

EFFICACY OF EGG-YOLK CITRATE EXTENDER FORTIFIED WITH AQUEOUS GARLIC EXTRACT ON ROOSTER SEMEN

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Abstract

An experiment was outlined to investigate the effect of aqueous extract of fresh and dry garlic inclusion in egg yolk citrate extender on rooster semen. Fresh and dry garlic were soaked in distilled water (1:4) and were included in 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). The extenders were mixed with semen at ratio 1:2 and evaluated for semen quality parameters. The results obtained showed that livability of sperm cells and semen pH was not adversely affected by both extenders (aqueous dry and fresh fortified extenders) at all inclusion levels. The percentage sperm motility was high and was 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. This revealed that garlic both fresh and dried aqueous extracts possess significantly contribute to the extending abilities of egg yolk citrate extender.

Introduction

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), (Twigg *et al.*, 1998; Bilodeau *et al.*, 2000; Ball *et al.*, 2001; Chateerjee and Gagnon, 2001; Baumber *et al.*, 2005).

Antioxidants are believed to play a very important role in the body defence system against reactive oxygen species or free radicals, which are harmful by-products generated during aerobic activity of normal cells. Increasing the intake of dietary antioxidant is believed to assist in maintaining an adequate antioxidant status and therefore, the normal physiological function of living system.

Garlic possess antioxidants that scavenge free radicals and its damage, thus preserving the body's healthy functioning (Munday *et al.*, 1999). 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 (Ohnishi and Ohnishi, 2001). Thus, an investigation of the effect of fortifying egg yolk with aqueous garlic extract on rooster semen extension was undertaken.

Material and Method

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.

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. Aqueous extracts of fresh and dried garlic was added at 0%, 1%, 2%, 3%, 4%, 5%, and 6% for treatments 2, 3, 4, 5, 6, 7 and 8 respectively, with unextended semen as control (T1) in a completely randomized design. The mixture was centrifuge at 4000rpm for 5 minutes.

10 roosters were purchased from a reputable breeding farm in Oyo State and were managed based on breeder's recommendations. Prior to collection of the semen, the cocks were trained for 2 weeks, after which semen was harvested twice a week and ejaculate was taken to the laboratory for *in-vitro* analysis. The roosters semen pool sample was divided into eight equal parts in two places, and were diluted with the constituted extenders in ratio 1:2.

The two extracts were added separately to the egg yolk citrate extender

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

Statistical analysis: The data obtained were subjected to 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 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. 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 (Al-Daraji, 2004). Agarwal and Said, (2005) 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 (Amara *et al.*, 2008) and protects the testis and spermatozoa against cadmium toxicity (Ola-Mudathir *et al.*, 2008), 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 (Hammami *et al.*, 2008). Some of these different results might be due to the type of preparations used like garlic powder (Oi *et al.*, 2001), water extract (Challa *et al.*, 1997) 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.

Conclusion

It is therefore conclusive 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.

References

- Agarwal, A. Said, T.M. (2005) Oxidative stress, DNA damage and apoptosis in male infertility: a clinical approach. *BJU Int.* Vol. 95: pp.503-507.
- Al-Daraji H.J. (2004). Diluent supplementation with vitamins A, C and E for improving fertilizing ability of indigenous roosters' semen. Patent No. 3195, issued from C. O. S. Q. C., Iraq
- Amara S, Abdelmelek H, Garrel C, Guiraud P, Douki T. (2008) Preventive effect of zinc against cadmium-induced oxidative stress in the rat testis. *J. Reprod. Dev.* 54(2):129-134.
- Ball, B.A., Vo, A.T., Baumber, J. (2001). Generation of reactive oxygen species by equine spermatozoa. *Am. J. Vet. Res.*, 62:508-515.
- Baumber, J., Ball, B.A., Linfor, J. (2005). Assessment of the cryopreservation of equine spermatozoa in the presence of enzyme scavengers and antioxidants. *Am. J. Vet. Res.*, 66:772-779.
- Bilodeau, J.F., Chateerjee, S., Serrard, M. (2000). Levels of antioxidant defenses are decreased in bovine spermatozoa after a cycle of freezing and thawing. *Mol. Reprod. Dev.*, 55:282-288.
- Challa, A., Rao, D.R., Reddy, B.S. (1997). Interactive suppression of aberrant crypt foci induced by azoxymethane in rat colon by phytic acid and green tea.

- Carcinogenesis* 18(10):2023-2026.
- Chateerjee, S., Gagnon, C. (2001). Production of reactive oxygen species by spermatozoa undergoing freezing and thawing. *Mol. Reprod. Dev.*, 59:451-458.
- Hammami, I., Nahdi, A., Mauduit, C., Benahmed M., Amri, M. (2008) The inhibitory effects on adult male reproductive functions of crude garlic (*Allium sativum*) feeding. *Asian journal of andrology*. 10(4):593-601.
- Munday, J.S., James, K.A., Fray, L.M., Kirkwood, S.W. and Thompson, K.G. (1999). Daily supplementation with aged garlic extract, but not raw garlic, protects low density lipoprotein against *in vitro* oxidation. *Atherosclerosis*, 143 (2): 399 – 404.
- Ohnishi, T. and Ohnishi, T. (2001). *In vitro* effects of aged garlic extract and other nutritional supplements on sickle erythrocytes. *J. Nutr.*, 131: 1085S – 1092S
- Oi Y, Imafuku M, Shishido C, Kominato Y, Nishimura S. (2001) Garlic supplementation increases testicular testosterone and decreases plasma corticosterone in rats fed a high protein diet. *The Journal of nutrition* 131(8):2150-2156.
- Ola-Mudathir, K.F., Suru, S.M., Fafunso, M.A., Obioha, U.E., Faremi, T.Y. (2008). Protective roles of onion and garlic extracts on cadmium-induced changes in sperm characteristics and testicular oxidative damage in rats. *Food and Chemical Toxicology*. 46 (12):3604-3611.
- Twigg, J., Irvine, D.S., Houston, P. (1998). Iatrogenic DNA damage induced in human spermatozoa during sperm preparation: protective significance of seminal plasma. *Mol. Hum. Reprod.*, 4:439-445.

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

	Unextended	Egg yolk extender	Egg yolk-Garlic inclusion					
	T1	T2	T3 (1%)	T4 (2%)	T5 (3%)	T6 (4%)	T7 (5%)	T8 (6%)
Aqueous extract of Fresh garlic								
Motility %	95.00 ^a	90.00 ^{ab}	80.00 ^b	87.50 ^{ab}	80.00 ^b	80.00 ^b	65.00 ^c	55.00 ^c
Liveability %	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Semen ph	6.05 ^a	5.91 ^b	5.92 ^b	5.83 ^c	5.82 ^c	5.97 ^b	5.95 ^b	5.95 ^b
Sperm concentration (x10 ⁸ sperm cells)	24.07 ^a	21.34 ^b	20.40 ^b	20.72 ^b	20.95 ^b	20.90 ^b	20.77 ^b	20.76 ^b
Aqueous extract of dry garlic								
Motility %	95.00 ^a	90.00 ^a	95.00 ^a	95.00 ^a	92.50 ^a	80.00 ^b	77.50 ^b	75.00 ^b
Liveability %	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Semen ph	6.05 ^a	5.91 ^b	5.89 ^b	5.95 ^b	5.89 ^b	5.92 ^b	5.93 ^b	5.71 ^c
Sperm concentration (x10 ⁸ sperm cells)	22.76	21.39	21.80	21.37	21.96	21.22	21.90	21.96