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INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/13140
DOI URL: <http://dx.doi.org/10.21474/IJAR01/13140>



RESEARCH ARTICLE

EFFECTS OF GUANIDINOACETIC ACID SUPPLEMENTATION IN BROILER CHICKEN DIET ON CARCASS CHARACTERISTICS

Ngazi Chaari Gabriel, Prof. Lucy Kabuage, Dr. Purity Nguhiu and Prof. Charles Gachuiiri
Kenyatta University, School Of Agriculture and Enterprise Development Nairobi, Kenya.

Manuscript Info

Manuscript History

Received: 10 May 2021
Final Accepted: 14 June 2021
Published: July 2021

Key words:-

Carcass Characteristics, Complete
Randomized Design, Guanidinoacetic
acid (GAA)

Abstract

Guanidinoacetic acid (GAA) is a natural organic acid in the body that acts as a precursor of creatine which plays the role of energy carrier in the cell. This study was conducted to determine the effects of supplementing broiler chicken feed with GAA on carcass characteristics. Two hundred-and forty, day old Cornish breeds were kept under a deep litter system. Six treatments with four replicates of 10 birds per replica were used. The experimental diets comprised of six treatments with varying levels of feed additive (GAA) supplemented in the diets; D1 (control) with no GAA, D2 was supplemented with (0.003% GAA/kg), D3 (0.006% GAA/kg), D4 (0.009% GAA/kg), D5 (0.012% GAA/kg), and D6 (0.015% GAA/kg). The treatments were laid down in a complete randomized design (CRD) with four replicates. Carcass characteristics were determined in the laboratory by analyzing the (abdominal fat, tissue protein, tissue fat and pH). There were statistically significant effects observed on abdominal fat, body tissue protein and body tissue fat of the carcass. The study concluded that GAA supplementation increased carcass characteristics, and provided better economic returns. The study recommends utilization of GAA supplementation at level of 0.12% GAA/Kg for rapid growth of broiler, enhanced abdominal fat, body tissue protein, body tissue fat, and utilization of GAA for better economic returns to the farmers.

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Introduction:-

One of the key components of poultry production is their diet, which is composed of feed. These feeds constitute the greatest proportion of cost associated with poultry production, with most of that cost relating to the high price of protein-furnishing ingredients. It is therefore important to look for alternative feed ingredients to use as substitutes. These alternative feed ingredients are categorized as co-products, by-products, and non-traditional feed ingredients [1].

However, these alternatives may have lower quality and higher variability of protein and amino acid concentrations compared with the original sources [4]. Therefore, to improve on feed efficiency with an aim of reducing the production cost, it is necessary to direct efforts on feed additives. One of the feed additives in use is Guanidinoacetic acid (GAA) which is a natural precursor of creatine (CREA) in the vertebrate body. According to Michiels et al. [8], GAA helps to improve the general performance of poultry in terms of growth, feed conversion rate (FCR) and feed intake (FI). Additionally, it is a precursor of creatine that is considered as more a stable molecule [3].

Corresponding Author:- Ngazi Chaari Gabriel

Address:- Kenyatta University, School of Agriculture and Enterprise Development.Nairobi, Kenya.

Most of the poultry industry aim at increasing the carcass yield and reduce carcass fatness especially abdominal since excessive fat in modern poultry strains has been one of the problems facing this industry[17]. In addition, excessive fat in modern poultry strains is a major problem facing poultry industry due to consumer's preference [17]. This has made consumers have preferences in terms of carcass characteristics (fat levels) hence affects consumer acceptance, which the Kenyan meat production, processing and distribution sectors must make enormous efforts to satisfy.

Whilst the immediate effect of guanidinoacetic acid on poultry has been well documented, the effects of guanidinoacetic acid supplementation in broiler chicken diet on growth performance and carcass characteristics on broilers production is poorly understood. This therefore formed the rationale of this study. Previous studies showed that GAA consumption reduced the amount of abdominal fat, which is directly correlated to the total body fat. Studies have shown that energy was deposited in the carcass of birds mainly as protein rather than fat during the early stages of chick's life [8], [12], [5].

Dietary supplementation with GAA resulted in a higher percentage of breast meat yields in the carcass [8]. However, [10] reported that the main effects of GAA supplementation and energy levels on carcass traits were not significant, although addition of GAA significantly reduced the liver percentage. This study scrutinized broilers growth and their carcass characteristics to ascertain if GAA inclusive levels had any considerable effect. The main purpose being to determine the effect of dietary inclusion of Guanidinoacetic acid on carcass characteristics of broiler chicken.

Materials and Methods:-

Study Animals

A total of 240-day-old Cornish breed chicks were obtained from a reputable firm and kept under the deep litter system a vaccine of Newcastle and infectious bronchitis was given a drop per eye at 7th and 21st days while Gumboro vaccine through drinking water was given at 14th and 28th days of age.

Experimental Treatments, Study Design and Study Layout

GAA and feed ingredients used for formulating the experimental diets were bought from a reputable feed manufacturing firm. The experiment had six formulated treatments as shown in Table 3.1 and Table 3.2. The feed additive (GAA) was supplemented in diet 2 to diet 5 with diet 1 being the control. That is Diet (D1) was the control with no GAA, diet 2 (D2) was supplemented with 0.003% GAA/kg, diet 3 (D3) was supplemented with 0.006% GAA/kg, diet 4 (D4) was supplemented with 0.009% GAA/kg, diet 5 (D5) was supplemented with 0.012% GAA/kg, and diet 6 (D6) was supplemented with 0.015% GAA/kg. The treatments were laid down in a complete randomized design (CRD) in four replicates.

The birds were randomly allocated to the six treatments and housed in groups of 10 birds of 7 females and 3 males per pen in 24 pens. The experiment was divided into two phases which were growing phase from 1st day to 21st day (Table1) followed by finishing phase from 22nd to 42nd day (Table2). The chicks were fed on broiler starter mash for the first 21 days and broiler finisher for the last 21 days. The feeding trial experiment lasted for 42 days.

The birds were divided into 6 groups of 40 chicks were assigned the six treatments. Each group was further subdivided into four replicates of ten chicks per replica. The birds were weighed and placed in 24 well ventilated pens of 1 m X 1 m where they were reared for 42 days. The chicks were fed on broiler starter for the first 21 days and broiler finisher for the next 21 days. The floor of each pen was covered with wood shavings to a depth of about 6 cm and maintained dry. The temperature in each pen was maintained by infra-red lights at 26-36°C up to 14 days of age, and then it was eventually reduced to room temperature by adjusting the height of the bulb. During the entire period of the study, the lighting program was 24 hrs light with free access to feed and water.

Table 1:- Broiler Starter Composition (1-21 days) diet in %.

Ingredient (Units)	D1	D2	D3	D4	D5	D6
Maize grain		48.05	48.02	47.99	47.96	47.93
Pollard	15	15	15	15	15	15
Corn oil		3	3	3	3	3
Soya bean meal		17	17	17	17	17
Fish meal		10	10	10	10	10

Corn gluten meal	5	5	5	5	5	5
Dicalcium Phosphate	0.10	0.10	0.10	0.10	0.10	0.10
Limestone	1	1	1	1	1	1
Broiler pre-mix	0.25	0.25	0.25	0.25	0.25	0.25
Coccidiostat	0.10	0.10	0.10	0.10	0.10	0.10
Iodized salt	0.50	0.50	0.50	0.50	0.50	0.50
GAA	0	0.03	0.06	0.09	0.12	0.15
Total composition of formulated						
Diet	100%	100%	100%	100%	100%	100%

D1= control with no GAA; **D2**= supplemented with 0.003% GAA/kg; **D3**= supplemented with 0.006% GAA/kg, **D4**= supplemented with 0.009% GAA/kg; **D5**= supplemented with 0.012% GAA/kg, and **D6**= supplemented with 0.015% GAA/kg.

Table 2:-Broiler Finisher Composition (22 -42 days) diet in %.

Ingredient	D1	D2	D3	D4	D5	D6
Maize grain	35.15	35.12	35.09	35.06	35.03	35
Pollard	20	20	20	20	20	
Wheat bran	23	23	23	23	23	23
Corn oil	3	3	3	3	3	3
Soya bean meal	17	17	17	17	17	17
Fish meal	10	10	10	10	10	10
Corn gluten meal	5	5	5	5	5	5
Dicalcium Phosphate	0.10	0.10	0.10	0.10	0.10	0.10
Limestone	1	1	1	1	1	1
Broiler pre-mix	0.25	0.25	0.25	0.25	0.25	0.25
Coccidiostat	0.10	0.10	0.10	0.10	0.10	0.10
Iodized salt	0.50	0.50	0.50	0.50	0.50	0.50
GAA	0	0.03	0.06	0.09	0.12	0.15
Total composition of formulated diet						
	100%	100%	100%	100%	100%	100%

Feed formulation

Two basal diets of broiler starter and broiler finisher diets were formulated basing on the Kenya Bureau Standards (KEBS) for nutrient requirements for broiler feeds [6]. Maize grain and pollard were used as the main sources of energy while soybean meal, fishmeal and corn gluten meal were used as protein sources. Broiler starter diet had 3000 kcal ME/kg of feed, protein content of 220 g/kg (DM basis), 1% calcium and available phosphorus levels at 0.4% in the diets while lysine and methionine levels were 0.11 % and 0.05% respectively (Table 3). Broiler finisher formulated contained 3400 Kcal ME/kg of feed, protein content of 180 grams per kilogram feed, calcium, and available phosphorous level of 1% and 0.4%, respectively while lysine and methionine levels will be 0.09 % and 0.04% respectively (Table4; as per NRC, 1994). For each diet, all the ingredients were weighed and mixed for 45 minutes using a small feed mixer.

Table 3:-Ingredient Composition (%) of the basal diets used in the study as per the Kenya Bureau Standards(KS EAS 90:2019).

Ingredient	Broiler starter %	Broiler Finisher %
Maize grain	48.05	35.15
Pollard	15	20
Wheat bran	0	23
Corn oil	3	6
Soybean meal	17	1
Fishmeal	10	9
Corn gluten meal (60%)	5	5

Dicalcium Phosphate	0.1	0
Limestone	1	0
Broiler premix	0.25	0.25
Coccidiostat	0.1	0.1
Iodized salt	0.5	0.5
Totals Composition of formulated diets	100%	100%

Table 4:-Composition of Formulated Diets as per the Kenya Bureau Standards(KS EAS 90:2019).

Broiler starter	Broiler finisher	
Composition		
Metabolizable energy (ME) Kcal/kg	3000	3400
Crude protein %	22	18
Calcium %	1	1
Available Phosphorus %	0.4	0.4
Crude fibre %	6	6
Lysine %	0.11	0.09
Methionine %	0.05	0.04

Table 5:-Composition of formulated diets.

Ingredient	Broiler starter %	Broiler Finisher %
Maize grain	48.05	35.15
Pollard	15	20
Wheat bran	0	23
Corn oil	3	6
Soybean meal	17	1
Fishmeal	10	9
Corn gluten meal (60%)	5	5
Dicalcium Phosphate	0.1	0
Limestone	1	0
Broiler premix	0.25	0.25
Coccidiostat	0.1	0.1
Iodized salt	0.5	0.5
Totals Composition of formulated diets	100%	100%

Animal health care

The chicks were vaccinated against Newcastle disease and Infectious bronchitis diseases (IBD) (B.NO :B1IBH20-004) at the age of 7 days and another repeat of Newcastle disease and IBD (B.NO :IN20-008 at 21st days of age of a drop per eye. The birds through drinking water were also vaccinated against Gumboro at 14th and 28th days of age (B.NO Georgian strain BQ 3020). All vaccinations were followed with a vitamin boost for 3 days. The birds were monitored daily to assess their health in terms of their activeness during feeding, watering, and their behavioral aspect like posture and movement within the cage.

Chemical analyses of the formulated diets

The experimental basal diets were subjected to proximate analysis using [2]. procedures while the Calcium and phosphorus contents were determined using atomic absorption spectrophotometry and calorimetry, respectively.

Broiler performance

The body weight gain (BWG) of the chicks and the feed intake (FI) was recorded on a weekly basis. Feed intake was the difference between the cumulative daily feed offered and left over at the end of every week. Body weight gain was the difference in weight at the beginning and end of each week. The weekly performance of the two parameters (BWG and FI) for each replicate were summed up at the end of the starter and finisher phase to determine the changes in each phase. The means for the four replicates per treatment were used to compute the

treatment performance per bird in terms of body weight gain and feed intake. Feed conversion ratio (FCR) was determined as a ratio between feed intake and body weight gain at the end of each phase [16].

$$FCR = FI / \Sigma WG$$

The feeds were weighed in bulky every week and offered daily ad libitum. The amount of feeds consumed and the weight of each birds in the cage was taken weekly in the morning after feeds and water have been withdrawn.

Carcass characteristics

At the end of the study (42 days) two birds from each replica to make eight per treatment, were randomly picked and tagged. The birds were starved off feed for 12 hours but provided with drinking water. The selected birds were sacrificed and abdominal fat pad (an indicator of carcass fat content) from each carcass was removed and weighed using an electronic weighing scale, while fresh and the mean weight for each treatment recorded. The abdominal fat was calculated as a percentage of the final body weight and used as an indicator of carcass fat content. The breast meat from each carcass was cut off and used to determine postmortem pH, tissue protein %, and tissue fat %.

Statistical analysis

The collected data on feed intake, feed conversion rate, body weight gain, abdominal fat body tissue fat and pH was recorded in Microsoft excel 2008 and later was subjected to analysis of variance (ANOVA) using GenStat software 15th edition. The level of statistical significance was preset at $p \leq 0.001$. The mean separation test was determined using the Turkey HSD test procedures.

Results:-

Effect of dietary inclusion of Guanidinoacetic acid on carcass characteristics of broiler chicken.

Abdominal fat, body tissue protein, body tissue fat and pH

Results revealed that there was significant effects ($P \leq 0.001$) exhibited on abdominal fat, body tissue protein and body tissue fat of the carcass. However there was no significant effect on pH of the carcass Table 6. The lowest abdominal fat (13.1), body tissue protein (30.5%) and body tissue fat (9.33%) was obtained in 0.00%GAA/Kg.

Table 6:- Effect of dietary inclusion of guanidinoacetic acid on abdominal fat, body tissue protein, body tissue fat and pH.

Treatment (%GAA/Kg)	Abdominal fat (%)	BTP (%)	BTF (%)	pH
0.00 %	13.10 ^c	30.50 ^b	9.33 ^d	6.60 ^a
0.09 %	16.05 ^{bc}	34.28 ^{ab}	12.48 ^{cd}	6.71 ^a
0.03 %	19.40 ^{ab}	32.07 ^b	17.65 ^b	6.77 ^a
0.06 %	20.40 ^a	39.58 ^a	18.43 ^{ab}	6.60 ^a
0.15%	20.43 ^a	32.45 ^b	15.16 ^{bc}	6.84 ^a
0.12 %	22.07 ^a	39.17 ^a	21.38 ^a	6.78 ^a
P value	≤ 0.001	≤ 0.001	≤ 0.001	0.44

Values followed with similar letters in the same column are not significantly different at $p \leq 0.001$.

Results revealed that both abdominal and body tissue fat were significantly influenced by GAA and they were much lower than the recorded levels of body tissue protein. This finding corroborates with the findings of other researchers [8], [12], [5]. whose studies showed that energy was deposited in the carcass of birds mainly as protein than fat during the early stages of chick's life. Generally, broiler chickens proportionally gain more carcass protein than fat in their early stage of growth. Inclusion of GAA in the feeds enhanced body tissue protein although the highest level 0.15% reduced it concentration. In this situation, it can be considered that the protein needs for optimum cell-mediated immune response may be reduced when diets contained optimum levels of GAA. Similar findings have been reported previously [9]. Also studies have suggested that GAA supplementation in feeds may spare arginine and help protein synthesis, which finally increases meat yield[3]. There were no significant differences exhibited in the pH. The findings differ with those of [9], who reported that dietary supplemental GAA significantly reduced pH of meat. Also [14]reported that increasing dietary supplementation of GAA could increase

the muscle pH value and water holding capacity in growing finishing pigs. This manifestation could be attributed to same muscle glycogen levels of the broilers at the time of slaughter which is known to determine the ultimate pH.

Carcass characteristics results revealed that significant effects was exhibited on abdominal fat, body tissue protein and body tissue fat of the carcass. The lowest abdominal fat (13.1%), body tissue protein (30.5%) and body tissue fat (9.33%) was obtained in 0.00% GAA/Kg.

Conclusion:-

The study concluded that:

Guanidinoacetic acid supplementation at level (0.12% GAA/Kg) influenced broilers growth characteristics positively such that it increased feed intake, both lowest average feed intake and total feed intake were recorded. Also it gave the highest feed conversion rates, body weight and average weight. This showed that GAA supplementation at level (0.12% GAA/Kg) gave the best results.

Dietary inclusion of Guanidinoacetic acid increased carcass characteristics of broiler chicken. Lowest abdominal fat, body tissue protein and body tissue fat of the carcass were obtained in 0.00% GAA/Kg while the maximum abdominal fat and body tissue fat were recorded at level 0.12% GAA/Kg. although the highest body tissue protein were recorded at level 0.06% GAA/Kg.

Recommendations:-

Based on this study the following recommendations were made:

1. Guanidinoacetic acid supplementation should be utilized by broiler farmers who should also feed their birds with standard feeds to gain more especially at level 0.12% GAA/kg since increases the growth performance of the birds. GAA should be utilized from the onset of broiler rearing.
2. Guanidinoacetic acid supplementation should be utilized at level 0.12% GAA/kg by broiler farmers since affects broilers carcass characteristics positively. The customers will therefore prefer this kind of birds due to their qualities.

Recommendations for further Study:-

For maximum benefit of broiler industry the following research areas of interest should be considered:

1. A study should be carried out to establish the long-term effects of GAA on broilers.
2. A study to establish the benefits of GAA supplementation in feeds meant for other chicken breeds.

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Appendix I: General requirements for compounded poultry feeds.

SNo	Characteristic	Required quantity in feed on 12 % moisture basis	Test method
i)	Moisture content (max.)	12.0	KS ISO 6496 ^{a)}
ii)	Crude protein (min.)	18-22	KS 01-63-1 ^{b)}
iii)	Acid insoluble ash (max.)	4.0	KS 01-63-1
iv)	Crude fibre (max.)	7.5	KS 01-63-1
v)	Sodium chloride (max.)	0.5	KS ISO 6495 ^{c)}
vi)	Crude fat (max.)	10.0	KS ISO 6492 ^{d)}
vii)	Calcium		KS 01-63-1
	a) Layers	3.5-4.5	
	b) Breeders	3.3-4.0	
	b) Others	0.8-1.2	
viii)	Phosphorus available		KS ISO 6491 ^{e)}
	a) Poults (min.)	0.6	
	b) Broilers and ducklings (min.)	0.45	
	c) Chicks, layers and breeders (min.)	0.40	
	d) Growers (min.)	0.35	

Source: Kenya Bureau Standards (KS EAS 90:2019)

Appendix II: Proximate analysis results for Experimental broiler starter and Broilerfinisher diets.

Component	Broiler starter	Broiler finisher
Dry matter	92.47	92.67

Ash	6.23	7.45
Crude protein	22	22
Ether extract	6.04	6.5
Crude fibre	6	6
NFE	57.94	7.82
Metabolizable energy (kcal/kg)	3000	3400

Appendix III:- Feed intake per week per treatment.

Treatment	Week					
	1	2	3	4	5	6
0.00 %GAA/Kg	29.45	447.70	755.50	1244.00	1250.00	1584.00
0.03 %GAA/Kg	28.26	361.40	701.60	1090.00	1326.00	1333.00
0.06 %GAA/Kg	26.28	353.20	533.20	955.00	1161.00	1182.00
0.09 %GAA/Kg	35.25	455.00	666.50	1297.00	1277.00	1402.00
0.12 %GAA/Kg	26.59	290.50	403.50	856.00	1045.00	1147.00
0.15 %GAA/Kg	25.99	421.80	663.50	1265.00	1370.00	1248.00