



Free Testosterone Level and Quality of Cauda Epididymis Sperm of Local Rabbit That Given Commercial Feed Supplemented by Cod Fish Liver Oil



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Article history:

Received: 18 December 2015

Accepted: 30 January 2016

Published: 31 March 2016

Keywords:

Cod fish liver oil;

Free testosterone;

Local rabbit;

Reproduction;

Sperm quality;

Abstract

This study was intended to determine the free testosterone levels and sperm quality of local rabbit that was given commercial feed supplemented cod fish liver oil. The experiment design that was used in this research was Complete Random Design (CRD) with four experiments of feed, i.e. commercial feed without cod fish liver oil (R-0) as control, commercial feed + 1,5% cod fish liver oil (R-1), commercial feed + cod fish liver oil 3% (R-2), commercial feed + cod fish liver oil 4,5% (R-3). Each experiment included eight rabbits and feed experiment was given starting by 13 weeks to 26 weeks years old. The variable that observed was free testosterone level and sperm quality of local rabbit. The data that was obtained to be analyzed with One Way ANOVA and if its contrast was done more test with Duncan's Multiple Range Test (DMRT). The result of this research was to show that supplementation of cod fish liver oil in the commercial feed was to show the result that a real distinction of ($P < 0,05$) towards free testosterone level and sperm quality of local rabbit.

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1. Introduction

The effort is to increase reproduction efficiency rabbit cattle so that needed a breakthrough reproduction technology. There was one real effort the Indonesian government was done by artificial insemination (AI) program. Especially to the rabbit cattle, AI technology has been benefited in limited access to one area of study. In order to enjoy the business of rabbit breeder, therefore, the utilization of AI technology should be shared with the society.

In AI center, the male function is as semen producer. The male that its semen collected for AI need is a healthy male, superior, and mastering a good reproduction perform. A good reproduction perform is able to evaluate with reference to cattle physically, sexual behavior and semen analysis. The semen that consists of well sperm quality was obtained by the male that physical health with their performance and maximum productivity. There is one of a factor in order to cattle becomes health is nutrition factor. Therefore, the improvement of male reproduction perform is needed by IA and is able to approach with intake refinement of feed nutrition.

Intake refinement of feed nutrition is able to be reached by showing the factor of feed given, included by showing quantity and commercial feed quality. Sometimes, commercial feed that is given to cattle in bad condition towards unsaturated fatty acids. [Tanuwiria *et al.*, \(2011\)](#) stated that insufficient supply acid unsaturated mainly essential acid was caused by low of cattle reproduction perform. In order to complete or increasing the availability of essential acid frequently the feed contents less than cattle needed, so that in commercial feed can be added fish oil. One of fish liver production that famous in the market is cod fish liver oil.

Codfish liver oil is a *Polyunsaturated Fatty Acid* (PUFA) source. In nutrition view, PUFA is not able to be synthesized by the body, therefore, it is a fatty acid. Essential fatty acid is a very needed for the physiological process in cattle body, then, n-3 PUFA (α -linolenic acid) i.e. DHA (*docosahexaenoic acid*) and EPA (*eicosapentaenoic acid*) is available needed if feed ([Wathes *et al.*, 2007](#)). There were some previously that was conducted to evaluate the impact of n-3 PUFA in male reproduction, there was a study towards of pig ([Maldjian *et al.*, 2005](#)), turkey ([Zaniboni *et al.*, 2006](#)) and rabbit ([Gliozzi *et al.*, 2009](#)).

In one time, commonly in AI technology application, the sperm that benefitted is to ejaculation result that accommodated by the artificial vagina. Another alternative that has benefitted as a sperm source, i.e. sperm of epididymis cauda. The sperm that leaves testis before doing a move, as well as dysfunctional (infertile). The most of development process occurs on epididymis ([Guyton, 1986](#)). Epididymis cauda is a place storing sperm before ejaculated and sperm that therein epididymis cauda is well sperm due to experience in gate and corpus field ([Toelihere, 1983](#)). The sperm maturation in epididymis depends on testosterone level ([Hafez, 1987](#)).

Testosterone is to the main hormone toward a male. Biologically, testosterone has effect in order to spur a growth and development next functional activity of male sex accessories organs, in order to look after the clue of secondary sexuality, the male sex behavior, to spur penis growth and scrotum, then structure maintain and commonly function of male reproduction system ([Junqueira *et al.*, 2004](#)). On another hand, testosterone as well role in spermatogenic cells development of testis seminiferous tubules, look after a gametogenesis and to spur spermatogenesis occurred ([Hadley, 1992](#)). The low testosterone level is able to decrease libido, a weak erection and fail to have descent.

In term of observation, therefore, the research at conducting is intended to know free testosterone level and sperm quality of local rabbit that is given commercial feed supplemented by cod fish liver oil. It is a hope to consume it as PUFA is able to be produced by a male rabbit that has a better reproduction perform. Genetically, choosing a superior male become very important to AI technology utilization.

2. Materials and Methods

Time and Research Location

This research was conducted from January to June 2015. It was done at Math Faculty and Natural Science in Udayana University Denpasar, Bali, Indonesia.

Livestock

The cattle that used was 32 local male rabbit immature, the weight was 1035,15 g on 12 weeks old, the rabbit was taken from seedling at Desa Riang Gede Tabanan, Bali, Indonesia. The experiment procedure which uses the rabbit as experiment animal has established *Ethical Clearance* by Ethics Commission the Animal Use in Research and Education, Faculty of Veterinary, Udayana University, Denpasar, Bali, Indonesia.

Feed

The feed that used is a pellet of rabbit commercial feed that is produced by P.T. Japfa Comfeed Indonesia. The commercial feed material is yellow corn, bran, soybean tuber, molasses and palm oil. Next, to commercial feed is supplemented cod fish liver oil of 1,5% (R-1), 3% (R-2) dan 4,5% (R-3) and commercial feed is not supplemented by cod fish liver oil as control (R-0). The experiment is done for three months.

Free Testosterone Level

Determining the content of testosterone hormone is done by Elisa method within using *Rabbit Free Testosterone Elisa Kit*. Determining procedure of testosterone content is done in accordance with guiding that there is in it, the procedure is as follows: into standard well plate is added 50 μL of *biotinylated detection ab*, is incubated on 37⁰C for 45 minutes, is washed for three times with *washing buffer*, and is added 100 μL HRP conjugate to each well, then is incubated towards 37⁰C for 30 minutes, is washed for five times with *washing buffer*, is added 90 μL substrate reagent, is incubated to the 37⁰C for 15 minutes, is added 50 μL stopping solution. The last step is done absorbance reading with a spectrophotometer at a wavelength of 450 nm. FT rate is in units of ng/mL.

Sperm Quality

The observation of sperm quality is done by making a sperm suspension of epididymis cauda. The right epididymis cauda is included into petri dish that consists of 1,0 mL saline 0,9%, preheated on 37-40⁰C then is cut with small scissors until delicate and is stirred with stirrer glass, therefore, it is obtained sperm suspension that homogeny. Sperm suspension that is obtained, is able to be used for analyzing sperm quality.

Sperm quality that is observed is motility, viability, and sperm normal morphology. The observation of sperm motility is done by adding a motile sperm up divided the amount of non-motile sperm. Sperm viability is done by smear method with eosin coloring 0,5%. Sperm morphology is done through smear method with eosin coloring 1% and 10% nigrosine. The quality observation is conducted under the microscope with 400X magnifications and the calculation is stated in percentage.

Data Analysis

The data that was obtained was to be analyzed statistically with *Analysis of Variance (ANOVA)* through using *One Way ANOVA* of Costas program. If, the data that was obtained real distinction so that will be continued by *Duncan's Multiple Range Test (DMRT)* toward 5% believe rate.

3. Results and Discussions

3.1 Free Testosterone (FT) Level

The result of statistical analysis a level means of free testosterone and sperm quality of rabbit local that is given commercial feed supplemented of cod fish liver oil, can be shown to table 1.

Table 1

The result of mean statistical analysis FT level and sperm quality of male rabbit local that is given commercial feed supplemented of cod fish liver oil

Variable	Treatment			
	R-0	R-1	R-2	R-3
FT Level (ng/mL)	0,75 ^a	0,77 ^a	0,87 ^b	1,03 ^c
Sperm Motility (%)	70,30 ^a	71,28 ^a	73,26 ^b	78,43 ^c
Sperm Viability (%)	84,10 ^a	84,32 ^a	87,15 ^b	93,27 ^c
Sperm Normal Morphology (%)	77,18 ^a	77,62 ^a	80,10 ^b	85,36 ^c

Descriptions: *One Way ANOVA* test is forwarded by *DMRT* test ($P < 0,05$). The number that is followed by a different letter with reference to the same line is to show a real distinction.

R-0 = commercial feed without cod fish liver oil

R-1 = commercial feed + cod fish liver oil 1,5%

R-2 = commercial feed + cod fish liver oil 3%

R-3 = commercial feed + cod fish liver oil 4,5%

Regarding the observation result that is listed in Table 1.1, is to show that in order to a mean of FT level of local male rabbit that is given commercial feed supplemented cod fish liver oil is to show a result which has the real distinction ($P < 0,05$) within controlling rabbit. The advance test as well as to show a real distinction result ($P < 0,05$) towards feed treatment. The rabbit that gets feed treatment R-1 is not a real distinction within rabbit R-0 (control), whereas the rabbit that gets feed treatment R-2 and R-3 has real distinction within R-0 (control). The highest mean of FT level is found in R-3 rabbit i.e. 1,03 ng/mL, is followed by R-2 rabbit i.e. 0,87 ng/mL and R-1 rabbit i.e. 0,77 ng/mL. The mean of FT level to the R-1, R-2, R-3 each rabbit is 2,60%, 13,79% and 27,18% higher than compared with R-0 rabbit (control).

The local of male rabbit sperm of motility percentage means that is given commercial feed supplemented of cod fish liver oil is to show the real distinction result ($P < 0,05$) with control rabbit (table 1.1). The advance test as well as to show the real distinction result of ($P < 0,05$), among R-0 with R-2 and R-3, among R-1 with R-2 and R-3, between R-2 with R-3, whereas between R-0 with R-1 are no real distinction (table 5.6). The rabbit that gets R-3 feed treatment is to produce the highest sperm motility of percentage mean i.e. 78,43%, is follow by R-2 rabbit i.e. 73,26% and R-1 rabbit i.e. 71,28%. The percentage mean of sperm motility to the R-1, R-2, R-3 each rabbit are 1,37%, 4,04%, and 10,36% higher than compared by R-0 rabbit (control).

Viability percentage mean of local male rabbit sperm that is given commercial feed supplemented by cod fish liver oil is to show the real distinction result of ($P < 0,05$) with control rabbit (table 1.1). The advance test as well as to show the real distinction result of ($P < 0,05$) towards feed treatment. Sperm normality percentage mean towards R-0 rabbit (control) is 77,18% meanwhile the percentage mean of morphological normality to the rabbit of R-1, R-2, and R-3 is higher i.e. 77,62%, 80,10%, and 85,36%. The sperm normality means toward rabbit of R-1, R-2, R-3 each of them is 0,57%, 3,64%, and 9,58% higher than compared with R-0 rabbit (control).

The testosterone secretion depends on pituitary LH secretion that there in the influence of hypothalamus GnRH (Guyton, 1986). Meanwhile, PUFA n-3 supplementation of fish oil is directly influential towards hypothalamus-hipofisis-testis (Ahluwalia *et al.*, 1966; Adibmoradi *et al.*, 2012). GnRH that is secreted by hypothalamus FSH and LH stimulated emission from lipolysis gland, its turn to be testosterone secreted by Leydig cell (Hadley, 1992). It is to show that steroidogenesis is produced by male rabbit depends on GnRH, FSH, LH, and testosterone. Steroidogenesis is influenced by PUFA because PUFA has an important role in arranging steroid synthesis (Wathes *et al.*, 2007) and as well as arranging reproduction endocrinology (Wang *et al.*, 2003).

Ahluwalia *et al.*, (1966) stated that degenerative changing occurs to the basofil cell from lipolysis grand toward rabbit that experiences fatty acid deficiency, it is caused by less of testosterone secretion. The result of this research is supported by (Yan, *et al.*, 2013) that stated occur increasing level of GnRH, LH, FSH and rat testosterone that is given essential fatty acid supplemented with PUFA n-3 dan n-6 ratio in balance. Esmaeili *et al.* (2014) stated fish oil is supplemented to the sheep produces the highest total of testosterone level be compared with palm oil supplementation and sunflower oil. In this research, the highest FT level belongs to R-3 rabbit i.e. 1,03 ng/mL. Sanni *et al.*, (2012) reported that the rabbit level of testosterone total is NZW of 7,17 ng/mL.

Sperm motility is an ability of sperm motility for forwarding to in front. The increasing of percentage occurred motility sperm and decreasing of no motility sperm is caused by increasing of testosterone level. Hafez (1987) stated glycolysis occurred in the center of sperm due to consist of mitochondrial as energy source i.e. ATP. Oxidative of metabolism towards sperm with testosterone substrate is able to support a motility. Thus, if testosterone level increase, there is the possibility of the glycolysis process increase that causes increasing of sperm energy, therefore, finally increasing sperm motility is occurred. Insides, DHA as well can increase sperm motility through increasing of membrane fluidity. The higher it is PUFA toward sperm membrane so that causes increasing of membrane fluidity (Connor *et al.*, 1998).

R-3 rabbit is to produce the highest of sperm motility mean i.e. 78,43% whereas R-3 rabbit of non-motility sperm means is 21,57%. In term of this, it is supported by the research that has been done by Castellini *et al.* (2005) which fish oil is supplemented to the 2% degrees are able to increase rabbit sperm motility up to 8,75%. Maldjian *et al.*, (2015) the fish oil is supplemented on 3% degrees of feed is able to increase pig sperm motility. Surai *et al.*, (2004) stated that PUFA intake is on the feed is able to increase sperm motility toward broilers. Samadian *et al.* (2000) the feed that is complicated with fish oil is able to increase a progressive sperm motility to the sheep. The results research is to report that rabbit sperm motility has variations, including the result is obtained 65,2% (El-Kholy *et al.*, 2008), 74,50% (Ogbuewu *et al.*, 2009), 50,00%-60,00%, 68,00%. (Ahemen *et al.*, (2013).

In one hand, sperm viability is an ability of sperm life motility. It is occurred increasing of viable percentages and decreasing of non-viable are caused by increasing of testosterone level. Partodihardjo (1992) stated that testosterone is needed for the vitality of spermatozoa in the epididymis. Epididymis has a function as a container of sperm production and testicular fluid. Furthermore, according to Partodihardjo (1992) epididymis as well is a warehouse of sperm because it is a place sperm hoarding. So that, it should ensure that is hoarding sperm has to keep a life and testosterone role is important because it is able to go on sperm life into the epididymis. On the other hand, sperm viability is most determined by the purity of the sperm membrane and the ability of the membrane transport system.

The highest variable of sperm means is produced by R-3 rabbit i.e. 93,27% whereas non-variable sperm means is 6,75%. Dolatpanah *et al.*, (2008) reported supplementation of fish oil towards 2,5% level is able to increase goat sperm viability. Zaniboni *et al.*, (2006) stated the fish oil supplementation of 2% level that was combined with E vitamin was able to increase sperm viability to the gobbler. Ahemen *et al.*, (2013) were to obtain the viability percentage of rabbit sperm was 84,00 %.

As well as, sperm morphology is a variable that is important to value the fertility of fertilizing power due to if sperm abnormality amount is too high so that the fertility will be decreased. According to Laing (1979), sperm abnormality occurred could be caused by sperm growing interferences in seminiferous and interferences of sperm maturation into the epididymis. Sperm morphology as well as related to sperm motility because sperm that has abnormal morphology will cause sperm motility interferences.

The highest normal morphology means is produced by rabbit R-3 about 85,36% whereas its abnormality means is 14,64%. Dolatpanah *et al.*, (2008) stated fish oil supplementation towards 2,5% level was able to increase normal morphology of goat sperm. DHA has been proved correlated significantly within increasing of sperm percentage with normal morphology and in decreasing of sperm percentage abnormal morphology.

Olubowale *et al.*, (2014) reported fish oil supplementation to the 3% level were able to increase chicken sperm normal morphology. The research results have been reported that there was a variation to the rabbit sperm normal morphology, included it was obtained 80,50% (Olugbenga and Samuel, 2007), 77,2% (El-Kholy *et al.*, 2008), 90,4%, (Ahemen *et al.*, 2013) and 81,8%, (Campos *et al.*, 2014).

In regards the sperm characteristics above, it can be stated that the sperm of local male rabbit that is given a commercial feed of cod fish liver oil supplementation mastering a good of quantity and sperm quality and in fulfilling requirements to be an advanced processed of IB needs. In term of this, it is in accordance with the minimum requirement of sperm quality that is decent used in IB program i.e. mastering at least 40%, 50% viability sperm, and 80% normal sperm (Toelihere, 1993). Meanwhile, Marco-Jiménez *et al.* (2010) stated that cement that was able to be used for IB mastering more than 70% motility sperm and less than sperm that was abnormal.

4. Conclusion

The cod fish liver oil supplementation towards 4,5% in the commercial feed is able to increase free testosterone level of local rabbit and was able to increase sperm quality of local rabbit epididymis cauda, i.e. motility can be increased, viability, therefore, in increasing a sperm with normally morphological.

Conflict of interest statement and funding sources

The authors declared that they have no competing interest. The study was financed by personal funding.

Statement of authorship

The authors have a responsibility for the conception and design of the study. The authors have approved the final article.

Acknowledgments

My deep and sincere gratitude were presented to God for having granted me the ability and the opportunity to complete this paper. I would also like to thank my friends for their support, their patience, their contribution, and their valuable input so that this paper could be completed. I would also thank Dr. A. Heidari, Ph.D., D.Sc. as editor of IJCU who has reviewed and approved this study to be published.

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