CTU Journal of Innovation and Sustainable Development
ISSN 2588-1418 | e-ISSN 2815-6412

DOI:10.22144/ctujoisd.2025.020

Effects of chive (*Allium schoenoprasum)* bulb extract on semen quality and blood biochemical profiles of roosters under heat stress conditions

Phan Vu Hai*, Nguyen Dinh Thuy Khuong, and Nguyen Xuan Hoa

Faculty of Animal Science, College of Agriculture and Forestry, Hue University, Viet Nam

**Corresponding author (phanvuhai@hueuni.edu.vn)*

Article info.

Received 12 Apr 2024
Revised 9 May 2024
Accepted 29 Oct 2024

Keywords

Chive bulb, heat stress, rooster, semen characteristics

ABSTRACT

Heat stress (HT) is a factor seriously affecting the semen quality and antioxidant status of roosters. This study was designed to evaluate the effect of chive bulb extract (CBE), as a natural antioxidant, on roosters exposed to heat stress (HT). Twenty-four 3F-Viet roosters were randomly divided into 3 treatments (CBE was added to drinking water at 3 doses: 0, 1 or 2 ml/l) with 4 replicates to determine the effect of CBE on semen characteristics and some blood biochemical parameters of roosters in the hot season. Treatments with CBE at concentrations of 1% or higher significantly improved semen characteristics, including semen volume, sperm concentration, and motility ($p > 0.05$), while significantly reducing sperm mortality and abnormalities ($p < 0.05$) compared to the control. In addition, the antioxidant malondialdehyde was significantly reduced ($p<0.05$), whereas TAC and serum levels of LH, FSH and testosterone significantly increased ($p<0.05$) in the treatment supplemented with 2% CBE. It can be concluded that CBE possessed fertility-promoting properties in roosters due to its antioxidant properties and increased reproductive hormone activity.

1. INTRODUCTION

Chickens lack sweat glands to dissipate heat and have insulating feathers, making them highly susceptible to heat stress (HT) during hot summers, which can negatively impact rooster fertility. (Attia et al., 2019; Karaca et al., 2003; Attia et al., 2019). The optimal expression of genetic potential in roosters lies in an average temperature range of 22–26°C (Cassuce et al., 2013). Reductions in testicular weight, semen volume, sperm concentration, percentage of viable sperm, motility, and an increase in the rate of ectropion caused by the excessive presence of reactive oxygen species (ROS, Reactive Oxygen Species) are produced when birds suffer from sneezing (Fouad et al., 2016). Unsaturated fatty acids contained in poultry sperm are susceptible to lipid peroxidation, generating many harmful ROS (Surai, 2016a). In this regard, recent attention has been given to the use of natural antioxidants to mitigate the adverse effects of HT on semen quality by reducing free radical generation, both affordable and without side effects (Attia et al., 2019). Natural feed sources, such as herbs and their extracts, overcome the adverse effects of HT on chicken growth (Mahmoud et al., 2014).

Chive bulb (*Allium schoenoprasum*) is a popular agricultural product in mountainous and sandy regions of Viet Nam's central provinces. Chive bulbs contain many medicinal substances with high biological activity, including allicin, diallyl disulfide, ajoene, organosulfur, polyphenols, saponins and fructo-oligosaccharides (Thanh, 2012). Our recent research shows that ethanol

extract from chive bulbs has antibacterial properties (Hai et al., 2020, 2019a) and prevents and treats diarrhoea caused by *E. coli* (Hai, 2019b) in broiler chicken. Additionally, many researches have highlighted a strong relationship between antioxidant activity and sulphur derivatives (OSC) in *Allium* species. Chemically, OSC functions by inhibiting the systemic production of ROS species, particularly nicotinamide adenine dinucleotide phosphate (NADPH), while also preventing the degradation of antioxidant enzymes such as glutathione S-transferase, causing reduced ROS levels (Yin et al., 2002; Kim et al., 2017).

Based on the potential active ingredients of chive bulbs, especially their antioxidant properties, we hypothesized that chive extract supplemented in daily drinking water would reduce the adverse effects of HT on semen quality and blood biochemical characteristics in roosters.

2. MATERIALS AND METHOD

2.1. Experimental animals

Twenty-four healthy 3F-104 roosters of 3FViet Company (24 weeks old, average weight: 1721±121g) were randomly kept in wire mesh cages (50x150x40 cm; 2 chickens/each cage The cages were hung 0.5 m high on a fecal tray system that was changed continuously every week. The barn was guaranteed to have good ventilation and a lighting schedule of 16 hours/day. Chickens were provided with a diet formulated to meet their nutritional requirements in accordance with Vietnamese Standards (TCVN 2265:2007). The feed composition included key ingredients such as corn, rice bran, anchovy meal, 48% soybean meal, oyster powder, vitamin premix, mineral premix, $CaCO_3$ powder, L-lysine, DL-methionine (Table 1). Throughout the experiment, chickens had ad libitum access to feed and clean drinking water. Experimental chickens were vaccinated against Marek's disease, Newcastle disease, chickpox, and Gumboro. Care was conducted according to the Guide for the Care and Use of Laboratory Animals (Clark et al., 1997).

Table 1. Nutritional composition of experimental diets

Nutritional ingredients	Unit	Value
Dry matter	%	92.29
Total energy	cal/kg	4064.83
Crude protein	% DM	21.30
Minerals insoluble in hydrochloric acid	% DM	1.99
Total minerals	% DM	6.06

The environmental temperature (°C) and humidity (%) inside the house were recorded daily with a Testo thermo-hygrometer (Model 608-H1) hung at a height of 0.3m in each barn. The information was automatically recorded every 5 minutes during the experiment using a HOBO data logger (Onset Computer Corporation, Pocasset, MA, USA). The temperature-humidity index (THI) during the adaptation and testing period was calculated according to Spencer (1995) as follows:

$$THI = T - \left[\left(0.31 - 0.31\left(\frac{RH}{100}\right)\right)(T - 14.4)\right]$$

Note: THI is the temperature and humidity index; T is the ambient temperature, and RH is the relative humidity.

THI values were classified as no HT (<27.8), moderate HT (27.8-28.8), severe HT (28.9–29.9), and very severe HT (>30.0). Results in Table 2 show that chickens raised in the testing months suffered from severe HT (June, July) and severe HT (August).

Table 2. Temperature, humidity and heat-humidity index (THI) throughout the experiment

Targets	June	July	August	SEM	P value
Temperature (°C)	31.26[a]	32.06[a]	30.18[b]	0.12	0.035
Humidity (%)	65.04	66.22	68.17	3.52	0.091
THI	29.43[a]	30.21[a]	28.62[b]	0.10	0.029

Note: Mean values with different superscripts (a,b) in each row indicate statistically significant differences (p<0.05).

2.2. Extract preperation

Chive bulbs were purchased at Dien Mon, Phong Dien, TT-Hue (grown according to Vietgap biosafety standards - TCVN 11892-1:2017). The chive samples were sequenced with the ITS1-4 gene segment showing that it is closely related to the *Allium schoenoprasum* species with GenBank ID is NC_057575.1. Chive bulb juice was prepared daily by washing an appropriate amount of peeled bulbs with an equal amount of distilled water (w/v). The juice obtained with a Panasonic juicer (model: MJ-SJ01WRA) was incubated at 37°C overnight and

then filtered to obtain the extract of chive bulbs (CBE) and used directly in drinking water.

2.3. Experimental design

The experiment was arranged according to the completely randomized method (CRD) with 24 roosters in 3 treatments, with 4 repetitions (2 birds/repetition). Treatments T1 and T2 were given water containing CBE daily at doses of 1 and 2 ml/l, respectively; the control treatment (CT) did not supplement CBE.

2.4. Tracking targets

2.4.1. Analysis of semen characteristics

Before starting to collect semen, the rooster was trained for 1 month to collect semen. Semen collection by abdominal massage is done when the bird is 6 months old. This was performed three times a week for 2 consecutive months into Eppendorf tubes (with graduated lines) before 08:00 am. A laboratory examination of semen was performed immediately after the extraction.

The semen volume was read directly from the Eppendorf tube. Samples on the Newbauer counting chamber and glass slides were observed under a microscope (Olympus Corp., Tokyo, Japan) to determine sperm concentration and motility as described by Peters et al. (2008). The percentage of dead and abnormal sperm was determined on slides stained with eosin-nigrosine staining, as described by Blesbois (2007). A JVC microscopic video system and Panasonic monitor (Victor Company, Japan) were used throughout the entire measurement process to improve the accuracy of sperm counting.

2.4.2. Blood biochemical characteristics

Blood samples were taken from the rooster's wing vein at the end of the experiment. Plasma was obtained by centrifuging the blood at 1500 × g for 20 min and stored at -20^{o}C until analysis.

Serum FSH and LH concentrations were determined in duplicate samples using Radioimmunoassay (RIA), Biocode-Belgium (Catalog number: 6107325 and RK-040-02, respectively), following the manufacturer's instructions. The detection sensitivity for FSH and LH per test tube was 0.2 ng/ml and 0.14 ng/ml, respectively. Total testosterone levels in serum were quantified using a dual-antibody RIA kit from Beckman Coulter ImmunoTechnology-USA (Catalog number: MG12191), with a detection sensitivity of 0.025 ng/ml per test tube.

Measurement of total antioxidant capacity (TAC) and Malondialdehyde (MDA) plasma concentrations were determined using the diagnostic kit (catalog number: MAK334 and 19160, respectively) and following the manufacturer's recommendations (Diamond Diagnostics, 23 EL-Montazah St. Heliopolis, Cairo, Egypt).

2.5. Statistical analysis

Data were statistically analyzed using the ANOVA method on SPSS (version 26.0) according to the general linear correlation model (GLM). Statistical algorithm: $yij = \mu + Ci + eij$; where: yij = dependent variable; Ci = influence of preparations eij = random error. The confidence interval was set at 95% ($p<0.05$). Compare percentage differences using the Chi-square test.

3. RESULTS AND DISCUSSION

3.1. Effects of CBE supplementation on semen quality

Overall, daily use of CBE in drinking water at concentrations of 1 ml (T1) and 2 ml/l (T2) improved semen volume, sperm concentration, motility, mortality, and morphology in males. Both experimental treatments were compared to the control treatment (CT) (Table 3). Specifically, compared to control, semen volume doubled ($p<0.05$) at T2 (0.72 vs 0.38 ml), significantly increased ($p<0.05$) sperm concentration at T2. T1 and T2 (3.74-4.88 vs 2.79 x 109 mL^{-1}), sperm activity in T1 and T2 (70.21-79.34 vs 61.10%), decreased significantly ($p<0.05$) at T1 and T2 in terms of death rate (19.27 and 12.12 vs 25.21%), abnormal rate (13.12 and 8.66 vs 17.98%).

Table 3. Effects of CBE on semen quality of roosters subjected to heat stress

Parameter	CT	T1 (CBE 1%)	T2 (CBE 2%)
Semen volume (ml)	0.38 ± 0.03^{b}	0.47 ± 0.03^{b}	0.72 ± 0.05^{a}
Sperm concentration (10^9 mL^{-1})	2.79 ± 0.43^{c}	3.74 ± 0.22^{b}	4.88 ± 0.2^{a}
Active potency (%)	61.10 ± 4.35^{c}	70.21 ± 2.33^{b}	79.34 ± 1.32^{a}
Dead rate (%)	25.21 ± 0.68^{a}	19.27 ± 1.23^{b}	12.12 ± 1.54^{c}
Abnormal rate (%)	17.98 ± 4.3^{a}	13.12 ± 1.43^{b}	8.66 ± 0.64^{c}

Note: Mean values with different superscripts (a,b,c) in each row indicate statistically significant differences ($p<0.05$).

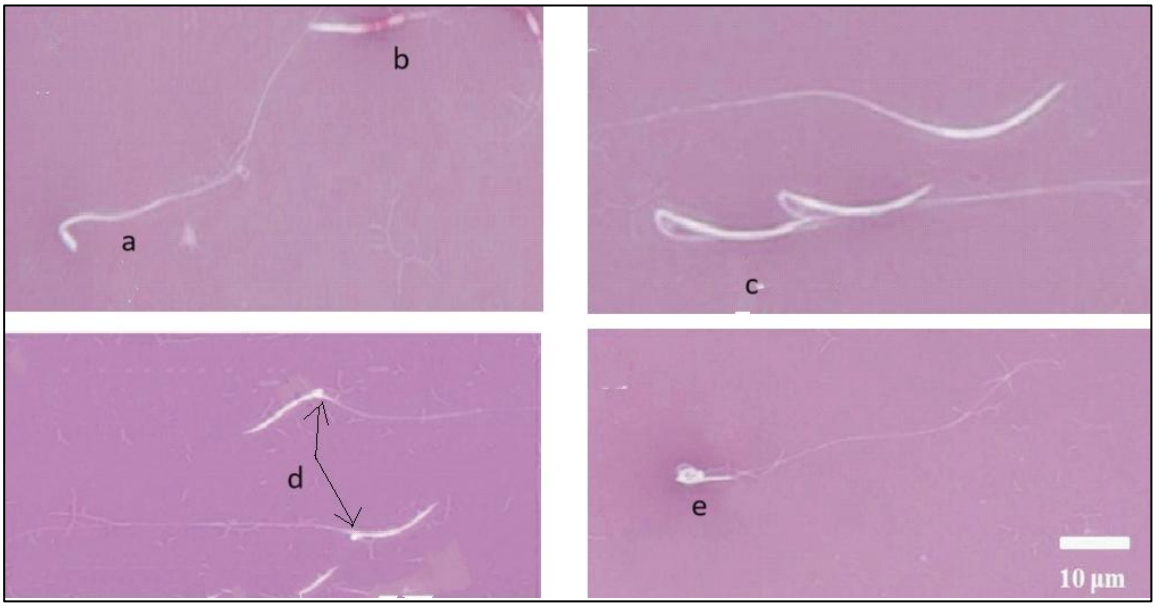

Figure 1. Morphology of dead and abnormal sperms in eosin-nigrosine smear: (a) spermatid-like sperm, (b) dead (stained) sperm, (c) bent-necked sperm, (d) drop-shaped sperm and (e) looped-headed sperm

ROS are essential for sperm maturation and capacitation, but high ROS production leads to sperm dysfunction. In addition, ROS generated during HT causes lipid peroxidation in the membrane of avian sperm, adversely affecting sperm viability and motility (Surai et al., 2001). However, high levels of ROS can trigger oxidative stress, which may cause damage to cells and potentially lead to their death (Poljsak et al., 2013). To counteract this, the antioxidants of chive bulbs may protect against ROS-related oxidative damage to sperm cells due to severe HT by enhancing antioxidant defense mechanisms' metabolism activity in the testicles (Banihani, 2019).

The results of Table 3 showed that CBE could significantly increase most semen quality parameters in roosters with HT. The ameliorating effect of HT damage in this study may be due to onion's antioxidant phytochemical compounds in CE. Chae et al. (2017) found that flavonoids, particularly quercetin in onion (*Allium cepa* L.) offer protection from oxidative stress and enhance several key attributes of sperm, such as their viability and movement capabilities. In addition, quercetin has a positive effect on the sperm membrane and acrosome integrity (El-Khawagah et al., 2020). Besides, the effects of phenolic compounds that are associated with antioxidant activity that reduce testicular oxidative stress to increase spermatogenesis (El-Gindy & Abu Hafsa, 2020).

3.2. Effects of CBE supplementation on some reproductive hormones

Using CBE at a concentration of 2% (T2) for 3 consecutive months was effective in improving serum LH, FSH and testosterone levels between treatments. Specifically, compared to control, 2% CBE supplementation significantly increased ($p<0.05$) the concentrations of LH (12.38 vs 9.67 mUI/ml), testosterone (2.33 vs 1.17 ng/ml) and FSH (12.37 vs 10.09 mUI/ml).

Table 4. Effects of CBE on some reproductive hormones in roosters subjected to heat stress

Parameter	CT	T1 (CBE 1%)	T2 (CBE 2%)
LH (mUI/ml)	9.66 ± 0.22^{b}	$9,67 \pm 0.21^{b}$	$12,38 \pm 0.34^{a}$
FSH (mUI/ml)	10.09 ± 0.24^{b}	11.83 ± 0.15^{ab}	12.37 ± 0.72^{a}
Testosterone (ng/ml)	1.17 ± 0.04^{b}	1.45 ± 0.05^{b}	2.33 ± 0.04^{a}

Note: Mean values with different superscripts (a,b) in each row indicate statistically significant differences (p<0.05).

HT negatively affects testicular function, which can inhibit testicular testosterone production (Chen et al., 2015) leading to reduced sperm cell quality and fertility. The possible mechanism by which CBE enhances testosterone production is mainly by improving the activities of 3βhydroxysteroid dehydrogenase, 17αhydroxylase and 17, 20 lyase and 17βhydroxysteroid dehydrogenase in the testicles (Chandra et al., 2013). Additionally, CBE increases LH release by increasing cholesterol levels and reducing lipid peroxidation in the testicles. Furthermore, CBE normalizes blood sugar levels, enhances nitric oxide production, and increases blood flow into Leydig cells, in addition to increasing testicular weight and recycling testosterone receptors (Banihani, 2018). This results in improved testosterone levels, which play an important role in maintaining and improving semen production in roosters. FSH is the key to determining the rate of spermatogenesis, a growth factor in the development and maintenance of sperm production in roosters (Gordon, 2017). The results of the present study parallel those in previous reports (Ige & Akhigbe, 2012) that onion has a positive effect on LH production.

3.3. Effects of CBE supplementation on some blood fat and antioxidant indicators

The mean concentration of MDA was significantly lower ($p<0.05$) in T2 and T1 compared to the control (0.18 and 0.18 vs 0.44 nmol/ml). Similarly, TAC concentrations were significantly higher ($p<0.05$) in T2 and T1 compared to control (1.18 and 0.94 vs 0.72 μmol) (Table 5). Meanwhile, the blood fat index, cholesterol and triglyceride did not change when supplementing with CBE, however HDL content increased significantly ($p<0.05$) in T2 compared to the control (60.39 vs 50.05 mg/dl).

Table 5. Effects of CBE on some blood fat and antioxidant parameters in roosters subjected to heat stress

Parameter	CT	T1 (CBE 1%)	T2 (CBE 2%)
MDA (nmol/ml)	0.44 ± 0.04^{a}	0.18 ± 0.01^{b}	0.18 ± 0.03^{b}
TAC (μmol)	0.73 ± 0.02^{c}	0.93 ± 0.04^{b}	1.17 ± 0.08^{a}
Cholesterol (mg/dl)	131.51 ± 1.26	128.25 ± 1.21	131.33 ± 1.31
HDL (mg/dl)	50.05 ± 1.12^{b}	51.37 ± 1.26^{b}	60.39 ± 1.1^{a}
Triglycerides (mg/dl)	58.85 ± 0.98	58.96 ± 0.87	56.57 ± 0.85

Note: Mean values with different superscripts (a,b,c) in each row indicate statistically significant differences (p<0.05).

The improvement in the above indicators is mainly related to the natural antioxidant properties of CBE. Rui et al. (2016) concluded that MDA causes functional impairment of chicken sperm under in vivo conditions. In this regard, CBE reduced MDA levels as a lipid peroxidation marker, while increasing TAC content. Reactive oxygen species (ROS) result from increased HT and ROS leading to lipid oxidation. MDA is the product of lipid oxidation by ROS. The reduction in MDA levels (end product of lipid peroxidation) by CBE in plasma is indicative of lower lipid peroxidation and has an potential enhancement on sperm function (Hsieh et al., 2006). Comparable findings were observed in an earlier study by Attia and Kamel (2012), where treatment with quercetin (the main flavonoid in onions) significantly improved the antioxidant status of semen (SOD) and GPx) with protective effects against oxidative stress.

4. CONCLUSION

The present study has demonstrated that the extract from the chive bulbs added to the drinking water of roosters at doses of 2% possesses fertility-promoting properties in roosters through its antioxidant effects and modulation of reproductive hormones under HT conditions in roosters. This research highlights CBE's potential as a natural supplement to mitigate HT's negative effects on rooster reproductive health.

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