis of Variance for Volume (Cm^3) of the Liver, Stomach, Pancreas, and Small Intestines

Organs	Sex		Mean Volume	p-values	Post-hock
Liver	Male	Group 1	37.75 ± 6.34	F=;P=0.015	All P values are below 0.05
		Group 2	20.50 ± 1.73		
		Group 3	28.75 ± 9.22		
	Female	Group 1	25.50 ± 11.03	F=;P=0.675	All P values are above 0.05
		Group 2	21.25 ± 2.63		
		Group 3	24.75 ± 4.65		
Stomach	Male	Group 1	41.75 ± 4.65	F=;P=0.054	All P values are above 0.05
		Group 2	40.75 ± 0.96		
		Group 3	60.75 ± 18.68		
	Female	Group 1	40.00 ± 9.13	F=;P=0.004	All P values are below 0.05
		Group 2	35.00 ± 2.94		
		Group 3	63.00 ± 12.36		
Pancreas	Male	Group 1	12.75 ± 2.22	F=;P=0.734	All P values are above 0.05
		Group 2	12.00 ± 5.10		
		Group 3	14.00 ± 2.71		
	Female	Group 1	11.50 ± 1.3	F=;P=0.025	All P values are below 0.05
		Group 2	11.75 ± 1.50		
		Group 3	14.25 ± 0.96		
Small intestines	Male	Group 1	51.75 ± 6.40	F=;P=0.005	All P values are below 0.05
		Group 2	27.00 ± 5.72		
		Group 3	29.50 ± 12.23		
	Female	Group 1	44.50 ± 1.00	F=;P=0.053	P values 0.046 for Group 2 and Group 3
		Group 2	30.25 ± 0.50		
		Group 3	52.75 ± 19.33		

The finding showed that the mean weight of the female and male liver, male stomach and female small intestines have P values above 0.05 the minimum threshold for differences between group 1, group 2, and group 3 of indigenous chicken to be statistically significant. The other mean weight between the three groups of chicken was statistically significant since their P values are below 0.05 (Table 2).

Table 2: Analysis of Variance for Weight (grams) of the Liver, Stomach, Pancreas, and Small Intestines

Organs	Sex		Mean Weight	p-values	Post-hock
Liver	Male	Group 1	41.88 ± 8.06	F=;P=0.11	All P values are above 0.05
		Group 2	22.13 ± 1.15		
		Group 3	28.99 ± 9.51		
	Female	Group 1	26.58 ± 12.25	F=;P=0.239	All P values are above 0.05
		Group 2	23.40 ± 3.40		
		Group 3	26.51 ± 2.01		
Stomach	Male	Group 1	42.78 ± 6.06	F=;P=0.109	All P values are above 0.05
		Group 2	44.95 ± 1.59		
		Group 3	58.43 ± 16.21		
	Female	Group 1	40.35 ± 9.91	F=;P=0.012	All P values are below 0.05
		Group 2	39.38 ± 2.30		
		Group 3	60.83 ± 11.33		
Pancreas	Male	Group 1	11.93 ± 2.84	F=;P=0.011	All P values are below 0.05
		Group 2	6.33 ± 2.69		
		Group 3	13.73 ± 2.85		
	Female	Group 1	8.03 ± 1.92	F=;P=0.016	All P values are below 0.05
		Group 2	8.01 ± 3.10		
		Group 3	13.32 ± 1.82		
Small intestines	Male	Group 1	49.85 ± 6.26	F=;P=0.000	All P values are below 0.05
		Group 2	25.95 ± 2.11		
		Group 3	26.90 ± 4.01		
	Female	Group 1	48.50 ± 4.74	F=;P=0.111	All P values are above 0.05
		Group 2	30.30 ± 1.61		
		Group 3	40.25 ± 18.08		

Concentration of Glucose, Lipid, Cholesterol, Protein, and Alkaline Phosphatase.

The glucose concentration for males is statistically significant, while for females, the differences between groups were not statistically significant (Table 3).

The lipid concentration showed that the differences between the mean for males and females are statistically significant (Table 3).

The Cholesterol concentration showed that there was no significant difference between group 1, group 2, and group 3 (Table 3). The result for the post hoc test, which makes multiple comparisons of the mean, also showed that their means were not significantly different.

The protein concentration showed that mean differences between groups 1, 2, and 3 were statistically significant (Table 3).

The alkaline phosphatase concentration showed that for males, the difference between groups is statistically significant, while for females, the difference between groups 1, 2, and 3 is statistically significant (Table 3).

Table 3: Analysis of Variance for Concentration of Biochemical Parameters of the Indigenous Chicken (Unit for Glucose = mmol/L, for lipid, cholesterol, protein, and alkaline phosphatase = u/L)

Biochemical parameters	Sex		Mean Concentration	p-values	Post-hock
Glucose	Male	Group 1	13.76 ± 3.56	F=;P=0.022	All P values are below
		Group 2	8.73 ± 0.44		0.05
		Group 3	10.27 ± 0.57		
	Female	Group 1	11.41 ± 1.46	F=;P=0.460	All P values are above
		Group 2	9.75 ± 2.77		0.05
		Group 3	10.02 ± 1.20		
Lipid	Male	Group 1	2.27 ± 0.18	F=;P=0.00	All P values are below
		Group 2	0.86 ± 0.10		0.05
		Group 3	1.80 ± 0.31		
	Female	Group 1	1.21 ± 0.12	F=;P=0.00	All P values are below
		Group 2	0.87 ± 0.03		0.05
		Group 3	1.98 ± 0.18		
Cholesterol	Male	Group 1	4.09 ± 0.89	F=;P=0.638	All P values are above
		Group 2	3.52 ± 0.64		0.05
		Group 3	3.62 ± 1.07		
	Female	Group 1	3.29 ± 0.15	F=;P=0.562	All P values are above
		Group 2	2.83 ± 0.37		0.05
		Group 3	3.28 ± 1.09		
Protein	Male	Group 1	42.27 ± 11.24	F=;P=0.045	All P values are below
		Group 2	52.13 ± 2.61		0.05
		Group 3	38.43 ± 0.67		
	Female	Group 1	41.05 ± 4.85	F=;P=0.001	All P values are below
		Group 2	52.08 ± 10.48		0.05
		Group 3	20.55 ± 6.02		
Alkaline Phosphatase	Male	Group 1	223.09 ± 23.91	F=;P=0.046	All P values are below
		Group 2	94.68 ± 95.90		0.05
		Group 3	636.55 ± 455.93		
	Female	Group 1	56.12 ± 24.71	F=;P=0.001	All P values are below
		Group 2	75.38 ± 62.38		0.05
		Group 3	557.68 ± 237.34		

Growth Performance of the Indigenous Chicken

The influence of short-term starvation on growth performance was measured through the weight gain and feed intake efficiency of the indigenous chicken. The result indicates that there was a slight decrease in group 1 from the initial weight measured compared to the weight measured after 60 hours in both male and female indigenous chickens. The average initial weight was 1.00375 g (females), and 1.1325 g (males) while the final average weight after 60 hours was 0.985 g (females),

and 1.1237 g (males) a reduction of 0.01875 g (females) (1.868% reduction) and 0.00875 g (males) (0.779% reduction). Therefore, there was no feed efficiency in the Group 1 indigenous chicken investigated in this study (Table 4).

Results for group 2 reveal that the initial average weight was 1.10875 g for females and 1.17500 g for males. This reduced to 1.01375 g (females) and 1.0225 g for males after 60 hours of starvation, a reduction of 0.095 g (females) (8.568% reduction) and 0.1525 g (males) (12.979% reduction). The reduction was greater than in Group 1 (Table 4).

Results for group 3 showed a reduction after 60 hours. When re-feeding was done for five days there was additional weight. The average initial weight for females was 1.1125 g and for males was 1.240 g, which reduced to 1.0275 g for females and 1.1413 g for males after 60 hours of starvation a reduction of 0.085 g for females (7.640% reduction) and 0.098 g for males (7.903% reduction) were recorded. Re-feeding of Group 3 resulted in an increase in average weight of 1.220 g for females and 1.261 g for males an increase of 0.1925 g for females (17.303% gain) and 0.1197 g for males (9.653% gain) (Table 4).

Table 4: Weight Analysis of the Indigenous Chicken After 60 hours and after re-feeding for 5 days

		Initial average weight (grams)	Average weight after 60 hours (grams)	Reduction in weight (grams)	Average weight after refeeding for 5 days (grams)	Additional weight (grams)
Group 1	Female	1.00375	0.985	0.01875		
	Male	1.1325	1.12375	0.00875		
Group 2	Female	1.10875	1.01375	0.095		
	Male	1.17500	1.0225	0.1525		
Group 3	Female	1.1125	1.0275	0.085	1.220	0.1925
	Male	1.240	1.1413	0.0987	1.261	0.1197

DISCUSSION

Size of Body Organs

The result showed that males had larger sizes of organs than females' Indigenous chicken, since their weight and volume had higher values. Similar results were observed by Badi, Khalid, and Asaad (2023) research on chicken investigated under similar conditions as the present study. The result showed that males recorded a high body weight compared to female chickens. This was attributed to differences in the concentration of Follicle-stimulating hormone (FSH). Males had higher concentrations of FSH in the blood serum compared to females. The FSH contributes to a faster growth rate, resulting in higher body weight in males.

The group 2 indigenous chicken had the lowest size of the organs because they had been starved for 60 hours. This usually occurs to save the cost of energy needed for maintaining physiological functions (Funes *et al.,* 2014). This correlates with the study conducted by Karasov et al. (2014) that investigated the anatomical changes in migrating Blackcaps and found that fasting led to more reduction in the size of the liver, pancreas, and small intestine than the overall decrease in body mass.

The chicken in group 3 were also starved for 60 hours. However, they were re-fed for five days. During the re-feeding period, they were able to attain more weight than those fed throughout due to the compensatory growth phenomenon (Dissanayake and David, 2017). According to Mahmoud *et al.* (2019), compensatory growth enables chicken subjected to short-term starvation to achieve the same growth performance or higher than chicken given food throughout. This is because when an animal whose growth has been retarded by dietary restriction is given adequate nutrition, it grows faster than an animal of the same age with no restriction. These results agree with those of a study conducted by Ribeiro *et al.* (2018) influence of fasting of female broiler chicken on the development of digestive organs. The results of the authors showed that fasting for 60 hours led to reduced weight and length of digestive organs, especially the small intestines, liver, proventriculus, and gizzard.

Concentration of Glucose, Lipid, Cholesterol, Protein, and Alkaline Phosphatase.

The mean concentration of glucose was highest in group 1 (13.76 mmol/L, equivalent to 247.93 mg/dL), followed by group 3 (10.27 mmol/L, equivalent to 185.05 mg/dL), and lowest in group 2 (8.73 mmol/L, equivalent to 157.29 mg/dL) (Table 3). The reason group 2 had the lowest concentration of glucose in their blood can be attributed to the 60-hour fasting. While the indigenous chicken in group 3 had also been starved for 60 hours, they were re-fed for five days, explaining the reason they had a higher glucose level than group 2. However, the range of glucose concentration is within the normal range, because according to Hernawa, Wahyuni, and Suprapti (2018), glucose concentration in broiler chicken should range between 180-250 mg / dL.

Glucose is the major circulating carbohydrate in chicken, and its level in the blood is used as a biometric indicator in various studies (Kawasaki, et al., 2020). The result of this study is in agreement with studies conducted (Smith, 2016) on the level of blood glucose in a chicken fasted for 31 hours. The result indicated that fasting chicken had reduced blood glucose levels compared to those fed throughout. This correlates to the current study since Group 2, which had the lowest glucose concentration, had been starved for 60 hours. The increase in levels of glucose in the blood of Group 3 chicken that were starved and re-fed relates to research conducted by Goodridge (2018) that showed that re-feeding of starved chicken increases blood glucose levels to normal or higher than normal levels.

The mean concentration of lipids showed that for males' Group 1 (2.27 u/L) had the highest concentration of lipids, followed by Group 3 (1.80 u/L) and Group 2 (0.86 u/L). The trend is contrary to lipid concentration in females, where the highest is in group 3 (1.98 u/L), followed by group 1(1.21 u/L), and lowest in group 2 (0.87 u/L) (Table 3). The common trend is that in both males and females, fasting led to a decrease in the concentration of lipids. Lipids are vital in the physiological functioning of the chicken, and their concentration is regulated by the spexin hormone. The study on the effect of short-term starvation on the concentration of spexin showed that it increased significantly, causing a change in lipid metabolism (Kołodziejski *et al.,* 2021). Fouad and El-Senousey (2014) opine that limiting feed intake and quantitative feed restriction in broiler chicken reduces fat content compared to full feeding.

The decrease in lipid concentration in the blood serum of group 2 indigenous chicken results correlates with a study conducted by Yu *et al.* (2016) investigating the effect of starvation on lipid metabolism in broiler chicken. The study revealed that starvation significantly ($p<0.01$) decreased the lipid contents of the chicken under study same to the result of this study. The lipid concentration in Group 3 indigenous chicken that was starved and re-fed is the highest due to the compensatory growth phenomenon sometimes referred to as catch-up growth. According to Radulovic et al. (2021), an animal that is restricted in food or under environmental or disease stress will exhibit an increased rate of gain and enhanced feed utilization when the stress is eliminated. This is in agreement with research by Wang, et al. (2021) that showed that food withdrawal and re-feeding improve the digestibility of food and increase the level of lipid substances in blood serum.

The mean concentration of cholesterol showed that both male and female indigenous chicken were highest in group 1, followed by group 3, and lowest in group 2. The reason Indigenous chicken in group 2 have the lowest concentration of cholesterol is that they were starved for 60 hours (Table 3). The Indigenous chicken in group 3 was also starved for 60 hours but re-fed for five days, thus why it had more cholesterol than group 2. The result correlates with a study conducted by Peeble *et al.* (2014) on the effect of fasting on serum cholesterol concentration in egg-laying hens. The result indicated that total serum cholesterol concentration decreased after fasting.

The mean concentration of protein showed that the starved indigenous chicken in Group 2 had the highest concentration of proteins, followed by the chicken in Group I, and the lowest in Group 3, which were starved and re-fed (Table 3). Fasting significantly affects the expression of structural proteins, proteins involved in lipid transport, those involved in stress response, and intestinal defense proteins. When these proteins are mobilized to do their functions during starvation, they increase the total protein concentration. This finding is in agreement with a study conducted by Simon *et al.* (2019) on the change of proteins in the small intestine of chicken subjected to fasting. It also correlates with a study conducted by Okumura and Tasaki (2019) that found that fasting for 72 hours caused a marked increase in plasma uric acid concentration, which increased 10 times the initial concentration. However, upon re-feeding after 6 hours, the concentration of uric acid in plasma started decreasing to normal, as in Group 3 of this study.

The mean concentration of alkaline phosphatase showed that Group 3 (starved and re-fed) (636.55 u/L) had the highest concentration of alkaline phosphatase, followed by Group I (control, fed throughout) (223.09 u/L), and the lowest in Group 2 (starved) (94.68 u/L) (Table 3). The reason for the decline of alkaline phosphatase activity

during starvation can be attributed to factors such as a fall in the rate of synthesis caused by lowered metabolic demands and an electrolyte imbalance caused by tissue overhydration (Shaffi, 2019). The results in this study are similar to research by Lone and Akhtar (1988) on starvation and re-feeding of Japanese Quail, which reported that Quails fasted for 3 days and those fasted for 5 days their alkaline phosphatase activity significantly decreased. After re-feeding the Alkaline phosphatase activity increased.

Growth Performance

This parameter was measured by weighing the chicken of the three groups after every 12 hours. Analysis of weight measured after 12 hours showed that in Group 1, there was a slight decrease in weight measured from the initial to the end of 60 hours. The average initial weight was 1.00375 g (females), and 1.1325 g (males), while the final average weight after 60 hours was 0.985 g (females), and 1.12375 g (males), a reduction of 0.01875 g (females) and 0.00875 g (males). Therefore, there was no feed efficiency in the Group 1 indigenous chicken investigated in this study.

The results revealed that the initial average weight of chicken in Group 2 was 1.10875 g for females and 1.17500 g for males. This reduced to 1.01375 g (females) and 1.0225 g for males after 60 hours of starvation, a reduction of 0.095 g for females and 0.1525 g for males. The reduction was greater than in Group 1 (Table 4). The result correlates with a study conducted by Xue *et al.* (2021) on the effect of fasting on the weight loss of broilers, the result revealed that feed withdrawal increased the weight loss of the chicken.

The chicken in Group 3 average weight declined after 60 hours of starvation. It was a reduction of 0.085 g for females, and 0.0987 g for males. Re-feeding of Group 3 increased average weight, an increase of 0.1925 g in females and 0.1197 g for males. The 8 Indigenous chicken were fed 1000 g of growers' mash per day, which translates to 125 grams per chicken. Feed efficiency, which is the weight gain of each chicken per gram of feed taken by the chicken, was calculated as the total weight of feed consumed / Total grams of weight gain. This translates to 125/0.1925, which is 649.351 for five days of re-feeding for females and 125/0.1197, which is 1044.277 for males. When divided per day, it is 129.870 (649.351/5) for females and 208.855 (1044.277/5) for males. The result is in agreement with the study by Wei *et al.* (2020), which found that starvation followed by re-feeding improves feed efficiency.

CONCLUSION

The following conclusions were made;

The pancreas and stomach are the most affected organs by starvation and re-feeding of indigenous chicken. This is because starvation followed by re-feeding increases the size of the stomach and pancreas faster than normal.

Short-term starvation decreases glucose, lipid, and cholesterol concentration, while it increases the concentration of protein and alkaline phosphatase in the blood. Glucose, cholesterol, and lipid concentrations decrease because they are oxidized to provide energy.

However, protein concentration increases because the type of protein involved in lipid transport, stress response, and intestinal defense is activated. Short-term starvation followed by re-feeding increases the activity of the alkaline phosphatase in the blood than normal.

Short-term starvation followed by re-feeding improves feed efficiency and increases weight gain.

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