Substitution of commercial feed with moringa leaf meal to improve the sperm quality of male rabbit

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Abstract. The aims of this study were to find out the effects of moringa leaf meal substitution in commercial feed on the sperm quality of male rabbits (bucks). Twenty 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 including motility, viability, morphology and the total of sperm. The results showed that there were significant differences (P <0.05) between all treatments (K1, K2, K3) and the control (K0). Substitution of moringa leaf meal up to 45% in commercial feed increase quality of sperm (motility, viability, morphology and the total of sperm. The optimal substitution of moringa leaf meal in commercial feed in this study was 30%. It was concluded that the moringa leaf meal might be substituted in commercial feed up to 45 % to improve the sperm quality of male rabbits (bucks). To get maximum sperm quality of male rabbits it should be better substituted the commercial feed with moringa leaf meal as much as 32.31% - 35.07%.

1. Introduction

The increasing need for meat in Indonesia is not accompanied by the amount of meat production of good quality. An alternative is needed to increase the amount of meat production and quality. Rabbits can be used as an alternative to the fulfilment of quality meat wholeness because rabbit meat contains high protein and low fat. Rabbits are very easy to breed, as long as the feed given in accordance with their needs. Rabbit breeding is largely determined by the quality of male rabbits as the quality of sperm produced. this will be achieved when the feed is given the high quality. In the market, there is much commercial feed available for rabbits whose nutrients have been adjusted but at an expensive price. Rabbit breeders still use a lot of grass like a rabbit feed, so the growth and development of rabbits are kept slower than rabbits fed commercial. Therefore it is necessary to find an alternative rabbit feed that is cheap, easy to obtain, and its availability is sustainable and high quality. Moringa is a leguminous plant whose production is sustainable and has more value in the content of protein, minerals, and vitamins so it can overcome the obstacle of feed availability throughout the year. The use of moringa leave meal as a source of protein in reinforcing feed has been widely applied. Ref. [1] stated that substitution of commercial feed with moringa leaf 15%, 30%, and 45% can improve the diameter of seminiferous tubule thick germinal epithelium, the number of germinal cells and number of Leydig cell. Based on the above, the research on the provision of commercial feed with moringa leaf meal to improve the quality of sperm done.
2. Materials and Method

2.1. 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.

2.2. Sampling collection and analysis

2.2.1. Sperm motility
The number of sperm motility observed with the Improved Neubauer-type hemocytometer, under a light microscope equipped with the digital device Optilab microscope with the 400x magnification. To obtain more accurate results, the motility was recorded with a digital device of Optilab microscope for 10 minutes. The motility was analysed on the recording by using the Image Raster 3.

2.2.2. Total of sperm
Calculations were performed by using digital devices of Optilab microscope and light microscope with 400x magnification. To simplify the calculation of hemocytometer that already contained sperm was photographed, then counting was done on a photograph obtained.

2.2.3. Abnormality of sperm morphology
Observations were made with 1% eosin staining in 70% alcohol. Observations were made under a microscope and digital devices of Optilab microscope with 400x magnification of 100 sperm, the results are expressed in percent (%). Observations were repeated 5 times.

2.2.4. Sperm viability.
Observations on the viability of sperm performed on preparations used for observation of morphology of sperm. Observation by light microscope and digital devices Optilab microscope with 400x magnification of 100 sperm live sperm will appear clear or colourless whereas non-viable sperm appear red coloured.

2.3. 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

The quality of the rabbit cauda epididymal sperm fed with substitution of commercial feed with moringa leaf meal can be seen in Table 1.

3.1. Sperm Motility
The results of this study indicated that sperm motility of rabbits fed with commercial feed substitution with moringa leaf meal was better than the control. Of the average value, it can be seen the highest increase occurred in the K2, then there was a decrease in the K3 (Table 1) but statistically showed significant differences with the K2, so it can be said that up to 45% of meal of moringa leaves in commercial feed can improve the cauda epididymal sperm motility. The pattern of the relationship between the content of moringa leaf meal in the feed with the male rabbit sperm motility follows the equation of \( Y = 86.13 + 0.16x +0.0024x^2 \) where \( Y = \) sperm motility and \( X = \) content of moringa leaf meal in the feed with \( R^2 = 0.90 \) and \( R = 0.95 \). From the equation, it was obtained the optimal moringa
leaf content in the feed was (33.4%) to get the maximum sperm motility so as to maximize the reproductive capacity of male rabbits (Figure 1).

The more of feed consumed can increase of sperm motility in this study. The increase sperm motility of rabbits treated with moringa leaf meal because the moringa leaves contain so many nutrients that can improve the quality, especially the sperm motility. Reference in [2] suggested that supplementation of vitamin C and E in the drinking water of rabbits can improve the sperm motility. Reference in [3] also suggested that, supplementation of vitamin B, vitamin E and selenium can reduce the levels of MDA and increase the motility of sperm. Dried moringa leaves contain vitamin C of 17.3 mg / 100g (12 x vitamin C in oranges, 2.64mg / 100g vitamin B1, 20.5 mg / 100g B2 and 8.2 mg / 100g B3 [4] which can improve the sperm motility in this study. Selenium is needed in small amounts but if inadequate, it will greatly affect the health and ultimately will affect the quality of reproduction.

Table 1. The quality of the rabbit cauda epididymal sperm fed with substitution of commercial feed with moringa leaf meal

| Variables                                      | Treatments |
|------------------------------------------------|------------|
|                                                | K0         | K1         | K2         | K3         |
| Motility (%)                                   | 86.28c     | 87.51bc    | 89.23a     | 88.34ab    |
| Viability (%)                                  | 88.48c     | 91.83b     | 93.38a     | 92.85a     |
| Abnormal sperm count (%)                       | 7.43a      | 6.95b      | 5.83c      | 6.50b      |
| Total sperm (million/cauda epididymal)         | 647.13d    | 663.07c    | 676.97a    | 670.17b    |

Note: 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.

Figure 1. Graph of the relationship between sperm motility of male rabbit with meal content of moringa leaves in feed.

3.2. Sperm Viability

The results showed that the viable sperm are: sperm of clear in colour (v) and the non-viable sperm is red (nv) as presented in (Figure 2). The average sperm viability of rabbits given commercial feed with substitution of moringa leaves is presented in (Table1). The average amount of sperm viability of the K2 was the highest among other treatments, because the average value of K3 is smaller than the K2 but still it is higher than K0 and K1. This is presumably because the highest feed consumption was at the K2 treatment so that nutrients from the moringa leaf meal was so complete that more was consumed by rabbits in this research. High content of antioxidants in moringa leaves can inhibit the negative effects of free radicals that substitution of commercial feed with leaf meal moringa in this study can reduce the
number of non-viable sperm. The red-coloured sperm found in this research showed that the sperm were already non-viable because the membrane of sperm were no longer intact as evidenced by the penetration of colour substance into sperm cells with ease. Sperm membrane rupture that caused the death of sperm caused by ROS either from the environment or from the feed. Ref. [5] suggest that ROS can directly damage sperm by inducing lipid peroxidation in the plasma. The occurrence membrane lipid peroxidation in the plasma membrane will reduce the integrity of the membrane.

![Figure 2](image)

**Figure 2.** Comparison of rabbit sperm, v = viable sperm and nv = non-viable sperm.

### 3.3. Abnormalities of sperm morphology

Some morphological abnormalities in sperm of rabbits were found in this study namely a tapered head, double head, big and round head, double tails, curled tail and the drops of cytoplasmic in the tails (Figure 3). Substitution of commercial feed with moringa leaf meal caused significant differences (P <0.05) between the control and the treatment groups. The abnormal cauda epididymal sperm count was found mostly at the lowest number of the control i.e. at the treatment of K2 with a decrease of 21.53% compared with the control (K0).

![Figure 3](image)

**Figure 3.** Various sperm morphological abnormalities found in rabbits after treated with the substitution of commercial feed with moringa leaf meal. A= normal sperm,,B= tapered head sperm,,C= double-headed sperm,D =sperm with the remaining drops of cytoplasm in the tail,E = sperm with double tails, F = sperm with large and round heads,G = sperm with coiled tail

Environmental conditions can be ascertained at this time that are not exempt from the effects of free radicals. This is proven by the highest number of abnormal sperm found in the controls (K0) although it can still be said to be within the normal limit of 7.43%. Abnormality is considered serious if the primary sperm abnormality found reaches 18-20% because it can reduce fertility [6]. If it is ignored, it will surely continue to increase the number of abnormal sperm because antioxidants found in the body of the rabbit no longer able to counteract free radical attacks continuously. The increasing number of abnormal sperm can reduce male fertility. Disorders or abnormal sperm can be caused by the presence of ROS in the process of maturation of sperm. Sperm morphological abnormalities like the rest of the disorder caused by the cytoplasmic sperm maturation process. The disorder is caused by the damage of
the DNA and the production of ROS is high. ROS production is associated with an abnormal number of sperm, because ROS can cause disruption of the normal process of apoptosis and DNA damage. Apoptosis is not normal and may interfere with DNA damage of spermatogenesis and sperm maturation [7]. The rise in ROS levels and reduced antioxidant, can cause damage to cellular DNA i.e. the cellular DNA fragmentation and morphological abnormalities (head, neck and tail) of sperm [8]. In this study, the substitution of commercial feed with moringa leaf meal to 45% can reduce the number of abnormal sperm. This is caused by the nutrients and antioxidants that are so full of moringa leaves that it can counteract free radicals that come from the environment and from food. 

3.4. Total of Sperm (millions / cauda epididymis).
In this study, the average number of rabbits’ cauda epididymal sperm were: 647.13 on the control, 663.07 in the K1, 670.17 on the K2 and 676.97 on the K3. There are significant differences (P <0.05) between the control and the treatment groups (Table 1). It could be argued that the substitution of commercial feed with moringa leaf meal to 45% can increase the rabbits’ cauda epididymal sperm. Optimal content of moringa leaf meal in the feed is determined by the equation of $Y= 646.2 + 1.69x - 0.025x^2$, where $Y$ = total of cauda epididymal sperm and $X$ = content of moringa leaf meal in the feed

$$R^2 = 0.96\text{ dan } R = 0.98$$

From equation, it was obtained the content of moringa leaf meal in feed was 33.8% which is optimal to get the maximum number of sperm thereby increasing the maximum reproductive capability of male rabbits (Figure 5).

The number of sperm stored in the rabbits’ epididymis around 1,000 x 106 where 275 x106 is in the head and body of the epididymis, 650 x 106 located in the cauda epididymis and 50 x 106 located in the ductus [19]. In this study, an increasing number of cauda epididymal of sperm by moringa leaf meal compared with the controls. The average number of cauda epididymal sperm of rabbits in this study was 64.13 million / cauda epididymis (the control), 663.07 million / cauda epididymis (K1), 679.97 million / cauda epididymis (K2) and 670.17 million / cauda epididymis (K3).

![Figure 4](image)

**Figure 4.** Graph of the relationship between the number of male rabbits’ cauda epididymal sperm with moringa leaf content in feed

Ref. [10] stated that there were no significant differences (P> 0.05) on sperm count after being fed with 5%, 10% and 15% of moringa leaf meal in the feed. But there was a positive correlation between increased 5%, 10% and 15% of moringa leaf meal in feed with the number of sperm, this indicates that the moringa leaf meal can increase the number of sperm of rabbits. The increasing number of sperm is because of the complete nutrient content of moringa leaves, one of which is Zn. Ref. [11] suggested that zinc is a trace element and more than 300 enzymes require zinc in their activities. Zinc is also important in the process of cell division and differentiation so that the process of spermatogenesis is indispensable. Ref. [12] stated that Zn has links with several enzymes in the body and it can prevent damage of the cells through activation of the antioxidant system. Ref. [13] stated that, Zn deficiency can increase lipid peroxidation and Zn supplementation can handle it. Ref. [14] also stated that the feeding of isoflavone-
rich soy meal, Zn and vitamin E in male rats in full produced testosterone serum levels and higher total spermatogenic cells.

Spermatogenesis is a complex process starting from the germinal cell proliferation and maturation of spermatogonia into sperm. During spermatogenesis, programmed non-viable cell (apoptosis) plays a critical role to eliminate defective germ cells that carry DNA mutations. This physiological process can occur so that the dysregulation of apoptosis of germ cells may cause infertility. Apoptosis is necessary for the survival of normal spermatogenesis and for cellular homeostasis and balance between germ cells with Sertoli cells [15].

4. Conclusion
Substitution of commercial feed with moringa leaf meal to 45% improved the quality of spermatozoa (motility, viability, total cauda epididymis sperm and lowered the amount of sperm morphological abnormalities of male rabbits. Optimal substitution of commercial feed with moringa leaf meal improved the sperm quality of male rabbits to the maximum analysed by regression namely between 32.31% - 35.07%.

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