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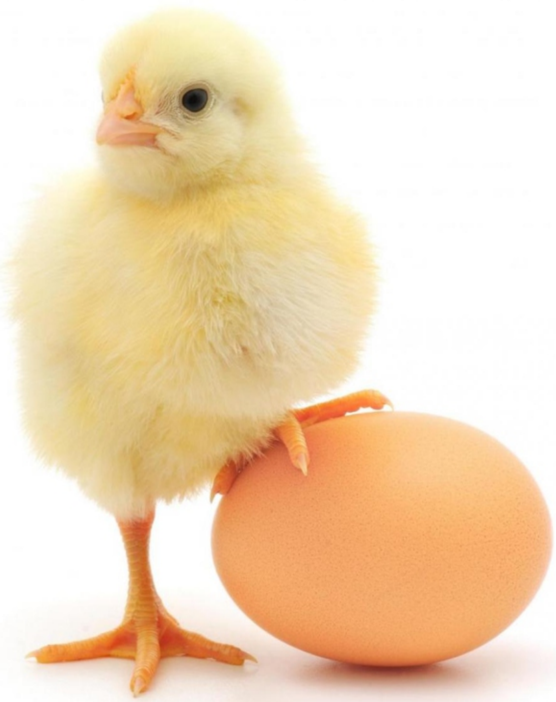

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Research Article
Effect of Pawpaw (*Carica papaya*) Seed Diets on Production Performance of Boiler Breeders and Hatching Parameter

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Abstract
Background and Objective: A major challenge in broiler breeder management is the nutritional requirement to optimize their reproductive performance. Additional levels of phytobiotics in the diet could improve broiler breeder reproductive performance and hatchability. The present study was conducted to evaluate the effects of Pawpaw seed meal in the diets on reproductive performance and egg hatching of Sasso breeder hens. **Materials and Methods:** Ninety hens and 12 males of Sasso strain were divided at random, into two groups of 45 hens and 6 cocks each. During the trial, feed intake, body weight, egg weight and egg component weights were recorded weekly. Blood samples were collected at 35 and 41 weeks of age to determine some serum parameter. At 31 and 43 week of age, respectively, 490 and 440, set table eggs were collected during 7 days and stored at 15°C and 70% relative humidity. Prior to setting for incubation, eggs were numbered, weighed, assigned into 4 replicates of 50 eggs in each dietary treatments. **Results:** Results indicated that breeders of *Carica papaya* seed diets group had heavier eggs (p<0.05) with higher ratio of albumen weight to egg weight (p<0.01) and feed conversion ratio than those of control group (p<0.05). In addition, day-old chicks from eggs of *Carica papaya* seed diet group were heavier (p<0.05) than those from eggs of control diet group. **Conclusion:** It was concluded that *Carica papaya* seed diets during lay period improved performance parameters and day-old chick weight. However, *Carica papaya* seed diets negatively influenced hatchability rate, ASAT, ALAT and Uric acid concentration.

Key words: Blood parameter, broiler breeder, broiler reproduction, *Carica papaya* seed, egg hatching, incubation event

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The nutrition of breeding hens has been a subject of research for many years and it is becoming clear that manipulation of their feed and feed intake are yielding results in their production performances. Furthermore, manipulating the feed of the parent stock to influence the growth of their offspring in a most economical way[1] has also been a subject of research. In the quest for alternative sources of feed materials, unconventional sources such as phytobiotics have been explored. Phytobiotics are rich sources of essential plant amino acids, vitamins, minerals and antioxidants[2]. Further to the rich contents mentioned, it has been established that phytobiotics are the cheapest and the most abundant sources of protein. Therefore, plant as a viable alternative of conventional feedstuffs should be explored as it saves cost. Botanical additives have shown the beneficial effects in poultry feeds, since its positive effects on feed intake, digestive secretions, immune stimulation, antibacterial, coccidiostatical, antiviral or anti-inflammatory activity have been reported[3].

Pawpaw is commonly known for its food and nutritional values throughout the world. The genus *Carica papayaLinn* is the most widely cultivated and best known species of the four genera that belongs to a small family of Caricaceae[4]. The seeds are numerous, small, black, round and covered with gelatinous aril. Medical research in animals and humans has confirmed the contraceptive and abortifacient capability of pawpaw seeds[5,6]. The seed of pawpaw has antimicrobial activity against Trichomonas vaginalis trophozoites. It could also be used in urinogenital disorder like trichomoniasis with care to avoid toxicity[7-6]. Pawpaw seed macerate has a clinical potential on conjugal R plasmid transfer from Salmonella typhimurium to Escherichia coli in vitro and in the digestive tract of genotobiotic mice[4]. The benzylisothiocynate present in seeds is the chief or sole antihelminthic[8].

Pawpaw analysis indicate high level of protein, energy and fat in seed. The mineral analysis also indicates the presence of major/macro elements which are required in large quantities in meals. Example of these are Na, K, Ca and some other. Few micro elements like Cu, Fe were also found[9]. All these afore-mentioned parameters are indication of good nutritional value of the pawpaw seeds. However, some anti-nutrient factors have been reported in pawpaw. These antinutrients-oxalate, tannins, phytic acide and phytic phosphorus are known for their ability to reduce the absorption of mineral elements[10]. Some other active substances in *Carica pawpaw*, like carpine and papain, have been found to be toxic[11] and/or with anti-fertility properties[12--14]; suggesting that the ingestion of pawpaw seeds may adversely affect the fertility status of mammals. Nideou *et al.*[15] studies the pawpaw seed and found significant differences in weight gain and better feed efficiency. The study revealed that dried pawpaw seed can be included in cockerel diets at 0.5% level. Bolu *et al.*[16] found that a 5% inclusion of pawpaw seed in the diet of broilers could improve performance parameters. However, the use of pawpaw seed in broiler breeder diet has received very little attention. Therefore, the objective of the current experiment was to investigate the effects of adding pawpaw seeds in the diets of broiler breeder on production and hatching performance of Sasso breeders.

MATERIALS AND METHODS

Experimental design: A total of 90 Sasso hens and 12 males were divided at random, into two groups each with three replicates. One group received the control feed designated as CONT. The second group received the experimental diet that contained pawpaw seed at 5% (CP). Diets' compositions and calculated nutritive values are shown in Table 1. In a previous study, 0.5% level was defined to give the best result of feeding trial[15]. Breeders' feed consumption, body weight, egg production rate and egg component weights were recorded weekly. Blood samples were collected at 31 and 43 weeks of age to determine protein, triglycerides, uric acid, aspartate aminotransferases and alanine aminotransferases concentrations. At 31 and 43 weeks of age, respectively 490 and 440 settable eggs were collected during 7 consecutive days and stored at 15°C and 70% relative humidity before setting for incubation. Sample of eggs were used to determine egg component weight according to treatment. Prior to setting for incubation, the eggs were numbered, weighed, assigned into 4 replications of 60 and 50 eggs each respectively according to feeding treatment and breeders age. The eggs were incubated in Petersime Vision®incubator (Headquartered in Olsene (Zulte), Belgium) at 37.6°C, relative humidity of 50% and turning each hour through an angle of

Table 1: Diets composition and macronutrient levels according to treatment

Feed stuffs	Control diet (%) (CONT)	Pawpaw seed diet (%) (CP)
Maize	55.00	54.50
Wheat bran	10.00	10.00
Fish meal 40%	11.00	11.00
Soya seed	15.00	15.00
Concentrate 5%	3.00	3.00
Oyster shell	6.00	6.00
Pawpaw seed	0.00	0.50
Calculated values		
EM (kcal kg^{-1})	2809.98	2802.65
CP	18.56	18.21

90°. At day 18 of incubation, eggs were weighed, candled and those with evidence of living embryos were transferred from turning trays to hatching baskets. During the last 2 day of incubation, hatching events were monitored and hatched chicks were recorded and weighed as well.

Pawpaw seeds: Pawpaw seeds were obtained from commercial fruit sellers at the local market in Lomé, Togo. The seeds were sun dried for some days until no traces of water was left. They were blended in a high speed blender into flour. The seed flour was stored in a cool place prior to use.

Management of chickens: In total, 90 hens and 12 males of Sasso brand chickens of 20 weeks of age were used for this experiment. The day-old chicks of these birds were provided by Incubel nv (Hoogstraten, Belgium). They reared under standard management condition until 20 weeks of age. Then the 20 weeks old birds were housed at 15 hens/2 cocks per pen with stocking density of 5 birds/m^2. Lighting and feeding throughout rearing period were provided according to primary breeder recommendations. Sasso hens were given restricted feed following the recommended daily allowances and water was provided *ad libitum*.

Egg component weights and egg weight loss during incubation: Prior to setting of the eggs for incubation, sample of 30 eggs per treatment were cracked and opened to collect meticulously and weigh shell, albumen and yolk. At day 18 of incubation, all incubated eggs were weighed. These weights and those recorded prior to incubation were used to calculate relative egg weight loss up to d 18 of incubation as Tona *et al*[17].:

$$WL = \frac{W0 - W18}{W0} \times 100$$

Where:
WL : Relative egg weight loss
W0 : Egg weight at setting
W18 : Egg weight at day 18 of incubation.

Blood samples: Blood samples were collected from 12 hens (4 birds/replicate) at 31 and 43 weeks of age. The whole blood was put aside for approximately 20 min and then centrifuged at 3,000×g for 10 min for serum collection at room temperature. Pure serum samples were aspirated with pipette and stored in 1.5 mL Eppendorf tubes and used for Triglyceride and total protein levels determination. Also, serum concentrations of total protein (TP),uric acid (UA), triglyceride (TG) and total cholesterol (T-CHO) were measured spectrophotometrically (UV-2000, UNICCO Instruments Co. Ltd., Shanghai, China) using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The Serum Uric acid, Glutamic Oxaloacetate Transaminase (GOT), Glutamic Phosphate Transaminase (GPT) level were computed as described by Scott[18].

Hatching parameters: Between 456-503 h of incubation, eggs transferred into the hatchers were checked individually every 3 h and the hatched chicks were recorded and weighed. At hatching stage incubation duration was defined as the time between setting and hatching for each egg. Spread of hatch was defined as the dispersion around the average incubation duration. After 503 h of incubation, the number of hatched chicks was recorded. Eggs that failed to hatch were broken for macroscopic analysis in order to distinguish infertile eggs from eggs containing dead embryos. These data were used to calculate hatchability in relation to the number of fertile eggs.

Statistical analysis: The data were analyzed using the statistical software package Graph Pad PRISM 5. GraphPad Prism is a commercial scientific 2D graphing and statistics software published by GraphPad Software, Inc., a privately held California (USA) corporation. The generalized linear regression model was used to analyze the effects of diets on egg production, egg weights and egg components; feed intake, feed conversion ratio, durations of incubation and post hatch weights. When the means of the general model were statistically different, the means were further compared using T-test. Differences were considered significant at $p<0.05$. In a second analysis, hatchability was considered as binomial in distribution. A 2-tailed test for comparison of variances was used to analyze the influence of diets on hatchability.

RESULTS

Daily average feed consumption, daily weight gain and feed conversion ratio: Table 2 shows daily average feed consumption, daily weight gain and feed conversion ratio. Daily feed consumption was similar for both control and CP seed groups. The feed conversion ratio in the CP group was lower ($p<0.05$) than that of control group. Overall, body weight increased with the age of breeders. From 22-40 weeks of age, body weight of control group was significantly ($p<0.05$) higher than that of CP seed group. Between 40-50 weeks of age body weights were similar for all groups.

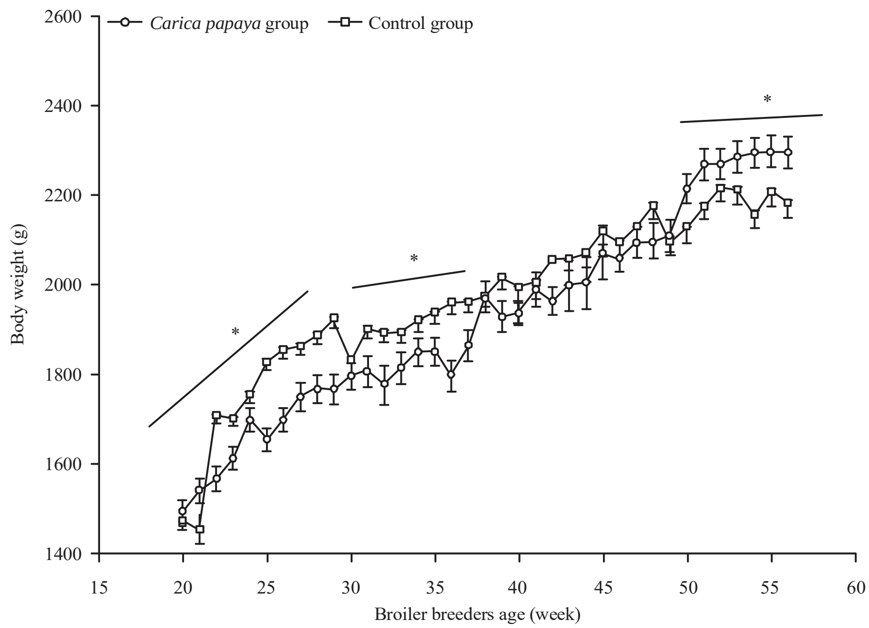

Fig. 1: Body weight according to age and treatments

Table 2: Daily average feed consumption, daily weight gain, feed conversion ratio according to treatments

Treatments	Age	Feed intake (g)	Daily weight gain	Feed conversion ratio
Control group	31.0000	125.35±0.33	32.64±1.48[b]	3.11±0.06[a]
CP	31.0000	127.55±0.21	28.11±1.04[a]	2.31±0.42[b]
p-value		0.5054	0.0048	0.0044
Control group	43.0000	126.66±1.18	74.43±0.87[a]	3.63±0.05[a]
CP	43.0000	126.20±1.32	40.25±0.99[b]	2.95±0.11[b]
p -value	0.2590	0.0372	0.0275	

[a,b]Data sharing no common letter within column are different ($p<0.05$) with age and between treatments

But from 51weeks to onward, body weights of breeders fed with CP seed diets were higher compared to those fed control feed ($p<0.05$) (Fig. 1).

Blood parameters: Blood serum concentrations of Uric acid, Glutamic Oxaloacetate Transaminase (GOT), Glutamic Phosphate Transaminase (GPT) triglyceride, total protein and cholesterol levels are shown respectively in Fig. 2-7.

At both ages of breeders, blood serum concentration of uric acid, Glutamic Oxaloacetate Transaminase (GOT), Glutamic Phosphate Transaminase (GPT) level of CP seed group were significantly higher than that of the control group ($p<0.05$). The concentrations of triglyceride and total protein of pawpaw seed diet (CP) were significantly lower than that of the control group ($p<0.05$) in both ages of the breeder. Cholesterol concentration was not affected by treatment and were similar between CP diet group and control group.

Egg weights and ratios egg components weights to egg weights: Table 3 shows, according to diet treatments, egg weights and ratios of egg components weights to egg weights. Hens of CP seed group had heavier eggs and higher ratio of albumen weight to egg weight ($p<0.01$) than that of the control group at both ages. At both ages, ratio of yolk weight to egg weight was not affected by the diet treatments. However, the ratio of eggshell weight of control group was significantly ($p<0.05$) higher than that of CP group.

Diets effect on incubation parameters and day-old chick weight: Up to day 18 of incubation, relative egg weight loss at age 31 and 43 weeks, egg weight from breeders fed with Control diet (7.89±0.32 and 9.1±0.37 %) was lower ($p<0.05$) than those of eggs from CP seed diet group (11.44±0.33 and 13.47±0.35) (Table 3). Figure 8 shows the spread of hatch according to treatment. In the control group, the hatching curves showed that the majority of eggs from the breeders of 31 weeks of age hatched between 470 and 482 h of incubation. Whereas, eggs laid by breeders on CP seed diet at 31 week of age hatched from 477 h and peak at 486 h. Figure 9 shows that the majority of eggs laid by breeders on control diet group at 43 weeks of age started increasing from 477h with two peaks at 483 and 496 h.

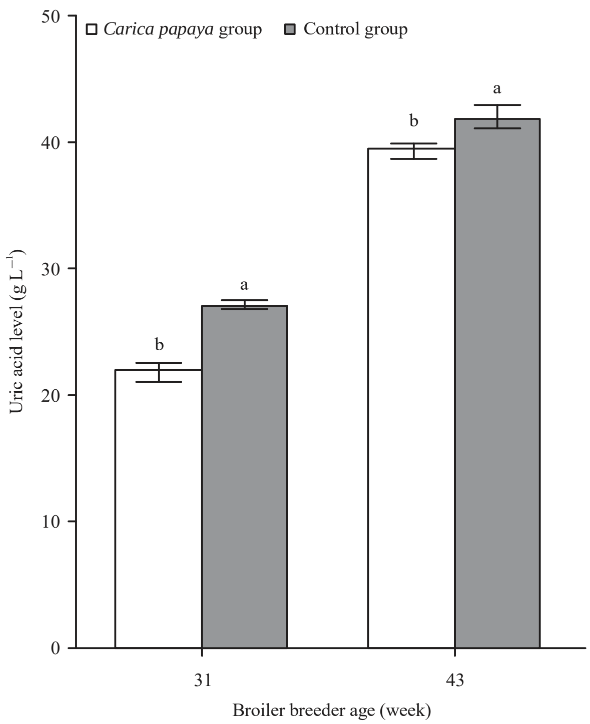

Fig. 2: Concentrations of uric acid according to age and treatments
Data sharing no common letter are different ($p<0.05$)

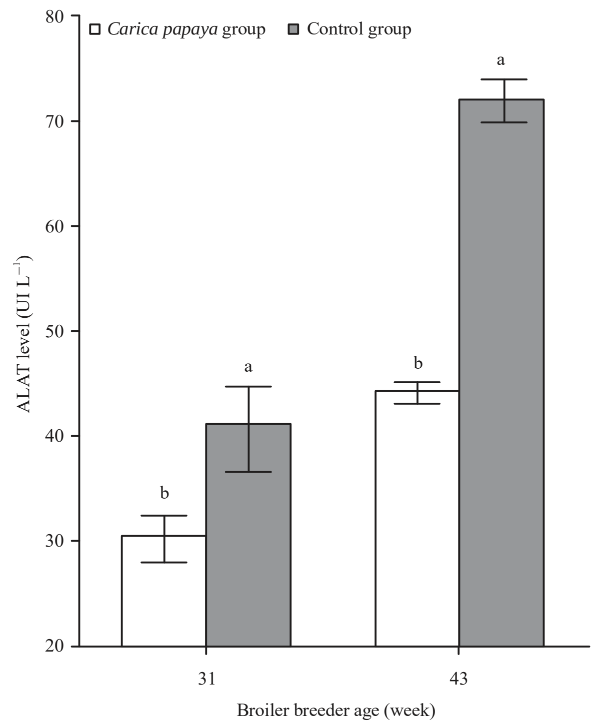

Fig. 4: Concentrations of ALAT according to age and treatments
Data sharing no common letter are different ($p<0.05$)

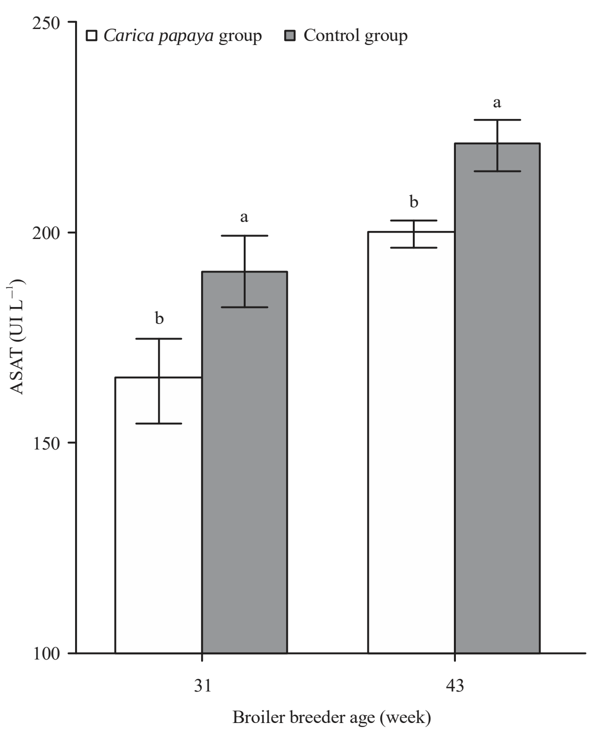

Fig. 3: Concentrations of ASAT according to age and treatments
Data sharing no common letter are different ($p<0.05$)

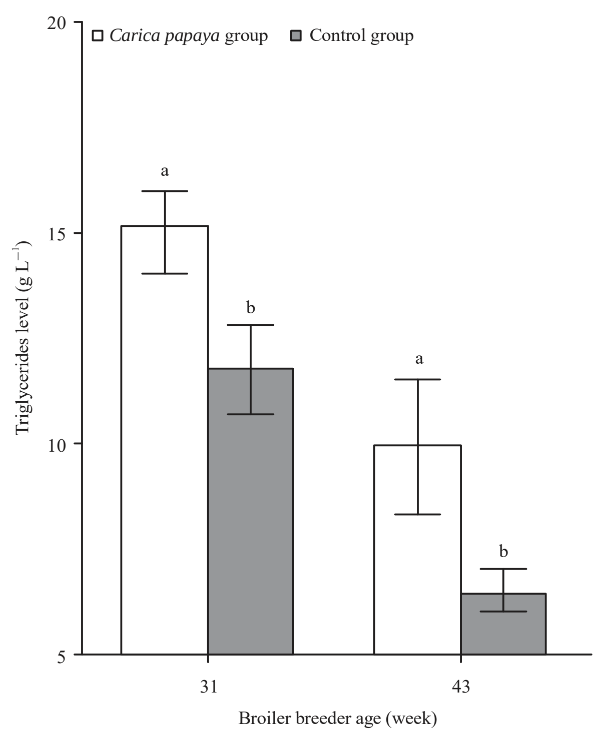

Fig. 5: Concentrations of triglycerides according to age and treatments
Data sharing no common letter are different ($p<0.05$)

Table 3: Egg components weights to egg weight ratios and egg weights loss up to 18 days of incubation according to treatments

Treatments	Age	Egg weight (g)	Egg weight loss (%)	Shell ratio (%)	Albumen ratio (%)	Yolk ratio (%)
Control group	31.0000	50.76±0.32[b]	7.89±0.32[b]	12.58±1.07[a]	59.97±7.44[b]	27.45±1.41
CP	31.0000	54.25±0.25[a]	11.44±0.33[a]	9.81±0.42[b]	63.83±0.88[a]	26.36±1.96
p-value		0.0170	0.0008	0.0056	0.0065	0.1868
Control group	43.0000	56.68±0.41[b]	9.10±0.37[b]	13.63±0.21[a]	59.48±7.24[b]	26.89±1.10
CP	43.0000	61.44±0.34[a]	13.47±0.35[a]	10.50±0.72[b]	63.26±7.87[a]	26.24±1.74
P-value	0.0036	p<0.0001	0.0044	0.0020	0.8305	

[a,b]Data sharing no common letter within column are different (p<0.05) with age and between treatments

Table 4: Incubation duration up to hatch, hatching performance and embryo mortality according to treatments

	31			43		
Hatching events	Control	CP	p-value	Control	CP	p-value
Incubation duration up to Hatch (h)	480.25±0.57	481.71±0.62	0.9693	480.71±0.67	478.02±0.52	0,1495
Hatchability (%)	82.06[a]	75.57[b]	p<0.0001	87.37[a]	73.63[b]	p<0.0001
Early mortality (%)	8.04	9.15	0.1767	5.47	7.85	0,5295
Late mortality (%)	9.90[a]	14.28[b]	0.0015	7.16[a]	16.52[b]	p<0.0001

[a,b]Data sharing no common letter within column are different (p<0.05) with age and between treatments

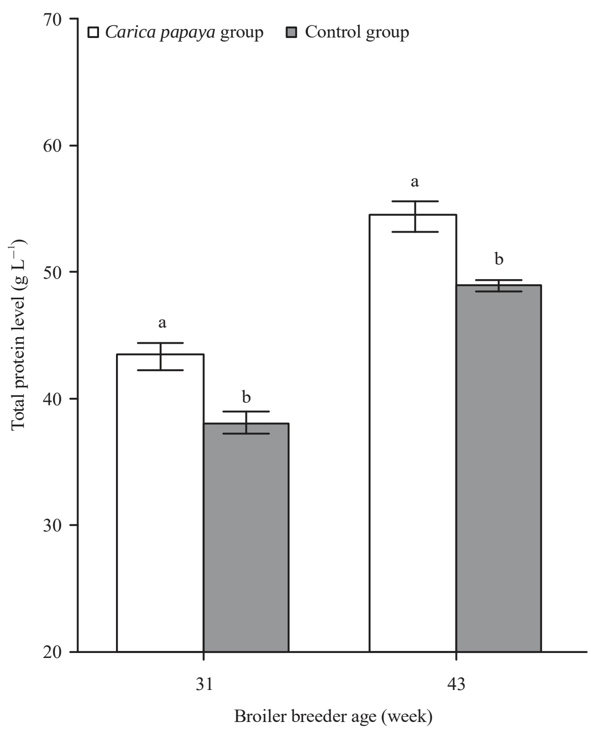

Fig. 6: Concentrations of protein according to age and treatments

Data sharing no common letter are different (p<0.05)

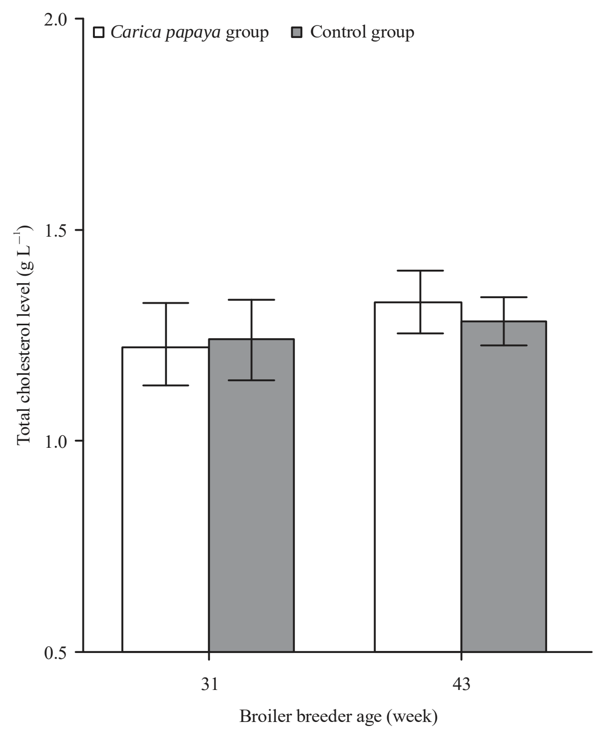

Fig. 7: Concentrations of Cholesterol according to age and treatments

Whereas, eggs on CP seed diet, started hatching from 475 h and peak at 490 h. Table 4 shows the incubation duration up to hatch, hatching performance and embryo mortality according to treatments. Total incubation duration did not differ between the feed treatment and control group within the ages. But in both ages, hatchability of CP group was lower and late mortality rate was higher than that of control group (p<0.01) while early mortality rate was not affected as in both groups and age. Figure 10 shows day-old chick weight according to treatments. Day-old chicks from eggs of CP seed diet were heavier (p<0.05) than those from eggs of control group diet at both ages.

DISCUSSION

Pawpaw seed incorporation at 0.5% level into the feed of broiler breeder enhanced feed efficiency, egg weight, egg

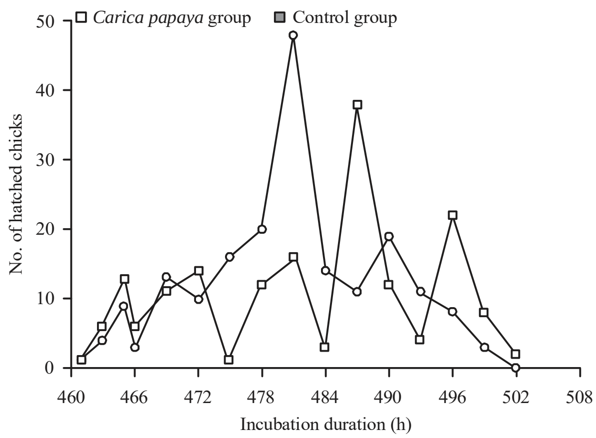

Fig. 8: Hatching curve in relation to the incubation duration and broiler breeders age (31 weeks)

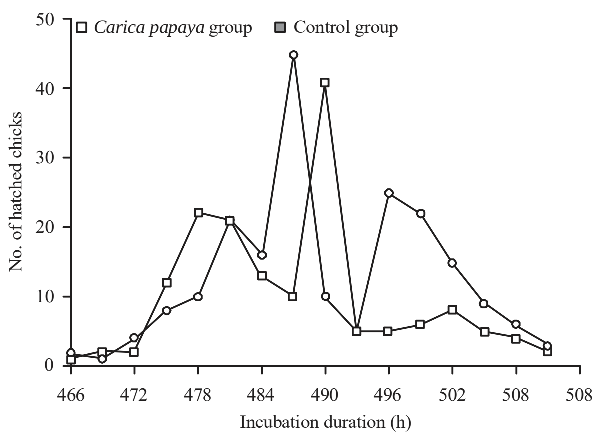

Fig. 9: Hatching curve in relation to the incubation duration and broiler breeder age (43 weeks)

production and chick weight but depressed the hatchability of the eggs and increased embryo mortality. These are very important breeder production parameters.

The increase observed in egg production and egg weight may be related to the better feed efficiency recorded in birds fed diet with pawpaw meal since there was no difference in feed intake. Furthermore, since the two diets contained similar levels of protein, it can be assumed that pawpaw seed might have provided some unidentified factors that enhanced protein utilization for albumen production. From the data, differences in egg shell ratio and albumen content between treatments for each age accounted for the difference in egg weight. This suggests that the enhanced protein utilization was diverted to oviduct egg albumen production in both 31 and 43 weeks old breeders. Also, more proteins are made available for follicle growth in the ovary to enhance more egg production. This interpretation is consistent with the

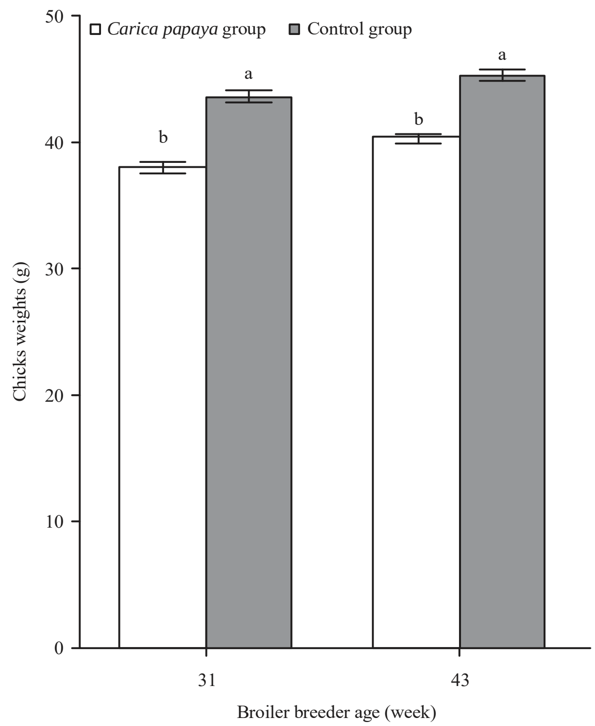

Fig. 10: Day-old chick weight according to treatments
Data sharing no common letter are different (p<0.05)

finding of Gunawardana *et al.*[19], Gunawardana *et al.*[20] and King'Ori *et al.*[21]. These authors reported increased egg production and egg components production by adjusting dietary protein or amino acid levels. It was interesting that the age of the breeders did not have effect on the response of birds to pawpaw seed meal or the control diet. Shell ratio to egg was significantly reduced by the incorporation of pawpaw seed meal in the feed. This is likely due to phytic acid content of pawpaw seeds. Adesuyi and Ipinmoroti[11] have reported that the acid inhibits calcium uptake.

Incubation parameters and hatchability were both affected by pawpaw seed meal. Pawpaw seed meal inclusion in feed caused higher weight loss of about 4% by breeders of both ages. However, absolute losses were higher in the eggs from older birds. The greater weight loss by eggs of older birds than younger birds is consistent with previous findings of Iqbal *et al.*[22]. Similarly, larger eggs lost more weight than small eggs. One factor has been advanced to be responsible for egg weight reduction through water loss, the nature of the egg cuticle which is composed large of lipids. This dictates the number of egg pores through which water evaporates[23]. Whether a component of pawpaw interferes with the structure of the cuticle needs to be ascertained. Peeble *et al.*[24] reported that weight loss by hatching eggs during incubation influenced embryogenesis. Total incubation duration did not differ between the feed treatment within the ages. However, hatchability was significantly reduced by pawpaw seed meal incorporation into the feed at both ages, the effect being greater in older birds. The reduction in hatchability can be ascribed to early and late mortality of embryos. Consistently, eggs from breeders fed pawpaw seed meal had higher embryo mortality during late development. Again, there was greater effect on the older birds. These levels of mortality could not be ascribed to water loss during incubation alone but may be due to some factors in the pawpaw seeds. These factors tended to affect the older birds more. Phytic acid which is a component of pawpaw seed is known to from complexes with lipids[25]. This, possibly, may interfere with yolk fat storage as an energy source for hatchability during late incubation. This effect may be greater in older birds where higher late mortality was recorded in this study.

Egg weight is one of the most influential factor on hatchability[19]. The results of the present study in the both age of broiler breeders indicate that egg weights and day-old chick weights of CP seed diet group were higher compared with the control group. This is consistent with previously reported study which indicated that the chick weight is positively correlated with egg weight[26].

Uric acid and creatinine values are measures of amino acid degradation. Higher uric acid concentration in the blood of animals fed on pawpaw seed diet points to depressed liver and kidney function[27]. High serum values of ALAT and ASAT in birds given pawpaw seed meal are indicative of initial stage of normal liver and kidney dysfunctions[28]. These effects could be attributed to the effect of some content in the pawpaw seeds. Serum triglyceride and Cholesterol concentrations may have reflected lipid absorption and metabolism. Lower concentration of triglycerides and Cholesterolmay be the consequence of low lipid profile in the blood reflecting a high amino acid transport and a better lipid metabolism with a consequent decrease in fat deposits[29].

CONCLUSION

In conclusion, pawpaw seed diets offered to broiler breeders during lay improved performance parameters like egg weight, egg production and feed conversion ratio and day-old chick weight. However, the diet had negative effects on hatchability and some serum parameter (ASAT, ALAT and uric acid).

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