

Dietary vitamin E and selenium yeast supplemented with L-arginine enhanced laying performance and egg quality attributes of guinea fowl (*Numida meleagris*)

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SUMMARY

The need to increase the commercialization and popularity of guinea fowl and better exploit their higher nutritional value compared to other poultry species has necessitated the search for feed additives that will enhance their egg laying potential. A 70 d study was carried out to investigate the laying response and egg quality attributes of guinea fowl fed diets containing vitamin E (vit. E) and selenium (Se) yeast with or without L-arginine (L-arg). A total of 240 helmeted guinea hens at the age of 20 weeks (average weight $2,11 \pm 0,02$ kg) were allotted to 8 dietary treatments. Each treatment was replicated three times, with 10 birds per replicate, in a completely randomized design. The dietary treatments were arranged in a 4×2 factorial system, with four combinations of additives, i.e. (1) no additive, (2) Se yeast (0,3 mg/kg), (3) vit. E (30 IU/kg), and (4) Se yeast + vit. E) and two variants of arginine supplementation: (1) no additive or (2) 1 g L-arg/kg of feed. Laying performance and egg quality were analysed by two-way ANOVA using a general linear model. Supplementation with L-arg improved ($P < 0,05$) the number of eggs collected per day (19,73 eggs) and daily production (65,76%) compared with the diet without L-arg supplementation (17,68 eggs, 58,90%, respectively). Birds fed a diet supplemented with vit. E + Se yeast had the highest number of eggs collected per day (19,40 eggs) compared to all other groups ($P < 0,05$). Also, birds fed various



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additives had higher ($P < 0,05$) hen-day production (63,33%) compared with those fed the control diet.

Birds fed the diet containing vit. E + Se yeast supplemented with L-arg had the highest ($P < 0,05$) total number of eggs collected per day (21,00 eggs) and hen-day production (70%). Supplementation with all tested additives had a significant effect ($P < 0,05$) on yolk weight (8,06-12,53 g), yolk height (10,49-16,73 mm), yolk percentage (24,62-35,17%) and albumen weight (14,04-17,06 g). Supplementation with all tested additives led to an increase in the number of eggs produced per day. It was concluded that the inclusion of vit. E and Se yeast supplemented with L-arg enhanced the laying performance and egg quality attributes of guinea fowl. Further studies should be conducted using other poultry species such as turkey, quail and broilers.

KEY WORDS: guinea fowl, selenium yeast, vitamin E, L-arginine, laying performance, egg quality

INTRODUCTION

The term 'guinea fowl' is the common name of the seven species of gallinaceous birds of the family Numididae, which is indigenous to Africa. It is well adapted to the African continent. The strains are descended from the helmeted guinea fowl, *Numida meleagris*. In many parts of the world, most people raise guinea fowl for their meat and eggs. Guinea fowl meat has a taste similar to that of other game birds and has many nutritional qualities that make it a valuable addition to the diet (Saidu et al., 2022). Guinea fowl eggs are an important source of energy, protein and other nutrients for humans, and their rational consumption stimulates metabolic functions in the body and increases resistance to disease. They are packed with minerals and vitamins even in their small size. Guinea fowl eggs are considered a delicacy and have good flavour (Lala et al., 2022). Nutritionally, the whole guinea fowl egg contains 13,56% crude protein; 12,09% fat; 1,02% carbohydrates; 0,04% crude fibre; 0,79% ash and 72,5% water (Oluwafemi and Udeh, 2016). However, there is a need for increased effort to improve and increase guinea fowl production.

Some reproductive problems of humans and other mammals, such as placenta retention, abortion, premature birth, cystic ovaries, metritis, and delayed conception can be caused by vitamin E and Se deficiency (Fu et al., 2022). Selenium (Se) and vitamin E play a significant role in improving immunity, antioxidant status, and reproduction in animals. Selenium is required for the development and expression of non-specific humoral and cell-mediated immune responses (Salmon et al., 2009). Vitamin E is an indispensable cellular antioxidant (Prasad et al., 2022) and may help the immune response in chickens by protecting lymphocytes, macrophages, and other cells against oxidative damage (Leeson, 2007). Additionally, Vlaicu et al. (2022) reported that an increase in the vitamin E concentration and the incorporation of tocopherols in hen diets prevents oxidative deterioration of lipids. Ciftci et al. (2005) also showed that vitamin E supplementation significantly increased egg production in laying hens exposed to heat stress.

L-arginine has been considered a powerful secretagogue that helps to increase the release of growth hormone and insulin, mediated by insulin-like growth factor IGF-I in the bloodstream (Nyawose et al., 2022), as well as synthesis of nitric oxide, a highly reactive and short-lived radical implicated in regulating reproductive functions in poultry (Manwar et al., 2003, Fouad et al. 2012).

L-arginine is required in poultry diets to avoid the harmful influences of excessive free radicals produced during normal metabolism (Atakisi et al., 2009). However, less attention has been given to arginine in comparison to other amino acids in commercial feed formulations for guinea fowl. The literature also contains little information on the influence of vitamin E and Se yeast in the diets of laying guinea hens. In most cases, only feed-grade amino acids, such as lysine, methionine, and threonine, are present in the ingredient matrix software that considers the least-cost feed formulation used by the poultry industry. Therefore, it is not yet clear whether the benefits of arginine supplementation outweigh the costs (Kidd et al., 2013). This study was therefore conducted to determine the laying performance and egg quality attributes of guinea hens fed diets containing vitamin E and selenium yeast with and without L-arginine.

MATERIALS AND METHODS

The study was carried out at the Poultry Unit, Institute of Food Security Environmental Resources and Agricultural Research (IFSERAR), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The farm is located in the derived savannah vegetation zone of South West Nigeria (7°13'35,48"N, 3°25'39,01"E) at an elevation of 415 meters and eye altitude of 700 feet (Google Earth, 2017).

Test ingredients

L-arginine (Shanghai TECH, Chemical Industry testing Co Ltd, Shanghai, China), vitamin E in the form of all-rac- α -tocopheryl acetate (Rovimix® E50-Adsorbate), and selenium yeast (Se yeast) (Alltech, Inc., Nicholasville, KY) were used in the study.

Experimental design and dietary treatments

Breeding helmeted guinea hens (n = 240) at the age of 20 weeks (body weight (mean + SD) 2,11 ± 0,02 kg) were allotted to 8 dietary groups (3 replicates of 10 birds per group) in a completely randomized design. Dietary treatments were arranged in a 4 × 2 factorial arrangement, with four variants of vitamin and mineral supplementation, i.e. 1) none, 2) Se yeast (0,3 mg/kg), 3) vit. E (30 IU/kg), 4) Se yeast + vit. E), and two variants of arginine supplementation: 1) none or 2) 1 g L-arg/kg of feed. The Se yeast contained 98% organic Se.

The levels of the supplements were based on manufacturer's specifications. Each pen was an open-sided unit which had a concrete floor littered with wood-shavings. The temperature during the study ranged between 29,7 and 31,9°C, and relative humidity was 80% with rainfall of about 1,037 mm (Google Earth, 2017). A natural 12 ± 0,5 h cycle (equal length of day and natural dark in Nigeria) was maintained throughout the trial. A standard compound feed for guinea fowl layers (Table 1) was formulated for the study. The experiment lasted for 10 weeks. Feed in mash form and drinking water were provided *ad libitum*.

Table 1

Ingredients and chemical composition of standard compound feed for guinea fowl layers

Ingredients	Component (g/kg)
Maize	63,00
Soybean meal	23,00
Wheat offal	8,80
Bone meal	1,70
Limestone	2,60
Lysine	0,15
Methionine	0,20
*Premix	0,30
Salt	0,25
Total	100,00
Calculated nutrients	
Crude protein (%)	17,26
Crude fibre (%)	3,24
Calcium (%)	3,67
Available phosphorus (%)	0,49
Metabolizable energy (MJ/kg)	10,77
Lysine (%)	0,71
Methionine+ lysine (%)	0,62

*Composition of vitamin premix per kg: vitamin A 8,000 IU; vitamin D₃ 1600 IU; vitamin E 5 IU, vitamin K 0,200 mg; B vitamins: thiamine B₁ 0,5 mg; riboflavin B₂ 4 mg; pyridoxine B₆ 0,015 mg; Niacin B₃ 0,015 mg; cobalamin B₁₂ 0,01 mg; pantothenic acid B₅ 0,5 mg; folic acid B₉ 0,5 mg and biotin B₇ 0,020 mg; chlorine chloride 0,02 mg; anti-oxidant 0,125 g and minerals (Mn, Zn, Fe, Cu, I, Si, Co) 0,156 g

Data collection

The following parameters were measured:

Laying performance

All laying performance parameters, i.e. number of eggs collected, age at first egg, weight of birds at first egg, and weight of first egg, were calculated according to the formula reported by Oluyemi and Roberts (2000) and Makinde et al. (2014).

Hen-day egg production

Hen-day egg production for the experimental period (70 days) was calculated as the percentage of the total number of eggs laid to the number of hens in the house per replicate.

$$\text{Hen - day production (\%)} = \frac{\text{Number of eggs laid}}{\text{Number of hens in house}} \times \frac{100}{1}$$

Egg quality assessment

Egg quality was assessed on a daily basis between days 49 and 63 of the study. Twelve eggs were sampled each day from each treatment (1,344 eggs in total) for morphological and physicochemical assessment. The eggs were stored and assessed within 24 h of lay.

a. External egg qualities

i. Egg weight

Egg weight was determined with an electronic balance (Mettler-Teledo® PB 3002) with accuracy of 0,01 g.

ii. Egg shape index (ESI)

Egg shape index was calculated from the measurements of egg length and egg breadth using a vernier calliper, as the ratio of egg breadth to egg length.

$$ESI = \frac{\text{Egg breadth}}{\text{Egg length}}$$

b. Internal egg quality

i. Albumen height

The height of the thick white was measured off the chalazae at a point above midway between the inner and outer circumference of the thick white with a p6085 spherometer with accuracy of 0,01 mm.

ii. Yolk weight

The yolk was separated from the albumen using a plastic egg separator, and its weight was measured using a sensitive electronic balance (Mettler-Teledo® PB 3002 with sensitivity of 0,01 g). The yolk weight was also expressed as a percentage of the egg weight and recorded for each egg sample.

$$\% \text{ Yolk} = \frac{\text{Yolk weight}}{\text{Egg weight}} \times \frac{100}{1}$$

iii. Yolk colour

The yolk colour was determined using the Roche yolk colour fan.

iv. Albumen weight

The albumen weight was determined as the difference between the egg weight and the combined weight of the yolk and dry egg shell for an individual egg sample. The albumen weight was also expressed as a percentage of the egg weight and recorded as percentage albumen for the individual egg sample.

$$\% \text{ Albumen} = \frac{\text{Albumen weight}}{\text{Egg weight}} \times \frac{100}{1}$$

v. Shell weight

Egg shells were air-dried for 3 days in egg trays, and individual shell weight was determined with an electronic balance (Mettler-Teledo® PB 3002 with sensitivity of 0,01g). The shell weight was also expressed as a percentage of the egg weight and recorded as percentage shell (% shell) for the individual egg sample.

$$\% \text{ Shell} = \frac{\text{Shell weight}}{\text{Egg weight}} \times \frac{100}{1}$$

vi. Shell thickness

The thickness of the shell for an individual dry shell was measured with micrometer screw to the nearest 0,01 mm. The mean of triplicate measurements was taken as the shell thickness.

The Haugh unit (HU) was calculated using the values obtained for the egg weight and albumen height as expressed by Haugh (1937) and Makinde et al. (2014) in the following formula:

$$\text{HU} = 100 \log (H - 1,7 W^{0,37} + 7,6),$$

where:

H = albumen height in mm; W = egg weight in g.

Statistical model

Data generated from the experiment were subjected to 4 x 2 factorial arrangements using the general linear model procedure of SAS (2003). Significant means were separated by Duncan's Multiple Range Test according to Makinde et al. (2022). The model used was as follows:

$$Y_{ijk} = \mu + T_i + A_j + (TA)_{ij} + \varepsilon_{ijk},$$

where:

Y_{ijk} = observed value of dependent variables

μ = population mean

T_i = main effects of various additives (vit. E, Se yeast)

A_j = main effects of L-arginine supplementation

$(TA)_{ij}$ = interaction effect of the various additives and L-arginine supplementation

ε_{ijk} = sampling error

RESULTS

Effect of L-arginine supplementation and additives on laying performance of guinea fowl

The main effect of L-arginine supplementation showed that birds fed a diet supplemented with L-arginine had a higher ($P < 0,05$) number of eggs collected per day and higher hen-day production than those fed a diet without L-arginine supplementation (Table 2).

Table 2

Main effects of L-arginine supplementation and additives on laying performance of guinea fowl

Parameter	-Arg.	+Arg.	SEM	Control	Se Yeast	Vit. E	Vit E+Se	SEM
Age at first egg (days)	173,50	173,00	0,25	176,00	174,00	171,00	172,00	2,10
Weight at first egg (kg)	2,12	2,13	0,01	2,08	2,15	2,15	2,11	0,07
Weight of first egg (g)	32,54	34,98	1,22	32,79	3,23	34,19	34,80	0,79
No of egg collected/day	17,68 ^b	19,73 ^a	3,25	17,35 ^c	19,05 ^b	19,00 ^b	19,40 ^a	5,25
Hen-d (%) production	58,90 ^b	65,76 ^a	0,15	57,83 ^b	63,50 ^a	63,33 ^a	64,67 ^a	0,15

^{abcd}Means in the same row with different superscripts differ significantly ($P < 0,05$)

SEM - Standard error of mean

The main effect of the additives showed that birds fed a diet supplemented with vitamin E + Se yeast had the highest ($P < 0,05$) number of eggs collected per day, followed by those fed diets containing Se yeast and vitamin E, while birds fed the control diet recorded the lowest ($P < 0,05$) number of eggs per day. Birds fed the diet with all tested additives had higher ($P < 0,05$) hen-day production compared with the control (Table 2).

Birds fed a diet supplemented with L-arginine had a higher ($P < 0,05$) total number of eggs collected per day and higher hen-day production (%) than those fed a diet without L-arginine supplementation. Birds fed a diet containing vit. E + Se yeast supplemented with L-arginine had the highest ($P < 0,05$) number of eggs per day and hen-day production (Table 3).

Table 3

Interaction effect of L-arginine supplementation and additives on laying performance of guinea fowl

Parameter	-Arg.				+Arg.				SEM
	Control	Se	Vit E	Vit E+Se	Control	Se	Vit E	Vit E+Se	
Age at first egg (days)	177,00	175,00	172,00	170,00	175,00	173,00	170,00	174,00	2,50
Weight at first egg (kg)	2,05	2,10	2,20	2,11	2,10	2,20	2,09	2,10	0,15
Weight of first egg (g)	31,84	31,25	33,40	33,65	33,75	35,20	34,99	35,95	2,35
Number of eggs collected/day	17,00 ^d	17,90 ^c	18,00 ^c	17,00 ^c	17,70 ^c	20,20 ^b	20,00 ^b	21,00 ^a	5,20
Hen-day (%)	56,67 ^d	59,70 ^c	60,00 ^c	56,67 ^d	59,00 ^c	67,33 ^b	66,67 ^b	70,00 ^a	6,72

^{abcd}Means in the same row with different superscripts differ significantly ($P < 0,05$)

SEM - Standard error of mean

Effect of L-arginine supplementation and various additives (selenium yeast and vitamin E) on egg parameters

Birds fed the basal diet supplemented with L-arginine had higher ($P < 0,05$) values for egg length (46,01), yolk height (15,64) and albumen weight (15,95), while those fed the basal diet without L-arginine had a higher ($P < 0,05$) egg shape index (0,87). Birds fed the basal diet supplemented with vit. E and Se yeast had higher values ($P < 0,05$) for egg length and yolk height, while albumen weight (16,43) was higher ($P < 0,05$) in birds fed a diet supplemented with vit. E + Se yeast (Table 4).

Table 4

Main effect of L-arginine supplementation and the addition of vitamin E and selenium yeast on egg quality attributes of guinea fowl

Parameters	- Arg.	+Arg.	SEM	Control	Se	Vit E	VitE+Se	SEM
Egg weight (g)	34,75	34,67	0,51	33,92	35,49	35,30	34,12	0,73
Egg length (mm)	42,16 ^b	46,01 ^a	2,94	44,02 ^{ab}	45,86 ^a	45,86 ^a	40,50 ^b	3,33
Egg breath	35,40	35,90	0,39	35,41	35,67	36,67	34,93	0,56
Egg shape index	0,87 ^a	0,78 ^b	0,03	0,82	0,78	0,80	0,91	0,04
Yolk color	6,71	6,50	0,32	7,00	6,50	6,42	6,50	0,45
Yolk height (mm)	13,96 ^b	15,64 ^a	2,32	14,58 ^{ab}	15,18 ^a	15,84 ^a	13,61 ^b	2,45
Yolk weight (g)	10,63	10,66	0,28	10,34	11,29	10,80	10,17	0,40
Yolk percentage	30,57	30,79	0,82	30,43	30,82	30,55	29,90	1,16
Albumen height (mm)	6,93	7,23	0,12	6,90	7,10	7,25	7,07	0,17
Albumen weight (g)	14,77 ^b	15,95 ^a	2,25	14,53 ^c	14,84 ^{bc}	15,63 ^{ab}	16,43 ^a	1,36
Albumen percentage	42,84 ^b	46,10 ^a	1,94	43,17 ^b	41,84 ^b	44,45 ^b	48,42 ^b	1,33
Shell weight (g)	5,72	5,52	0,15	5,44	5,70	5,56	5,79	0,22
Shell thickness (mm)	0,84	0,81	0,02	0,86	0,79	0,86	0,80	0,02
Shell percentage	16,64	15,99	0,55	16,19	16,09	15,78	17,19	0,78
Haugh unit	91,29	92,95	0,68	91,40	92,00	92,82	92,29	1,33

^{ab}Means in the same row with different superscripts differ significantly ($P < 0,05$)

SEM= Pooled standard error of means.

Birds fed the basal diet supplemented with vit. E without L-arginine had higher ($P < 0,05$) yolk weight (12,53) and yolk height (16,12) than birds in the other groups. Yolk percentage (35,17) and albumen weight (17,06) were higher ($P < 0,05$) in birds fed the control diet supplemented with vit. E+Se and L-arginine compared to those fed other diets (Table 5).

Table 5

Interactive effect of L-arginine supplementation and the addition of vitamin E and selenium yeast on egg quality attributes of guinea fowl

Parameters	-Arg.				+Arg.				SEM
	Control	Se	Vit E	Vit E+Se	Control	Se	Vit E	VitE+Se	
Egg weight (g)	33,76	35,82	36,17	33,24	34,08	35,16	34,43	34,99	0,36
Egg length (mm)	41,82	45,86	45,49	35,47	46,22	46,06	46,22	45,54	0,80
Egg breath	34,84	35,15	36,42	35,20	35,97	36,06	36,91	34,65	0,28
Egg shape index	0,86 ^b	0,77 ^b	0,80 ^b	1,06 ^a	0,78 ^b	0,78 ^b	0,80 ^b	0,76 ^b	0,22
Yolk colour	7,00	6,67	6,67	6,50	7,00	6,33	6,17	6,50	0,21
Yolk height (mm)	13,87 ^c	15,36 ^{ab}	16,12 ^a	12,49 ^c	15,29 ^{ab}	14,99 ^{ab}	15,55 ^{ab}	16,73 ^a	3,33
Yolk weight (g)	9,95 ^c	11,99 ^{ab}	12,53 ^a	9,06 ^c	10,73 ^{bc}	10,58 ^{bc}	10,07 ^{bc}	12,28 ^{ab}	2,28
Yolk percentage	29,48 ^{bcd}	33,51 ^{ab}	34,65 ^{ab}	28,62 ^{bc}	31,39 ^{abc}	30,13 ^{abc}	29,46 ^{bc}	35,17 ^a	2,74
Albumen height (mm)	6,48	6,96	7,25	7,03	7,32	7,24	7,24	7,11	0,86
Albumen weight (g)	14,04 ^d	14,42 ^{cd}	14,81 ^{cd}	15,79 ^{abc}	15,02 ^{bcd}	15,26 ^{bcd}	16,45 ^{ab}	17,06 ^a	3,22
Albumen percentage	42,07	40,27	40,97	48,04	44,26	43,41	47,93	48,80	0,77
Shell weight (g)	5,26	5,76	5,65	6,21	5,61	5,64	5,47	5,36	0,11
Shell thickness (mm)	0,88	0,80	0,86	0,82	0,83	0,79	0,85	0,78	0,01
Shell percentage	15,85	16,11	15,62	18,98	16,53	16,06	15,95	15,40	0,39
Haugh Unit	89,18	91,13	92,56	92,33	93,62	92,87	93,08	92,25	0,50

^{ab}Means in the same row with different superscripts differ significantly ($P < 0,05$)
SEM - Pooled standard error of means

DISCUSSION

Several studies have reported on the laying performance of different varieties of guinea fowl in the tropics (Avornyo et al., 2016; Shoyombo et al., 2021). In Nigeria, Onunkwo and Okoro (2015) reported that Black helmeted guinea hens start laying at 28 weeks of age, yielding an average of 147 eggs per production cycle. Yakubu et al. (2014) reported overall average egg production of 79 eggs per year in backyard production, while in Ghana, Avornyo et al. (2016) reported a range of 41-114 eggs/hen.

In the current study, the increase observed in the total number of eggs among birds fed a diet supplemented with L-arginine could be attributed to the unique role of the digestion and absorption of arginine in promoting the utilization of dietary protein and its conversion to egg protein in breeding guinea fowl (Youssef et al., 2015). The increase could also be due to the acquired immunity of the birds following intake of arginine. According to Khajali and Wideman (2010) and Silva et al. (2012), arginine improves production by increasing the release of insulin, GH and IGF-I into the bloodstream. Corzo and Kidd (2013) and (Munir et al., 2009) observed a significant requirement for dietary arginine in chicks, associated with immune system development and early microbial challenges. Yu et al. (2018) reported improved body weight in chickens fed a diet supplemented with 2% or 4% arginine. The reduced number of eggs observed in the –Arg + Vit E + Se yeast groups showed the benefit of Arg supplementation in the diets of laying birds.

The increase observed in the number of eggs among birds fed diets supplemented with vit. E + Se yeast supports the earlier report of De Moura et al. (2022) of a synergistic relationship between selenium and vitamin E which increases performance in laying birds and protects against cellular damage by reactive oxygen species. Bilal et al. (2022) reported that combined supplementation with both vitamin E and selenium yeast led to synergistic enhancement of the immune response and disease resistance in birds. Singh et al. (2006) reported that vitamin E is known to regulate haem biosynthesis, stimulate immune responses, and play specific roles in the essential transport of amino acids (Singh et al., 2006), while Se is required for normal growth and reproduction (Bilal et al., 2022). The increase in egg numbers among birds fed a dietary combination of arginine, selenium yeast and vitamin E implies positive interactions among these additives. This is in agreement with previous studies (Kirunda et al., 2001; Puthongsiriporn et al., 2001).

Supplementation of diets with the tested additives significantly influenced the egg shape index, yolk height, yolk weight, yolk percentage and albumen weight. Birds fed diets supplemented with L-arginine had higher egg length, yolk height and albumen weight than the other groups. Egg weight has been reported to improve in hens receiving high-protein diets, especially diets supplemented with crystalline amino acids (Novak et al., 2006). The improvement observed in yolk height and albumen weight may be due to the improved amino acid profile of the diet following arginine supplementation. Yolk percentage and albumen weight were higher in eggs laid by birds fed a diet supplemented with vitamin E + selenium yeast and L-arginine. This could be due to the interactive effect of the three additives. However, Ciftci et al. (2005) and Irandoust et al. (2012) reported that supplementation of feed with 250 mg/kg of vitamin E had no significant effect on egg quality, while egg weight was reported to increase following Se yeast inclusion in the diet of laying birds (Natasha, 2012). Reis et al. (2012) observed that various arginine levels had no effect on egg quality differences. Silva et al. (2012) and Yang et al. (2016) reported that egg quality parameters were not influenced by different L-Arg levels. Further studies are needed to evaluate the combined effect of the additives tested in this study.

CONCLUSION AND RECOMMENDATIONS

The results of this study indicate that inclusion of vitamin E and selenium yeast supplemented with L-arginine enhanced the laying performance and egg quality attributes of guinea fowl. Further studies should be conducted using other poultry species such as turkey, quail and broilers.

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