

**SPERM MEMBRANE INTEGRITY AND MDA LEVEL OF MALE RABBIT  
FED COMMERCIAL FEED SUBSTITUTED  
BY *Moringa oleifera* LEAF MEAL**

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**Abstract**

*Moringa oleifera* is a clump plant that many of them could be found in Indonesia as a plant that used for fence and has many benefit like vegetables and substance of traditional medicine. This research was intended to know commercial feed substituted with *Moringa oleifera* leaf meal effect towards sperm membrane integrity and *Malondialdehyde* (MDA) level of male rabbit blood. There were used 24 male rabbit on four months years in weight average of 1200 gram. There were four treatments on this study i.e. 05 (KO), 15% (K1), 30% (K2), and 45% (K3) *Moringa oleifera* leaf meal in commercial feed, the each treatment included of 6 repetition. The treatment was conducted for 2 months. The blood that was used for measuring MDA level was taken from vena which found in ears. Referring to membrane integrity, it was used caudal epididymal sperm. The result saw that there was significantly ( $P < 0,05$ ) between controlling within way of treating toward sperm membrane integrity and MDA level of rabbit blood. Commercial feed substituted by *Moringa oleifera* leaf meal up to 45% was able to increase sperm membrane integrity and decrease rabbit MDA level in real. It was concluded that *Moringa oleifera* leaf meal up to 45% was able to be used as commercial feed substituted to the rabbit.

**Key words:** *Moringa oleifera*, rabbit, membrane integrity, sperm, MDA

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## I. Introduction

Normally condition, the living things may not deny toward the free radical effect from environment and food. The free radical is able to decrease a quality of male reproduction, especially sperm membrane integrity. Fuglie (1999), Sidduraju and Becker (2003) stated that Moringa consists of 46 antioxidants i.e. compositional grading that protects a body from the effect of free radical. Antioxidants that is consist of Moringa leaf is able to prevent the attack from free radical towards sperm wall (sperm membrane integrity). *Malondialdehyde* (MDA) is to a product from free radical reaction. In this research, it is done the analysis of blood MDA level and sperm membrane integrity of rabbit that is treatment of commercial feed substituted with Moringa leaf meal. In order to increase a quality of male reproduction is needed feed with completely nutrient. Moringa consist of all those nutrients so that it is hoped is able to substitute commercial feed that it has been completely contents and has common in used.

## II. Material and Method

### 2.1 Material and Animal Testing

Testing material is Moringa leaf that is obtained from five regency in Bali, they are: Badung, Denpasar, Tabanan, Karangasem, Negara. The rabbits that used are 24 local male on four months years old, in weight average 1200 gram, and good condition. The rabbit is injected by 0,2 *ivomec* ml per head before doing a treatment, in order to prevent *endoparasites* and *ectoparasites* (Hon *et al*, 2009). Rabbit acclimation is conducted before the research for two weeks in order to stable their weight. The treatment in this study is done for two months and take place in Banjar Gelogor Carik, Denpasar. At the time, in this research it is applied hutch graded individually made of bamboo, plywood and wire with measurement 75x60x50 cm. Zoology Laboratory of FMIPA UNUD, as a place does operation towards animal for taking a sample of cauda epididymal sperm that used as observation to the sperm membrane integrity and taking a blood for analyzing MDA level. Animal Physiology Faculty of Medicine Laboratory of Brawijaya University in Malang, as a place for measuring MDA level of blood plasma.

### 2.2 Feed

The feed that is used in this research is commercial feed. As a feed treatment it is substituted by 15%, 30%, and 45% Moringa leaf meal. The material composition of feed is to show at Table 1. It will be presented into two tables:

The composition of the material making up feed experiment

Table 1

Material (%)	K0	K1	K2	K3
Commercial				
fee	100	85	70	55
Moringa leaf				
meal	0	15	30	45
Total	100	100	100	100

Descriptions:

K0: 100% commercial feed (pellet)

K1: substituted pellet 15% of Moringa leaf meal

K2: substituted pellet 30% of Moringa leaf meal

K3: substituted pellet 45% of Moringa leaf meal

Nutrient substance in feed experiment

Table 2

Nutrient (%)	K0	K1	K2	K3
CP	13,64	15,90	19,02	21,84
CF	10,35	9,57	8,92	8,09
Fatty	5,56	5,68	6,04	5,91
Energy	3938	3990	4001	4017
Calcium	0,97	1,30	1,44	1,72
Phosphorus	0,49	0,45	0,39	0,50

Descriptions:

CP: Crude Protein, CF: Crude Fiber

K0: 100% commercial feed (pellet)

K1: substituted commercial feed 15% of Moringa leaf meal

K2: substituted commercial feed 30% of Moringa leaf meal

K3: substituted commercial feed 45% of Moringa leaf meal

Feed and water are given by *ad libitum*, and it is done three times a day i.e. 10 am, 1 pm, and 6 pm (Subroto, 2010).

### 2.3 Research Design

The design that is used in this research is complete random design. In this research there are four treatments i.e. Control (KO) is given 100% commercial feed. K1 is given 15% a powder of Moringa leaf in commercial feed, K2 is given 30% a meal of Moringa leaf in commercial feed, and K3 is given 45% a meal of Moringa leaf in commercial feed. The each treatment is conducted six testing, therefore, 24 male rabbit is used in this study.

## III. Analysis, Discussion, and Result

### 3.1 The analysis of plasma membrane integrity of sperm

In order to know the sperm membrane integrity, therefore, it is needed done a specific testing which is called HOS Test (Hypo Osmotic Swelling Test). Sperm suspensions of 0,5 ml is added 4,5 ml swollen solution 0,735 gram Sodium Citrate Dehydrate ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ ) and 1,351 gram fructose in 100 ml distillation water and mixing carefully with straw, then, is incubated in incubator CO<sub>2</sub> temperature 37 °C for 60 minutes. After the solution is incubated then taking one drops and put on the glass, the next object is added one drops of eosin solution and 10% *nigrosine*. Next, it is made a smear preparations and observed under the light of microscope and digital tool of optilab microscope by 400x enlargement. Sperm membrane integrity is calculated by sperm number that bulging or tail coiled (crooked) every 100% sperm (WHO, 1999).

### 3.2 The analysis of blood MDA level

One day before the male rabbit is operated conducted taking a blood in vena of ears by syringe that has cleared with heparin and pin number 23 G. The blood that is obtained centrifugation for 15 minutes of 1000rpm. The plasma that is obtained next to be analyzed by *Enzyme-linked Immunosorbent Assay (Elisa) Kit for MDA*.

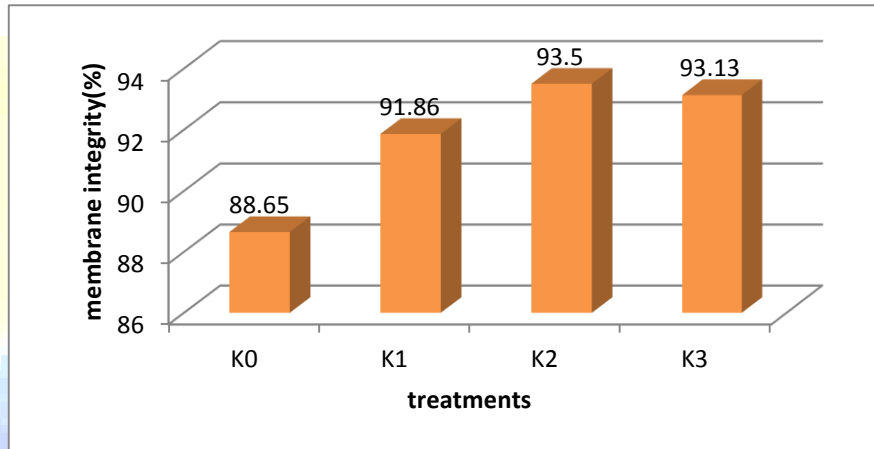
### 3.3 The analysis of the Data

The data that was obtained so that analyzed by *costat*. In order to know there is in real a different using One Way Anova, in order to know the difference between on treatment to another next to use *Duncan's Multiple Range Test* by believing level 5% ( $P < 0,05$ )

### 3.4 The result of sperm membrane integrity of rabbit *cauda epididymis*

In this research, commercial feed substituted with Moringa leaf meal up to 45% is able to increase sperm membrane integrity of rabbit. The highest sperm membrane integrity occur on K2 treatment i.e. 93,5%, it is occurred increasing 4,85% is compared by control.

**Figure 1**



The comparison sperm membrane integrity of male rabbit that is conducted treatments commercial feed substituted by of Moringa leaf meal.

**Figure 2**

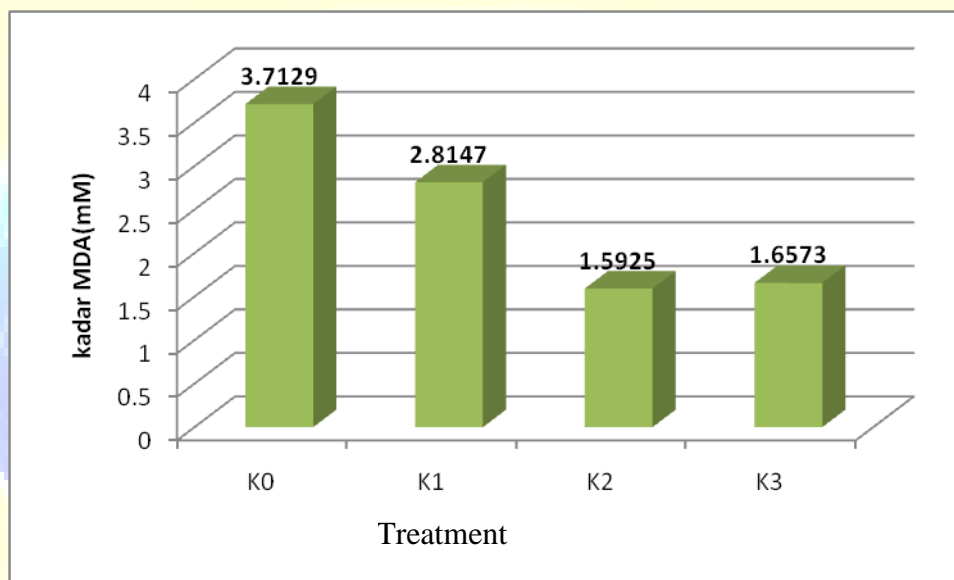


Rabbit sperm membrane integrity is to show that sperm membrane still intact (white arrow) appear curled tail and enlarged head and sperm is not curled (black arrow) membrane is unwell.

### 3.5 The result of blood MDA level

MDA level of rabbit blood that has given commercial feed substituted with Moringa leaf meal is presented on (table 3 and figure 3). The result of study is to show that there is in real differentiation ( $P < 0,05$ ) towards its treatment. Commercial feed substituted with Moringa leaf meal is able to decrease MDA level of rabbit blood on this study. The highest of decreasing of MDA level occur to the K2 treatment in the amount of 57,37% then K3 55,76%, and K1 24,66% are compared by control.

Figure 3



MDA level of plasma of male rabbit blood that is given treatment a commercial feed substituted with meal of Moringa leaf

Table 3

Variable	Treatments			
	K0	K1	K2	K3
Sperm membrane integrity (%)	88,65c	92b	93,5a	93,13a
MDA level ( $\mu\text{m}$ )	3,73 a	2,81 b	1,59 c	1,65 c

Sperm membrane integrity and MDA level of male rabbit blood that is given commercial feed substituted with meal of Moringa leaf.

#### Descriptions:

- 1). The number that is followed by in different alphabet is not the same towards the same line is to show a different result in 5% test level.
- 2). K0: 100% commercial feed (pellet)  
K1: substituted commercial feed 15% of Moringa leaf meal  
K2: substituted commercial feed 30% of Moringa leaf meal  
K3: substituted commercial feed 45% of Moringa leaf meal

#### IV. Discussion

HOS test is used to determine whether sperm membrane is still intact or not intact by positive or negative observation to the test itself. Sperm head that bulging or sperm tails that curl is to show a positive test which means sperm membrane is still intact (see figure 2). It shows that HOS test result is negative which means sperm membrane is not intact anymore and surely its sperm is dead (see figure 2).

Testis is as a place for spermatogenesis occur is highly vulnerable towards oxidation process by free radical. There is free radical to the testis is able to change stabilization and membrane function, it is caused by further lipid oxidation. Lipid oxidation process is reported by Sancka and Kuspizs (2004) is to occur a trouble of spermatogenesis. According to Sikka (2004), radical scavenger will clean free radical to the system that produce a sperm. The immune system that can be used to fight free radicals to be influenced by the availability of nutrients derived from ingredients feed that have potential as antioxidants.

In this research, in fact, the lowest of sperm membrane integrity is 88,65% to the control (KO), then is followed by K1 (92%), K3 (93,13) and the highest is K2 (93,5). It is a vice versa to the MDA level of blood wherein the highest MDA level is to the control (KO) i.e. 3.73. It is to prove that is in normal condition, the animal does not free from free radical that is able to influence sexual quality. Commercial feed integrity with Moringa leaf meal, in fact, it can be prevented free radical by fitting sperm membrane integrity and decrease MDA level to the rabbit blood.

Some of Moringa substance have specific influence toward male reproduction system, as like D, A vitamin and zinc. A vitamin as well is able to prevent the attack of free radical towards sperm wall (sperm membrane integrity) (Bunmi, 2012). Bey (2010) stated that A

vitamin substance in dried Moringa leaf ten times is more than carrot. The highest vitamin substance is as a strongest anti-oxidant so that is able to prevent free radical, ever, which comes from environment or feed. There is synergy effect between E vitamin and iso-flavones is considered to strength both as phenolic antioxidant therefore is able to stop the chain reaction of lipid peroxidation of unsaturated fatty acids in the cell membrane phospholipids testis, the free radical accumulation has been helped prevented to the system that produce a sperm, and sperm function is protected. In term of this, as well as it is supported by Zn role in maintaining sell membrane integrity (Corah, 1996). Flavonoid has an ability as antioxidants and prevent to occur a damage caused by free radical, whereby, flavonoid acts as scavenger about directly free radical (Nijveldt *et al.*, 2001).

MDA is a result of processing of lipid oxidation (one of damage process of plasma membrane caused oxidative stressing that is caused by free radical. Oxidative stress is one of main factor that causes male infertilities. It is occurred by ROS (Reactive Oxygen Species) increasing that will make a damage for DNA and finally sperm apoptosis happens. Plasma membrane and cytoplasm of sperm sell consist of unsaturated fatty acids in the big number, therefore, ROS is able to be easy entry in the plasma membrane. The main mechanism in the process sperm membrane damage is by ROS towards lipid peroxidation reaction or LPO (lipid peroxidation).

Antioxidant compound is substances that body needs for naturalizing free radical and damage prevented that is caused by free radical to the normal sell, protein, and fatty. This compound has molecule structure that the electron can be given to the free radical molecule without disturbing its function and chain reaction can be passed by free radical. Antioxidant has important role as sperm protector towards ROS. Antioxidant itself includes *Superoxide Dismutase (SOD)*, *catalase*, and *Glutathione Peroxidase (GPX)*. The other side, as well as, there is non-enzymatic antioxidant like C vitamin, E vitamin, pyruvate, glutathione, and *carnitine* (Saleh et al, 2003).

Moringa has antioxidant about 46 and it is to be one of sources of most natural antioxidant. Moringa is rich in Flavonoids, beta carotene which has function as antioxidants. Antioxidants will have maximum function when working with several other antioxidants and some nutrients (Bey, 2010). Moringa leaf nutrient has complete content and it has 46



antioxidants, therefore, in this study substituted commercial feed up to 45%, MDA level can be decreased and sperm membrane of rabbit integrity can be increase.

## V. Conclusion

Commercial feed Substituted with Moringa leaf meal up to 45% can improve sperm membrane integrity and MDA level of male rabbit blood can be reduced. It can be said that Moringa leaf powder can substitute up to 45% of commercial feed, however, the optimal dose is 30%.

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