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# Effects of Dietary Crude Protein Levels on Crossbred Buck Rabbit Semen Characteristics and Reproductive Performance of Does

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**Abstract** | The study aimed to evaluate the effects of dietary crude protein levels on rabbit semen characteristics. Thirty crossbred male rabbits (New Zealand White x local), with an average weight of 2.59 ± 0.14 kg, were completely randomized design consisted of five treatments and six replications, with one buck rabbit as an experimental unit. The five experimental treatments were diets containing 14%, 16%, 18%, 20%, and 22% crude protein corresponding CP14, CP16, CP18, CP20 and CP22 treatment, respectively. Feedstuffs used in the experiment included fermented soya waste, soybean extraction meal, *Operculina turpethum*, and *Pennisetum purpureum*. The experiment was carried out for 12 weeks in which sperm of the experimental rabbits was collected and analyzed weekly. After 12 weeks of semen collection, 1 bucks per treatment with stable semen quality were used for mating with does to evaluate reproductive performance. Six does were used for each treatment (total 30 doe rabbits). The results of the study showed that there was a fluctuating tendency in sperm concentration over time ($P < 0.05$) among treatments. The sperm concentration of CP14, CP16 CP20, and CP22 tended to decrease during 12 sampling weeks, however, this result increased for the CP18. When the ratio of CP increasing in the diet gave higher results in terms of sperm concentration, live sperm rate, total motile sperm, and gross motility ($P < 0.05$) compared to 14% treatment. Those results gave higher values for the 18% treatment at 314.1 x $10^6$/mL, 60.4%, 139.4 x $10^6$, and 59.5%, respectively. There was no impact ($P > 0.05$) of dietary crude protein on the weight of male rabbits and reproductive performance in doe rabbits in terms of litter size at birth, mean weight at birth, and litter size at weaning. Results of this study showed that among the different diets, the 18% CP diet was suitable for the reproduction of male crossbred rabbits.

**Keywords** | Crossbred rabbits, Dietary crude protein, Membrane integrity, Motility, Testosterone, Sperm quality

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## INTRODUCTION

Rabbit production had enormous potential to alleviate the problem of animal protein supply in developing countries (Oseni, 2012) and in some peri-urban parts of developed nations (Attia *et al.*, 2011). Rabbits could digest fiber and other nutrients well through fermentation in the caecum (Leng, 2006) and had the ability to convert 20% of

the protein in the feed into protein that accumulates in the body (Lebas *et al*., 1997; Dalle Zotte, 2014), thus reducing competition for feed and aligning with the current trend of sustainable livestock production. Additionally, low levels of technical and management skills in rabbit production, climatic factors, availability, and quality of forage were constraints in developing countries, which were mostly located in hot climatic regions (Lukefahr, 2007; Oseni, 2012). In the Mekong Delta of Vietnam, 9 out of 13 provinces have developed rabbit raising (Chau and Thu, 2014). Local people fed rabbits by utilizing natural plants, forages, and available local agro-industrial by-products (Trung *et al*., 2022). In recent years, the application of artificial insemination techniques in rabbit breeding has also greatly developed. This technique requires bucks to have superior sperm characteristics and higher reproductive productivity.

In tropical and subtropical areas such as Vietnam, heat stress is the major constraint to livestock production (Marai *et al*., 2002; Ganaie *et al*., 2013). Not only affects livestock efficiency, but stress also negatively affects animal health and reduces reproductive performances (Abdulrashid and Juniper, 2016). Jimoh and Ewuola (2018) reported that the semen quality (motility and viability) of buck rabbits was partially affected by heat stress (36°C). Supplementation of vitamins, minerals, prebiotics, and probiotics in the diet could reduce the negative effects of heat stress (Daader *et al*., 2018; Ayyat *et al*., 2021a; Trung *et al*., 2022). Additionally, the increasing of protein and energy content in the diet was also a common solution (Ayyat and Marai, 1997; Ayyat and El-Aasar, 2008). Protein plays a key role in metabolism, tissue development, and the composition of enzymes and hormones (Ayyat *et al*., 2021b) and affects semen characteristics (Abdulrashid and Juniper, 2016). Therefore, to develop rabbit farming in the Mekong Delta, special attention should be paid to the protein content in the diet. It was indicated that buck rabbits fed 14% dietary protein levels had reduced testicular size with a decline in sperm production (Ladokun *et al*., 2006). However, it has been shown that feeding diets with an excessive amount of protein can have deleterious effects on semen characteristics and testicular tissues (Elmaz *et al*., 2007). The NRC (1997) recommended that a diet content of 16% CP was suitable for reproduction buck. Abdulrashid and Juniper (2016) stated that the dietary of the buck was 18% CP. The recommendations for CP content in the buck diets mainly utilized pellets, and the natural conditions differed in the Mekong Delta in Vietnam.

In general, there was very limited research on the optimum protein content in the diet of buck rabbits used for artificial insemination under the conditions of the Mekong Delta. The objective of this study was to determine the optimal crude protein level in the diet for suitable semen characteristics in buck rabbits.

## MATERIALS AND METHODS

The study was conducted with approval for animal care, housing, and sample collection under the Animal Welfare Assessments of The Animal Ethics Committee of Can Tho University (CTU-AEC24002).

The experiment was carried out from April to July 2023 at the experimental farm of the Faculty of Animal Science, Can Tho University. The experimental rabbits were raised in open cages consistent with natural conditions in the Mekong Delta.

### Experimental Design

The experiment involved 30 crossbred (New Zealand White x local) buck rabbits (2.59 ± 0.14 kg/buck, 8.5 months of age) and was arranged completely randomly with 5 treatments (corresponding to 5 levels of protein in the diet: 14, 16, 18, 20, and 22% CP (dry matter basis), respectively) and six replications (one buck per trial unit).

**Table 1:** Ingredients and chemical composition of experimental diets, %DM.

| Feeds | Crude protein (%) | | | | |
|---|---|---|---|---|---|
| | **14** | **16** | **18** | **20** | **22** |
| Fermented soya waste | 26 | 28 | 34 | 30 | 29 |
| Soybean extraction meal | 0 | 5 | 11 | 19 | 27 |
| *Pennisetum purpureum* | 26 | 19 | 18 | 21 | 26 |
| *Operculina turpethum* | 48 | 48 | 37 | 30 | 18 |
| Total | 100 | 100 | 100 | 100 | 100 |
| OM | 89.8 | 90.0 | 91.0 | 91.4 | 92.1 |
| CP | 14.2 | 16.0 | 18.0 | 20.0 | 22.0 |
| EE | 5.5 | 5.3 | 5.1 | 4.8 | 4.6 |
| NDF | 44.5 | 41.7 | 40.2 | 40.3 | 40.8 |
| ADF | 29.8 | 28.6 | 27.6 | 27.5 | 27.4 |
| CF | 21.4 | 19.8 | 18.3 | 17.8 | 17.2 |
| Ash | 10.2 | 10.0 | 9.0 | 8.6 | 7.9 |
| ME, MJ/kgDM | 9.60 | 9.90 | 10.0 | 10.0 | 10.0 |

**OM:** Organic Matter; **CP:** crude protein; **EE:** Ether Extract; **NDF:** Neutral Detergent Fiber; **ADF:** Acid Detergent Fiber; **CF:** Crude Fiber; **Ash:** Total mineral; **ME:** Metabolizable Energy.

Before starting the experimental diet, rabbits were fed *ad libitum* for 1 week to determine the amount of DM intake. The chemical composition of the feed used in the experiment was analyzed and calculated according to the AOAC (2000) and Maertens *et al*. (2002). The ingredients formula and chemical composition of experimental diets were shown in Table 1. The feedstuffs used in the experiment included *Operculina turpethum*, *Pennisetum purpureum*,

fermented soya waste, and soybean extraction meal. Soya waste was fermented according to the method of Trung *et al.* (2024). The fermented soya waste, soybean extraction meal, and *Operculina Turpethum* were fed twice, once in the morning (07.00 am), and once in the afternoon (02.00 pm). Following this, in the afternoon (6.00 pm) the animal was fed with the *ad libitum Pennisetum purpureum*. Feed offers and refusal was weighed daily to calculate nutrient intake.

The experiment lasted 12 weeks. The sperm of the experimental rabbits were collected and analyzed for both quantity and quality weekly (a total of 360 semen samples of experimental bucks were analyzed).

After 12 weeks of semen collection, 1 bucks per treatment with stable semen quality (sperm concentration, motility, live sperm, and membrane integrity) were chosen for natural mating with does to evaluate reproductive performance. Each buck would naturally mate with six does rabbits (a total of 30 doe rabbits were used). During this time, the buck would maintain a diet consistent with the experimental design. Thirty crossbred doe rabbits (New Zealand White x local) at 10 months of age, with an average weight of 2.89 ± 0.21kg (first-time reproduction), were used in a completely randomized design with 5 treatments (corresponding to 5 levels of protein in the buck's diet for mating: 14, 16, 18, 20, and 22% CP, respectively) and six replications (one doe per experimental unit). The reproductive performance trial was conducted with one litter. All does rabbits in the experiment were provided with the same diet containing 24 g CP/day and 1.00 MJ ME/day (Trung and Truong, 2020) including 240g fermented soya waste, 33g soybean extraction meal and 150g *Pennisetum purpureum*.

The buck and does rabbits were individually housed in separate cages with dimensions of 50 x 50 x 30cm (width x length x height), under the same environmental conditions of temperature, relative humidity, lighting time, and with free access to drinking water from nipple drinkers. The rabbits were vaccinated against common diseases before the start of the experiment.

## Measurements Taken

**Semen Collection (Ejaculate Sampling):** Semen samples were collected weekly and continuously throughout the 12-week experiment period. Semen samples were collected from individual bucks using an artificial vagina (Ewuola *et al.*, 2014), made of a plastic cylinder with a rubber lining fixed around the rim to warm the water. The artificial vagina (AV) was pre-warmed in water at 50 to 55°C, ensuring a temperature of 40-42°C at the time of collection. The inner sleeve was lubricated with glycerol, and then a teaser doe was introduced to the buck's pen at the time of collection as the buck mounted the teaser, the AV was introduced and the ejaculate was collected. Semen was evaluated for color, ejaculation volume, and pH (after 30 minutes of semen collection). Fresh semen samples were mixed into the medium at a ratio of 1:10 in solution (cold stored at 12-17°C) and analyzed for sperm characteristics.

Rabbit libido was evaluated by determining the buck's reaction time. Reaction time was defined as the duration between the introduction of the teaser doe and the ejaculation time following copulation.

**Semen Evaluation:** Semen was evaluated as described by Hafez and Hafez (2000). Semen color was evaluated visually by observing the appearance of the ejaculate contained within the graduated tubes. The ejaculate volume was determined by reading the volume directly from the calibrated collecting tube and the gel-free ejaculate volume recorded. Ejaculate pH was determined immediately following collection using pH paper (SpezialIndikatorpapier pH 5.5-9.0, Macherey-Nagel, Germany) as recommended by the World Health Organization (WHO, 2021). Viscosity was determined according to the method of Bootwalla and Froman (1988) with sperm diluted with Ostwald viscometers (funnel diameter 0.8).

**Microscopic Analysis:** The sample was diluted with a ratio of 1:10 (Tris-Citric-Glucose (TCG) medium: Acid citric 1,69 g, Tris-hydroxymethyl-aminomethane 3,0285 g, Glucose 0,8469 g, L-Gentamycin 200 µL and NaOH 0,124 g), cold stored immediately at 12-17 °C to be transported to the analysis site. The semen samples were slowly warmed to 37°C. The concentration of spermatozoa in semen was determined by haemocytometric counts using a Neubauer haemocytometer (Neubauer Improved – Marinfeld, Germany), under 400x magnification. The sample was diluted 1:4 with 5% $NaHCO_3$ solution, 15µL of the semen mixture was taken to the counting chamber, counting on 2 counting chambers. Sperm concentration was estimated using the standard formula of Hafez and Hafez (2000) C = N * D * 50,000, where C = concentration of spermatozoa per ml (x$10^6$/ml). N - number of spermatozoa counted and D - dilution factor = 20.

**Membrane Integrity:** Take 10 µL of semen mixed with 90 µL of the hypoosmotic swelling (HOS) solution (100 mL of HOS solution, 70 mOsmol: content sodium citrate 0.343 g and D-fructose 0.630 g). Count the samples on the slide with 3 replicates, the number of spermatozoa per 100 individuals is the sperm membrane integrity rate. Sperm morphology abnormalities were assessed using the stained semen smears. At least 200 spermatozoa were manually counted on each slide and examined at x400 magnification. Sperm motility (%) is the number of active sperms out of the total number of sperm present in each microsphere. Percentage live/dead spermatozoa were determined using eosin 1% nigrosin 10% stained smears. Semen smears were

**Table 2:** The chemical composition of feed used in the experiment.

| Feed | DM | OM | CP | EE | NDF | ADF | CF | Ash | ME, MJ/kgDM |
|---|---|---|---|---|---|---|---|---|---|
| *Pennisetum purpureum* | 17.8 | 90.5 | 10.5 | 6.10 | 67.5 | 38.9 | 30.7 | 9.50 | 7.87 |
| *Operculina turpethum* | 13.5 | 86.3 | 14.0 | 5.32 | 38.8 | 28.9 | 21.1 | 13.7 | 9.77 |
| Soybean extraction meal | 89.0 | 93.8 | 42.3 | 1.98 | 25.8 | 20.6 | 6.60 | 6.20 | 11.2 |
| Fermented soya waste | 16.9 | 95.5 | 18.4 | 5.23 | 31.9 | 22.5 | 12.5 | 4.50 | 11.1 |

**DM:** Dry Matter; **OM:** Organic Matter; **CP:** Crude Protein; **EE:** Ether Extract; **NDF:** Neutral Detergent Fiber; **ADF:** Acid Detergent Fiber; **CF:** Crude Fiber; **Ash:** Total mineral; **ME:** Metabolizable Energy.

prepared using one drop of eosin stain on a clean glass slide which was then dried at room temperature. The slide was examined at 400 magnification, using an Olympus CX21 microscope, (Olympus Corporation, Tokyo, Japan). At least 100 cells were counted and the percentage was calculated.

**Testicular Measurements:** Testicular measurements were recorded at the end of the experiment period from each buck and averaged over the measurements of the two testicles. Testis length (TL) and width (TW) were measured using a flexible measuring tape calibrated in centimeters and millimeters. Testis weight (TM) and testis volume (TV) were estimated using the mathematical model of Bailey *et al*. (1996), where

$TM = 0.5533 \times (TL) \times (TW)^2$ and $TV = 0.5236 \times (TL) \times (TW)^2$.

**Testosterone:** Thirty-buck rabbits were collected blood samples in the morning before feeding to test blood testosterone levels. Testosterone levels were determined by luminescence measurement on AutoLumo A1000.

**Reaction Time:** The doe rabbit will be put into the buck rabbit's cage and time will begin to be recorded. The period from when a buck comes into contact with a doe until mating occurs.

**Temperature and Humidity Index (THI):** THI is calculated by the formula of Marai *et al*. (2002):

$$THI = t - (0.31 - 0.31 \times RH) \times (t - 14.4)$$

while t is the temperature (°C) in degrees Celsius and RH is relative humidity (%).

### Statistical Analysis

Data were analyzed by ANOVA using the General Linear Model of the Minitab 13.21 program (Minitab, 2016). The results will be presented as Least Squares Means with their pooled standard errors. Pair-wise comparisons with a confidence level of 95 will be used to determine the effects of dietary treatment between groups by Tukey posttest. Significance was declared at $P < 0.05$. The model applied for the analysis of buck rabbits was:

$$Y_{ij} = \mu + t_i + e_{ij}$$

where: $Y_{ij}$ was the observation
$\mu$ was the overall mean for each parameter
$t_i$ was the effect of the $_i$th level of CP ($_i$: 1 to 5)
$e_{ij}$ was residual error
For the doe rabbits was:

$$Y_{ij} = \mu + t_i + e_{ij}$$

where:
$Y_{ij}$ was the observation
$\mu$ was the overall mean for each parameter
$t_i$ was the effect of the $_i$th level CP in the buck diet ($_i$: 1 to 5)
$e_{ij}$ was residual error

## RESULTS AND DISCUSSION

### Chemical Composition of Feeds

The composition of feed in the present study is shown in Table 2. The materials used in the experiment were locally available. The fermented soya waste had high organic matter (95.5%) and crude protein (18.4%) levels, but low dry matter (DM). Soybean extraction meal was also used as a supplement protein source, with a crude protein content of 42.3%. The *Operculina turpethum* and *Pennisetum purpureum* was used in the experiment for fiber supplementation.

### Temperature and Humidity Index, Feed and Nutrient Intakes

Effects of dietary crude protein difference levels and the ambient condition on testicular measurements of bucks.

The temperature-humidity index (THI) recorded in the study ranged from 27.0 to 32.0 (Table 3). The results in Table 3 showed that rabbits suffered from severe heat stress during the last 8 weeks of the experiment. As reviewed by Marai *et al*. (2002), rabbits had severe heat stress when the THI index was $29.8 < THI < 30.0$ and very severe heat stress when $THI > 30.0$. When high stress lasts for a long time, it will affect feed intake, leading to a lack of nutrients and deterioration of the rabbit's health. The CP levels in the diet did not affect the DM, OM, Ash, and ME intakes among treatments (Table 4; $P > 0.05$). The highestCP intake was 17.9 g/day with the CP22 diet, which was 1.57 times higher than the 11.3 g/day of CP14 ($P < 0.05$). The NDF

**Table 3:** Temperature and humidity index (THI) through 12 weeks of the experiment.

| Items | 1th week | 2th week | 3th week | 4th week | 5th week | 6th week | 7th week | 8th week | 9th week | 10th week | 11th week | 12th week |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Temperature (°C) | 28.5 | 28.9 | 30.0 | 29.7 | 32.8 | 27.6 | 32.3 | 30.9 | 29.7 | 29.7 | 32.4 | 32.7 |
| Relative humidity (%) | 84.4 | 78.8 | 88.6 | 88.5 | 82.0 | 85.0 | 84.0 | 86.0 | 85.1 | 87.6 | 86.6 | 88.0 |
| THI | 27.8 | 27.9 | 29.5 | 29.1 | 31.7 | 27.0 | 31.4 | 30.1 | 29.0 | 29.2 | 31.3 | 32.0 |

**THI:** temperature-humidity index.

intake decreased (35.2g for the 14% treatment and 33.2g for the 22%CP treatment) as the %CP in the diet increased ($P < 0.05$). On the other hand, reducing fiber and increasing CP in the diet was a solution to improve nutrition and reduce the effects of heat stress on rabbits (Ayyat and El-Aasar, 2008).

**Table 4:** Nutrient intake (g/buck/day) in the experiment period.

| Feed | Crude protein (%) | | | | | SEM | P |
|---|---|---|---|---|---|---|---|
| | 14 | 16 | 18 | 20 | 22 | | |
| Fermented soya waste | 22.3$^{b}$ | 27.2$^{ab}$ | 29.6$^{ab}$ | 30.8$^{a}$ | 31.6$^{a}$ | 1.64 | 0.020 |
| Soybean extraction meal | 0.15$^{e}$ | 3.91$^{d}$ | 7.45$^{c}$ | 12.1$^{b}$ | 18.2$^{a}$ | 0.65 | 0.001 |
| *Pennisetum purpureum* | 26.7$^{a}$ | 19.1$^{b}$ | 15.2$^{bc}$ | 11.5$^{c}$ | 10.4$^{c}$ | 1.50 | 0.001 |
| *Operculina turpethum* | 30.0$^{a}$ | 20.6$^{b}$ | 27.1$^{ab}$ | 25.2$^{ab}$ | 21.4$^{b}$ | 1.48 | 0.010 |
| DM | 79.2 | 70.8 | 79.5 | 79.6 | 81.5 | 4.40 | 0.380 |
| OM | 71.1 | 63.8 | 72.4 | 72.7 | 75.0 | 3.68 | 0.360 |
| CP | 11.3$^{c}$ | 11.3$^{c}$ | 14.3$^{bc}$ | 15.9$^{ab}$ | 17.9$^{a}$ | 0.74 | 0.001 |
| EE | 4.40 | 3.74 | 4.02 | 3.84 | 3.74 | 0.21 | 0.190 |
| NDF | 35.2 | 29.5 | 32.0 | 32.1 | 33.2 | 1.69 | 0.051 |
| ADF | 23.6 | 20.2 | 22.0 | 21.9 | 22.3 | 1.13 | 0.190 |
| CF | 16.9$^{a}$ | 14.0$^{b}$ | 14.6$^{ab}$ | 14.2$^{b}$ | 14.0$^{b}$ | 0.76 | 0.010 |
| Ash | 8.10$^{a}$ | 7.00$^{ab}$ | 7.10$^{ab}$ | 6.90$^{ab}$ | 6.50$^{b}$ | 0.35 | 0.050 |
| ME, MJ | 0.76 | 0.70 | 0.80 | 0.80 | 0.82 | 0.04 | 0.120 |

**a,b,c,d,e:** Values within rows with different superscripts are different; $p < 0.05$; **DM:** dry matter; **OM:** organic matter; **CP:** crude protein; **EE:** ether extract; **NDF**: neutral detergent fiber; **ADF:** acid detergent fiber; **CF:** Crude Fiber; **Ash:** total mineral; **ME:** Metabolizable Energy; **SEM:** Standard error of the mean.

**Table 5:** The growth performance of buck rabbits during the experiment period .

| Item | Crude protein (%) | | | | | SEM | P |
|---|---|---|---|---|---|---|---|
| | 14 | 16 | 18 | 20 | 22 | | |
| The initial live weight (kg) | 2.55 | 2.68 | 2.57 | 2.56 | 2.61 | 0.09 | 0.80 |
| The final live weight (kg) | 2.55 | 2.68 | 2.59 | 2.56 | 2.61 | 0.09 | 0.86 |

**SEM:** Standard error of the mean.

## The Growth Performance of Bucks

There was no significant difference in the live weight of experimental bucks before and after the trials among all treatments (Table 5; $P > 0.05$). This indicates that the experimental diets adequately met the basic and spermatogenesis needs of bucks. The change in the live weight of rabbits before and after the experiment ($P>0.05$) showed that the 14% CP content still meets the energy needs needed to maintain and production activities.

## Effects of Testicular Measurements

The results of testicular measurements were presented in Table 6, indicating the influence of %CP in the diet on the length, and volume of testicular measurements. The length of the testicle was higher in CP18 and CP20 compared to CP14 ($P < 0.05$) (7.23, 7.33 and 5.85 cm, respectively). There was no significant difference in testicular width among all treatments ($P > 0.05$). However, the volume and weight of the testicle were higher ($P < 0.05$) in CP18 compared to CP14 being 8.09cc vs 4.27cc and 7.66g vs 4.04g, respectively.

**Table 6:** Testicular measurement results of bucks during the experiment period.

| Item | Crude protein (%) | | | | | SEM | P |
|---|---|---|---|---|---|---|---|
| | 14 | 16 | 18 | 20 | 22 | | |
| Testis length (cm) | 5.85$^{b}$ | 6.73$^{ab}$ | 7.23$^{a}$ | 7.33$^{a}$ | 6.78$^{ab}$ | 0.23 | 0.003 |
| Testis width (cm) | 1.13 | 1.35 | 1.42 | 1.31 | 1.34 | 0.07 | 0.132 |
| Testis weight (g) | 4.04$^{b}$ | 6.47$^{ab}$ | 7.66$^{a}$ | 6.70$^{ab}$ | 6.35$^{ab}$ | 0.78 | 0.050 |
| Testis volume (cc) | 4.27$^{b}$ | 6.84$^{ab}$ | 8.09$^{a}$ | 7.08$^{ab}$ | 6.71$^{ab}$ | 0.82 | 0.050 |
| Testosterone (ng/dL) | 4108$^{b}$ | 4875$^{ab}$ | 5049$^{a}$ | 5208$^{a}$ | 4559$^{ab}$ | 181.9 | 0.011 |

**a,b:** Values within rows with different superscripts are different; $p < 0.05$; **SEM:** Standard error of the mean.

The length, and volume of testicular measurements were affected by the levels of crude protein in the buck's diet (Table 6; $P > 0.05$). Larger testicular size held greater promises in terms of spermogenesis and storage capacity of bucks. This was supported by testosterone concentration evaluation; the testosterone concentration was higher ($P < 0.05$) in both CP18 (5049 ng/dL) and CP20 (5208 ng/dL) compared to CP14 (4108 ng/dL), corresponding to different testicular lengths among all treatments. The changes in testicular size from 14% CP to 18% CP, with no improvement observed in diets with higher protein content, indicated that supplementation of 18% CP in the diet could meet the essential needs for spermogenesis.

This result was consistent with the study of Ladokun *et al.* (2006) when supplementing 14% CP in the diet of buck rabbits reduced testicular size. Many previous studies have shown that increased dietary protein was correlated with increased testicular size (Ladokun *et al.*, 2006; Abdulrashid and Juniper, 2016). On the other hand, testicular size could be partly influenced by the live weight of the animal (Elmaz *et al.*, 2007; Oyeyemi and Okediran, 2007). Testicular size was an important indicator of sperm production ability (Perry and Petterson, 2001; Ogbuewu *et al.*, 2009). Increasing testicular size was associated with increased diameter of the seminiferous tubules, increased Sertoli cells, increased testosterone production, and semen production (Thompson and Berndtson, 1993; Bailey *et al.*, 1996). This was completely consistent with the results of testosterone concentrations of buck rabbits in the experiment. Thus, the change of crude protein content in the diet affected the size of buck rabbit testicles, thereby affecting testosterone secretion and sperm production. The 18% CP diet gave a positive result in the initial assessments of buck selection for artificial insemination.

**Table 7:** Overall assessment results.

| Item | Crude protein (%) | | | | | SEM | P |
|---|---|---|---|---|---|---|---|
| | 14 | 16 | 18 | 20 | 22 | | |
| Ejaculate frequency (times) | 11.7 | 12.0 | 11.0 | 10.8 | 11.8 | 0.39 | 0.169 |
| Reaction time (s) | 7.14$^{a}$ | 6.57$^{ab}$ | 5.63$^{ab}$ | 5.64$^{ab}$ | 5.10$^{b}$ | 0.26 | 0.026 |
| Volume (mL) | 0.56 | 0.57 | 0.62 | 0.62 | 0.53 | 0.05 | 0.596 |
| pH | 7.13 | 7.31 | 7.35 | 6.99 | 7.21 | 0.12 | 0.251 |
| Viscosity (cp) | 1.14$^{a}$ | 1.06$^{c}$ | 1.11$^{ab}$ | 1.10$^{bc}$ | 1.12$^{ab}$ | 0.01 | 0.001 |

$^{a,b,c}$: Values within rows with different superscripts are different; p < 0.05; **SEM:** Standard error of the mean.

Based on those results, attention needs to be paid to the environmental conditions for breeding bucks for semen collection in the Mekong Delta, particularly the impact of heat stress. A high-protein diet could mitigate negative effects by reducing heat stress. A diet containing 18% crude protein yielded optimal results in terms of meeting nutritional demands and semen concentration in bucks.

## Effects on Semen Characteristics of Bucks

The results in Table 7 showed that there is no difference in ejaculation, semen volume, and pH value among diets with different CP levels (P > 0.05). Reaction time decreased gradually (P<0.05) from CP14 (7.14 s) to CP22 (5.10 s). Semen viscosity varied significantly between treatments (P < 0.05).

The results of sperm quality (Table 8) showed that there was an influence of %CP in the diet on sperm concentration, live rate, sperm membrane integrity, motility and sperm abnormality (P < 0.05). The diet containing 18% CP gave the best sperm quality results such as sperm concentration (314.1 x$10^6$/mL), motility (59.5%), and live sperm (60.4%), while the lowest evaluation results were in the diet containing 14% CP (202.1 x$10^6$/mL, 38.4% and 41.3%, respectively) (P<0.05). This result is consistent with the statement in Table 6, the diet containing 18% CP is suitable for raising male rabbits for sperm extraction in the climatic conditions of the Mekong Delta.

In rabbits, reaction time is considered an important criterion for choosing a buck in breeding. Reaction time tended to decrease with increasing crude protein levels in the diet (Table 7; P < 0.05). Fast reaction time reflects the male's excitement level, which is consistent with the results of the experiment's testosterone concentration assessment (Table 6). Additionally, rabbits are food for many animals in nature, so rapid mating behavior is a survival characteristic of the species. Semen viscosity increased the membrane stability of sperm, enhancing the ability of sperm to move and penetrate egg cells (Coy *et al.*, 2009). However, high viscosity may interfere with the determination of sperm motility, concentration, and antibody coating of spermatozoa (Vasan, 2011). In current studies, there is no information available to assess this criterion. The pH values ranged from 6.99 to 7.35, which was similar to the results of Mohamed (2021) (6.38 - 7.95). Increasing semen pH values could cause changes in the seminal plasma ion balance, affecting the cytosol, the integrity of the sperm membrane, and the sperm's ability to store nutrients (Abdulrashid and Juniper, 2016). The low pH value could be due to glycolysis producing lactic acid, which reduced semen pH values (Rigau *et al.*, 1996), leading to the inhibition of sperm motility (Gadea, 2003).

According to Oyeyemi *et al.* (1998), concentrate with high protein diets was reported to increase sperm motility and sperm concentration. Previously, Ahemen *et al.* (2013) found similar results when using diets containing 18% CP with the addition of *Ipomoea aquatica* leaf powder. Sperm motility was an essential factor for sperm's ability to fertilize (Evans and Maxwell, 1987). Higher sperm concentration leaded to better fertility (Oyeyemi and Okediran, 2007). Additionally, the average sperm mortality was about 25% (Arthur *et al.*, 1975), and the rate of abnormal sperm ranged from 6-16% (Ajayi *et al.*, 2009), ensuring high fertility in both natural mating and artificial insemination (Oyeyemi and Okediran, 2007). However, the results of the live sperm and sperm abnormality (Table 8) were lower than in previous studies, possibly due to the effects of prolonged heat stress in the experimental conditions. Temperatures of 30 - 32$^{o}$C have been shown to increase sperm abnormalities in rams (Marai *et al.*, 2009) and rabbits (El-Raffa, 2004), thereby reducing semen quality.

**Table 8:** Results of semen characteristics.

| Item | Crude protein (%) | | | | | SEM | P |
|---|---|---|---|---|---|---|---|
| | 14 | 16 | 18 | 20 | 22 | | |
| Sperm concentration (x10$^6$/mL) | 202.1$^b$ | 208.8$^{ab}$ | 314.1$^a$ | 283.2$^{ab}$ | 216.9$^{ab}$ | 23.9 | 0.026 |
| Motility (%) | 38.4$^b$ | 52.2$^{ab}$ | 59.5$^a$ | 46.4$^{ab}$ | 43.2$^{ab}$ | 3.91 | 0.027 |
| Sperm abnormality (%) | 37.5$^{ab}$ | 36.6$^{ab}$ | 30.6$^b$ | 53.0$^{ab}$ | 60.6$^a$ | 5.88 | 0.023 |
| The total motile sperm (x10$^6$/mL) | 86.9$^b$ | 116.7$^{ab}$ | 139.4$^a$ | 110.7$^{ab}$ | 104.9$^{ab}$ | 9.15 | 0.027 |
| Live sperm (%) | 41.3$^b$ | 54.5$^a$ | 60.4$^a$ | 53.0$^{ab}$ | 49.8$^{ab}$ | 2.74 | 0.007 |
| Membrane integrity (%) | 45.5$^b$ | 55.3$^{ab}$ | 65.6$^a$ | 55.1$^{ab}$ | 54.2$^{ab}$ | 2.36 | 0.002 |

$^{a,b}$: Values within rows with different superscripts are different; $p < 0.05$; **SEM:** Standard error of the mean.

**Table 9:** Results of reproductive performance in doe rabbits.

| Item | Crude protein (%) | | | | | SEM | P |
|---|---|---|---|---|---|---|---|
| | 14 | 16 | 18 | 20 | 22 | | |
| Conception rate (%) | 100 | 79.2 | 89.6 | 86.1 | 86.1 | 9.00 | 0.590 |
| Litter size at birth (kits) | 4.75 | 5.17 | 4.96 | 5.17 | 5.17 | 0.36 | 0.896 |
| Alive litter size at birth (kits) | 4.33 | 5.17 | 4.96 | 4.33 | 5.17 | 0.44 | 0.445 |
| Mean weight at birth (g/kit) | 56.3 | 51.9 | 59.6 | 52.9 | 50.5 | 2.79 | 0.174 |
| Litter size at weaning (kits) | 3.92 | 4.33 | 4.54 | 4.13 | 4.33 | 0.46 | 0.893 |
| The kits survival rate until weaning (%) | 91.7 | 84.0 | 92.4 | 96.5 | 84.4 | 5.32 | 0.405 |
| Mean weight at weaning (g/kit) | 294 | 246 | 266 | 277 | 269 | 24.2 | 0.729 |

**SEM:** Standard error of the mean.

### Effects on Reproductive of Doe Rabbits

The reproductive results of doe rabbits mated directly by the corresponding experimental bucks were presented in Table 9, showing no difference in conception rate, litter size at birth, alive litter size at birth, mean weight at birth, and litter size at weaning among treatments ($P > 0.05$). The litter size at birth ranged 4.75 to 5.17 kits while litter size at weaning was 3.92 – 4.54 kits. This result was consistent with the prediction of sperm quality analysis results (Table 8). Although the number of sperm between treatments had a significant difference, in practice, it was still sufficient for reproduction in natural mating. The total number of motile sperm required for one insemination dose is $1.6\times10^6$ (Chen *et al*., 1989) and $6\times10^6$ sperm (Lavara *et al*., 2005). This could explain the reproductive results of doe rabbits when mated naturally, with no difference in conception rate and litter size at birth between treatments (Table 9; $P > 0.05$). The litter size at birth in the experiment ranged from 4.75 - 5.17, consistent with previously published survey data in the Mekong Delta by Chau and Thu (2014) which reported 4.84 ± 0.103. The difference in sperm quality and quantity of experimental bucks would bring greater significance when serving the needs of artificial semen application. Therefore, depending on the purpose of using the buck, choose an appropriate diet to ensure economic efficiency and serve long-term use.

## CONCLUSION AND RECOMMENDATIONS

The results of this study provided new assessments of the impact of dietary protein on the buck's reproductive performance under severe heat stress conditions of the Mekong Delta, Vietnam. The diet containing 18% crude protein gave great results in terms of testicular size improvement, the sensory value of sperm motility, microscopic evaluation of concentration, motility, live sperm rate, sperm membrane integrity, and morphological traits. To achieve high reproductive performance in bucks for artificial insemination, the supplementation of 18% crude protein in the diet should be utilized to rear buck rabbits under heat-stress conditions. Rabbit producers should implement the findings in practice. However, attention should be given to the feedstuffs used for rabbits and long-term research on the productivity of buck and doe rabbits.

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## NOVELTY STATEMENT

Determining the optimum protein content in the diet of buck rabbits used for artificial insemination under the con-

ditions of the Mekong Delta is a new result.

## AUTHOR'S CONTRIBUTIONS

Trung Thanh Truong: Conceptualization and design of the experiment, investigation, supervision, editing, and finalization.
Hai Long Tran: Investigation, methodology, formal analysis, manuscript preparation, editing, and finalization.

### Conflict of Interest

We certify that there is no conflict of interest

## REFERENCES


Abdulrashid M, Juniper DT (2016). Effect of dietary protein, selenium and temperature humidity index on reproductive traits of male rabbits in a, trop. Environ. J. of Anim. Prod. Res., 28: 61-65.

Ahemen T, Abu A, Orakaanya T (2013). Sperm quality and testicular morphometry of rabbits fed dietary levels of water spinach (Ipomoea aquatica) leaf meal. Agric. Biol. J. N. Am., 4: 352-357. https://doi.org/10.5251/abjna.2013.4.3.352.357

Ajayi AF, Raji Y, Togun V, Oyewopo A (2009). Caudal epididymal sperm characteristics and testicular morphometrics of rabbits fed graded levels of a blood-wild sunflower leaf meal (BWSLM) mixture diet. J. Complement. Integr. Med., 6. https://doi.org/10.2202/1553-3840.1232

AOAC (2000). Official methods of analysis. 17th edn. Association of Official Analytical Chemists: Washington, DC, USA.

Arthur GH, Noakes DE, Pearson H (1975). Veterinary reproduction and obstetrics. London: Baillere Tindal.

Attia YA, Al-Hanoun A, Bovera F (2011). Effect of different levels of bee pollen on performance and blood profile of New Zealand White bucks and growth performance of their offspring during summer and winter months. J. Anim. Physiol. Anim. Nutr., 95(1), 17-26. https://doi.org/10.1111/j.1439-0396.2009.00967.x

Ayyat MS, Abd El-Latif KM, Helal AA, Al-Sagheer AA (2021a). Interaction of supplementary L-carnitine and dietary energy levels on feed utilization and blood constituents in New Zealand White rabbits reared under summer conditions. Trop. Anim. Health Prod., 53: 279. https://doi.org/10.1007/s11250-021-02723-1

Ayyat M, El-Aasar T (2008). Effect of season of the year and dietary zinc supplementation on doe and buck performance of New Zealand white rabbits under Egyptian conditions. Egypt. J. Rabbit Sci., 18: 1-14.

Ayyat M, Marai I (1997). Effects of heat stress on growth, carcass traits and blood components of New Zealand White rabbits fed various dietary energy–fibre levels, under Egyptian conditions. J. Arid Environ., 37: 557-568. https://doi.org/10.1006/jare.1997.0308

Ayyat M, El-Latif AK, Helal A, Al-Sagheer A (2021b). New Zealand White rabbits tolerance to chronic thermal stress at different dietary energy/protein levels. Anim. Feed Sci. Technol., 278: 114992. https://doi.org/10.1016/j.anifeedsci.2021.114992

Bailey TL, Monke D, Hudson RS, Wolfe DF, Carson RL, Riddell MG (1996). Testicular shape and its relationship to sperm production in mature Holstein bulls. Theriogenology, 46: 881–887. https://doi.org/10.1016/S0093-691X(96)00245-2

Bootwalla S, Froman D (1988). Effect of extender viscosity on the insemination dose for chickens. Poult. Sci., 67: 1218-1221. https://doi.org/10.3382/ps.0671218

Chau NTV, Thu NV (2014). Current status of rabbit production in the Mekong Delta of Vietnam. Can Tho Univ. J. Sci., 32: 1-8.

Chen Y, Li J, Simkin M, Yang X, Foote R (1989). Fertility of Fresh and Frozen Rabbit Semen Inseminated at Different Times is Indicative of Male Differences in Capacitatlon Time. Biol. Reprod., 41: 848-853. https://doi.org/10.1095/biolreprod41.5.848

Coy P, Gadea J, Rath D, Hunter R (2009). Differing sperm ability to penetrate the oocyte in vivo and in vitro as revealed using colloidal preparations. Theriogenology, 72: 1171-1179. https://doi.org/10.1016/j.theriogenology.2009.07.011

Daader AH, Al-Sagheer AA, Gabr HA, El-Moniem EA (2018). Alleviation of heat-stress-related physiological perturbations in growing rabbits using natural antioxidants. Span. J. Agric. Res., 16. https://doi.org/10.5424/sjar/2018163-13184

Dalle Zotte A (2014). Rabbit farming for meat purposes. Anim. Front., 4: 62-67. https://doi.org/10.2527/af.2014-0035

Elmaz O Cirit U Keser O Gurbulak K Guvenc K Kutay C (2007). Effect of two dietary protein levels on testosterone, testicular parameters and semen quality in ram lambs during pubertal development. Med. Weter., 63: 1177-1180.

Ewuola EO, Lawanson AA, Adeyemi AA (2014). An improvised artificial vagina for rabbit semen collection and the characteristics of the extended rabbit semen as panacea for artificial insemination. Trop. Anim. Prod. Investig., 17: 19-24.

Evans G, Maxwell WMC (1987). Salamon's Artificial Insemination of Sheep and Goats. ed. Butterworths, London.

El-Raffa AM (2004). Rabbit production in hot climates. Proceedings of the 8thWorld Rabbit Congress, Puebla, Mexico, 1172-1180.

Gadea J (2003). Semen extenders used in the artificial inseminarion of swine. Span. J. Agric. Res., 1: 17-27. https://doi.org/10.5424/sjar/2003012-17

Ganaie A, Ghasura R, Mir N, Bumla N, Sankar G, Wani S (2013). Biochemical and physiological changes during thermal stress in bovines: A review. Iran. J. Appl. Anim. Sci., 3: 423-430.

Hafez B, Hafez ESE (2000). Semen Evaluation. Williams L, Wilkins (Editor), Reproduction in farm animals. USA: Philadelphia, Pennsylvania. https://doi.org/10.1002/9781119265306

Jimoh OA, Ewuola EO (2018). Semen characteristics, seminal biochemical and oxidative stress markers in rabbits during heat stress. J. Vet. Androl., 3: 35-44.

Ladokun AO, Egbunike GN, Adejumo DO, Sokunbi OA (2006). The effect of three dietary crude protein levels on digestibility and tests function in male pubertal rabbits. Tropicultura, 24: 3-6.

Lavara R, Mocé E, Lavara ,F de Castro MPV, Vicente JS (2005). Do parameters of seminal quality correlate with the results

of on-farm inseminations in rabbits? Theriogenology, 64: 1130-1141. https://doi.org/10.1016/j.theriogenology.2005.01.009

Lebas F, Coudert P, Rouvier R, De Rochambeau H (1997). The rabbit: husbandry, health, and production. Food Agric. Organ. U. Nations Rome,

Leng RA (2006). Digestion in the rabbit-a new look at the effects of their feeding and digestive strategies. Workshop-seminar" Forages for Pigs and Rabbits" MEKARN-CelAgrid, Cambodia, Phnom Penh, 22-24.

Lukefahr S (2007). The small-scale rabbit production model: intermediate factors. Livestock Res. Rural Dev., 19.

Maertens L, Perez JM, Villamide M, Cervera C, Gidenne T, Xiccato G (2002). Nutritive value of raw materials for rabbits: Egran tables 2002. World Rabbit Sci., 10: 157-166. https://doi.org/10.4995/wrs.2002.488

Marai IFM, El-Darawany AH, Ismail ESF, Abdel-Hafez MAM (2009). Reproductive and physiological traits of Egyptian Suffolk rams as affected by selenium dietary supplementation and housing heat radiation effects during winter of the sub-tropical environment of Egypt. Arch. Anim. Breeding, 52: 402-409. https://doi.org/10.5194/aab-52-402-2009

Marai IFM, Habeeb AAM, Gad AE (2002). Rabbits' productive, reproductive and physiological performance traits as affected by heat stress: a review. Livestock Prod. Sci., 78: 71-90. https://doi.org/10.1016/S0301-6226(02)00091-X

Minitab, 2016. Minitab reference manual release 16.1.0. Minitab Inc.

Mohamed B (2021). Improving reproductive buck rabbits by administrating citrus oil during summer condition. World Rabbit Science Association, Proceedings 12th World Rabbit Congress, Nantes, France, R-05, 04 pp.

NRC (1997). Nutrient requirements of rabbits. USA: Second revised edition, Washington DC.

Ogbuewu IP, Okoli IC, Iloeje M (2009). Semen quality characteristics, reaction time, testis weight and seminiferous tubule diameter of buck rabbits fed neem (Azadiractita indica A juss) leaf meal based diet. Iran. J. Reprod. Med., 7: 23-28.

Oseni S (2012). Rabbit production in low-input systems in Africa: prospects, challenges and opportunities. World Rabbit Science Association, Proceedings 10th World Rabbit Congress – September 3 - 6, 2012– Sharm El- Sheikh – Egypt, 719 – 731.

Oyeyemi MO, Okediran BS (2007). Testicular parameters and sperm morphology of chinchilla rabbit fed with different planes of soyameal. Int. J. Morphol., 25: 139-144. https://doi.org/10.4067/S0717-95022007000100021

Oyeyemi MO, Ajala O, Akusu M, Agbesola O (1998). Effects of Starvation on semen characteristics of West African dwarf bucks. Conf. Anim. Sci. Assoc. Niger., 22-24.

Perry GA, Patterson DJ (2001). Determining reproductive fertility in herd bulls. Univ. Mo. Agric. Publ., (2011): 1-8.

Rigau T, Piedrafita J, Reverter A, Canal M, Rodríguez-Gil J (1996). The rate of L-lactate production: a feasible parameter for the fresh diluted boar semen quality analysis. Anim. Reprod. Sci., 43: 161-172. https://doi.org/10.1016/0378-4320(96)01496-0

Thompson TL, Berndtson WE (1993). Testicular weight, Sertoli cell number, daily sperm production, and sperm output of sexually mature rabbits after neonatal or prepubertal hemicastration. Biol. Reprod., 48: 952-957. https://doi.org/10.1095/biolreprod48.5.952

Trung TT, Hai TL, Nhung PTC (2022). Effects of vitamin C supplementation in the drinking water on hematological indicators of growing and reproductive doe rabbits. J. Anim. Husbandry Sci. Tech., 279: 62-69.

Trung TT, Hai TL, Nghia NK, Tam NT (2024). Effects of the fermented soya waste supplementation with various probiotic sources on growth performance of crossbred rabbits. Vet. Integr. Sci., 22: 787–803. https://doi.org/10.12982/VIS.2024.052

Trung TT, Truong NB (2020). Effects of vitamin C supplement levels in diets on reproductive performances of female crossbred rabbits. J. Anim. Husbandry Sci. Tech., 259 (9): 2020.

Vasan SS (2011). Semen analysis and sperm function tests: How much to test? Indian J. Urol., 27:41-8. https://doi.org/10.4103/0970-1591.78424

World Health Organization (2021). WHO laboratory manual for the examination and processing of human semen. World Health Organization.