



# Reproductive performance, semen quality, and fatty acid profile of spermatozoa in senescent broiler breeder roosters as enhanced by the long-term feeding of dried apple pomace

A. Akhlaghi<sup>a,\*</sup>, Y. Jafari Ahangari<sup>b</sup>, M. Zhandi<sup>c</sup>, E.D. Peebles<sup>d</sup>

<sup>a</sup> Department of Animal Science, College of Agriculture, Shiraz University, 71441-65186 Shiraz, Iran

<sup>b</sup> Faculty of Animal Science, Gorgan University of Agricultural Science and Natural Resources, 49189-43464 Gorgan, Iran

<sup>c</sup> Department of Animal Science, University College of Agriculture and Natural Resources, University of Tehran, Karaj 31587-77871, Iran

<sup>d</sup> Department of Poultry Science, Mississippi State University, MS 39762, USA

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## ABSTRACT

Avian spermatozoa are characteristically high in polyunsaturated fatty acids which predispose them to lipoperoxidation, thereby diminishing their fertility. As a by-product, well-known for antioxidative properties, dried apple pomace (AP) was fed to aging Ross 308 breeder roosters ( $n = 80$ ) at the dietary inclusion levels of 0 (AP<sub>0</sub>), 10 (AP<sub>10</sub>), 20 (AP<sub>20</sub>), or 25 (AP<sub>25</sub>) percent for 14 consecutive weeks. Seminal traits were studied every other week. At the end of the trial, sperm fatty acids profile, seminal plasma total antioxidant capacity (TAC), fertility, hatchability, and hatchling quality were evaluated, using 240 artificially-inseminated young hens. Dietary AP increased sperm motility and livability and decreased the seminal content of thiobarbituric acid reactive species. Dietary treatment and age interacted to positively affect sperm concentration and sperm membrane integrity. Feeding AP was associated with an increase in C20:4( $n-6$ ), C22:4( $n-6$ ), and total unsaturated fatty acids percentages. Birds in the AP<sub>25</sub> and AP<sub>20</sub> treatment groups respectively showed 6 and 7% increases in fertility rate. Interestingly, a higher hatchability rate was found for AP<sub>20</sub> group, associated with a higher number of sperm penetration holes in the perivitelline membrane and a lower rate of early embryonic mortality. However, hatchling quality was not affected by dietary AP. Overall, these data suggest that AP could remarkably improve several sperm characteristics, seminal TAC, fertility, and hatchability rate in aging breeder roosters. These improvements were also associated with a higher content of total unsaturated FA in the sperm plasma membrane. Future studies are needed to disclose the causal mechanisms involved.

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## 1. Introduction

A major concern in the poultry industry is the production of higher numbers of embryonated broiler hatching eggs. Both males and females contribute to the overall fer-

tility rate of a broiler breeder flock. However, the male contributes more than each female to overall flock fertility because the ratio of males to females in a flock is very low (Ommati et al., 2013). Nevertheless, the female also plays a critical role toward the production of eggs and in providing spermatozoa with a suitable microenvironment in the sperm storage tubules of its oviduct (Bramwell et al., 1996).

A central factor in the fertilizing capability of sperm is the lipid content of their plasma membranes (Cerolini

\* Corresponding author. Tel.: +98 711 6138358; fax: +98 711 2286073.

E-mail addresses: [aakhlaghi@shirazu.ac.ir](mailto:aakhlaghi@shirazu.ac.ir),  
[amirakhlaghi837@gmail.com](mailto:amirakhlaghi837@gmail.com) (A. Akhlaghi).

et al., 1997). Because sperm plasma membranes are high in polyunsaturated fatty acids (PUFA), spermatozoa are susceptible to lipoperoxidation by reactive oxygen species (ROS). An increased production of ROS is associated with male infertility (Surai et al., 2001). Lipoperoxidation irreversibly abolishes the fructolytic and respiratory activity, which may thereby result in a considerable decline in their respiratory rate and motility (Surai et al., 2001). Although the antioxidant capacity of sperm is low, enzymatic and non-enzymatic anti-oxidative compounds in seminal plasma are capable of protecting sperm against ROS (Zhao et al., 2011). Additionally, results of human studies suggest that a lower level of total antioxidant capacity (TAC) occurs in the seminal plasma of infertile men when compared with fertile men (Lewis et al., 1995). In light of the results of these studies, several approaches have been introduced to enhance semen quality and the anti-oxidative capacity of seminal plasma, including the use of dietary lycopene (Türk et al., 2007), carnitine (Neuman et al., 2002), sage extract (Ommati et al., 2013), dried tomato pomace (Saemi et al., 2012), and dried ginger rhizome (Akhlaghi et al., 2014). Nevertheless, further research is needed to develop efficient alternative methods by which to utilize these dietary sources for the improvement of the reproductive performance of male broiler breeders, particularly those that normally exhibit age-related sub-fertility (Bramwell et al., 1996).

Apples are a major global agricultural product, with total world apple production in 2011 reaching 75,484,671 tons (FAOSTAT, 2011). Fruit juice production occupies a large proportion of the worldwide apple product industry. Therefore, apple pomace (AP) by-product is produced in massive amounts by the apple industry. Attempts have been made to include the AP in the diets fed to dairy cows (Abdollahzadeh et al., 2010), sheep (Alibes et al., 1984), goats (Ahn et al., 2002), and finishing pigs (Cho et al., 2012). A limited number of workers tried to use AP in poultry feeds to provide more cost-effective diets. It has been suggested that up to 10% of dried AP may be safely used in broiler diets as a source of energy (Zafar et al., 2005; Ayhan et al., 2009). However, information on feeding AP to male birds and its effects on their reproductive performance is lacking in the literature.

Apples are well known for their anti-oxidative characteristics, which is due in large part to the polyphenolic compounds they contain (Lu and Yeap Foo, 2000). Apple polyphenols are mainly located in the peel and seeds of the fruit, which are retained in the pomace after juice extraction (Schieber et al., 2003). Therefore, AP should be considered as more than a major dietary energy source (e.g. maize) substitute. Because the high anti-oxidative activity of AP may be beneficial to the reproductive performance of male poultry, the current study was conducted to determine the effects of the long-term feeding of dried AP on the semen quality, sperm FA, and reproductive performance of aging broiler breeder roosters. Furthermore, in light of reports suggesting a pro-oxidative rather than an anti-oxidative effect of higher levels of some well-known antioxidants such as vitamins C (Podmore et al., 1998) and E (Chen et al., 1998), while considering the presence of some anti-nutrient compounds found in AP (e.g. tannins

and pectins; Givens and Barber, 1987), a group of roosters was assigned to be fed on the higher inclusion levels of AP to reveal plausible detrimental effects on the traits evaluated.

## 2. Materials and methods

### 2.1. Apple pomace

Fresh AP was obtained from a local fruit juice processing factory where the mechanical procedure was implemented for juice extraction. The pomace was then dried (40 °C; Apex, Kent, UK), ground by a disk mill (Skiold A/S, Jutland, Denmark), screened (300 µm), and stored (22–25 °C) away from sunlight prior to dietary inclusion. The dried AP supplied 5.49, 4.65, 18.23, and 1.85% crude protein, ether extract, crude fiber, and crude ash, respectively. The pomace contained 5.21 mg total phenolics, 4.73 mg total flavan-3-ols, and 0.84 mg total flavonoids per gram.

### 2.2. Birds and dietary treatments

A total of eighty 54-wk-old Ross 308 breeder roosters, each weighing 4650 g, were randomly allotted to 4 treatment groups. For each treatment, there were 4 replicate groups, with each group containing 5 birds. The birds were housed in individual cages and received either 0 (AP<sub>0</sub>), 10 (AP<sub>10</sub>), 20 (AP<sub>20</sub>), or 25 (AP<sub>25</sub>) g dried AP per 100 g of the diet (mainly as a substitute of maize and wheat bran). The treatment diets were prepared fresh every 2 wk, were fed for 14 successive wk [54–68 wk of age (woa)], and were formulated to meet or exceed National Research Council (NRC, 1994) recommendations (Table 1). From hanging feeders at the same time each day (0445 h), the roosters were fed 146 g diet/day per bird at 54 woa up to 154 g/day per bird at 68 woa. The birds were reared under similar conditions (a 15.5L:8.5D photoschedule photoperiod, and 21 °C ambient temperature) and were weighed every other wk. Ross 308 breeder hens (*n* = 240) which had no previous contact with male birds, were also used for sperm penetration assay, fertility, and hatchability determinations (see below). To avoid possible age-related sub-fertility issues in hens as well as to obtain the highest number of hatching eggs during a 14-d-long collection period, hens in peak production (32 woa) were used. The hens were fed a standard pelleted diet (2695 kcal of metabolizable energy/kg, 14.5% crude protein, 3.08% calcium, and 0.41% phosphorus) in trap-nested floor pens bedded with wood shavings.

### 2.3. Semen quality indices

#### 2.3.1. Gross evaluations

The roosters were habituated by abdominal massage for semen collection (52–54 woa) before the start of semen collection and evaluation. Semen samples were collected and evaluated every 2 wk between 54 and 68 woa. The seminal attributes did not include analyses of sperm fatty acids (FA) or seminal plasma TAC. These two variables were evaluated as single measurements at the end of the trial (68 woa). The ejaculates obtained from the 5 birds

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