APRW -11

Meta-Analysis of the Effect of Ginger (*Zingiber officinale*) on Semen Quality Characteristics of Breeder Cocks

I.P. Ogbuewu, O.O., Emenalom, I.C. Okoli and M.U. Iloeje
Department of Animal Science and Technology, Federal University of Technology, P.M.B. 1526, Owerri, Nigeria
Corresponding author: I.P. Ogbuewu; E-mail: dr.ogbuewu@gmail.com

Abstract
Some authors have reported positive associations between dietary ginger and semen quality in birds, whereas others have not confirmed this. The objective of this study was to evaluate semen quality of breeders cocks fed diets supplemented with different levels of ginger using meta-analysis. The database was created on articles published in peer-reviewed journals and 20 peer-reviewed journal articles on the effect of ginger on semen quality of chickens were identified using Google search engine. A total of 5 peer-reviewed articles were selected after meeting apriori quality criteria. Data for each study were extracted and analyzed using OpenMEE software. The estimated effect size of ginger was calculated for semen volume (SV), sperm motility (SM), sperm concentration (SC), live sperm (LS) and abnormal sperm (AS). The pooled estimate for SV was 0.905 ml, with a 95% confidence interval (CI) from 0.189 to 1.621 and the pooled estimate for SM was 0.026 with a 95% CI from -1.668 to 1.720. The pooled estimates for SC and LS were 1.209 and 0.173 with a 95% CI from -1.899 to 2.245 and -1.953 to -0.948, respectively. Four out of the 5 outcomes displayed high heterogeneity of ginger response (I^2, which is a measure of variation beyond chance, I^2 = 74.28% for SV, 96.34% for SM, 99.06% for SC and 97.33% for LS). AS had moderate heterogeneity (I^2 = 43.89%). These findings confirm that dietary ginger supplementation in chicken significantly improved semen volume and reduced the percentage of sperm with abnormal cells but had no significant effect on sperm concentration, motility, and live sperm.

Keywords: Meta-analysis, ginger, *Zingiber officinale*, semen, cock

Introduction

In poultry, cocks play a vital role in flock fertility because in poultry breeding the ratio of male to female is low (Ommati *et al*., 2013), and most importantly when hens are inseminated artificially. In poultry, sperm membranes are rich in polyunsaturated fatty acids (PUFA) that play an important role in influencing sperm fertilizing ability. PUFA act as a possible source of energy during *in vitro* storage under aerobic conditions and they are also involved in a series of biochemical and functional changes required in pre and post-fertilization stage. Conversely, PUFA makes sperm prone to lipid peroxidation caused by reactive oxygen species (Surai *et al.,* 2000). Sperm is low in antioxidant capacity, but enzymatic and non-enzymatic antioxidants in the seminal portion of the semen protect the sperm by scavenging the free radicals (Zhao *et al*., 2011). In chickens, high concentrations of PUFA in sperm membrane render them more vulnerable to lipid peroxidation (Cerolini *et al*., 1997). As a result of this, sperm membranes must be protected by a highly efficient antioxidant system to prevent lipid peroxidation damage during *in vitro* and *in vivo* storage. In view of this, several attempts have been made to enhance semen quality and the antioxidative capacity of seminal plasma in chicken via dietary manipulations using phytogenics with known antioxidant abilities (Saemi *et al*., 2012; Akhlaghi *et al*., 2014). Ginger, one of such phytogenics belongs to the family Zingiberaceae. It is rich in essential oils, beneficial trace minerals and bioactive principles (Ogbuewu, 2013). Currently, there are mixed reports regarding the effects of ginger on semen quality of chickens (Khan *et al*., 2012; Nahed *et al*., 2014).

This study, therefore, aimed to determine the effect of dietary ginger supplementation levels on semen quality of chickens using meta-analytical techniques.

Materials and Methods

Literature search and inclusion criteria: A literature search and screening process were initially conducted using Google search engine to create a dataset of ginger articles using the keywords: "ginger and cock semen", "dietary ginger and avian semen", "ginger and poultry semen". No date restrictions were placed in the search engine, thus including all the articles on the effects of ginger on poultry semen. Furthermore, reference lists of retrieved articles were reviewed to search for other related studies. Randomized trials that examined the effects of ginger on semen quality of chickens were used for this study and all the studies were published in peer-reviewed journals. The quality of the papers was assessed using quality assessment criteria developed by the first author of this study. Criteria examined included randomization of study groups, recording of production outcomes (semen volume, sperm concentration, sperm motility, live sperm and abnormal sperm), statistical analysis, and comparability of treatment groups at entry to each trial. A dataset was created to compile data on effect of ginger on semen composition of chickens according to the following inclusion criteria: an adequate description of randomization procedures used, number of animals in each group, sufficient data to determine the effect size for semen quality outcomes, a measure of effect amenable to calculation of a standardized mean difference (SMD) for continuous data, and a measure of variance (SE or SD) or P-value for each effect estimate or treatment and control comparisons. Standard deviation (SD) is an important input variable to a meta-analysis. Hence, in situations where standard error (SE) of the outcomes (semen or blood lipid) has been provided instead of the SD, the SD values were calculated from SE using Equation (1) (Higgins and Deeks, 2011).

$$SD = SE\times\sqrt{n} \qquad (1)$$

In the current study, *Hedges*' *d*, the SMD between ginger in control and treatment groups was used (Borenstein et al. 2009). Currently, *Hedges*'*d* is reported to be the most common and preferred metric in meta-analysis (Koricheva *et al.,* 2013). *Hedges*' *d* is used for studies with small sample sizes, is not affected by unequal sampling variance in paired groups, in contrast to the response-ratio metric (Morales and Traveset, 2009). Moreover, when studies report *X* and SDs, and for our purposes of comparing the control and treatment groups in terms of continues response variable, *Hedges*' *d* is among the preferred metric of effect sizes (Borenstein *et al*., 2009). *Hedges*'*d* was computed by using Equation (2) (Rosenberg *et al*., 2013).

$$d = \frac{\overline{\mathrm{X}}\mathrm{e}-\overline{\mathrm{X}}\mathrm{c}}{S}\mathrm{J} \qquad (2)$$

where, $\overline{\mathrm{X}}\mathrm{e}$ and $\overline{\mathrm{X}}\mathrm{c}$ are the mean of the experimental group and mean of the control group, respectively.

$$J = 1-x = \frac{3}{4\ (\mathrm{Ne}+\mathrm{Nc}-2)-1}$$

is a correction term for sample size and,

$$S = \sqrt{\frac{(Ne+Nc)s_c^2+(Nc-1)s_c^2}{Ne+Nc-2}}$$

is the pooled standard deviation. The variance of *Hedges' d* given by equation (3).

$$V_d = \frac{Ne+Nc}{NeNc}+\frac{\mathrm{d}^2}{2(Ne+Nc)} \qquad (3)$$

where Ne and Nc are the sample size of the experimental group and the control group, respectively (Rosenberg et al. 2013). The following data were extracted from the studies when the information was present; duration of feeding and inclusion level of ginger. The required inclusion criteria for a data set were met by five studies.

Statistical analysis, assessment of heterogeneity and publication bias: The inability to obtain additional information from the authors and inconsistency in analytics were the reasons for the rejection of 15 candidate studies. A meta-analysis was conducted on the extracted production outcomes using the OpenMEE software. Heterogeneity of results among the trials was quantified using the I^2 statistic. Negative values of I^2 were assigned a value of zero; consequently, I^2 lies between 0 and 100%. An I^2 value >50% may be considered indicative of substantial heterogeneity.

Results and Discussion

The overall pooled estimate for sperm production was 0.152 with a 95% confidence interval (CI) from -0.732 to 1.035 (Table 1). The results of the forest plots of subgroup analysis of sperm quality of cocks fed diets supplemented with ginger are presented in Figures 1. The analysis of abnormal sperm (I^2 = 43.89%) showed a moderate level of heterogeneity. Other semen outcomes measured showed high levels of heterogeneity, 74.28% for SV, 96.34% for SM, 99.06% for SC and 97.33% for LS. Analysis for heterogeneity is important in the meta-analysis because it tests the amount of variance within the group of studies compared with the within-study variation. A high I^2 suggests the difference between individual study outcomes is greater (or more variable) than expected. The difference in treatment response may actually be caused by differences due to other factors, including age, breed responses such as exotic birds versus local birds, dietary form of ginger and inclusion level. The meta-analysis of SV, SM, SC, and LS were compiled from five studies and altogether included that 384 of exotic birds and 180 local birds.

Table 1: Pooled estimated effect of dietary ginger on semen quality characteristics of chickens

Studies	Estimate	Lower bound	Upper bound	Std. error	p-value
Subgroup volume (mL)	0.905	0.189	1.621	0.365	0.013
Subgroup motility (%)	0.026	-1.668	1.720	0.864	0.976
Subgroup concentration ($\times10_9$/ ml)	1.209	-2.485	4.903	1.885	0.521
Subgroup live sperm (%)	0.173	-1.899	2.245	1.057	0.870
Subgroup abnormal sperm (%)	-1.450	-1.953	-0.948	0.257	<0.001
Overall	0.152	-0.732	1.035	0.451	0.736

The overall pooled result for SV and SM were 0.905 and 0.026 with a 95% CI from 0.189 to 1.621 and -1.668 to 1.720, respectively. SC and LS had overall pooled estimate of 1.209 and 0.173 with a 95% CI ranging from -2.485 to 4.903 and -1.899 to 2.245. Data used the meta-analysis of AS was compiled from four studies that included 384 of exotic birds. The overall pooled result for AS was -1.450 with a 95% CI from -0.953 to -0.948. The results indicate that ginger reduced the percentage sperm with abnormal cells in chickens. Studies have shown that chicken sperm membrane is high in PUFA which makes them vulnerable to lipid peroxidation (Cerolini *et al*., 1997). The significant reduction in the percentage of sperm with abnormal cells in the current study could be attributed to the ability of antioxidants contained in ginger to protect sperm membranes free radical attack during *in vitro* and *in vivo* storage.

Figure I shows the forest plot of the random effect of ginger on semen volume, motility, concentration, live sperm and abnormal sperm of chickens The standardized mean difference was standardized using the z-statistic. Thus, points to the left of the line represent a reduction in the trait and points to the right of the line indicate an increase in the variable. Each square represents the mean effect size for that study. The upper and lower limit of the line connected to the square represents the upper and lower 95% CI for the effect size. The dotted vertical line represents the overall effect size estimate. The diamond at the bottom represents the 95% CI for the overall estimate. The solid vertical line represents a mean difference of 0 or no effect.

Acknowledgments
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors but this publication was made possible by the Agriculture for Food Security 2030 (AgriFoSe2030) training fellowship on meta-analysis granted to IPO. The AgriFoSe2030 programme is funded by the Swedish International Development Cooperation Agency (Sida).

References

Akhlaghi, A., Ahangari, Y.J., Navidshad, B., Pirsaraei Z.A., Zhandi, M., Deldar,H., Rezvani, M.R., Dadpasand,M., Hashemi, S.R., Poureslami, R. and Peebles, E.D. (2014). Improvements in semen quality, sperm fatty acids, and reproductive performance in aged Cobb 500 breeder roosters fed diets containing dried ginger rhizomes. *Poult. Sci.,*93:1236–1243.

Borenstein, M., Hedges, L.V., Higgins, J.P.T. and Rothstein, H.R. (2009). Introduction to Meta-Analysis. Wiley, Chichester, England.

Koricheva, J., Gurevitch, J. and Mengersen, K. (2013). Handbook of Meta-Analysis in Ecology and Evolution. Princeton University Press, Princeton and Oxford.

Cerolini, S., Kelso, K.A., Noble, R.C., Speake, B.K., Pizzi, F. and Cavalchini. L.G. (1997). The relationship between spermatozoan lipid composition and fertility during aging of chickens. *Biol. Reprod.,* 57:976–980.

Ezzat, W., El-Slamony, A.E., Bealish, A.M.A., Ouda, M.M.M. and Sabry, M.M. (2017).Effect of adding dried ginger rhizomes to diets on semen quality and fertility rate in aged local cocks under Egyptian hot summer condition. *Egypt Poult. Sci.,*37(I): 233-249.

Higgins, J.P.T. and Deeks, J.J. (2011). Chapter 7: Selecting studies and collecting data. In: Higgins, J.P., Green, S., (Eds), Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011.

Khan, R.U., Naz, S., Nikousefat, Z., Tufarelli, V., Javdani, M., Qureshi, M.S. and Laudadio, V. (2012). Potential applications of ginger in poultry diets. *World's Poult. Sci. J.,* 68:245- 252.

Morales, C.L. and Traveset, A., 2009. A meta-analysis of impacts of alien vs. native plants on pollinator visitation and reproductive success of co-occuring native plants. Ecol. Let. 12, 716–728.

Nahed, S., Tamer, A., Amera, A. and Emad, G. (2014). The effects of dietary supplementation of different levels of thyme and ginger essential oils on performance, hematological, biochemical and immunological parameters of broiler chickens. *Global Veterinaria,* 12: 736-744.

Ogbuewu, I.P. (2013). Studies on the physiological responses of rabbits to ginger rhizome powder. Ph.D. Thesis, Department of Animal Science and Technology, Federal University of Technology, Owerri, Nigeria.

Ommati, MM, Zamiri, MJ., Akhlaghi, A., Atashi, H., Jafarzadeh, M.R., Rezvani, M.R. and Saemi, F. (2013). Seminal characteristics, sperm fatty acids, and blood biochemical attributes in breeder roosters orally administered with sage (*Salvia officinalis*) extract. *Anim. Prod. Sci.,* 53: 548-554.

Rosenberg, M.S., Rothstein, H.R. and Gurevitch, J. (2013). Chapter 6: effect sizes: conventional choices and calculations. In: Koricheva, J., Gurevitch, J., Mengersen, K. (Eds.), Handbook of Meta-analysis in Ecology and Evolution. Princeton University Press, Princeton and Oxford.

Saeid J.M., Shanoon, A.K. and Marbut, M.M. (2011). Effect of *Zingiber officinale* aqueous extract on semen characteristics and blood serum sex hormones concentration in broilers breeder male. *Int. J. Poult. Sci.,* 10(8): 629-633.

Saemi, F., Zamiri, M.J., Akhlaghi A., Niakousari, M. Dadpasand, M. and Ommati, M.M. (2012). Dietary inclusion of dried tomato pomace improves the seminal characteristics in Iranian native rooster. *Poult. Sci.,* 91: 2310-2315.

Shanoon, A.K. (2011). Effect of *Zingiber officinale* powder on semen characteristics and blood serum sex hormones concentration in broilers breeder male. *Int. J. Poult. Sci.,* 10(11): 863-866.

Shanoon, A.K. and Jassim, S.M. (2012). Effect of *Thymus vulgaris* and *Zingiber officinale* aqueous extract on semen parameters, testes weight and histology measurements of broilers breeder male. *Int. J. Poult. Sci.,* 11 (9): 594 - 598.

Surai, P.F., Brillard,J.P.,Speake, B.K., Blesbois, E., Seigneurin, F. and Sparks, N.H. (2000). Phospholipid fatty acid composition, vitamin E content, and susceptibility to lipid peroxidation of duck spermatozoa. *Theriogenology,* 53: 1025-1039.

Zhao, X., Yang, Z.B., Yang, W.R., Wang, Y., Jiang, S.Z. and Zhang, G.G.(2011). Effects of ginger root on laying performance and antioxidant status of laying hens and on dietary oxidation stability. *Poult. Sci.,* 90: 1720-1727.

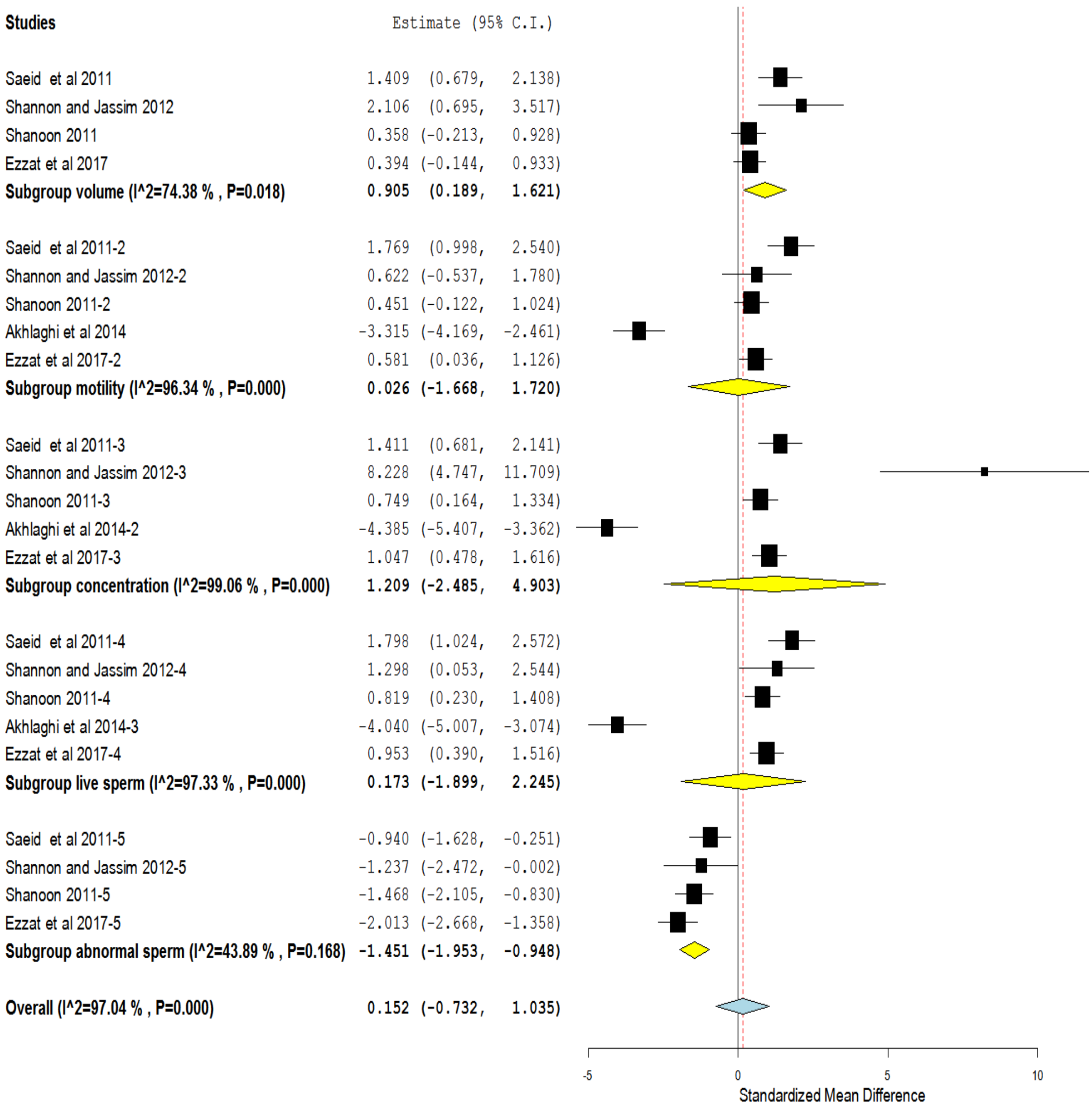

Fig. I: Forest plot of the random effect of ginger on semen volume, motility, concentration, live sperm and abnormal sperm of chickens.