

Efficacy of egg-yolk citrate extender fortified with aqueous garlic extract on rooster semen for artificial insemination

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Target audience: Reproductive Physiologist, Poultry breeders, farmers

Abstract

An experiment was designed to harness the potential of aqueous fresh and dried garlic extracts supplement in egg yolk extender for rooster semen to improve fertility and hatchability of egg. Aqueous extract of fresh and dry garlic were obtained and supplemented into egg yolk-citrate buffer at 0, 1, 2, 3, 4, 5, 6 and 7% for treatments 2, 3, 4, 5, 6, 7 and 8, respectively and unextended semen as control (T1). A total of 20 Marshal roosters and 160 hens of 40 weeks old were used for the experiment. The ejaculate pool of the marshal roosters was collected and pooled, divided into eight equal parts and randomly allotted to the treatment groups, the extenders were diluted with the semen in ratio 2:1 and evaluated for semen quality parameters. The hens were inseminated with the constituted extenders for three weeks and eggs were collected for the period of two weeks. The eggs were assessed for fertility and hatchability. The results obtained showed that livability of sperm cells and semen pH were not adversely affected by the treatments. The percentage sperm motility was high and within 80% to 100% in all aqueous dry garlic inclusive extended semen compare to aqueous fresh garlic extended semen which recorded 60% at 5% and 6% fresh garlic inclusion in egg yolk extender. The result of fertility of hens inseminated with egg yolk-aqueous dry garlic extracts extended semen showed that treatments 7 (5%) compare favourably with treatment 1 (unextended) with highest fertility percentage value of 80.0%. Aqueous fresh garlic extract inclusions of 2% and 6% had apparently better percentage values compare to unextended treatment. Inclusion of aqueous fresh garlic extracts at 6% in egg-yolk extenders, and 5% inclusion of aqueous dry garlic extract to egg yolk extender are recommended as an extender in roosters' semen extension.

Keywords: Egg-yolk citrate extender, aqueous garlic extract, rooster semen, artificial insemination

Description of problem

The increasing use of artificial insemination (AI) in the poultry industry emphasizes the need for the distribution

of good quality sperm cells. In order for the poultry industry to take advantage of modern AI techniques, proper storage of poultry semen is necessary. Chicken

semen is characterized by low volume and high concentration, several factors play a role in maintaining the quality of semen before use. The diluents used for semen extension and storage conditions such as time, aeration, and holding temperature play a major role (1 Dumpala *et al.*, 2006). The dilution of semen is associated with a reduction in cell viability and fertilizing capacity. The quality of extended and stored semen is affected by handling procedures such as dilution, centrifugation, dilution in semen extender and freezing. These procedures are associated with the generation of and imbalance among reactive oxygen species (ROS), (2; 3; 4; 5; 6).

Garlic possess antioxidants that scavenge free radicals and its damage, thus preserving the body's healthy functioning (7). Extracts of fresh garlic contain antioxidant phytochemicals that prevent oxidant damage. These include unique water-soluble organosulfur compounds, lipid-soluble organosulfur components and flavonoids, notably allixin and selenium (8). Garlic has been reported to possess tremendous health benefit and mitigate free radical accumulations. The property of garlic has been accredited to its antioxidant action (7).

The most common method of increasing poultry semen volume is by adding appropriate diluents to retain their viability *in-vivo*. Comparison of diluted and undiluted stored semen showed that applying extenders is necessary to sustain good-quality sperm (9). Antioxidant play an important role in

scavenging free radicals which otherwise may cause lipid peroxidation of sperm membrane (6). An extender must contain an antioxidant source to offer protection against lipid peroxidation. This experiment examines the potentials of fortifying egg yolk-citrate with aqueous garlic extract on rooster semen extension on fertility of hen artificially inseminated.

Materials and Methods

The fresh and dried garlic was purchased and the clove and bulb were separated, the garlic was crushed separately with blender. 40g of fresh and dried garlic sample were soaked with distilled water at ratio 1:4 Garlic to water for three days and was decanted, the filtrate was obtained from the residues.

20 roosters and 180 hens were purchased from a reputable breeding farm in Oyo State and were housed in battery cage containing one (1) rooster per cell and two (2) hens per cell. The birds were managed based on breeders' recommendations. Semen collection was carried out according to the modified semen collection procedure as outlined in (10), two times a week throughout the study. Care was taken to avoid any contamination of semen with cloacal products such as faeces. After which semen was harvested twice a week and ejaculate was taken to the laboratory for *in-vitro* analysis.

160 marshal pullets were randomly allotted to experimental groups, 5 replicates per treatment, 10 birds per treatment in a random manner. Fresh eggs were collected, sterilized the shell with alcohol, they were broken and

separation of yolk from albumen. Egg yolk and sodium citrate buffer solution were added and mixed together vigorously.

Semen pool of roosters divided into 8 equal parts and allotted their respective extenders in a completely randomized design. The treatments were diluted with the constituted extenders in ratio 1:2. For each of dry and fresh garlic extract their inclusion levels were 1% (T3), 2% (T4), 3% (T5), 4% (T6), 5% (T7) and 6% (T8) of egg yolk citrate, Unextended Semen (T1) and Egg Yolk citrate Extender (0%, T2).

Extended semen according to treatments were evaluated for motility, live-dead, abnormality and semen pH.

The hen insemination took place immediately after the assessment of semen quality. Each hen was everted and semen is deposited into the intra vagina of the hen through the use of tuberculin syringe in which tubular glass rod is attached to its mouth with the aid of rubber cork. The hens were inseminated twice every week in the evening for a period of 3 weeks. And the insemination doses were 0.05ml per hen. Eggs were collected for two weeks after the first week of insemination. Eggs collected were assessed for fertility and hatchability.

Statistical analysis: The data obtained were subjected to descriptive statistics,

analysis of variance (ANOVA) at $P = 0.05$. Differences in mean value are considered significant at 5% probability level.

Results and Discussion

The result of the extended semen quality is shown in Table 1. Egg yolk citrate fortified with dried garlic aqueous extract extended semen showed that across the treatments sperm motility ranged from 75% (T6) to 95% (T1), while sperm motility of egg-yolk fortified with aqueous fresh garlic extract extended semen showed that treatment means ranged from 55% (T8) to 95% (T1). It was observed that aqueous fresh garlic inclusion in egg yolk citrate extender slightly lowered the pH of extended semen. The highest semen pH (6.02) was observed in unextended treatment, while treatments 2-8 had values ranging from 5.82 to 5.92. The result of the egg yolk fortified with aqueous dried garlic extract extended semen indicated that control has the highest value of 6.05 and was significantly different from other treatments (2, 3, 4, 5, 6, 7 and 8) with values ranging from 5.95 to 5.70. The result of sperm livability in the treatments reveal that the fortified extended did not significantly ($p > 0.05$) influence livability of the sperm cells and had apparently similar structural membrane integrity.

Table 1: Semen characteristics of rooster extended with aqueous garlic extract fortified egg yolk citrate extender

	Unextended		Egg yolk-Garlic inclusion							
	T1	T2	T3 (1%)	T4 (2%)	T5 (3%)	T6 (4%)	T7 (5%)	T8 (6%)		
Motility %	95.00 ^a	90.00 ^{ab}	Aqueous extract of Fresh garlic							
Liveability %	100.00	100.00	80.00 ^b	87.50 ^{ab}	80.00 ^b	80.00 ^b	65.00 ^c	55.00 ^c		
Semen pH	6.05 ^a	5.91 ^b	100.00	100.00	100.00	100.00	100.00	100.00		
Sperm Concentration (X10 ⁸ Sperm Cells)	24.07 ^a	21.34 ^b	5.92 ^b	5.83 ^c	5.82 ^c	5.97 ^b	5.95 ^b	5.95 ^b		
			20.40 ^b	20.72 ^b	20.95 ^b	20.90 ^b	20.77 ^b	20.76 ^b		
Motility %	95.00 ^a	90.00 ^a	Aqueous extract of dry garlic							
Liveability %	100.00	100.00	95.00 ^a	95.00 ^a	92.50 ^a	80.00 ^b	77.50 ^b	75.00 ^b		
Semen pH	6.05 ^a	5.91 ^b	100.00	100.00	100.00	100.00	100.00	100.00		
Sperm Concentration (X10 ⁸ Sperm Cells)	22.76	21.39	5.89 ^b	5.95 ^b	5.89 ^b	5.92 ^b	5.93 ^b	5.71 ^c		
			21.80	21.37	21.96	21.22	21.90	21.96		

abc: means in the same row with different superscripts are significantly (P<0.05) different.

It was evident that the percentage sperm motility was high and was within 75% to 95% in all aqueous dry garlic inclusive extended semen compare to aqueous fresh garlic extended semen which recorded 65% and 55% at 5% and 6% fresh garlic inclusion respectively in egg yolk extender. Since motility of sperm cells is one of the factors that greatly determine the success of insemination. It can therefore be deduced from this result that dried garlic aqueous extracts has a good antioxidant capability on semen quality than fresh garlic aqueous extracts. Chain breaking antioxidants like Vitamin E can speed up the termination by catching free radicals (11) (12) reported that overproduction of ROS and decreased antioxidant defense activity cause low sperm motility and viability.

Furthermore it was observed that livability of sperm and semen pH was not adversely affected by both extenders (aqueous dry and fresh fortified extenders) at all inclusion levels. This suggests that the fortification of aqueous garlic extract does not possess adverse/lethal effect on liveability of the sperm cells. Aqueous extracts of garlic caused an increase in the catalase activity (13) and protects the testis and spermatozoa against cadmium toxicity (14), this could have contributed to result obtained in this study. Contrary reports in some studies have indicated that garlic harms the function of testes and has spermicidal effects on spermatozoa but some others demonstrated the opposite effect (15). Some of these different results might be due to the type of preparations used like

garlic powder (16), water extract (17) or the doses and the method of administration (gavage, i.p. injection, ad libitum). This study has revealed the contributions of aqueous garlic fortified extender to the enhancement of sperm cells activities of extended Rooster semen.

Fertility and hatchability of eggs from hens inseminated the various extended semen is shown in Table 2. The trend of result showed that inclusion of aqueous fresh garlic extracts to egg yolk citrate enhanced the fertility of eggs. The result revealed that the treatment 4, (2% inclusion) had the highest fertility of 83.9%, while inclusion of different levels of aqueous fresh garlic extract based extender had higher fertility values than ordinary egg-yolk extender, except for treatment 5 and 6 (3% and 4% inclusion). Aqueous fresh garlic extract inclusions of 2% and 6% had apparently better percentage values compare to unextended. The percentage hatchability of fertile eggs of hen inseminated with aqueous fresh garlic extract extended semen showed treatment 2 had the highest percentage value of 100.0, while treatments 5 and 6 recorded 91.67% and 90% respectively. The percentage hatchability of hen inseminated with aqueous fresh garlic extract extended semen showed that Treatment 5 had the highest percentage hatchability of 88.0%.

The result of fertility of hens inseminated with egg yolk-aqueous dry garlic extracts extended semen showed that treatments 7 (5%) compare favourably with treatment 1 (unextended) with highest fertility

percentage value of 80.0. The hatchability record of fertile egg sets of hens inseminated with aqueous dry garlic extracts extended semen revealed that treatment 2 and 6 had 100.0%, which was followed by treatments 8, 7, 3, 1, 5 and 4 with values of 93.3%, 91.67%, 88.24%, 87.5%, 84.62% and 80.0% respectively. The result of

hatchability of eggs set of hens inseminated with egg-yolk aqueous dry garlic extract extended semen revealed that treatment 7 had the highest value of 73.3, although treatment 1 had a close hatchability value of 70.0% which was followed by treatment 8, 3, 6, 2, 5 and 4 with values of 66.7%, 62.5%, 55.6%, 45.8% and 42.1% respectively.

Table 2: Fertility and hatchability of eggs as influenced by aqueous garlic fortification in egg yolk extender in rooster semen

	Unextended T1	Egg yolk extender T2	Egg yolk-Garlic inclusion					
			T3 (1%)	T4 (2%)	T5 (3%)	T6 (4%)	T7 (5%)	T8 (6%)
Aqueous extract of Fresh garlic								
% Hatchability	87.5	100	81.25	80.77	91.67	90	88.25	85.71
Hatchability of egg set%	70.00	55.56	61.90	67.74	88	40.91	68.18	70.59
Fertility%	80.00	55.60	76.20	83.90	56.00	45.50	77.30	83.30
Aqueous extract of Dry garlic								
% Hatchability	87.50	100.00	88.24	80.00	84.62	100.00	91.67	93.33
Hatchability of egg set %	70.00	55.56	62.50	42.11	45.83	60.00	73.33	66.67
Fertility%	80.00	55.60	70.80	52.60	54.30	60.00	80.00	71.40

The diluents used in poultry semen should be properly constituted and be evenly screened of any ingredient that may be deleterious to the maintenance and survival of the sperm cells, and adjudge not to hinder fertility. Impressively the fertilizing ability of aqueous fresh garlic extracts extended semen was better than unextended and 0% inclusions at 6% inclusion, and similarly 5% of aqueous dry garlic inclusion had overall performance better than unextended and pure egg yolk based extender. This is a good indication that aqueous garlic at 5% and 6% in dry and fresh garlic forms respectively, can perform optimally in extension of poultry semen for artificial insemination which could positively result into an encouraging fertilizing ability by the

breeders hen. (18) pointed out that antioxidant activity in seminal plasma and sperm is not high enough to prevent lipid peroxide damage after extension and *in vitro* storage, and that supplemental antioxidants could improve semen shelf life. Garlic inhibits lipid peroxidation, reducing ischemic/reperfusion damage and inhibiting oxidative modification of LDL, thus protecting endothelial cells from the injury by the oxidized molecules (19). However, phytochemicals from plant-rich diets, including garlic, provide important additional protection against oxidant damage (20). Interestingly, some of the treatments in the two garlic extracts formulated extenders performed comparable and

above the unextended semen in percentage fertility, this can be observed in treatments 4 and 8 for aqueous fresh garlic based extended semen and treatment 7 for aqueous dry garlic based extended semen. The performance of garlic based extenders could be attributed to the antioxidant abilities of this two garlic extracts formulated extenders. Expectedly, the hatchability of inseminated egg set was also favourable for the two formulated extenders across the treatments and still partially follows the same pattern as exhibited for fertility. It is a normal phenomenon to experience better hatchability subsequently following fertility provided candling is properly carried out.

Conclusion

It can be concluded that aqueous extracts of garlic (both fresh and dried garlic) significantly contribute to the extending abilities of egg yolk extender for rooster semen. It is recommended that fresh garlic aqueous extract can be supplemented up to 4% inclusion level in egg-yolk citrate extender, while dried garlic aqueous extracts can be used up to 6% level without degradable loss in semen quality.

The fortification of egg yolk citrate extender with aqueous extracts of fresh and dry garlic enhances the fertility and hatchability of pullet eggs. Inclusion of aqueous fresh garlic extracts at 6% in egg-yolk extenders, and 5% inclusion of aqueous dry garlic extract to egg yolk extender are recommended as an extender in roosters' semen extension which can give a better fertility and

subsequently good hatchability results. Poultry breeding farms should therefore endeavor to employ the use of aqueous garlic extracts as a supplement in their egg-yolk citrate extenders.

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