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Research Article

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Effect of Photoperiod on Growth and Immune Responses of the Pearl Guinea Fowl in Hot Humid Environment

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ABSTRACT

This study examines the photoperiodic effect on growth and immune responses of the pearl Guinea fowl. Keets used for this investigation were subjected to different photoperiod levels: 12HL:12HD, 14HL:10HD, 16HL: 8HD, and 18HL:6HD using a white bright LED Energy saving bulbs of 120 watts with light intensity of 5.60 lux and kept from day-old to eight weeks in a completely randomized design. Findings from this study showed that, at starter and grower phases the weight of the birds and feed consumed increased (P<0.05) with increasing levels of photoperiod. Birds subjected to 12HL: 12HD had the poorest (P<0.05) feed conversion ratio as compared to all the other treatment groups at all stages of growth. Birds subjected to 18HL: 6HD had the lowest (P<0.05) mortality rate followed by 16HL: 8HD and 14HL:10HD, while birds on the 12HL:12HD treatment recorded the highest (P<0.05) percentage mortality rate. Basophil level was highest (P<0.05) among birds subjected to 16HL: 8HD and 12HL: 12HD recorded the highest (P<0.05) monocyte levels in the blood. The levels of PCV, RBC, WBC, albumin, cholesterol, globin, and total serum protein in the blood increased (P<0.05) with increasing levels of photoperiod. As the levels of photoperiod increased, blood pH, Cl⁻ and K⁺ also increased. In conclusion, increasing photoperiodic levels up to 18HL: 6HD promotes rapid growth, ensures better feed utilization, reduces mortality rate and maintains good health in Guinea fowls.

Key words: Photoperiod, survivability, mortality, growth performance, immune responses

INTRODUCTION

Guinea fowl production is gaining much attention and recognition across the globe. The meat of the bird is an important source of protein for humans worldwide (Okyere et al. 2020). In recent times, the micro environmental factors for growing Guinea fowls and broilers are attracting a lot of scientific inquiries due to the fact that environmental factors have both positive and negative impacts on feed and water consumption, feed conversion efficiency (Lewis 2010), growth performance, hormonal production, physiological adaptations, and meat quality (Wu et al. 2022). Duration and source of light, temperature, humidity, air movement, and sanitation are the most important environmental factors influencing Guinea fowl and broiler production in modern farms (Wu et al. 2022).

Studies conducted by De-Oliveira and Lara (2016) and Wu et al. (2022), manipulation of light at different regimes for broilers influence feed and water intake with higher feed conversion efficiency. Hajrasouliha and Kaplan (2012) also reported that light supplementation to broiler chickens modulates systematic immune responses, reduces physiological aggressive behaviors, and improves health and welfare (Riber 2015). All over the world, birds are known for their seasonal production performance, and they mostly lay eggs during the rainy season (Kyere et al. 2017). Unfortunately, in the rainy season, Guinea fowls lay more fertilized eggs, with few eggs produced in the dry season. The higher demand for Guinea fowl meat and eggs is not met in Ghana and other countries due to the low productivity of the bird (Avornyo et al. 2016).

The bird's inability to lay all year round is one of the key challenges to commercialization and intensive production in the country and other African countries. This situation has called for extensive research to delve into the factors responsible for the seasonal breeding behavior of

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Guinea fowls and ensure that the birds lay eggs throughout the year. Among all the modern techniques for growing Guinea fowls, such as proper feeding, regular medication, proper housing, artificial insemination, and appropriate sex ratio, among others, manipulation of light is regarded as the most effective technology in broiler production which can control seasonality in birds and ensure successful breeding all year round (Dawson et al. 2001). Dawson et al. (2001) reported that photoperiod controls seasonality in birds and ensures maximum production.

In poultry birds especially chicken and Guinea fowl, the eyes receive light through the pineal gland (Dawson et al. 2001; Kuenzel et al. 2015). These mediators affect poultry birds' ability to detect light. Once the light is detected, information can be converted into biological signals, affecting the neuroendocrine system, especially the hypothalamic-pituitary-gonadal axis, which consequently exerts on the circadian rhythms and changes in the levels of the immune components in the blood, leading to a lot of physiological changes.

Hence, a light regime in poultry production appears to be indispensable to maximize the growth potential of poultry birds for the economic benefits of the farmer and the country at large (Wu et al. 2022). Unfortunately, this innovative technology has not been studied extensively in the case of Guinea fowls in Ghana and worldwide. With the increasing concern on maximizing Guinea fowl production efficiency, immunity, and behavior, it is important to research to delve into the problem of off-season laying in Guinea fowls to maximize the productivity and economic potential of the bird. Hence, there is a need to research the growth and immune responses of Guinea fowls subjected to photoperiod.

MATERIALS AND METHODS

Ethical approval

This research was developed from an ongoing PhD research work in reproductive physiology (animal science) with approval from the Animal Care and Ethics Committee of the Department of Animal Science, Kwame Nkrumah University of Science and Technology, Kumasi-Ghana. This research also strictly followed the ethics and animal handling guidelines and procedures outline in the Guide for the care and management and use of Poultry Birds in Research and Teaching by the Federation of Animal Science Societies (American Poultry Science Association, 2020).

Information about the study area

This study was set up at on-farm at Dawu near Jamasi in the Ashanti Region of Ghana, between 27th June, 2023 and 17th October, 2023. This study lasted for 16 weeks. Dawu is located in the Sekyere South District and on the Kumasi-Ejura Road in the Ashanti Region of Ghana. Dawu is 293 meters above sea level in the transitional zone characterized by abundant tropical rain forest and well known for its poultry farming activities in the Ashanti Region. The town coordinates are 6°43'60" N and 1°31'60" W in degrees minutes seconds (DMS). The latitudes and longitude of the area are 07o 04' and 01o 24' degrees respectively. The average minimum and maximum temperatures recorded during the study period were 20.89 and 30.4oC respectively (Ghana Meteorological Agency 2023). The town experiences a bimodal rainfall pattern between April-July and August-November and also experiences a dry season which occurs within December-March.

Experimental animals, design and treatments

Pearl Guinea fowls totaling 120 were randomly selected at day old and put on four different lighting treatment groups. Each treatment was replicated 3 times with ten birds per replicate in a completely randomized design. The treatment groups were: 12HL:12HD, 14HL:10HD, 16HL: 8HD, and 18HL:6HD using white bright LED Energy saving bulbs of 120 watts and a rechargeable lamp of 120 watts with a combined mean light intensity of 5.60 lux. Where HL: Hours of light and HD: Hours of darkness.

Experimental diets

A single diet was formulated and used for feeding the experimental birds (Table 1).

Table 1	1:F	eed	ingred	ients	and	nutrient	compositions

Feed ingredients	Starter	Grower			
	(0-8 weeks)	(9-16 weeks)			
Maize	56.2	58.00			
Wheat bran	7.00	13.20			
Soya bean meal	16.00	14.50			
Tuna fish meal	18.00	11.50			
Oyster shell	0.70	0.70			
Dicalcium phosphate	0.70	0.70			
Vitamin premix	0.70	0.70			
Common salt	0.70	0.70			
Total	100	100			
Nutrient compositions of the diets					
Ash, %					
Crude protein, %	22.10	19.45			
Crude fiber, %	3.37	3.78			
Moisture, %	10.01	10.08			
Ether extract, %	4.53	4.52			
Dry matter, %	90.44	91.21			
Metabolizable energy, kcal/kg	2789.50	2892.50			

Vitamins: A (8100 I.U); B2 (2.00mg); B12 (5.10mg); (D3 (1511 I.U); E (2.60mg) and K (1.50mg); Folic acid (0.52mg); Nicotimic acid (8.11mg); Calcium Panthotenate (2.11mg); Choline Cloruro (49mg); Trace elements: Cu (4.61mg); Co (0.11mg); mg (49mg); Se (0.12mg); Antioxidant Butylated Hydroxytoluene (10.10mg); Carrier; Calcium carbonate q.s.p (2.61kg)

Feed and clean water were made available at all times without restrictions. Health management practices were strictly followed at all stages of growth. The various compositions at the starter and grower phases are presented in Table 1. The nutrient levels met the NRC (1994) nutrient requirement for growing poultry.

Parameters measured

At day-old and each stage of growth, the experimental birds were weighed to obtain their body weight and weight gain respectively. Daily, feed given and feed leftovers were weighed and recorded. In computing the feed conversion ratio, feed consumed by the birds in each treatment was divided by the weight gain.

Prior to the final stage of the studies, nine birds from each treatment group were randomly selected for blood collection and analysis. Blood from the experimental birds was collected early in the morning between 7-8 a.m. from the brachial wing vein. A 5mL of blood was drawn from each bird for hematological, biochemical, and hormonal analysis. In 3ml blood, Ethylene-Diamine-Tetra-Acid (2mg) was mixed and used for hematological studies. Other 2mL blood sample was allowed to coagulate, serum was collected for the biochemical analysis. The procedures and methods described by Okeudo et al. (2003) were followed to determine all the hematological components of the blood. A digital photo colorimeter (Model 312E) manufactured by Digital Photo Instruments company; in Germany was used to determine the hemoglobin content in the blood. Hematocrit levels were estimated by using the Wintrobe microhematocrit technique while RBC counts were determined using a coulter Electronic Counter (Model ZF) manufactured by Coulter Electronic Company Ltd. London. All the other hematological parameters were determined using a Neubauer hemocytometer.

The clotted blood samples at room temperature were spun in the centrifuge to isolate the serum from the blood cells and used for the analysis as follows; the method for determining total blood protein described by Keller (1984) was followed and the levels recorded. Bromocresol Green (BCG) and CHOP-PAP methods were used to determine blood albumin and cholesterol levels respectively. The difference between the blood albumin and total blood protein was estimated as the globulin content. Furthermore, blood pH, Ca2+, K+, Cl-, and Na+ levels in the blood were determined following the procedures described by Keller (1984).

Statistical analysis

GenStat version 11.1 was used to analyze all the data collected during the conduct of this study. The average treatment means were separated using the LSD programmed in the GenStat at a 5% significant probability level (Steel and Torrie, 1980).

 $Yij = \mu + \alpha i + eij$

 μ = The mean of the entire population

 αi = The main effect of photoperiod

eij = Is the error associated with rep k (Photoperiod)

RESULTS

Growth performance of guinea fowls subjected to photoperiod

The growth performance trend of the birds presented in Table 2 revealed that, the initial body weights of the birds were similar (P>0.05) across all the photoperiodic levels. This study's findings revealed that, at starter phase of growth, body weight, weight gain, and feed consumption significantly (P<0.05) increased with increasing levels of photoperiod. Again, increasing the levels of photoperiod ensured better (P<0.05) feed utilization (Table 2). Hence, birds on the 12HL:12HD had the poorest (P<0.05) feed conversion ratio as compared to the other treatment groups. At grower phase, initial and final body weights, feed consumption and conversion were highest (P<0.05) among birds on the 18HL: 6HD treatment and lowest (P<0.05) among birds on the control treatment. Body weight and weight gain increased (P<0.05) with decreasing levels of photoperiod. Birds on the 18HL: 6HD had the lowest

(P<0.05) mortality rate followed by 16HL: 8HD and 14HL: 10HD. While birds on the 12HL: 12HD recorded the highest (P<0.05) percentage mortality rate.

Blood profile of the pearl guinea fowls subjected to photoperiod

Results on the blood parameters presented in Table 3 revealed no significant (P>0.05) differences in the levels of eosinophil, hemoglobin, lymphocytes, mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH), mean cell volume (MCV), neutrophil and platelets across all the levels of photoperiod considered in this study. However, photoperiod had significant (P<0.05) effects on basophil, monocytes, packed cell volume (PCV), red blood cells (RBC), and white blood cells (WBC), albumin, cholesterol, globulin and total serum protein levels in the blood (Table 3). Basophil level was highest (P<0.05) among birds subjected to 12HL: 12HD and lowest among all the other levels which had similar mean values. Birds subjected to 16HL: 8HD and 12HL: 12HD levels of photoperiod recorded the highest (P<0.05) monocyte levels in the blood followed by birds subjected to 14HL: 10HD. While, birds subjected to 18HL: 6HD had the lowest monocytes levels in the blood. The levels of PCV, RBC, WBC, albumin, cholesterol, globin, and total serum protein in the blood increased (P<0.05) with increasing levels of photoperiod.

Effect of photoperiod on guinea fowls metabolite levels

The metabolite levels of the pearl Guinea fowls as influenced by photoperiod are presented in Table 4. Table 4 revealed that blood pH, Cl- and K+ were significantly (P<0.05) influenced by the photoperiod. However, photoperiod had little (P>0.05) effect on Ca2+ and Na+ levels in the blood. Results from this study revealed that, as the levels of photoperiod increased, blood pH, Cl- and K+ also increased.

DISCUSSION

Photoperiod effect on growth performance of the pearl guinea fowls

Lighting program is among the most important environmental factors that affect poultry production and play a critical role in birds' metabolism and similar effects were realized in this study. The increased in body weight, higher feed consumption with better feed conversion ratio with increasing levels of photoperiod could be associated with improvement in the bird's metabolism which is responsible for effective feed utilization, normal physiological functioning, and normal growth (Abo Ghanima et al. 2021). Hence, this explains why in this study, increasing photoperiod led to a significant increase in body weight, feed intake, and conversion ratio. Reduction in photoperiodic levels caused a reduction in body weight and the reasons for this reduction could be as a result of the reduction in the time of feeding (Abo Ghanima et al. 2021; Wu et al. 2022). Light stimulates higher production of thyroid hormones more specifically triiodothyronine which regulates metabolic activities in birds and ensures normal growth and development (Ozkanlar et al. 2021; Riaz et al. 2021).

Table 2: Effect of photoperiod on growth performance

Starter phase, g/bird	12HL:12HD	14HL:10HD	16HL:8HD	18HL:6HD	SEM	P value
Initial body weight	23.40	22.90	23.30	22.80	0.32	0.281
Final body weight	379.50 ^d	454.70 ^c	480.30 ^b	580.90 ^a	5.98	0.001
Body weight gain	356.20 ^d	431.80 ^c	457.00 ^b	558.10 ^b	6.03	0.001
Daily weight gain	12.50 ^a	15.40 ^c	16.30 ^b	19.90 ^a	0.26	0.001
Total feed intake	907.30 ^d	994.90 ^c	1074.50 ^b	1138.20 ^a	1.88	0.001
Daily feed intake	16.20 ^d	17.80 ^c	19.20 ^b	20.30 ^a	0.03	0.001
Feed conversion ratio	1.32 ^a	1.16 ^b	1.18 ^b	1.02 ^c	0.03	0.001
Grower phase, g/bird						
Initial body weight	379.50 ^d	454.70 ^c	480.30 ^b	580.90 ^a	5.98	0.001
Final body weight	1245.80 ^d	1282.10 ^c	1328.60 ^b	1368.20 ^a	2.85	0.001
Body weight gain	866.30 ^a	827.40 ^c	848.30 ^b	787.30 ^d	6.00	0.001
Daily weight gain	15.50 ^a	14.80 ^c	15.20 ^b	14.10 ^d	0.11	0.001
Total feed intake	2580.80 ^c	2565.20 ^d	2673.40 ^b	2634.10 ^a	7.24	0.001
Daily feed intake	46.10 ^c	45.80 ^d	47.70 ^a	47.04 ^b	0.13	0.001
Feed conversion ratio	2.98 ^d	3.10 ^c	3.15 ^b	3.35 ^a	0.02	0.001
Keets survivability						
Mortality, %	41.20 ^a	31.40 ^b	21.60 ^c	19.60 ^d	0.68	0.01
Survivability, %	58.80 ^c	68.60 ^b	78.40 ^a	80.40 ^a	4.80	0.01

Values with different superscripts in the same row are significantly (P<0.05) different. HL= Hours of light, HD = Hours of light, SEM = Standard error, P = Probability of main effect

Table 3: Effect of photoperiod on Guinea fowls' blood components responses	Table 3: Effect of	otoperiod on Guinea fowls' blood components response	es
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Hematological Parameters	12HL:12HD	14HL:10HD	16HL:8HD	18HL:6HD	SEM	P value
Basophil (%)	2.33 ^a	1.57 ^b	1.56 ^b	1.56 ^b	0.27	0.015
Eosinophil (%)	3.22	2.19	3.27	2.00	0.59	0.068
Hemoglobin (g/dL)	11.00	11.50	11.20	11.30	0.31	0.604
Lymphocytes (%)	45.80	51.70	42.50	47.40	4.79	0.295
MCHC (g/dL)	43.90	42.20	44.40	43.50	1.73	0.626
MCH (pg)	69.30	75.30	71.50	59.30	7.87	0.233
MCV (fl)	162.50	170.00	170.60	173.40	5.74	0.281
Monocytes (%)	5.52 ^a	4.37 ^b	5.89 ^a	3.40 ^c	0.76	0.010
Neutrophil (%)	45.50	42.80	48.40	48.30	4.08	0.484
PCV (%)	27.20 ^d	30.20 ^c	32.80 ^b	36.90 ^a	0.55	0.001
Platelets $(x10^{9}/L)$	19.20	18.90	20.10	22.10	1.95	0.368
RBC (x10 ⁹ /L)	1.87 ^d	2.29 ^c	3.12 ^b	4.46 ^a	0.21	0.001
WBC (x10 ⁹ /L)	3.06 ^d	4.73 ^c	6.17 ^b	6.77 ^a	0.22	0.001
Biochemical parameters						
Albumin (g/dl)	22.50 ^b	24.60 ^b	31.00 ^{ab}	45.70 ^a	7.75	0.023
Cholesterol (mmol/l)	4.90 ^d	5.59°	6.55 ^b	7.18 ^a	0.14	0.001
Globulin (g/dl)	21.80 ^c	27.30 ^b	31.20 ^a	33.50 ^a	1.38	0.001
Total serum protein (g/dl)	26.50 ^d	29.90 ^c	33.60 ^b	40.40 ^a	0.55	0.001

Values with different superscripts in the same row are significantly (P<0.05) different.

Table 4: Effect of photoperiod on Guinea fowls' metabolite levels

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Parameters	12HL:12HD	14HL:10HD	16HL:8HD	18HL:6HD	SEM	P value
Blood pH	7.16 ^d	7.25°	7.31 ^b	7.38 ^a	0.013	0.001
Ca ²⁺ , nmol/L	4.18	4.15	4.25	4.53	0.165	0.107
Cl ⁻ , nmol/L	110.90 ^{ab}	114.22 ^a	106.88 ^b	100.04 ^c	2.180	0.001
K ⁺ , nmol/L	4.50 ^b	3.71°	6.27 ^a	6.61 ^a	0.534	0.001
Na+, nmol/L	163.31	161.47	159.82	161.84	2.288	0.511
37.1		• • • • • •	(D 0 0 5) 1'66			

Values with different superscripts in the same row are significantly (P<0.05) different.

Increasing the lighting regime in poultry production triggers the hypothalamus to stimulate higher secretion of hormones such as insulin, cortisol, corticosterone, and progesterone which are responsible for weight management (Ozkanlar et al. 2021). Hence, this explains why in this study body weight and feed consumption increased with increasing levels of photoperiod. These findings were similar to the works done by Okyere et al. (2020), Abo Ghanima et al. (2021) and Wu et al. (2022).

The reduction trend in mortality with increasing levels of photoperiod and the rapid increased in survivability with increasing levels of photoperiod could be ascribed to the long duration of light that the birds were exposed to. Again, the differences observed could be attributed to the higher levels of basophil, monocytes, and packed cell volume in the blood observed among birds subjected to higher photoperiod levels as compared to 12HL: 12HD. Moreover, the levels of basophil, monocytes, and packed cell volume in the blood determine the health status of the birds and all these levels were within the normal range showing that birds do not have any kind of infection and other diseases that can possibly cause death (Abdelazeem 2019; Wu et al. 2022). The levels of albumin, globulin, and total serum protein (TSP) also explain why birds subjected to 12HL: 12HD recorded the highest percentage of mortality and the lowest percentage of survivability. The nutritional conditions and immune status of birds are determined by the levels of TSP (Bharadwaj et al. 2016; Diaz de Bustamante et al. 2018; Inoue et al. 2022). Low TSP is associated with higher mortality (Inoue et al. 2022) due to weak immune system functioning while normal TSP is associated with building a strong immune system capable of fighting pathogens that cause infections leading to higher survivability.

Blood profile of the pearl guinea fowls subjected to photoperiod

Basophil is a type of white blood cell capable of protecting the body from infections (Abdelazeem 2019). However, a higher basophil level is an indication that the birds are suffering from autoimmune disease and also an indication that the birds are suffering from severe infections (Abdelazeem 2019; Wu et al. 2022). Basophil levels recorded in this study were within the normal range (1.58-6.90%) reported by the NRC (1994) and Kokore et al. (2021) for Guinea fowls. It can be concluded that the birds were healthy and free from infection despite being subjected to different levels of photoperiod.

Monocyte levels were significantly influenced by photoperiod and the mean values recorded were within the normal range (1.0-11%) reported by the NRC (1994) and Kokore et al. (2021) for Guinea fowls. Monocytes play a key role in building a strong immune system capable of fighting against germs infection in the blood tissues (Abo Ghanima et al. 2021; Ozkanlar et al. 2021). This explains why in this study percentage survivability was very high with increasing levels of photoperiod.

The levels of PCV, RBC, WBC, albumin, cholesterol, globin, and total serum protein in the blood confirm why all the treatment groups except birds subjected to 12HL:12HD recorded few mortalities with the highest survivability rate. The levels observed are an indication that there will be normal blood volume (Tóthová et al. 2018) in the birds, improve blood oxygen-carrying capacity (Ozkanlar et al. 2021) and ensure rapid diffusion and normal functioning of the body. Again, the normal levels of RBC and WBC recorded in this study were indication of good transport of carbon dioxide and oxygen in the lungs and other parts of the body and eliminate dehydration and anemia thereby, maintaining the good health condition of the birds (Belinskaia et al. 2021). The level of cholesterol observed in this study is an indication of the proper functioning of cell membranes with high integrity and fluidity required for the proper synthesis of steroid hormones, vitamin D, and bile acids (Zampelas and Magriplis 2019). Hence, an adequate supply of light improves the proper functioning of the bile to be able to detoxify the body.

Albumin, globin, and total serum protein play an important function in the transport of ions, neutral molecules and maintain constant colloidal osmotic blood pressure (Tejero and Gladwin 2014; Tóthová et al. 2018; Belinskaia et al. 2021). This means that increasing in photoperiod increased the production of albumin, globin, and total serum protein due to the fact that increased in the duration of light triggers the hypothalamus and the pituitary gland to produce insulin, glucagon, thyrocalcitonin among other hormones responsible for protein production in the blood. Hence, it was not surprising that the survivability rate among birds used for this research increased with increasing levels of photoperiod.

Effect of photoperiod on guinea fowls metabolite levels

Physiological reactions in birds are very important for the maintenance of homeostasis. Hence the observed blood pH, Cl⁻ and K⁺ levels with increasing levels of photoperiod show that the blood levels were slightly alkaline (Hopkins et al. 2022) which will ensure good blood oxygenation, an indication of good health, a sign of normal body functioning and maintenance of normal acidbase balance. This could be further explained that the levels of blood pH, Cl⁻ and K⁺ will facilitate and improve many biological activities such as fluid retention and acidbase balance (Zampelas and Magriplis 2019) in the birds. Chlorine and potassium are necessary to ensure the normal functioning of the cell, promote growth, and regulate the functions of nerves and muscles (Zheng et al. 2013; Ozkanlar et al. 2021). Hence, it is not surprising that birds subjected to higher photoperiodic levels in this study gained higher body weight due to proper nerve and muscle functioning.

Conclusion

This study concludes that increasing photoperiodic levels up to 18HL: 6HD promotes rapid growth, ensures better feed absorption and utilization, and reduces mortality in Guinea fowls. Birds subjected to higher photoperiodic levels responded well physiologically by producing normal blood components and maintaining good health.

Authors contributions

All authors contributed to the study conception and design. Author Clement Gyeabour Kyere (CGK) is the main and corresponding author respectively. Authors Clement Gyeabour Kyere, Stephen Alfred Osei, Michael Boateng and Yaw Oppong Frimpong designed the study, wrote the protocol and wrote the first draft of the manuscript. Author Clement Gyeabour Kyere, Michael Boateng, Yaw Oppong Frimpong, Patrick Atta Poku Jnr. and Okyere Korankye performed the statistical analysis, managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

Conflict of interest

There are no conflicts of interest in connection with this paper, and the material described is not under publication or consideration for publication elsewhere.

Ethical standards

The manuscript does not contain clinical studies or patient data.

Data availability

All data that were evaluated during the research are available in the manuscript.

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Competing interest

The authors have no competing interests to declare that are relevant to the content of this article.

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