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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Effect of adding orange juice into semen diluents on quality and storage ability of cocks' semen

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Abstract

The effect of adding orange juice into the extender of cocks' semen on mass motility, individual motility, dead and abnormal spermatozoa and acrosomal abnormalities were studied. A total of 60 White layer cocks, 32 weeks of age, were randomly divided into 6 groups of 10 cocks in this experiment. Semen samples for each treatment group were collected on a weekly basis during the whole experimental period which lasted 8 weeks. The treatment groups were; T1- the control (fresh semen); T2- the semen extended 1 : 1 with Al-Daraji 2 extender (AD2E) alone, whereas T3, T4, T5 and T6 represented semen samples extended with AD2E extender and supplemented with 1, 4, 7 or 10 ml of orange juice/100 ml extender. Results revealed that after 0, 24, 48 or 72 h *in vitro* storage, the supplementation of cocks semen extender with 7 and 10 ml orange juice/100 ml of extender (T5, T6) caused significant ($P<0.05$) increases in mass motility and individual motility of spermatozoa and significant decreases ($P<0.05$) in percentages of dead spermatozoa, abnormal spermatozoa and acrosomal abnormalities compared with control group. However, T2, T3 and T4 groups showed significant improvements in all of theses parameters in comparison with control group. Furthermore, there were no significant differences between T5 and T6 and between T2, T3 and T4 as regards all characteristics included in this study. In conclusion, the supplementation of orange juice into semen extender plays an important role in protecting spermatozoa against the harmful effects of lipid peroxidation during *in vitro* storage of cocks' semen for up to 72 h.

Keywords: Orange juice; semen quality; liquid storage; cocks

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Introduction

An important reason for the decrease in motility and viability during the storage of semen is the formation of lipid peroxides from oxygen radicals (Jones and Mann, 1977). The sperm plasma membranes contain a high amount of unsaturated fatty acids. Therefore, it is particularly susceptible to peroxidative damage, the lipid peroxidation destroys the structure of the lipid matrix in the membranes of spermatozoa, and it is associated with a loss of motility and membrane integrity (Sharma and Agarwal, 1996).

System that satisfy the metabolic requirement for oxygenation of avian spermatozoa during storage have improved the maintenance of fertilizing ability, but have led to consideration that the limiting factor of sperm maintenance may now be the deleterious effects of oxygen free-radicals and resultant lipid peroxidation. This has been identified as significant and problematic for both chicken and turkey spermatozoa, which having a high proportion of polyunsaturated fatty acids (PUFAs), are therefore considered to be likely to be particularly susceptible to lipid peroxidation (Surai et al., 2001).

Indeed, even at low temperatures, spermatozoa of both chickens and turkey were suffering from lipid peroxide during liquid storage (Donghue and Wishart, 2000). However, a correlation between semen quality and fertilizing ability and lipid peroxides following storage has yet to be made.

Blesbois et al. (1993) pointed out that in chicken peroxides are already present at the time of ejaculation,

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in equal concentration between spermatozoa and seminal plasma (2 to 4 × 10^{-2} nM Malonaldehyde/10^{9} spermatozoa). However, sperms are subject to oxygen toxicity resulting from lipid peroxidation, which can result in membrane damage, reduced motility and lower fertility (Donghue and Donghue, 1997).

Semen contains appreciable amounts of antioxidants that balance lipid peroxidation and prevent excessive peroxide formation (Lewis et al., 1997). However, the endogenous antioxidative capacity of semen may be insufficient during storage or dilution (Maxwell and Salamon, 1993). *In vitro* studies suggested that the addition of some antioxidants to diluted semen could improve the motility and survival of spermatozoa (Molinia et al., 1994; Sanchez-partida et al., 1997).

Recently, natural foods and food-derived antioxidants such as vitamin C and phenolic phytochemicals have received growing attention, because they are known to function as chemopreventive agents against oxidative damage (Kiwon et al., 2003). Gardener et al. (2000) studying the relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices, including orange, grapefruit, pink grapefruit, apple, pineapple and vegetable-juices and found that both vitamin concentrate-ions and total phenolic contents strongly correlated with antioxidant capacity. Antioxidant capacities of different juices varied markedly, orange juice being 5-7 folds more active than the vegetable juice and had the highest antioxidant power.

The objective of this study was to investigate the effect of adding different levels of natural orange juice to semen extender on quality of rooters' semen after liquid storage for up to 72 h.

Materials and Methods

The experiment was carried out to determine the effect of adding orange juice into semen diluent on quality and storage ability of cocks' semen. A total of 60 White Layer cocks, 32 weeks of age were randomly divided into 6 groups (10 cocks each). Birds were housed in separate terrestrial pens. Cocks were fed a commercial ration (16% protein and 2850 kcal metabolic energy/kg of diet) *ad libitum*. The semen was collected from all cocks manually by dorsal–abdominal massage (Lake and Stewart, 1978), on weekly basis, for 8 consecutive weeks (32-40 weeks of age). Semen samples for each treatment pen were divided into 3 test tubes of 1 ml each to provide 3 replicates pooled samples per each treatment. In order to maximize the quality and quantity of collected semen, collection was always performed under the same conditions (environment, time, persons, and massage method). Only clean ejaculates were used for treatments and evaluating. The experimental groups were as follows: T1 = fresh semen (control group); T2 = semen extended 1:1 with Al-Daraji 2 extender (AD2E) (Al-Daraji, 2004) alone; T3 = semen extended with AD2E and supplemented with 1 ml of orange juice (orange juice) / 100 ml of extender; T4 = semen extended with AD2E and supplemented with 4 ml of orange juice/100 ml of extender; T5 = semen extended with AD2E and supplemented with 7 ml of orange juice/100 ml of extender and T6 = semen extended with AD2E and supplemented with 10 ml orange juice/100 ml of extender. However, the levels of orange juice involved in the AD2E were choose on the basis of results of the preliminary experiment that conducted before the initiation of present study (unpublished data). The pH of extenders was adjusted to be 6.8–7.2 by using phosphate buffer solutions. Semen samples were then stored at the refrigerator temperature (4-6 $^{\circ}$C) for certain storage periods (24, 48 and 72 h). Orange juice were extracted from fresh orange and then the juice filtrated by filter paper before it supplemented to the extenders.

An aliquot of semen from each treatment group was evaluated directly after collection and then after *in vitro* storage for 24, 48 and 72 h for mass motility, individual motility, percentages of dead spermatozoa, abnormal spermatozoa and acrosomal abnormalities. Spermatozoa motility (movement in a forward) was estimated on a percentage basis by using the microscopic method of Sexton (Sexton, 1976). The measurement of dead spermatozoa was achieved by using a fast green stain–Eosin B stain–glutamate extender (Al-Daraji et al., 2002). Percentage of abnormal spermatozoa was determined by using a Gentian Violet–Eosin stain (Al-Daraji, 1998). As an alternative to evaluate acrosomal abnormalities in birds, staining procedure for fixed samples have been developed to distinguish which spermatozoa have retained or lost the acrosome (Al-Daraji, 2001). Data of experiment were evaluated by analysis of variance. Differences between experimental groups means were analyzed by Duncan's multiple rang test, using the ANOVA procedure in Statistical Analysis System (SAS, 2000).

Results and Discussion

The results obtained from this experiment confirm that the supplementation of the AD2E extender with 7 or 10 ml orange juice/100 ml of extender (T5, T6) improved significantly ($P<0.05$) mass motility, individual motility, percentage of live and normal spermatozoa and intact acrosomes in comparison with control (T1), T2, T3 and T4 groups (Tables 1, 2, 3, 4 and 5) when semen samples evaluated directly after collecting or after *in vitro* storage for 24, 48 and 72 h. Also T2, T3 and T4 surpass control group with respect

Table 1: Effect of adding orange juice into AD2E on mass motility (Mean ± SE) of cocks' semen stored for certain storage periods

Treatments	Storage periods (hours)			
	0	24	48	72
T1	83.1[(1)] ± 4.0[c]	39.0 ± 3.6[c]	18.3 ± 2.0[c]	2.1 ± 0.9[c]
T2	88.2 ± 3.6[b]	81.6 ± 4.0[b]	75.6 ± 1.7[b]	63.0 ± 4.3[b]
T3	89.1 ± 1.7[b]	83.3 ± 3.0[b]	77.2 ± 3.9[b]	64.2 ± 3.9[b]
T4	90.0 ± 3.3[b]	84.9 ± 2.2[b]	78.0 ± 6.0[b]	65.3 ± 2.6[b]
T5	95.3 ± 1.6[a]	90.3 ± 1.8[a]	87.3 ± 3.6[a]	79.6 ± 1.3[a]
T6	96.2 ± 5.3[a]	93.1 ± 3.3[a]	90.9 ± 5.5[a]	80.9 ± 5.0[a]

T1 = Fresh semen, T2 = semen diluted with AD2E alone, and T3–T6 = semen diluted with AD2E and supplemented with 1, 4, 7 and 10 ml of orange juice / 100 ml of extender, respectively; [(1)] Each value represented the mean of 8 consecutive measurements; [a, b, c] Means followed by different letters on the same column are significantly different at 5% level.

Table 2: Effect of adding orange juice into AD2E on individual motility (Mean ± SE) of cocks' semen stored for certain storage periods

Treatments	Storage periods (hours)			
	0	24	48	72
T1	84.6[(1)] ± 3.3[c]	40.3 ± 3.3[c]	19.9 ± 3.3[c]	4.9 ± 1.0[c]
T2	89.9 ± 5.7[b]	82.9 ± 5.5[b]	76.7 ± 3.8[b]	64.2 ± 4.3[b]
T3	90.3 ± 3.6[b]	84.0 ± 2.9[b]	78.1 ± 5.0[b]	65.8 ± 3.9[b]
T4	91.8 ± 2.7[b]	85.9 ± 3.6[b]	79.3 ± 4.2[b]	67.3 ± 2.6[b]
T5	96.0 ± 3.9[a]	91.6 ± 2.7[a]	88.1 ± 3.3[a]	81.3 ± 4.0[a]
T6	98.1 ± 4.4[a]	93.8 ± 3.0[a]	91.9 ± 5.6[a]	83.7 ± 2.9[a]

T1 = Fresh semen, T2 = semen diluted with AD2E alone, and T3–T6 = semen diluted with AD2E and supplemented with 1, 4, 7 and 10 ml of orange juice / 100 ml of extender, respectively; [(1)] Each value represented the mean of 8 consecutive measurements; [a,b,c] Means followed by different letters on the same column are significantly different at 5% level.

Table 3: Effect of adding orange juice into AD2E on percentage of dead spermatozoa (Mean±SE) of cocks' semen stored for certain storage periods

Treatments	Storage periods (hours)			
	0	24	48	72
T1	23.6[(1)] ± 2.2[a]	60.3 ± 3.0[a]	85.6 ± 1.3[a]	98.0 ± 5.8[a]
T2	16.0 ± 1.9[b]	30.1 ± 2.8[b]	43.9 ± 2.0[b]	53.6 ± 2.9[b]
T3	15.1 ± 2.0[b]	28.2 ± 1.3[b]	41.2 ± 1.3[b]	50.0 ± 1.7[b]
T4	13.3 ± 1.3[b]	27.0 ± 2.2[b]	40.0 ± 2.3[b]	50.9 ± 2.3[b]
T5	5.1 ± 2.0[c]	13.7 ± 1.6[c]	23.8 ± 1.6[c]	33.0 ± 1.7[c]
T6	3.0 ± 1.8[c]	11.2 ± 2.0[c]	20.3 ± 2.2[c]	30.8 ± 2.2[c]

T1 = Fresh semen, T2 = semen diluted with AD2E alone, and T3–T6 = semen diluted with AD2E and supplemented with 1, 4, 7 and 10 ml of orange juice / 100 ml of extender, respectively; [(1)]Each value represented the mean of 8 consecutive measurements; [a,b]Means followed by different letters on the same column are significantly different at 5% level.

to these characteristics. Furthermore, there were no significant differences (P> 0.05) between T2, T3 and T4, and between T5 and T6 concerning these spermatozoa traits.

The wholesome effects in semen quality that accompanied with enrichment of semen extender with

Table 4: Effect of adding orange juice into AD2E on percentage of abnormal spermatozoa (Mean±SE) of cocks' semen stored for certain storage periods

Treatments	Storage periods (hours)			
	0	24	48	72
T1	23.6[(1)] ± 2.6[a]	60.9 ± 3.7[a]	90.2 ± 6.7[a]	100.0 ± 0.0[a]
T2	14.0 ± 1.7[b]	29.0 ± 2.9[b]	50.3 ± 3.9[b]	63.2 ± 5.1[b]
T3	12.3 ± 2.0[b]	26.7 ± 1.3[b]	48.9 ± 2.2[b]	60.3 ± 3.9[b]
T4	11.9 ± 1.0[b]	26.0 ± 2.9[b]	47.0 ± 1.7[b]	60.9 ± 2.8[b]
T5	4.1 ± 2.9[c]	13.0 ± 1.7[c]	30.3 ± 2.6[c]	41.8 ± 4.0[c]
T6	3.3 ± 0.8[c]	10.8 ± 3.3[c]	27.8 ± 1.9[c]	39.3 ± 5.1[c]

T1 = Fresh semen, T2 = semen diluted with AD2E alone, and T3–T6 = semen diluted with AD2E and supplemented with 1, 4, 7 and 10 ml of orange juice / 100 ml of extender, respectively; [(1)] Each value represented the mean of 8 consecutive measurements; [a,b]Means followed by different letters on the same column are significantly different at 5% level.

Table 5: Effect of adding orange juice into AD2E on percentage of acrosomal abnormalities (Mean ± SE) of cocks' semen stored for certain storage periods

Treatments	Storage periods (hours)			
	0	24	48	72
T1	22.0[(1)] ± 2.7[a]	70.8 ± 3.5[a]	90.2 ± 2.9[a]	99.7 ± 6.5[a]
T2	17.6 ± 1.3[b]	35.0 ± 2.2[b]	48.8 ± 3.9[b]	60.3 ± 5.8[b]
T3	15.1 ± 2.0[b]	33.6 ± 4.3[b]	45.3 ± 1.7[b]	59.0 ± 3.6[b]
T4	14.7 ± 3.9[b]	33.9 ± 1.8[b]	45.0 ± 4.0[b]	58.1 ± 2.7[b]
T5	5.0 ± 4.4[c]	12.1 ± 1.4[c]	26.7 ± 3.9[c]	37.3 ± 4.8[c]
T6	2.3 ± 1.3[c]	13.9 ± 3.0[c]	23.9 ± 2.6[c]	35.9 ± 3.0[c]

T1 = Fresh semen, T2 = semen diluted with AD2E alone, and T3–T6 = semen diluted with AD2E and supplemented with 1, 4, 7 and 10 ml of orange juice / 100 ml of extender, respectively; [(1)] Each value represented the mean of 8 consecutive measurements; [a,b]Means followed by different letters on the same column are significantly different at 5% level.

orange juice may be account for it excellent source of vitamin C (Mermeistein, 1999; Martin et al., 2002), which acts as water–soluble antioxidant to scavenge aqueous peroxel radicals before these destructive substances have a chance to damage the lipids (Wainer et al., 1986). On the other hand, the major finding in recent years was the possibility of vitamin E in recycling from its oxidized (radical) form by means of vitamin C. However, vitamin C works synergistically with vitamin E (fat–soluble antioxidant) and the antioxidant enzyme glutathione peroxidase, performing their protective effects against lipid peroxidation and preserving cell membrane integrity (Chan et al., 1991; Donghue and Wishart, 2000; Friesleben and Packer, 1993). Furthermore, vitamin C can participate with vitamin E to protect molecules such as DNA from oxidative damage (Panpipe and Vanden Berg, 1997).

Many studies confirm our conclusions; Surai et al. (1998) reported that vitamin C as a water-soluble antioxidant was found in avian seminal plasma and it was negatively correlated with accumulation of reactive

oxygen species and positively correlated with the percentage of spermatozoa displaying normal morphology. Moreover, vitamin C is important for the spermatozoa since it has been shown to restore the physiological constitution of PUFA in cell membrane under certain conditions (Lenzi et al., 1996). Andrzej et al. (1999) found that the lack or low levels of ascorbic acid in semen highly correlated with the damage to male germ cells. Theses results are in agreement with Al-Daraji (2000) and El-Nasry et al. (2004) , who found that the supplementation of some antioxidants (vitamins A, C or E) to the semen extenders resulted in significant improvement in motility, survivability, morphology and fertilizing ability of cocks semen after *in vitro* storage for different periods. Other works reported that addition of ascorbic acid to sperm preparation medium did provide sperm with complete protection against H_2O_2– induced DNA damage and generation of H_2O_2– induced radical oxygen species (ROS) and was also significantly reduced after treatment with ascorbic acid (Cmhughes et al., 1998; Eilish et al., 1999). The antioxidant capacity of orange juice was not only vitamin C content of juice but may also arise from some of the phenolic compounds (Spanos and Wrolstad, 2004). The major phenolic compound in orange juice is ferulic acid (Augustin and Williams, 2000), it accounts especially good at neutralizing the free radicals known as superoxide, hydroxil radical and nitric oxide, and it acts synergistically with other antioxidant giving them extra potency and greatly reduces free radical damage to the external and internal membranes of cells (Zuo et al., 2002). This information gives us an indicator, that all improvements in semen quality when treated with orange juice belong to another agent (Ferulic acid) found in juice with vitamin C and they act together to protect spermatozoa from lipid peroxidation during storage. Our conclusion confirmed Zheng and Zhang (1997) who reported that ferulic acid was beneficial to sperm viability and motility in both fertile and infertile individuals, and that reduction of lipid peroxidative damage to sperm membranes and increase of intracellular cAMP and cGMP my be involved in these benefits, and it is possible that ferulic acid may be used for cure of asthenozoosperm infertility.

Conclusions

The present results lead to conclude that orange juice components especially vitamin C and ferulic acid produce good repression against lipid peroxidation during liquid storage of cocks semen. Thus, the addition of orange juice to semen extender was suitable agent for preserving semen quality when, semen stored at the refrigerator for up to 72 h.

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