Neuroendocrine and behavioural responses of Japanese quails to dietary *Aspilia africana* leaf meal and extracts

O.O.K. Oko*[1], O.R. Asuquo[2], E.A. Agiang[1] and E.E. Osim[3]

[1]Department of Animal Science, Faculty of Agriculture, [2]Department of Human Anatomy, Faculty of Basic Medical Sciences, [3]Department of Physiology, Faculty of Basic Medical Sciences, University of Calabar, PMB 1115, Cross River State, 540004 Nigeria

*Corresponding author E-mail: oluwatosin.kennedyoko@gmail.com, Ph. +234 8052299139

Journal of Livestock Science (ISSN online 2277-6214) 8: 43-51
Received on 19/11/2016; Accepted on 18/1/2017

Abstract

Impacts of dietary *Aspilia africana* leaf (AaL) as endocrine disruptors on some developmental landmarks and sexual behavioural traits in Japanese quails were evaluated in a 45 d period. 1050 unsexed one wk-old chicks were assigned to 14 dietary treatments comprising of 75 chicks per treatment (15chicks/replicate). Treatments included; basal diet (0%) and 0.02% terramycin+basal diet, basal diet plus 2%, 5%, 7.5% and 10% of meal (M), aqueous extract (Ae) or ethanolic extract (Ee) of AaL. At d 21, five males and females were housed in individually per replicate and given 3 days acclimatization period prior to data collection. Onset of egg lay, female calling, foam production, male crowing cloacal gland size, sexual behaviour and stressogenic responses were monitored from d 24 - 45. Kruskal-Wallis ANOVA and two-tailed Mann-Whitney U-tests were used in data analysis. Early onset of egg lay (36 vs 40 days) and female calling (27 vs 34 days) were observed in quails fed 5% AaLM and 5% EeAaL, respectively while, 4 days delayed of lay was recorded in quails on 7.5 % AaLM. Foam production and male crowing commenced earlier (P<0.001) in quails fed 5% AeAaL and 7.5% EeAaL diets (36 vs 42 days and 26 vs 32 days). Cloacal gland size was larger in 5-7.5% AeAaL and 7.5% EeAaL groups (2.8/2.4cm) but smaller (2.2cm) in birds fed 7.5 – 10% AaLM. Birds on 5 -7.5% extracts had higher frequencies of copulation success than those on AaLM and 10% extracts. Frequencies of flight were higher in the group fed 7.5 – 10% AaLM diets. Impacts of *Aspilia africana* leaf on quail behaviour were form and dose-sensitive, indicating the presence of some Endocrine Disrupting Chemicals (EDCs) in *Aspilia africana* leaf. For enhanced sexual characteristics and performances, 5% aqueous extract of *Aspilia africana* leaf should be supplemented into quail diet.

Keywords: Sexual Behaviour; Toxicity; *Aspilia africana*; Fear Behaviour; Stress; Quails

Introduction

Herbs, spices and plant extracts contain several active components with intrinsic biological activities on animal physiology and metabolism. *Aspilia africana* leaf is gaining interest, in the research community, as a valuable plant with several medicinal and nutritional benefits (Oko, 2011; Oko et al., 2011b; Oko et al., 2014a). Its phytochemical, nutritional and toxicological properties are well documented (Okoli et al., 2007; Oko and Agiang, 2011a and b; Oko et al., 2014). Its pharmacological properties have been extensively studied but it has recently found new application as a phytobiotic in animal production (Oko et al., 2011a; Oko et al., 2012a and b; Oko et al., 2014b). Reports on its growth promoting effects indicated that different forms exhibit varied effects in poultry (Agiang et al., 2011). At prolonged and higher levels of inclusion (10%) growth and egg depressing effects were observed.

According to Ottinger et al. (2002), gonadal steroids modulate sexual differentiation of accessory sex structures and endocrine system which in turn regulate endocrine and behavioural characteristics in animals. These sexual behavioural responses can be altered under exposure to adverse environmental conditions (Touart, 2005) Thus in order to determine its impacts on the reproductive fitness and survivability of the species, impact assessments of novel feeds are required.

Developmental landmarks are indicators of the physiological age of a growing animal (Ottinger and Brinkley, 1979), as well as the stage of maturation (Touart, 2005). Age at onset of first egg lay, age at first foam production and age at development of sexually-dimorphic plumage are critical landmarks in birds.

The quail species is considered as the accepted model for neuroendocrine studies because it utilizes relative concentration of sex hormones required for endocrine and behavioural expressions. Testosterone and estrogens are the main hormones that regulate avian sexual behaviours.

In male Japanese quail, the preoptic area of the central nervous system modulates sexual behavior with clear courtship and mating behaviors (Watson and Adkins-Regan, 1989) while in the females, estrogens generally modulate their sexual characteristics.

Behavioural responses and onset of sexual maturation are critical landmarks useful in measuring endocrine disrupting chemicals (EDCs) exposure. Chemicals exposures are linked to delayed reproductive functions and behaviours (Becker et al., 1993; Morgan and Tromborg, 2007). In birds, EDC-associated effects could also include: population decline, growth impairment and uncharacteristic behavior (Fairbrother et al., 1998; Colborn et al., 1993).

Methanolic extracts of *Aspilia africana* leaf had shown dose-dependent reduction in the duration of estrus cycle with inhibitory effects on uterine tissues of rats (Oluyemi et al., 2007), ovulatory and fertility status in Wistar rat (Okwuonu et al., 2007) suggesting that *Aspilia africana* leaf could possess some endocrine disrupting chemicals. However, no published reports are available on its effects in birds.

This work assessed the effects of *Aspilia africana* leaf meal and extracts on the pattern of sexual differentiation and behavior of growing Japanese quails.

Materials and Methods

Location of Study

This study was conducted at the Poultry Unit, Department of Animal Science, Teaching and Research Farm, University of Calabar, Calabar, Nigeria.

Formulation of Experimental Diets

Feed ingredients including; maize, soybean, wheat offal, palm kernel cake, vitamin premix, salt, lysine, methionine, bone meal and limestone were bought from the local markets in Calabar Municipality and Calabar South Local Government Areas of Cross River State, Nigeria. The basal (antibiotic-free) diets were formulated in mash form (Table 1) to meet the nutrient requirement of Japanese quails during the growing (24% CP, 2750 ME) period according to the recommendations of the Central Avian Research Institute (CARI, 1989).

Three supplementation procedures were employed (Smith et al., 2001) to determine whether different forms of *Aspilia africana* leaf contain EDCs and to assess their impacts on sexual differentiation in Japanese quails. In the first, *Aspilia africana* leaf meal (AaLM) diets were formulated (as recommended by Fasuyi, 2005) such that 2%, 5%, 7.5% and 10% of AaLM were supplemented into the basal diet. In the second procedure, two extracts of *Aspilia africana* leaf, (aqueous extract of *Aspilia africana* leaf – AeAaL) and ethanolic extract of *Aspilia africana* leaf - EeAaL) were supplemented into the basal diets with estimated equivalent of 2%, 5%, 7.5% and 10% of AaLM (Table 2). Aqueous and ethanolic extractions (using distilled water and industrial ethanol) were carried out at the Physiology Department, University of Calabar.

In the last procedure, two control diets were formulated and compared with the experimental diets. According to the methods of Denli et al. (2004), the basal diet (0%) and 0.02% terramycin plus basal diet served as the negative and positive control, respectively. Therefore, a total of fourteen (14) dietary treatments were studied. Basal diets were stored in tightly sealed containers, while on a weekly basis, experimental diets were mixed such that an estimated quantity of the substance was added per kg of basal diet.

Table 1. Composition of Basal Diet (%)

Ingredients	**%**
Maize	40.80
Soyabean meal	40.70
Wheat offal	10.00
Palm kernel cake	4.00
Bone meal	2.50
Limestone	0.00
*Vitamin premix	0.50
Salt	0.50
Lysine	0.50
Methionine	0.50
Total	**100**
Crude protein (%)	24.00
Calcium (%)	0.80
Metabolisable energy (Kcal/kg)	2,750.00

*Vitamin BCP (premix; 0.25% vitamins and Trace elements) Each 2.5kg contained;
Vitamin A 10,000,000 I.U, Vitamin D_3 2,000,000 I.U, Vitamin E 20,000I.U, Vitamin K 2,250mgr, Thiamine 1,750mgr, Riboflavin $B_2$5,000mgr, Pyridoxine B_6 2,750 mgr, Niacin 27,500 mgr, Vitamin B_{12} 15 mgr, Pantothenic acid 7,500 mgr, Biotin 50 mgr, Cholin chloride400gr, Antioxidant125 gr, Manganese 80 gr, Zinc 50 gr, Iron 20 gr, Copper 5 gr, Iodine1.20 gr, Selenium200 gr, Cobalt 200 gr; Synthetic antibiotic used was terramycin soluble powder and each contains; Oxytetracycline HCL B_p (Vet) 50mg; A broad spectrum antibiotic (Vetindia Pharmaceutical Limited, India).

Table 2. Estimate of extract added

Level of supplementation	**Statement**
Negative control	Basal diet
Positive contol	Basal diet + 0.02g/kg terramycin
2% AeAaL	Basal diet + 2.04 g/kg AeAaL
5% AeAaL	Basal diet + 5.10 g/kg AeAaL
7.5% AeAaL	Basal diet + 7.65 g/kg AeAaL
10% AeAaL	Basal diet + 10.20 g/kg AeAaL
2% AaLM	Basal diet + 20.00 g/kg AaLM
5% AaLM	Basal diet + 50.00 g/kg AaLM
7.5% AaLM	Basal diet + 75.00 g/kg AaLM
10% AaLM	Basal diet + 100.00 g/kg AaLM
2% EeAaL	Basal diet + 3.40 g/kg EeAaL
5% EeAaL	Basal diet + 8.49 g/kg EeAaL
7.5% EeAaL	Basal diet + 12.74 g/kg EeAaL
10% EeAaL	Basal diet + 16.98 g/kg EeAaL

AeAal - aqueous extract of *Aspilia africana* leaf ; AaLM - *Aspilia africana* leaf meal
EeAaL - ethanolic extract of *Aspilia africana* leaf

Experimental Animals and Management

One thousand and fifty (1050) unsexed Japanese quail chicks were assigned to 14 dietary treatments comprising of seventy-five (75) chicks per treatment. Each treatment had five (5) replicates of fifteen (15) chicks per replicate. The experiment lasted for 45 days and each treatment was exposed to the diet from day 1 to 45. All chicks had *ad libitum* access to feed and water daily at 0900hr. Temperature in the experimental unit was kept at 32^{o}C for the first 7 days of experiment and reduced gradually until a temperature of 22^{o}C was achieved. Continuous artificial lighting was provided throughout the experiment. Developmental landmarks (time of onset; of lay, foam production and initiation of sexual behaviour) were monitored from day 28 to 45 of the experiment.

At day 21, five males and five females were picked from each replicate/treatment, separated and housed in individual cages at room temperature, on a 16L: 8D photoperiod so as to monitor the onset of puberty. Prior to data collection, the birds were allowed 3 days to adjust to their cages. The first day of egg lay and female calling were used as measures of onset of puberty. In the male quails, the first day of foam production (from the proctodeal gland) and crowing was noted as the onset of puberty.

At d 42 impact of treatments on male copulatory behaviour was assessed. Fifteen males per treatment were individually tested on three consecutive days. In each test, male behaviour was observed for 3 mins following introduction of a sexually receptive female into the cage. The number of mount attempts and the number of successful cloacal contacts were recorded. Cloacal gland size (length × width) was determined at day 42 of experiment, a time that 90% of the control quails were sexually matured to serve as a comparison of the status of maturation across the treatments as highlighted by Quinn (2005).

To evaluate stress response, frequencies of defeacation and flight made by individuals within 3 mins of testing were also monitored as described by Ottinger et al. (2002) and Quinn (2005).

Data collection and analysis

Data collected on behavior of quails were based on visual inspection per group. Differences in the percentages of the birds emitting calls among the groups were analyzed by the two-tailed Fisher Exact Probability Test and differences in frequencies of calls were analyzed by the Kruskal -Wallis analysis of variance followed by two-tailed Mann-Whitney U-tests. Onset of egg lay, foam production and cloacal gland size were analyzed by one-way analysis of variance (ANOVA). Treatment means were separated using Tukey tests according to methods described by Chiba and Fujiwara (2013).

All animal experiments comply with the ARRIVE guidelines (Animal Research: Reporting of in vivo experiments of the National Centre for the Replacement, Refinement and Reduction of Animals in Research, UK) and were conducted according to the guidelines approved by the Animal Ethics Committee of The University of Calabar (Nigeria) and the NIH standards described in the NRC (2011) Guide for the Care and Use of Laboratory Animals.

Results

The impact of different forms and levels of *Aspilia africana* leaf on sexual differentiation of the gonads and brain as measured by onset of puberty, cloacal gland size and reproductive behaviours in male and female Japanese quails are presented in Figs.1 to 8.

Onset of lay was initiated 7 days earlier ($P<0.001$) in females from the 5% AaLM compared to other AaL treatments and control groups (Fig 1, $P<0.001$). The addition of 2 - 10% AeAaL or EeAaL led to early initiation of egg lay comparable to those on the control diets, whereas egg lay was delayed up to four days in female Japanese quails fed 2%, 7.5% and 10% of AaLM diets.

Fig. 2. Shows that the addition of 5% of any form of AaL supplement initiated early female calling ($P<0.001$) compared to other experimental groups. Increasing AaL supplementations (7.5 - 10%) delayed calling by about 2 days, whereas calling was recorded 7 days later in groups fed control diets.

In the male Japanese quails, highly significant ($P<0.001$) effect of dietary treatments were observed for onset of puberty, cloacal gland size and copulatory behaviour. Onset of puberty measured by the initiation of foam production (Fig. 3) and male crowing (Fig. 4), was delayed by 7 days in quails fed increasing levels (7.5 - 10.0%) of AaLM diet. Comparable rates were observed in quails on the control diets as against those on the other experimental groups. Foam production and male crowing were initiated earlier ($P<0.001$) in quails fed 2 - 7.5% AeAaL or 5 - 7.5% EeAaL diets.

Fig. 5 revealed that cloacal gland area was approximately 8% smaller in the 7.5-10% AaLM treatment and 17% larger ($P<0.001$) in the AeAaL (5 - 7.5%) or EeAaL (7.5%) groups than in those on the control groups. Cloacal gland size, comparable to that of the terramycin group, was recorded in groups fed low levels (2% - 5%) of AaL.

There were significant variations in copulatory behaviour (Figs. 6 and 7) between treatments. Results revealed that increasing AaL supplementations from 2% to 10% (except 10% AaLM) significantly ($P<0.001$) reduced mount attempts in male Japanese quails comparable to the terramycin groups. Males fed AaL supplements (2 - 10%) attempted to mount females about 28.69 - 64.24% less than the number of times by quails on the control diets.

Fig. 7 illustrates that males fed 2 - 7.5% AeAaL or EeAaL diets had higher ($P<0.001$) copulation success; comparable to those on the negative control diet; whereas, quails on 7.5 - 10% AaLM and 10% EeAaL groups achieved 33% less copulatory success than quails on the control diet. Terramycin treated quails achieved similar copulation success as quails on 10% AeAaL, 5% AaLm diets. Fig. 8 reveals that quails on 10% AaLM diets were as fearful as those on the control but more fearful than those on other AaL diets and terramycin diets.

Table 3 presents the interactions between developmental and behavioural responses as biomarkers of the neuroendocrine system, responsible for sexual differentiation in Japanese quails. Significant ($P<0.05$) to highly significant ($P<0.01$, $P<0.001$) interrelationships were observed in all parameters studied, aside onset of egg lay.

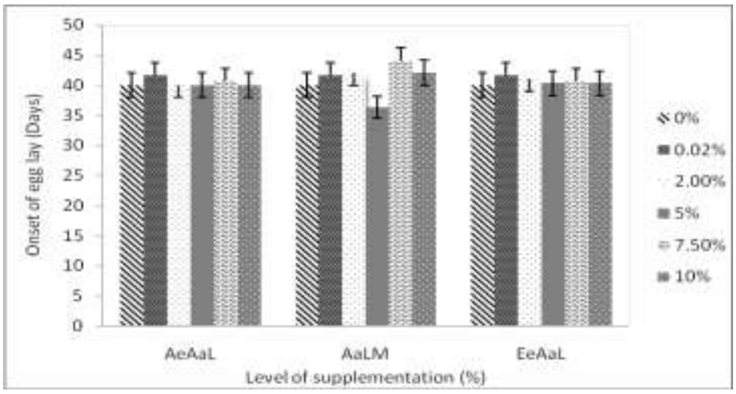

Fig. 1. Effects of *Aspilia africana* leaf supplementations on the onset of egg lay in Japanese quails

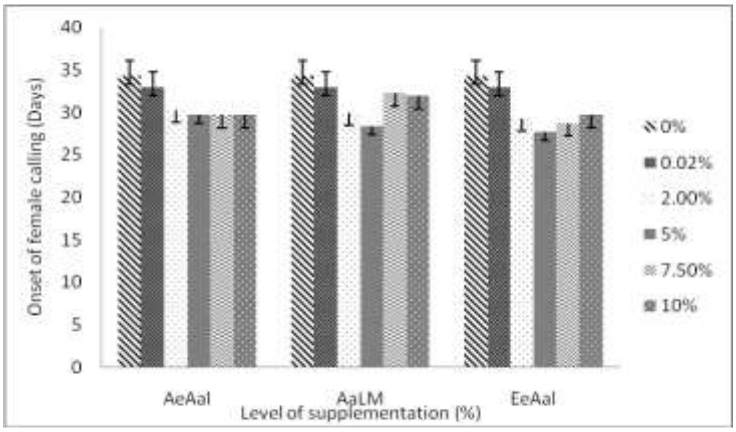

Fig. 2. Effects of *Aspilia africana* leaf supplementations on the onset of female calling

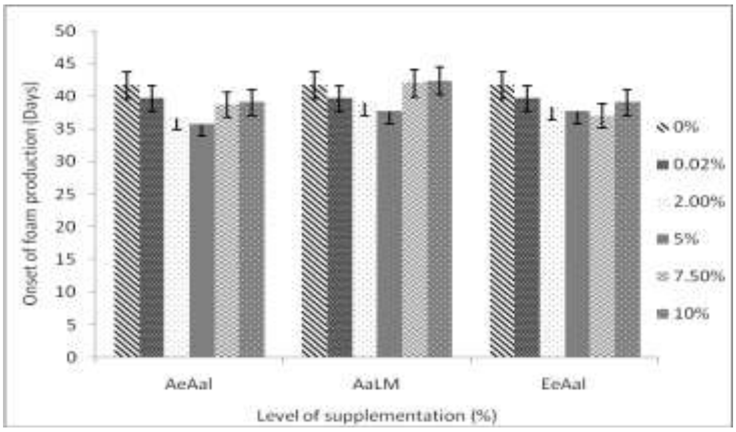

Fig. 3. Effects of *Aspilia africana* leaf supplementations on onset of foam production

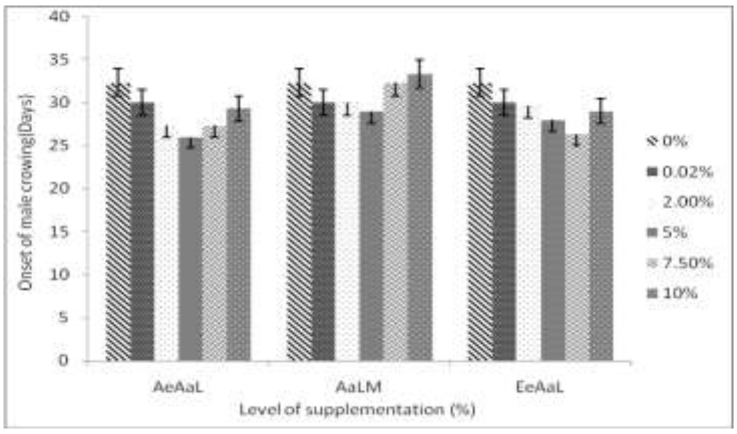

Fig. 4. Effects of *Aspilia africana* leaf supplementations on the onset of crowing in male Japanese quails

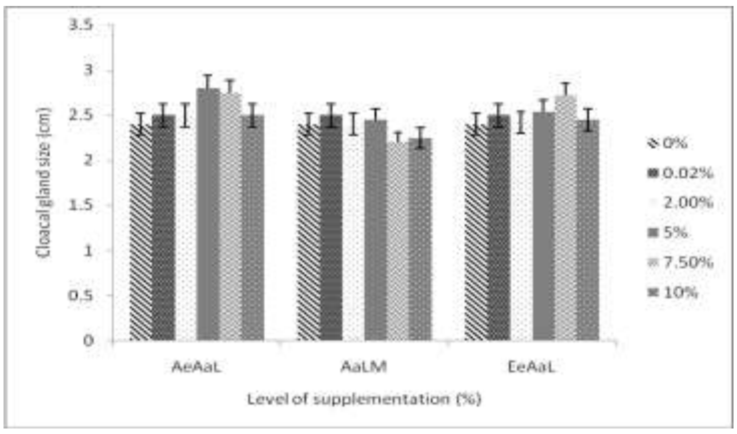

Fig. 5. Effects of *Aspilia africana* leaf supplementations on cloacal gland size

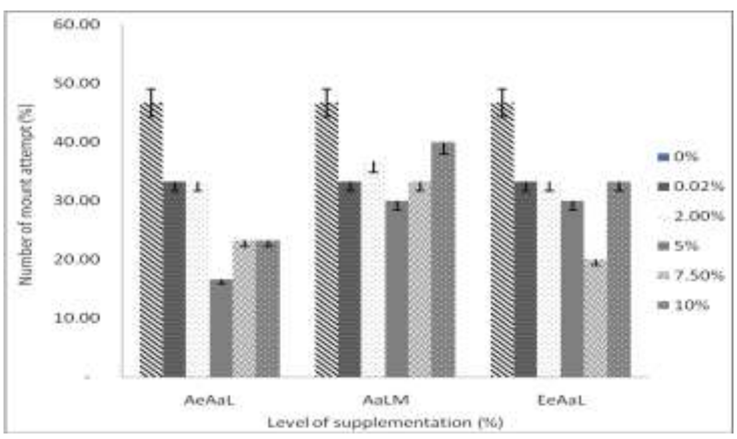

Fig. 6. Effects of *Aspilia africana* leaf supplementations on mount attempts in Japanese quails

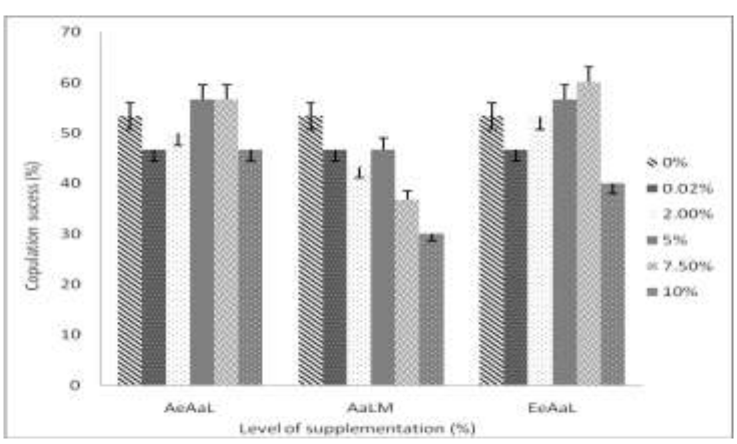

Fig.7. Effects of *Aspilia africana* leaf supplementations on copulation success in Japanese quails

Stress genic response measured in terms of frequencies of defecation and flight were highly significantly ($P<0.001$) correlated with onset of puberty and copulatory behaviour in Japanese quails. Results indicated that, under stress conditions, onsets of foam production, and male and female callings were significantly ($P<0.001$) delayed; the frequencies of defecation and mount attempts increased; while copulation success was fewer with smaller cloacal gland size. Whereas, onset of egg lay was insignificantly ($P>0.05$) delayed.

The frequency of copulation success achieved was positive and highly significantly ($P<0.001$) correlated with cloacal gland size but negatively correlated with other responses. This implied that higher copulation success would be achieved in male Japanese quails with larger cloacal gland that exhibit earlier sexual characteristics and with lower mount attempts. Results further revealed that the degree of copulatory success achieved was independent

(P>0.05) of the age of the female quails at sexual maturity. In addition, about 37.2% copulatory success could be based on the experience (observation and activity) of the growing male Japanese quails. Similarly, male attempts to mount the females revealed positive and highly significant (P<0.001) relationships with onsets of male calling, foam production and female calling but negatively correlated with cloacal gland size.

Table 3. Correlation between developmental and stress responses

Landmark	FF	ND	FCS	NMA	CGS	OMC	OFP	OFC	OEL
OEL	0.452	0.236	-0.380	0.247	-0.375	0.421	0.528*	0.455	---
OFC	0.778**	0.742**	-0.397	0.637**	-0.473	0.705**	0.744**	---	
OFP	0.928***	0.896***	-0.634**	0.712**	-0.775**	0.946***	---		
OMC	0.888***	0.926***	-0.743***	0.810***	-0.895***	---			
CGS	-0.708**	-0.759**	0.810***	-0.782***	---				
NMA	0.650**	0.773**	-0.508*	---					
FCS	-0.719**	-0.744**	---						
ND	0.920***	---							
FF	-----								

OEL– Onset of egg lay, OFC - Onset of female calling, OFP - Onset of foam production, OMC –Onset of male crowing, CGS - Cloacal gland size, NMA – Number of mount attempt, FCS - Frequency of copulation success, ND– Number of 235 Defeacation, FF– Frequency of flight

Discussion

This result indicated that the addition of 2 - 10% AaLM supplement could contain endocrine disruptive chemicals that alter the endocrine functions and causes decline in sexual maturity in female Japanese quails. The acceleration of onset of egg lay in females fed 7.5% AaLM might have both positive as well as potentially negative impacts on the reproductive performance of the individual. Joseph et al. (2002) had demonstrated that early egg stimulation in young hens impairs egg weight and might cause reductions in chick weight.

Conversely, acceleration of the onset of reproductive ability is reported to create the potential for infected individuals to reproduce earlier and gain a reproductive advantage (Touart, 2005). The results were consistent with the findings of Ottinger and Brinkley (1979) who reported the initiation of egg lay from 42 - 77 days in Japanese quails. Results further indicated that, dietary supplementation with *Aspilia africana* leaf meal or extracts, especially at 5%, stimulate early female secondary sex characteristics probably due to the enhancement of endocrine functions.

Results suggest that antibiotics could stimulate gonadal growth, in line with reports of Touart (2005) and Quinn (2005). Cloacal gland size of 22 - 57g and onset of foam production (32-50 days) had been reported (Ottinger et al., 2002; Touart, 2005). Thus, 5 – 7.5% *Aspilia africana* leaf extracts enhance cloacal gland growth indicative of improved reproductive function. Copulation success of about 37 - 81% had been reported in male Japanese quails (Touart, 2005). Results of present study fall within this range. Results demonstrated that dietary supplementations with 2 - 7.5% *Aspilia africana* leaf extracts could stimulate male sexual behaviour in line with earlier reports of improved reproductive performances (Oko et al., 2011a).

Kilgour and Dalton (1983) opined that observed external behaviour is indicative of the welfare and internal state of an animal and any deviation from normal behaviour could be indicative of ill health, stress, hormonal or nutrient imbalances. Frequencies of defecation and flight are generally accepted as indicators of the relative fearfulness of an individual quail, with more fearful quails showing higher frequencies of defecation and flight (Quinn, 2005).

Increasing dose (10%) of *Aspilia africana* leaf supplement insignificantly (P>0.05) increased fearfulness in Japanese quails. Several factors including stocking rate, illumination intensity, toxins, malnutrition, season, body mass and trauma have been reported to influence the fear response in birds (Jones, 1986; Morgan and Tromborg, 2007).

Guo (2007) noted that the potential performance of animal could be reduced when under stress condition like; high temperature, low levels of infections and diseases, high stocking density, high fibre diets and poor handling. This report could imply that feeding of increasing levels (10%) of AaL supplements to quails could lead to stress probably due to higher amount of EDCs present. However, the addition of 2 - 7.5% *Aspilia africana* leaf supplements into the diet may not be sufficient to trigger stress in Japanese quails.

Reports of Wellock et al. (2006) had earlier indicated that stressogenic responses could suppress reproductive functions by reducing the rate of maturity and maturity parameters in birds. Results hypothesized that exposure of Japanese quails to 7.5-10% AaL supplements could disrupt endocrine functions and subsequent sexual differentiation of the gonads and brain, consistent with the findings of Wada (1982) and Halldin et al. (1999). These results thus, explained the responses of Japanese quails to chemical insults at critical developmental stages and provide a clue on the long term consequences of Aspilia africana exposure on quail reproductive system critical to fitness and survival of the population as highlighted by Ottinger et al. (2002).

Correlation results demonstrated significant influence of the active principles in *Aspilia africana* leaf induced-stressors on the neuro-endocrine and reproductive systems which are responsible for behavioural and sexual developments in Japanese quails in line with Quinn (2005) observations on quails exposed to chemicals. Scott and Slomann (2004) reported that behavioural measurements are non-lethal methods of measuring alterations in brain development as well as links between physiological functions and ecological processes. Our results agree with their report and suggested that stress could impair reproductive performance especially in male Japanese quails as indicated by delayed initiation of puberty and lower copulatory intensity. These results suggested that the intensity of copulatory behaviour in male Japanese quails was greatly influenced by age at sexual maturity as well as by gonadal morphology as reported in earlier studies (Ottinger et al., 2002).

Balthazart et al. (2004) report showed that the copulatory behaviour in male Japanese quail is controlled by the sexually dimorphic medial preoptic area (POA) or nucleus of the brain. Exposure to adverse environmental influences could cause morphological and functional differences in the POA which could upset hormonal balance and consequently disrupt normal development (Quinn, 2005). Ottinger et al. (2001a) on the other hand opined that disruption of the formation of hypothalamic – pituitary-gonadal axis (HPG axis), affects its activation and could impact on the onset of puberty, alter development and behavioural patterns of birds.

A sexually dimorphic pattern of gonadal steroids is reported to modulate the sexual differentiation of accessory sex structures and endocrine systems which regulate both endocrine and behavioural components of reproduction (Ottinger et al., 2001a and b). These steroidal hormones are said to also activate many sex-specific endocrine and behavioural responses during puberty which can be altered under exposure to adverse environmental conditions (Touart, 2005). Consequently, their proper activation at puberty and adulthood are depressed.

Specifically, testosterone and estrogens have been shown to affect the vocal neural system during development, modulate the amplitude, frequency and behaviour of adult calling. Results obtained are consistent with earlier findings and suggest that dietary supplementation with 2 - 5% AaL could stimulate higher level of circulating endocrine hormones. Cloacal gland size often used as an indicator of male reproductive development in quails (Mohan et al., 2002) was significantly correlated with onset of puberty. The smaller the size of the gland, the longer it took for males to reach sexual maturity as indicated by delayed onsets of male calling and foam production ($P<0.001$). It is noteworthy that early initiation of female calling could greatly influence the development of larger cloacal gland ($P<0.05$). This might suggest that female reproductive behaviour could stimulate gonadal growth in male Japanese quails. The observation could be supported by the findings of Mohan et al. (2002) who reported that cloacal gland size is androgen-sensitive and could be stimulated in the presence and by the behaviour of the female quails.

Zelenka et al. (1984) noted that the size of accumulated reserve energy at the juvenile stage could determine the interval between somatic and sexual growth. Therefore, environmental variables that could alter growth rate, especially in the female quails, could influence onset of sexual maturity. Results however revealed higher influences in male rather than female quails following AaL supplementations. This could imply that the type of environmental conditions exposed to could affect male and female quails separately.

Results from correlation analysis have shown the implications of supplementation of varying levels and forms of *Aspilia africana* leaf in quail diets at the different sensitive phases of quail development. It also depicts the long-term consequences of dietary AaL exposure as an endocrine disruptive chemical on the reproductive performances in Japanese quails.

Conclusion From the present results, it can be concluded that impacts of *Aspilia africana* leaf on quail behaviour were form and dose-sensitive and for enhanced sexual characteristics and performances, 5% aqueous extract of *Aspilia africana* leaf should be supplemented into quail diet.

Acknowledgement The authors are grateful for the financial support received for this work from the International Foundation for Science, Stockholm, Sweden and the Organisation for the prohibition of Chemical Weapons, The Hague, The Netherlands; Grant number "IFS Gt B/4719 Olawoyin".

Conflict of interest The authors declare that there is no conflict of interest.

References

1) Agiang EA, Oko OOK, Essien GE, 2011. Quails response to Aqueous extract of bush marigold (*Aspilia africana*) leaf. American Journal of Animal and Veterinary Sciences 6(4):130–134.

2) Balthazart J, Baillien M, Cornil CA, Ball GF, 2004. Preoptic aromatase modulates male sexual behavior: Slow and fast mechanisms of action. Physiology and Behaviour 83: 247–270.

3) Becker PH, Schuhmann S, Koepff C, 1993. Hatching failure in common terns (*Sterna hirundo*) in relation to environmental chemicals. Environment and Pollution 79: 207–213.

4) CARI, 1989. Nutrient Requirements in Quails. Central Avian Research Institute, Izatnagar-India.

5) Chiba A, Fujiwara M, 2013. Roles of sex steroids in the crowings with sexual and non-sexual motivations in female Japanese quail, *Coturnix japonica*. Journal of Behavioral and Brain Science 3: 289-295.

6) Colborn T, VomSaal FS, Soto AM, 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environmental Health Perspectives 101(5): 378–384.

7) Denli M, Okan F, Uluocak AN, 2004. Effects of dietary supplementation of herb essential oils on the growth peformance, carcass and intestinal characteristics of quail. South African Journal of Animal Science 34(3): 174–179.

8) Fairbrother A, Landis WG, Dominquez S, Shiroyama T, Buchholz P, Roze MJ, Matthews GB, 1998. A novel nonmetric multivariate approach to the evaluation of biomarkers in terrestrial field studies. Ecotoxicology 7: 1– 10.

9) Fasuyi AO, Fayemilehin KSO, Aro SO, 2005. Nutritional potentials of Siam weed (*Chromolaenaodorata*) leaf meal (SWLM) on laying hens: Biochemical and haematological implications. Pakistan Journal of Nutrition 4(5): 336-341.

10) Guo RM, 2007. Predicting nutrient responses in poultry: Future challenges. Animal 1(1): 57- 65.

11) Halldin K, Berg C, Brandt I, Brunstrom B, 1999. Sexual behaviour in Japanese quail as test end-point for endocrine disruption: effects of in ovo exposure to ethinylestradiol and diethylstilbestrol. Environmental Health Perspectives 107(11): 861–866.

12) Jones RB, 1986. The tonic immobility reaction of the domestic fowl: A review. World's Poultry Science Journal 42: 82 - 96.

13) Joseph NS, Dulanley AA, Robinson FE, Renema RA, Zuidhof MJ, 2002. The effects of age at photostimulation and dietary protein intake on reproductive efficiency in three strains of broiler breeders varying in breast yield. Poultry Science 81: 597–607.

14) Kilgour R, Dalton DC, 1983. Livestock Behaviour, Granada Publishing, London.

15) Mohan J, Mooudgal RP, Venkata K, Sastry H, Tyagi J, Singh R, 2002. Effects of hemicastration and castration on foam production and its relationship with fertility in male Japanese quail. Theriology 58: 29–39.

16) Morgan KN, Tromborg CT, 2007. Sources of stress in captivity. Applied Animal Behaviour Science 102: 262–302.

17) NRC, 2011. Guide for the Care and Use of Laboratory Animals, 8th ed. National Research Council of the National Academies. National Academy Press, 246, Washington DC.

18) Oko OOK, 2011. Comparative efficacy of meal and extracts of *Aspilia 51fricana* leaf in laying quails. Journal of Animal Science 89(E-Suppl. 1), 759.

19) Oko OOK, Agiang EA, 2011a. Phytochemical activities of *Aspilia africana* leaf using different extractants. Indian Journal of Animal Science 81(8): 814–818.

20) Oko OOK, Agiang EA, 2011b. Phytochemical properties of *Aspilia africana* leaf. Planta Medica 12 (77): 1307.

21) Oko OOK, Agiang EA, Afolabi AF, 2011a. *Aspilia africana* leaf as egg booster and yolk colourant in quails. International Journal of Agriculture 3(1): 103 – 108.

22) Oko OOK, Agiang EA, Eneji CA, 2012a. Alterations in lipid profile of quails following *Aspilia africana* leaf extracts. World's Poultry Science Journal 68(Suppl. 1): 136.

23) Oko OOK, Agiang EA, Iso IE, 2012b. Dietary *Aspilia africana* leaf on nutrient digestibility and physic-chemical properties of intestinal segments in quails. Journal of Animal Science 90(suppl.3):136.

24) Oko OOK, Agiang EA, Osim EE, 2014a. Pharmacognosy of *Aspilia africana* plant: phytochemistry and activities, in: Gupta, V. K. (Eds.), Bioactive Phytochemicals: Perspectives for Modern Medicine. Vol. 2. Daya publishing House, New Delhi, pp. 383 – 409.

25) Oko OOK, Agiang EA, Osim EE, Asuquo OR, 2011b. Toxicological evaluation of crude extracts of *Aspilia africana* leaf. American Journal of Pharmacology and Toxicology 6(3)**:** 96 – 101.

26) Oko OOK, Agiang EA, Agwunobi LN, Eyoh GD, Ozung PO, Asuquo BO, 2014b. Effects of Feed form on dietary *Aspilia africana* leaf utilization by broilers Science into Practice, Conference on Animal Science. BSAS/AVTRW/EBLEX/WPSA/ KTN, Jubilee Campus Nottingham University, April 14–19, Notthingham, UK.

27) Okoli CO, Akah PA, Nwafor SV, Anisiobi AJ, Ibegbunam IN, Erojikwe O, 2007. Anti-inflammatory activity of hexane leaf extract of *Aspilia africana*. Journal of Ethnopharmacology 109(2): 219 - 225.

28) Okwuonu CU, Oluyemi KA, Baxter GD, Adesanya OA, Ukwenya VO, Odion BI, Ofusori DA, 2007. Effects of methanolic extract of *Aspilia africana* leaf on the ovarian tissue and weights of wistar rats. Internet Journal of Alternative Medicine 5(1): 12 – 16.

29) Oluyemi KA, Okwuonu UC, Baxter PG, Oyesola TO, 2007. Toxic effects of methanolic extract of *Aspilia africana* leaf meal on the estrus cycle and uterine tissue of wistar rats. International Journal of Morphology 25(3): 609 - 614.

30) Ottinger MA, Abdelnabi MA, Henry PFP, McGary S, Thompson N, Wu J, 2001a. Neuroendocrine and behavioral implications of endocrine disrupting chemicals in quail. Hormone and Behaviour 40: 234 – 247.

31) Ottinger MA, Brinkley HJ, 1979. Testosterone and sex-related physical characteristics during maturation of the male Japanese quail (*Coturnix coturnix japonica*). Biology of Reproduction 20: 905 - 909.

32) Ottinger MA, Abdelnabi M, Quinn M, Golden N, Wu J, Thompson N, 2002. Reproductive consequences of EDCs in birds; What do laboratory effects mean in field species? Neurotoxicology and Teratology 24: 17 – 28.

33) Ottinger MA, Pitts S, Abdelnabi MA 2001b. Steroid hormones during embryonic development in Japanese quail: Plasma, gonadal, and adrenal levels. Poultry Science 80: 795 – 799.

34) Oyesola TO, Oyesola OA, Okoye CS, 2010. Effects of aqueous extract of *Aspilia africana* on reproductive functions of female Wistar rats. Pakistan Journal of Biological Sciences 13(3): 126 – 131.

35) Quinn MJ, 2005. Effects of embryonic exposure to androgen-active endocrine disrupting chemicals in Japanese quail. Ph.D. Dissertation, Department of Animal and Avian Sciences, University of Maryland, Maryland.

36) Scott GR, Slomann KA, 2004. The effects of environmental pollutants on complex fish behaviour: integrating behaviour and physiological indicators of toxicity. Aquatic Toxicology 68: 369 - 392.

37) Smith AH, Odenyo AA, Osuji PO, Wallig MA, Kandil FE, Siegler DS, Mackie RI, 2001. Evaluation of toxicity of *Acacia angustissima* in a rat bioassay. Animal Feed Science and Technology 91, 41 - 57.

38) Touart L. 2005. Review paper for Avian two-generation toxicity test. Submitted to the US Environmental protection agency endocrine disruptor screening program, Washington DC. EPA Contract number 68-W-01-203.

39) Wada, M., 1982. Effects of sex steroids on calling, locomotor-activity and sexual behaviour in castrate male Japanese quail. Hormone and Behaviour 16(2): 147 – 157.

40) Watson JT, Adkins-Regan E, 1989. Activation of sexual behavior by implantation of testosterone propionate and estradiol benzoate into the preoptic area of the male Japanese quail (*Coturnix japonica*). Hormone and Behaviour 23(2): 251 – 268.

41) Wellock IJ, Emmans GC, Kyriazakis I, 2006. The effects of social stressors on the performance of growing pigs, in: Gous, R.M., Morris, T.R., Fisher, C. (Eds.), Mechanistic Modelling in Pig and Poultry Production. CABI, Wallingford, pp. 54 - 75.

42) Zelenka DJ, Cherry JA, Nir I, Siegel PB, 1984. Body weight and composition of Japanese quail (*Coturnix coturnix japonica*) at sexual maturity. Growth 48: 16 – 28.