Effects of soy isoflavones on intake, body weight, sex hormones, antioxidant performance, and semen quality in Xinong Saanen goats

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ABSTRACT
Efficacy of soy isoflavones to promote semen quality by improving antioxidant parameters in Xinong Saanen goats was explored. Soy isoflavones (SI), regulator of steroid hormone synthesis and antioxidant in organism, was supplemented in a total mixed ration of male Xinong Saanen dairy goats to study their effects on intake, body weight, level of testosterone and oestradiol, antioxidant performance, and semen quality. Supplementation with SI increased (P < 0.05) dry matter intake expressed as kg/day or % of body weight, with body weight remaining unchanged. In animals receiving SI supplementation, we observed increases (P < 0.05) in serum superoxide dismutase and total antioxidant capacity and decreases in oestradiol and malondialdehyde concentrations in comparison to the Control, while testosterone and glutathione peroxidase remained unchanged. An increase (p < 0.05) in plasma membrane integrity of sperm were observed in bucks received SI supplementation with no other effects on semen quality such as seminal pH, ejaculate volume, viability, motility, and acrosome integrity. The results indicated that SI supplementation improved semen characteristics via enhancing antioxidant parameters.

Highlights
- Supplementation of soy isoflavones increased feed intake in Xinong Saanen bucks.
- Soy isoflavones decreased serum oestradiol but not testosterone concentrations.
- Soy isoflavones could be used as a safe, effective, and natural antioxidant in dairy goats. It alleviated oxidative stress, without negative influence on sperm quality of Xinong Saanen bucks.

1. Introduction

Breeding bucks with desired characteristics are useful for improving the productive performance in dairy goats. Semen quality has economic significance to assure efficient and successful breeding via artificial insemination. Manipulations to increase semen quality in goats may be helpful if the semen quantity is not adversely affected.

Oxidative stress causes tissue injury by invading of inflammatory cell when the animals suffer from various harmful stimulations resulting in breaking the balance of the oxidation and antioxidation. Vital biological activities rely on ATP produced by oxidative phosphorylation involving metabolic processes of oxygen metabolites and peroxided molecule, which are described as reactive oxygen species (ROS). Oxidative stress occurs when ROS or oxidants exceed the capability of the cell to mount an effective antioxidant response. Consequently physiological functions of cells are impaired that could result in eventual death of cells (Ray et al. 2012; Sarikhani et al. 2018). There are sufficient evidences (Clyne 2011; Ranganathan et al. 2018) indicated the importance of low level of endogenous ROS in the regulation of the sperm functions such as capacitation, acrosome reaction, and the fusion of sperm and oocyte (Chandra et al. 2012). Over production of ROS may also damage sperm and the antioxidant system of seminal plasma and cause oxidative stress reaction with pathological effects (Homa et al. 2015). Oxidative stress influenced male reproductive function negatively by impairing structure and function of semen, increasing the apoptosis of germ cells, and disrupting the synthesis of steroid hormone (Lanzafame et al. 2009).

Soy isoflavones (SI), the polyphenol mixture with 3-chromone as the mother nucleus, found in the cotyledon and plamellar axis of soybean seed principally, includes daidzein, genistin and glycitin and their corresponding binding forms of glycoside, acetyl and malonyl glucoside (Munro 2003). Positive effects of SI, such as antioxidant performance (Wei et al. 1995; Wang and Wu 2017), antitumor action (Ono et al. 2017; Imai-Sumida et al. 2017), antiaging effect (Wang et al. 2013), prevention of osteoporosis (Akhalghi et al. 2019), obesity (Wang et al. 2017) and cardiovascular disease (Hanwell et al. 2009; Lu et al. 2019), and improving climacteric symptoms (Chedraui 2011) had been reported.
The application of SI had implications in livestock and poultry production, especially in the enhancement of immune function, bone metabolism, antioxidant ability, and reducing heat stress (Lee et al. 2005; Nuraini et al., 2017; Zhang et al. 2017). The SI contains phenolic hydroxyl groups which bind with free radical to enhance antioxidative function as well as chelate metal ions and promote antioxidative ability via the catalytic function of metal ions (Chun et al. 2005). The supplement of SI in fodder increased the expression of antioxidative protein and the activity of antioxidantase (Barbosa et al. 2011). SI, the physiological activator with function of weakening oestrogen, affected the level of hormone in vivo by influencing synthesis of protein and secretion of sex hormone (Zhu et al. 2016).

The objectives of the reported research were to study the effects of incorporation of SI into the diet as antioxidants and active substances of oestrogen on feed intake, body condition, secretion of steroid hormone, antioxidant ability and sperm quality in dairy goats.

2. Materials and methods

2.1. Animals and experimental diets

Twelve male Xinong Saanen dairy goats (2–3 years old, 72.5 ± 6.33 kg body weight) at Xinong Experimental Station of Northwest A&F University in Yangling (34°14′ N; 107°59′ E), Shaanxi, China, were equally divided into two groups and randomly assigned to one of the two experimental diets. The study was carried out from August to October. After analysis of the SI amount reported in animals (Masilamani et al. 2012), we confirmed that supplementation of additional 100 mg of SI could be effective without adverse effect/nontoxic. The control group (SI−) received a basic total mixed ration (Table 1) while the treatment group (SI+) received the same TMR plus a supplement of SI (Taiyuan Yongyao Biotechnological Limited Company, Taiyuan, Shanxi, China) at the rate of 100 mg/day. The TMR was formulated according to NRC (2007). The animals were adjusted to the feeding pens and TMR for two weeks prior to the experimental period and the experimental period lasted for 10 weeks. The goat pens were disinfected thoroughly prior to the initiation of the experiment while animals went through immunoprophylaxis and disinsec-
tization. The inactivated vaccines of goats (caprine infectious pleuro pneumonia, combined with ovine/caprine clostridial diseases vaccine) and abamectin (0.2 mg/kg) were subcu-
taneously injected. Animals were screened for quality of semen with emphasis on sperm counts, mobility and abnor-
mality prior to the initiation of the experiment. There was no difference (P > 0.05) in age, weight and sperm quality between the two groups as confirmed by the statistical analysis prior to the initiation of the experiment.

2.2. Feeding, intake and body weight measurements

Animals were divided into two groups and fed twice daily at 08:30 and 15:30 in two pens. The residual feed was recorded prior to the morning feeding daily. Average daily feed dry matter (DM) intake was calculated. Weekly empty body weight was recorded for each animal prior to the morning feeding.

2.3. Steroid hormone secretion and antioxidant index analysis

The jugular blood samples were collected from each animal once every two weeks. Alternate sampling from bilateral jugular vein was employed to minimize animal stress, reaction and vasculitis. The blood samples were incubated at 37°C for 0.5 h then centrifuged (3000 rpm, 10 mins, 4°C). The separated serum was stored at −20°C for further analyses.

Level of testosterone, oestradiol, glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), total antioxidant capacity (T-AOC) and malondialdehyde (MDA) in the serum were analysed by an ELISA kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

2.4. Semen quality analysis

Semen was collected every two weeks after morning feeding via artificial vagina. Does in oestrus were used as teasers. The volume and pH of semen were assessed immediately after collection. The volume was measured by a disposable syringe and pH was determined using pH test papers. Fresh semen was diluted in semen diluent (375 nM tromethamine, 124 mM citric acid and 41 mM glucose) at 1–4 at 37°C then transferred to vacuum cup within 1 h for further analysis.

Sperm sample (10 μl) was observed under the microscope (X400) to assess the semen viability and motility with three randomly selected microscopic fields and repeated three times.

The percent of sperm with intact plasma membranes (VIA) was detected by Hypoosmotic Swelling Test (HOST) as described (Irez et al. 2012) with minor modification. The sperms were placed in the glass dish containing hypoosmotic solution. Then the dish was placed in the incubator at 37°C for 30 min. In our study, HOST 3 and HOST 4 subgroups were considered as sperms with intact plasma membrane. At least 200 sperms were counted using microscope (X400) and

| Table 1. Composition and nutrient content of the concentrate (air-dry basis) |
|-----------------------------|------------------|
| **Ingredients (%) (as fed basis)** | **Content** |
| Corn | 60 |
| Wheat bran | 12 |
| Soybean meal | 20 |
| Rapeseed meal | 3 |
| CaHPO4 | 1.5 |
| NaCl | 1.5 |
| Premix | 2 |
| **Nutrient levels** | **Content** |
| Net energy (MJ/kg) | 5.87 |
| SE (MJ/kg) | 133.4 |
| Dry matter (%) | 95.2 |
| Crude protein (%) | 18.8 |
| Crude fat (%) | 11.7 |
| Crude ash (%) | 14.4 |
| **Vitamins (IU/kg)** | **Content** |
| A | 20000 |
| D | 4000 |
| E | 400 |
replicated three times. The differentiation was based on the criteria that the sperms with intact plasma membranes had bended and swelling tail and took in water to keep cellular balance of osmotic pressure. The sperms with no morphological alteration and inability to balance osmotic pressure indicated the damage of plasma membranes.

The acrosome integrity of sperms was evaluated by Trypan-blue Giemsa stain (Serafini et al. 2013) of sperm morphology. After smearing and staining preparation, acrosome integrity was detected under the microscope using oil immersion. The number of total sperms would have to be at least 200. Every analysis was repeated three times.

2.5. Statistical analysis

Data obtained from six animals were expressed as mean ± SD. One-way ANOVA for repeated measures was used to determine the treatment effects of SI in body weight and dry matter intake for each week. For the time course of the hormone and semen quality parameters, a two-way ANOVA with repeated measures was used. The Tukey–Kramer post hoc analysis was used to identify significant differences between treatments (SPSS 19.0). The significant differences between the means were at P < 0.05.

3. Results

Feed DM intakes of SI+ group were higher (P < 0.05) than those in SI-group during Week 2, 4, 5, 6, 7, and 8 (Figure 1(A)), while there is no difference (P > 0.05) during Week 1 between groups. There were no differences (P > 0.05) in empty body weight between groups throughout the entire experiment period (Figure 1(B)). Average feed DM intake expressed as % of empty body weight was higher (P < 0.05) in SI+ than SI−, 5.03 and 4.65%, respectively (Figure 2).

There were no differences (P > 0.05) in serum testosterone between groups during the entire experimental period (Table 2). Serum oestradiol were lower (P < 0.05) in SI+ than SI− during Week 2, 6, 8, 10 of the experimental period (Table 2). The oestradiol concentration also decreased in Week 2, 4, 6, 8, 10 in SI+ group. There were no differences (P > 0.05) in serum GSH-Px between SI+ and SI− during the entire experimental period (Table 3). The SOD was higher (P < 0.05) in SI+ than SI− during Week 6 and 10 of the experimental period. The T-AOC was higher (P < 0.05) while MDA was lower (P < 0.05) in SI+ than SI− during Week 4, 6, 8, and 10 of the experimental period.

Supplementation of 100 mg/day had no effect (P > 0.05) on serum volume and pH, and viability, motility and acrosome integrity of sperm (Table 4). However, the data seemed to indicate that SI promoted (P < 0.05) plasma membrane integrity during Week 4 and 10 (Table 4) of the experimental period.

4. Discussion

Our study demonstrated that supplementation of soy isoflavones at the rate of 100 mg/day increased feed DM intake and improved oxidation resistance and semen quality in Xinong Saanen bucks.

Figure 2. Mean (±SEM) for intake/weight for male Xinong Saanen dairy goats fed diet with supplementation of soy isoflavones (SI+) versus control (SI−). The treatment value was different (P < 0.05) as compared to the control.

It has been reported that supplementation of SI increased animal (pigs and rats) intake (Greiner et al. 2001; Payne et al. 2001; Watkins et al. 2005). We found similar increases in feed DM intake with the supplementation of 100 mg/day in Xinong Saanen bucks. Specifically the increases were observed in Week 2, 4, 5, 6, 7, and 8. The soy flavones with soy fragrance stimulate animals’ appetite to induce the increase of intake. In bucks, the situation would potentially occur as goats had a higher threshold for bitter tastes (Robertson et al. 2006) to overcome the bitter tastes from SI (Meinlschmidt et al. 2016).

Palatability of fodder, genetic environment, feeding, management, and disease all could influence animals’ intake which was one of the major factors affecting animal
reproductive performance (Decruyenaere et al. 2009). The onset of the experimental period was in August and completed in October, which might be a factor of intake onset of the experimental period was in August and completed in the early part of the experiment (Lu1989). The strong libido exhibited by the bucks during breeding period prior to and in during the initial period of the experiment might have resulted in the loss of appetite (Walkden-Brown et al. 1994; Zarazaga et al. 2009).

The significant difference observed in intake expressed as % of empty body weight between SI+ and SI− could also be attributed to the beneficial effect of SI on reducing oxidative stress and accelerating immunity (Ryan-Borchers et al. 2006). SI supplement kept animals from oxidative stress and heat stress that was induced by intensive feeding under higher ambient temperature (Xu et al. 2009).

SI, a promoter of glucose and lipid metabolism, could help animals maintain the body weight (Silva et al. 2018). Body weights of dairy goats were in during the initial period of the experiment might have been influenced by factors such as age, dietary energy density, dietary protein, age, breed, environment and management (Lu and Potchoiba 1990). In our study, the variability of body weight in SI+ bucks was unstable appetite contributed to the variability in body weight which was in unstable appetite contributed to the variability in body weight among animals, which accompanied the changes in hormone secretion. Seasonality of buck’s reproductive performance was obviously reflected by changes detectable behaviour, testicular morphologic characteristics and hormone secretion, which followed the seasonal activity of the ewes (Sarlós et al. 2013). Our study was carried out during breeding period when the blood plasma testosterone concentration remained relatively high level. The testosterone concentration was directly influenced by frequently stimulation from female effect. Oestradiol, enzymatic product of testosterone, was converted from testosterone by cytochrome P450 aromatase in testis (Liguori et al. 2018). In our study, supplement of SI inhibited oestradiol secretion which would produce feedback effect on testosterone. We considered that further molecular experiment in testicular cells should be applied to explain the SI effect on the relationship of testosterone and oestradiol.

Generally, it is known that reproductive hormones that regulate reproductive process can have a direct effect on animal reproductive performance. Testosterone promotes the development of sexual organs and secondary sex characteristic, accelerates the synthesis of protein and reduces urinary nitrogen (Eidelsberg et al. 1942; Wilson et al. 1973), and maintains normal sexual function (Walker 2011). Testosterone was an important hormone which could be used to measure breeding stock reproductive performance (Tyagi et al. 2017).

It has been reported that SI, with a weak oestrogen function (Barnes 2004), affected male neuroendocrine system, promoted the synthesis of GH in pituitary and IGF-1 in liver (Antonella 2007), via oestradiol receptors in hypothalamus and pituitary (Setchell 2001; Sá 2014). In our study we did not observe any changes in testosterone concentration as a result of SI supplementation. Other studies also reported no effects of SI on testosterone concentrations in roosters (Gjorgovska and Filev 2013) and rabbits (Yousef et al. 2003; Yousef et al. 2004). A seasonal variation in reproductive performance can be observed in goats, which accompanied the changes in hormone secretion. Seasonality of buck’s reproductive performance was obviously reflected by changes detectable behaviour, testicular morphologic characteristics and hormone secretion, which followed the breeding activity of the ewes (Sarlós et al. 2013). Our study was carried out during breeding period when the blood plasma testosterone concentration remained relatively high level. The testosterone concentration was directly influenced by frequently stimulation from female effect. Oestradiol, enzymatic product of testosterone, was converted from testosterone by cytochrome P450 aromatase in testis (Liguori et al. 2018). In our study, supplement of SI inhibited oestradiol secretion which would produce feedback effect on testosterone. We considered that further molecular experiment in testicular cells should be applied to explain the SI effect on the relationship of testosterone and oestradiol.

\[\text{Table 2. Effect of supplementation of soy isoflavones on serum testosterone and oestradiol concentrations} \]

| Week | SI− (pg/ml) | SI+ (pg/ml) | SI− (pg/ml) | SI+ (pg/ml) |
|------|-------------|-------------|-------------|-------------|
| 0    | 380.82 ± 97.12 | 373.91 ± 107.61 | 92.69 ± 20.1 | 90.85 ± 19.28 |
| 2    | 394.64 ± 47.53 | 391.65 ± 76.09 | 97.72 ± 12.48 | 64.57 ± 14.18 |
| 4    | 373.67 ± 73.36 | 431.10 ± 92.40 | 94.61 ± 25.10 | 71.32 ± 14.18 |
| 6    | 391.4 ± 95.86 | 407.25 ± 75.09 | 101.54 ± 17.44 | 71.15 ± 7.59 |
| 8    | 392.87 ± 63.37 | 472.97 ± 41.86 | 96.86 ± 18.94 | 76.92 ± 11.9 |
| 10   | 363.49 ± 86.33 | 460.82 ± 80.91 | 75.01 ± 13.93 | 75.91 ± 16.28 |

\[\text{Mean (±SEM).} \]

\[\text{ab and AB P < 0.05, AB showed the differences in same treatment group, ab showed treatment differences within the same week .} \]

\[\text{Table 3. Effect of supplementation of soy isoflavones (SI+) versus control (SI−) on serum glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), total antioxidant capacity (T-AOC) and malondialdehyde (MDA) concentrations} \]

| Week | GSH-Px (U/mL) | SOD (U/mL) | T-AOC (U/mL) | MDA (mmol/mL) |
|------|--------------|------------|--------------|---------------|
| 0    | 209.21 ± 31.17 | 216.06 ± 26.19 | 76.31 ± 10.24 | 77.06 ± 9.51 |
| 2    | 216.73 ± 29.19 | 223.37 ± 21.62 | 79.60 ± 9.39 | 84.58 ± 7.47 |
| 4    | 220.51 ± 16.36 | 227.76 ± 19.16 | 80.96 ± 7.85 | 91.15 ± 7.11 |
| 6    | 219.14 ± 18.46 | 214.91 ± 14.33 | 78.21 ± 7.90 | 99.07 ± 6.14 |
| 8    | 199.86 ± 33.29 | 217.06 ± 23.51 | 79.34 ± 9.18 | 89.44 ± 8.31 |
| 10   | 203.11 ± 19.01 | 231.17 ± 21.88 | 75.36 ± 6.55 | 97.81 ± 7.64 |

\[\text{ab and AB P < 0.05, treatment within the same week differs.} \]
It had been reported that effects of supplementation of SI on serum oestradiol concentration was inhibitory (Kao et al. 1998; Xu et al. 1998; Kumar Rajan et al. 2017). With $1/10^{-5}$ to $1/10^{-3}$ activity as that of oestradiol, SI bond with oestradiol receptor beta to inhibit the intact function and feedback regulation of oestradiol (Setchell 2001). Another reason for the reduction of oestradiol by SI supplementation is that SI act as inhibitors of steriodogenic enzymes (aromatase and 17β-hydroxysteroid oxidoreductase) which caused oestradiol level to decrease (Deluca et al. 2005; Wang et al. 2008). In our study we observed a consistent decrease in serum oestradiol concentration with SI supplementation throughout the experimental period, with the exception of Week 4. The data showed the obvious inhibition effect of SI on oestradiol following but no influence on testosterone. The supplement of SI occupied the oestradiol receptor, weakening activity of aromatase to cause the decrease of oestradiol (Carreau et al. 2006). It is also possible that effects of SI supplementation on serum testosterone and oestradiol concentrations in male bucks could be multifactorial, as the interactions may permeate throughout reproductive organs. In addition, it could also be related to animal species, age, sex, the amount of SI supplementation, and the length of the experiment.

The antioxidative factors in serum, such as T-AOC, SOD, MDA, GSH-Px, were important parameters to evaluate oxidation resistance of SI in vivo (Tao et al. 2005). It has been proved that dietary SI enhanced antioxidant properties in different species (Wei et al. 1995; Amigo-Benavent et al. 2008; Dixit et al. 2012). In our study, we observed SI intake stimulated accumulation of SOD and T-AOC and inhibited the serum MDA concentration. MDA, end product from fatty peroxide formation, caused cross-linking polymerization of protein resulting in cytotoxicity effect (Ulbrecht et al. 2019). SOD was a highly conserved enzyme that played an important role in the balance of oxidation and anti-oxidation by dismutation of superoxide into oxygen and hydrogen peroxide (Tsang 2014). T-AOC was the parameter which reflected the whole anti-oxidation performance including antioxidant and antioxidase (Pisoschi et al. 2015). It seemed to confirm that SI supplementation enhanced enzymatic action of antioxidant system and reduced the degree of lipid peroxidation (Akitha Devi and Giridhar 2015). There were reports that the likely mechanism of which SI intake influenced antioxidant action of male bucks was increasing the expression of antioxidant genes via oestrogen receptors, ERK1/2 (Kang et al. 2007), and NFκB (Davis 2001). It is advisable that further study on molecular mechanism of SI oxidation resistance could be carried out in vivo and in vitro. It had been reported that after treated with high dose SI for 12 months, morphological and histopathologic assessment of testis, sperm quality remained unchanged in rats (Faqi et al. 2004), humans (Beaton 2010), rabbit (Yousef et al. 2004; Cardoso 2007), mouse (Jung et al. 2004), and monkey (Sharpe et al. 2002). In our study, there were no changes observed in pH, volume, viabiliity, motility and acrosome integrity in bucks with SI supplementation. However, plasma membrane integrity of sperm was significantly increased in bucks with SI supplementation. Several studies reported that SI had no adverse effects on reproductive performance of animals (Faqi et al. 2004; Cardoso 2007; Cardoso and Bao 2009). Similar results

| Table 4. Effect of supplementation of soy isoflavones (SI+) versus control (SI−) on semen quality |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Week | Ejaculation volume (mL) | pH | Survival ratio (%) | Motility (%) | Plasma membrane integrity (%) | Acrosome integrity (%) |
| SI− | SI+ | SI− | SI+ | SI− | SI+ | SI− | SI+ | SI− | SI+ |
| 0  | 1.13 ± 0.69 | 1.44 ± 0.36 | 6.73 ± 0.19 | 6.76 ± 0.21 | 87.19 ± 5.19 | 86.16 ± 4.95 | 69.44 ± 2.83 | 68.91 ± 4.42 | 64.62 ± 3.16 | 66.62 ± 5.58 |
| 2  | 1.24 ± 0.49 | 1.37 ± 0.58 | 6.79 ± 0.15 | 6.87 ± 0.06 | 85.34 ± 3.60 | 84.54 ± 3.60 | 71.23 ± 5.26 | 71.31 ± 5.07 | 65.54 ± 3.16 | 66.62 ± 3.65 |
| 4  | 1.38 ± 0.66 | 1.46 ± 0.34 | 6.77 ± 0.16 | 6.76 ± 0.16 | 84.23 ± 3.77 | 83.59 ± 3.89 | 69.21 ± 8.62 | 69.18 ± 8.62 | 66.24 ± 4.18 | 66.24 ± 4.18 |
| 6  | 1.37 ± 0.51 | 1.46 ± 0.34 | 6.78 ± 0.09 | 6.76 ± 0.09 | 83.59 ± 3.86 | 83.59 ± 3.86 | 69.21 ± 8.62 | 69.18 ± 8.62 | 66.24 ± 4.18 | 66.24 ± 4.18 |
| 8  | 1.29 ± 0.72 | 1.46 ± 0.34 | 6.65 ± 0.08 | 6.70 ± 0.19 | 83.69 ± 9.23 | 83.69 ± 9.23 | 69.21 ± 8.62 | 69.18 ± 8.62 | 66.24 ± 4.18 | 66.24 ± 4.18 |
| 10 | 1.07 ± 0.45 | 1.23 ± 0.19 | 6.69 ± 0.17 | 6.69 ± 0.17 | 86.05 ± 5.21 | 86.05 ± 5.21 | 62.71 ± 4.31 | 62.71 ± 4.31 | 57.64 ± 9.72 | 57.64 ± 9.72 |

*P < 0.05, treatment within the same week differs.
were also obtained in our study. We concluded that increase of plasma membrane integrity of sperm had a high correlation with antioxidant performance induced by SI (Paul and Smk 2017). The HOST was described as a classical assay to evaluate function of sperm and integrity of physiological function of sperm plasma membrane (Lechniak et al. 2003). Sperm plasma membrane, an important part of spermatozoan, is related to most of reproductive process, such as energy metabolism, capacitation, acrosomal reaction and fusion of sperm and oocyte (Jeyendran et al. 1984; Grieblova et al. 2017).

In conclusion, the study indicated that SI affected the levels of the endogenous hormone and antioxidant parameters and can be considered as a safe, effective, and natural antioxidant with no negative influence on reproductive organs and sperm quality of bucks.

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