Testosterone level and mating capability of male rabbit fed commercial feed substituted with moringa oleifera leaf meal

N M R Suarni, I G L Oka, I G Mahardika and I P Suyadnya

1 Study Program of Biology, Faculty of Mathematic and Natural Sciences
2-3 Faculty of Animal Husbandry, Udayana University, Bali-Indonesia
4 Corresponding author: rai_suarni@unud.ac.id

Abstract. Rabbit is an alternative livestock that have a chance of being the source of healthy and high quality animal protein. One way to improve the productivity of rabbit meat is to improve the quality of male rabbits. Moringa leaves have androgenic effects by increasing testosterone, increasing blood flow to the male reproductive organs and stimulating the central nervous system to increase libido. The aims of this study were to find out the effects of moringa leaf meal substitution in commercial feed on the testosterone levels and mating capability of male rabbits. Twenty four of four months old male rabbits with an average live weight of 1200g were used in this study. There were four treatments in this study including 0% (K0) as control and 15% (K1), 30% (K2), 45% (K3) substitution of moringa leaf meal in commercial feed and each treatment consisted of six replicates. The treatments were carried out for two months. The variables measured were mating capability and testosterone levels in the blood. The results showed that there were significant differences (P <0.05) between all treatments (K1, K2, K3) and the control (K0). Substitution of commercial feed with moringa leaf meal in this study was able to increase the testosterone levels in the blood and mating capability of male rabbits. It was concluded that the moringa leaf meal able to substitute the commercial feed up to 45% to improve the testosterone levels in the blood and mating capability of male rabbits. To get maximum the testosterone levels in the blood and mating capability of male rabbits it should be better substituted the commercial feed with moringa leaf meal as much as 35.07%

1. Introduction
Rabbit is an alternative livestock that have a chance of being the source of healthy and high quality animal protein. Rabbit is very suitable to be developed in the densely populated countries such as Indonesia. Efforts to increase the production of rabbit meat need to be done, because the demands for rabbit meat in Indonesia will certainly increase. One way to improve the productivity of rabbit meat is to improve the quality of male rabbits. This will be achieved, one of them by improving the quality of the male reproductive system such as quality of reproductive hormones and mating capability that will affect the fertility of male eventually also affect the reproductive capabilities.

Male quality improvement can be achieved by improvements in livestock feed quality [1]. Ref. [2] stated that the quality of the feed greatly affects the quality of rabbit’s spermatozoa. A good quality feed is necessary for increasing the quality of male rabbits. Commercial feed materials for rabbits whose nutrient contents have been adjusted are widely produced and sold, but the price is not cheap. Therefore, it is necessary to find alternative feed rabbits that are cheap, easy to get, continuously available and high quality. Ref. [3] reported that treatment with 10g/kg body weight of moringa leaf extract can increase the number of mounting and ejaculation in white wistar rats, without significantly influence their testosterone.
Moringa is a leguminous plant with sustainable production and has a high value in protein, minerals and vitamins so as to overcome the constraints of food availability throughout the year. The use of moringa leaf meal as a protein source in concentrate has been widely applied. Since the complete nutrients content of the moringa, it may be used as a substitution part of commercial feed to obtain a cheaper feed, continuously availabilities and with complete nutrient contents. There was no research on how many percent of moringa can be used to substitute part of commercial feed and can produce an optimal male reproductive quality. Based on the above background a study on the effects of substitution of commercial feed with moringa leaf meal on the testosterone levels and mating capability of male rabbits need to be conducted.

2. Materials and Methods

2.1. Animal feed
Moringa leaf meal made from all leaves that are dark green and obtained from several regencies in Bali such as: Badung, Denpasar, Tabanan, Karangasem, Negara. Moringa leaf meal is made by separating the dark green moringa leaves from the stalk and then drying it until the weight is constant for about one week. Moringa leaves that have been dried are pureed into meal. This study use rabbit commercial feed which were mashed into flour. Both ingredients, namely Moringa leaf meal and commercial feed flour were mixed by a mixer and put into a pelleting machine. The pellet were dried with a freeze dryer for 8 hours. Feed were store in a cool and dry place.

2.2. Treatments
Experimental design used in this study was Completely Randomized Design (CRD). There were four treatments consisted of control (K0) fed 100% commercial feed, K1 fed 15% substitution of moringa leaf meal, K2 fed 30% substitution of moringa leaf meal as a substitute for commercial feed and K3 by 45% meal of moringa leaf meal. Each treatment consisted of six replicates, so that the total number of animal were 24 male rabbits. The treatment was conducted for two months. For mating tests 120 female rabbits were used and fed with commercial feed only.

Before treatments, each rabbit were injected with Ivomek 0.2 mL to prevent parasite attacks. The rabbit were adapted to housing and feeding for one week. The rabbit were housed individually under a rabbit standard laboratory conditions (23-25°C temperature, 50-60% relative humidity and 12 hours light/dark cycle). The feed was given 150g/day (twice a day in the morning and evening). Rabbits were given drinking water ad libitum. The experiment was approved by The Animal Ethical Committee, Faculty Veterinary Medicine, Udayana University

2.3. Sampling collection and analysis
The day before the mating capability test, the blood taken from the vein on the male rabbit's ears with the rinsed syringe with heparin with needle 23 G. The blood obtained was centrifuged for 15 minutes at 1000rpm. Plasma obtained then analysed by the Rabbit F TEST (Free Testosterone) ELISA Kit (Elabscience). The mating test was carried out at the end. The trick is to include five female rabbits who are ready to mate to the male rabbit cage, then observed: 1. Introduction (approach): how many times do male rabbits approach such as sniffing and kissing each female rabbit. 2. Mount (how to ride): how many times do male rabbits ride every female rabbit before copulation. 3. Copulation: how many times can male rabbits be copulated.

Observation of mating capability is done using CCTV (Closed Circular Television) so that the results obtained are more effective and thorough. The mating test is carried out for 2 hours. After that the female rabbit was removed from the male rabbit cage. Rabbit mating tests should be done when the air temperature is low, that is, the morning before eight o'clock in the morning and evening. The results are then compared between (K0), (K1), (K2) and (K3).
2.4. Data Analysis
Data were analysed by analysis of variance (ANOVA). To determine the differences between treatments including the control group, it was conducted using Duncan's Multiple Range test with a confidence level of 5% (P <0.05).

3. Results and Discussion

3.1. Testosterone hormone levels
The results of the male rabbit testosterone level analysis which are treated by the substitution of commercial feed with moringa leaf meal are presented in (Table 1). In this study, the substitution of commercial feed with moringa leaf meal was able to increase the testosterone levels in the blood of male rabbits. When it was analysed by One Way ANOVA analysis showed significant differences (P <0.05) between the control and the treatment groups. This means the substitution of commercial feed up to 45% with moringa leaf meal was able to increase testosterone levels. The highest testosterone levels found at the K2 treatment and for the highest consumption per day also found on the K2 treatment and consumption decline again in the treatment of K3. The relationship between the content of moringa leaf meal in feed with high levels of testosterone has the following equation of \( Y = 1.74 + 0.7x - 0.0067x^2 \), where \( Y \) = testosterone and \( X \) = content of moringa leaf meal in the feed with \( R^2 = 0.71 \) and \( R = 0.84 \).

From the equation, it was obtained that the content of moringa leaf meal in an optimal feed (35.07%) to get the maximum testosterone levels so as to maximize the reproductive capacity of male rabbits (Figure 1).

Testosterone production is strongly influenced by the amount and quality of feed. Reduced feed intake in the treatment of K3 compared to the treatment of K2, may cause the amount of nutrients absorbed decrease so that the production of testosterone levels in the treatment of K3 became lower than the K2 treatment.

Moringa leaf meal contains over 90 nutrients such as zinc, vitamin D, vitamin C, protein and 46 antioxidants. The high content of nutrients in moringa leaves can improve the treatment feed quality. Vitamin D may increase testosterone levels. Male with vitamin D levels of 30 ng / ml in the blood, testosterone levels are very significantly higher than the levels of vitamin D in the blood of less than 30 ng / ml [4] The vitamin D content of moringa leaves in this study were able to increase testosterone levels.
levels in the blood of rabbits. This is evidenced by the growing plasma of testosterone levels of all treatments, K1, K2 and K3, and difference significantly increasing compared with the controls. Ref. [5] states that at 12–week old male rats which were fed for six weeks of Zn deficiency, increased the levels of FSH and LH. The decrease of LH caused an impaired in testosterone secretion that resulted in low levels of testosterone. It was further reported that if the feed of Zn deficiency, testicular cells are capable of transporting cholesterol which is a steroid hormone precursor, but was unable to convert them into a steroid hormone, causing retention of the process of spermatogenesis. Moringa leaf Zn content in the research was proven to increase the levels of Zn in animal experiments that can increase the testosterone levels in the blood of rabbits.

3.2. Mating Capability

Substitution of commercial feed with moringa leaf meal up to 45% increased significantly (P <0.05) the mating capability of male rabbit as presented in (Table 1). Mating capability of male rabbits in this study was influenced by the levels of testosterone in the blood. The highest levels of the testosterone in the study was in treatment K2, then decreased in the K3, but still higher than the control and K1. This was because the levels of testosterone in the blood of rabbits K2 was also the highest. Decreased reproductive capability in treatment K3 compared with K2 because the levels of testosterone in the blood of K3 was lower than K2. The pattern of testosterone levels in each treatment were significantly affect the mating capability of male rabbit in the same treatment. The higher the levels of testosterone produced the higher the mating capability of male rabbit in this study. Ref. [6] also suggested that the sexual capability of males is influenced by testosterone. Ref. [7] stated that rabbits reproduction capability is influenced by the testosterone hormone which can perform very fast copulation of rabbits, when fed the ready to mate female, and it can perform copulation many times. Reference in [8] also stated that the mating capabilities of male mammal animals depend on the condition of the animal androgen hormones.

In this study the substitution of commercial feed with moringa leaf meal can increase mating capability in male rabbits. This is because of moringa leaves have androgenic effects by increasing testosterone, increase blood flow of the male reproductive organs and stimulate the central nervous system to increase libido so that mating capability increases. The results of this study are supported by the research of Ref. [3] which suggested that moringa leaves can improve male sexual function such as libido, quality of spermatozoa treating erectile dysfunction. The results of their research on rats, at a dose of 10mg / kg extract of leaves of moringa very significantly increased the number of introduction and mount in mating capability. So it can be concluded that moringa is a feed supplement to improve sexual function.

| Variables  | Treatments                  |
|-----------|-----------------------------|
|           | K0             | K1    | K2             | K3    |
| Introduction | 4.33d    | 5.67c | 10.50a         | 6.83b |
| Mount       | 5.67c    | 6.83b | 7.50a          | 7.33ab |
| Copulation  | 2.67b    | 4.33a | 5.00a          | 4.50a |

Different letter at the same row indicates significantly different results (p>0.05). K0 = commercial feed of 100%, K1 = commercial feed substituted with 15% of moringa leaf meal, K2 = commercial feed substituted 30% moringa leaf meal, K3 = commercial feed substituted 45% moringa leaf meal.

Moringa can suppress Monoamine Oxidase B (MAOB) so that dopamine increase, then it works to increase libido and mating capability such as the number of introduction, mount and copulation [9]. Reference in [10] suggest that dopamine plays a very important role in sexual capability by way of the oxytocin of paraventricular nucleus (PVN) in the hypothalamus which induces nitric oxide (NO) of cavernosum nerve thus improving cyclic guanosine monophosphate (cGMP) on penis. The increase in cGMP induced by moringa leaves and decreased levels of phosphodiesterase type 5 (PDE-5) causes the
blood flow to the penis then erection. This is what causes male rabbits in this study experienced improvement in the process introduction, mount and copulation.

4. Conclusions
Substitution of commercial feed with moringa leaf meal up to 45% increase the blood levels of testosterone and mating capability, such as (introduction, mount and copulation) in male rabbits. To get maximum the testosterone levels in the blood and mating capability of male rabbits it should be better substituted the commercial feed with moringa leaf meal as much as 35.07%.

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