Effect of Cod Liver Oil Supplementation in Feed on the Haematological Values and Concentration of Epididymis Spermatozoa of Local Rabbits

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Abstract. This the study aimed to determine the effect of cod liver oil supplementation in commercial feed on the haematological values and concentration of epididymis spermatozoa of local rabbits. The experimental design used was Completely Randomized Design with four feed treatments, i.e. commercial feed without supplementation of cod liver oil (P0) as control, commercial feed supplemented by 1.5% (R1), 3% (R2), and 4.5% (R3) of cod liver oil. Each trial consisted of eight rabbits and feed experiment was given starting by 13 weeks to 26 weeks of years old. The variables measured were the count of red blood cell (RBC), white blood cell (WBC), platelets, haemoglobin (Hb), packed cell volume (PCV), and concentration of epididymis spermatozoa of local rabbits. The results showed that supplementation of cod liver oil with a different grade in feed commercial had a significant effect (P<0.05) on the haematological values and concentration of epididymis spermatozoa of local rabbits. It can be concluded that cod liver oil supplementation in commercial feed at 4.5% level capable maintain normal haematological values and can increase the concentration of epididymis spermatozoa of local rabbits.

1. Introduction

Reproductive biotechnology encompasses powerful tools to enhance the efficiency and profitability of livestock reproduction, production, and product quality. These technologies include genomic selection, evaluation of semen (from bulls, bucks, and rams) using advanced cell and molecular technologies, semen cryopreservation and artificial insemination (AI) estrus synchronization, superovulation of the females (cows, dams, and ewes), ovum pick-up, in vitro fertilization and embryo culture, embryo transfer, and pregnancy detection [1]. In Indonesia, especially in Bali, raising rabbits is something new and have not been common since most major livestock are reared in Bali is pork, beef, and chicken. In an effort to increase the efficiency of livestock reproduction of rabbits, we need a breakthrough in reproductive technologies, among which is the AI. Especially for rabbits, AI technology has not been popular in the community and even for local rabbit breeders Bali has never been done.

The efficiency of AI (fertility rate and prolificacy) is directly dependent on the quality of semen doses and on the number of spermatozoa used for insemination [2]. Various laboratory methods techniques are used to evaluate sperm quality, such as sperm concentration, motility, viability, and morphology. However, there is no single semen assay that provides complete information about semen quality [3,4,5]. Sperm concentration is an important parameter affecting fertility. Animal species of agricultural interest are mainly produced by AI which contributes highly to the development of...
worldwide swine production, making the impact of the male in reproductive efficiency of the pig herds more crucial [6]. Until now, the spermatozoa collected in the application of AI technology were ejaculated spermatozoa that were accommodated with artificial vagina. Another alternative that can be used as a source of spermatozoa is the spermatozoa collected from the caudal epididymis.

Sperm production is affected by many environmental factors (temperature, light, age, health status and feeding strategies) [7]. Feed is an important aspect of livestock production. The importance of feed supplementation in animal production has increased in the last few years [8]. Blood pictures of animals might be influenced by certain factors, one of which is nutrition [9], nutrition affects blood values of animals [10,11], haematological values of farm animals are influenced by nutritional status [12]. Processing of feed could have effect on haematological parameters of farm animals [13]. Dietary content affect the blood profile of healthy animals [14,15,16,17]. Haematological components such as red blood cells, white blood cells or leucocytes, Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentration are valuable in monitoring feed toxicity, especially, with feed constituents that affect the blood as well as the health status of farm animals [18].

Fish oil is a source of polyunsaturated fatty acids (PUFAs) such as the EPA and DHA. PUFAs affect the male in reproductive efficiency of the pig herds more crucial [6]. Until now, the spermatozoa collected in the application of AI technology were ejaculated spermatozoa that were accommodated with artificial vagina. Another alternative that can be used as a source of spermatozoa is the spermatozoa collected from the caudal epididymis.

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Fish oil is a source of polyunsaturated fatty acids (PUFAs) such as the EPA and DHA. PUFAs cannot be synthesized by the body so it is an essential fatty acid [19]. Since 1970, regular consumption of fish has been approved to have a positive effect on heart health [20]. The content of EPA and DHA in fish oil is a good nutrient for health. Some research results have reported the benefits of fish oil, cod liver oil supplementation up to 4.5% could increase testosterone levels and the quality (motility, viability, and morphology) of caustive spermatozoa of local rabbits epididymis [21], fish oil diet positively affect testes developments and spermatogenesis in the goat [22], DHA and EPA in fish oil have anti-inflammatory effects and reduce thrombotic processes [23].

Based on the above, a paper is made which contains an evaluation of procedures to be used in applied reproduction trials performed with rabbits. This study aimed to determine the effect of cod liver oil supplementation in commercial feed on the haematological values and concentration of epididymis spermatozoa of local rabbits.

2. Materials and Methods

2.1. Time and Research Location
The research was conducted from January to June 2015. The study was conducted at the Faculty of Mathematics and Natural Sciences, Udayana University Bali, Indonesia.

2.2. Livestock
The animal used is 32 male local rabbits aged 12 weeks with an average body weight of 1035.34 to 10.35.41 g. They were kept under a controlled light-darkness cycle (12 h light; 12 h darkness). Air temperature in the cage was 27.05°C and the air humidity in the enclosure was 75.40%. The experimental procedure using rabbits as experimental animals has been declared Eligible Ethics (Ethical Clearance) by the Ethics Committee Animal Use in Research and Education, Faculty of Veterinary Medicine Udayana University Bali, Indonesia.

2.3. Administration of feed
Feed is given in ad libitum. The feed used is commercial feed pellets for rabbits. Commercial feed constituents are yellow corn, bran, soybean meal, molasses and palm oil. Furthermore, commercial feed supplemented with cod liver oil level of 1.5% (R1), 3% (R2) and 4.5% (R3) and commercial feed without supplemented with cod liver oil as a control (R0). The experiment lasted three months, from rabbits aged 13 weeks to 26 weeks of age.

2.4. Collection of Blood
Blood is collected from the auricular vein with a syringe and then blood is poured into a glass tube for analysis. The count of RBC, WBC, and platelets do with the type of improved Neubauer haemocytometer method. Haemoglobin levels were calculated using the acid haematin-Sahli
haemoglobin and its value expressed in g/dl. PCV are determined using the microhematocrit and its value is expressed in % [24].

2.5. Collection of Spermatozoa
Spermatozoa collected from caudal epididymis. After the rabbits collected their blood, the rabbits were sacrificed and dissected. Caudal epididymis was put into a petri dish containing 1.0 mL of physiological solution 0.9% at 37°C. Caudal epididymis is cut into pieces so that it forms a homogeneous suspension of spermatozoa. The spermatozoa suspension was analysed to measure the concentration of spermatozoa. Measurement of spermatozoa concentration was carried out by the Improved Neubauer haemocytometer method [25].

2.6. Data Analysis
The data obtained were statistically analysed by one-way analysis of variance (ANOVA). If the data obtained significantly different it will be followed by Duncan's Multiple Range Test (DMRT) at the level of 5%.

3. Results and Discussions

3.1. Haematological Values
Results of statistical analysis of the haematological values and spermatozoa concentration of local rabbits fed commercial feed with cod liver oil supplementation can be seen in Table 1.

| Variable                        | Treatment |
|---------------------------------|-----------|
|                                 | R0        | R1        | R2        | R3        |
| RBC count (10⁶/µl)              | 5.80a     | 5.80a     | 5.81a     | 5.84b     |
| WBC count (10³/µl)              | 9.20a     | 9.20a     | 9.19a     | 9.15b     |
| Platelet count (10⁹/µl)         | 630.00a   | 629.63a   | 629.00a   | 625.00b   |
| Haemoglobin level (g/dl)        | 10.55a    | 10.55a    | 10.56a    | 10.60b    |
| PCV (%)                         | 32.37a    | 32.37a    | 32.38a    | 32.46b    |
| Spermatozoa concentration (10⁶/ml) | 310.16a  | 310.41a   | 320.37b   | 340.33c   |

The values followed by different letters in the same row show significantly different results (P<0.05). R0=0% (control), R1=1.5%, R2=3%, R3=4.5%.

The average haematological values (count of RBC, WBC, platelets, PCV and haemoglobin levels) of local rabbits given commercial feed supplemented with cod liver oil showed significantly different results (P<0.05) with control rabbits. Further tests also showed significantly different results (P<0.05) between feed treatments (Table 1). The R3 group produced the highest average RBC count of 5.84x10⁶/µl, followed by R2 group of 5.81x10⁶/µl and R1 group of 5.80x10⁶/µl. The average count of RBC in the R0 group was 0.17% lower than in the R2 group and 0.68% lower with the R3 group. The R3 group produced the lowest average WBC count of 9.15x10³/µl, followed by the R2 group of 9.19x10³/µl and R1 group of 9.20x10³/µl. The average WBC count in group R0 was 0.11% higher compared to group R2 and 0.54% higher with group R3. The R0 group produced the highest average platelet count of 630x10⁹/µl and the lowest was in the R3 group of 625x10⁹/µl, followed by the R2 group of 629x10⁹/µl and R1 which was 629.63x10⁹/µl. R3 is 0.06%, 0.16% and 0.80% lower than the R0 group (control), respectively. The R0 and R1 groups produced the lowest average haemoglobin level of 10.55 g/dl while the average haemoglobin level in the R2 and R3 groups was higher at 10.56 g/dl and 10.60 g/dl. The average haemoglobin level in the R0 group was 0.09% lower than the R2 group and lower 0.47% with the R3 group. The average PCV in the R0 group (control) was 32.37% while the average PCV in the R1, R2, and R3 groups were 32.37%, 32.38% and 32.46%, respectively. The average PCV in the R0 group was 0.03% lower than the R2 group and 0.28% lower with the R3 group.

Aspects of the red blood cell function that affect haemoglobin function (transport and exchange of respiratory gases in the blood: haemoglobin) are particularly important from the respiratory [26].
PUFA can increase the deformity of erythrocyte membranes so that it can easily carry nutrients and oxygen throughout the body [27]. Cod liver oil supplementation in commercial feed can increase the RBC count of rabbits even though the increase is still within the normal range. The increase in the count of RBC evident in R3 rabbits may be caused by an increase in oxygen use by R3 rabbits so that the release of erythropoietin which stimulates erythropoiesis. Reference in [28] stated that a diet containing 8% cod liver oil can increase the count of RBC in *Catla catla* fingerlings. In this study, the mean count of RBC in the control and treatment of rabbits ranged between 5.80-5.84x10^9/ml. The results of this study, it is still within the normal range is 4.0 to 7x10^9/ml [29,30], 4.0-6.7x10^9/ml [31], 4.08-6.96x10^9/ml [32], 4.00-8.60x10^9/mm^3 [33], and 5.0-8.0x10^9/mm^3 [34].

White blood cells (WBC) play a significant role in the immune system by protecting the body from infectious disease and foreign invaders [35]. Cod liver oil supplementation in commercial feed can reduce the count of rabbit WBC even though the decline is still within the normal range. Although there is a marked decrease in WBC count in P3 rabbits, it may be due to the ability of DHA and EPA to reduce plasma MPO (myeloperoxidase). Reference in [36] stated that the decrease in MPO showed a reduction in leukocytes, especially PMN activation. Ref. [37] stated that the high WBC number was related to the extent of infarction that occurred, impaired left ventricular function and death after acute myocardial infarction. The inflammatory or inflammatory process that occurs in acute myocardial infarction is often characterized by peripheral leukocytosis and relatively often is neutrophils. Reference in [38] states that n-3 PUFA functions as an anti-inflammatory, by inhibiting the action of inflammatory cytokines. In this study, the mean number of leukocytes in control rabbits and feed treatment ranged from 9.15-9.20x10^9/µl. The results of this study are still in the normal range of 5.43x10^9/µl [28], 5-12x10^9/µl [29], 5.2-12x10^9/µl [30], 3.0-12.5x10^9/mm^3 [34], and 5-13x10^9/µl [39].

Platelets play an important role in the vessel and primarily function as regulators of hemostasis and thrombosis. Following vascular insult or injury, platelets become activated in the blood resulting in adhesion to the exposed extracellular matrix underlying the endothelium, formation of a platelet plug, and finaly formation and consolidation of a thrombus consisting of both a core and shell [40]. Cod liver oil supplementation in commercial rations can reduce rabbit platelet counts even though the decline is still within the normal range. A marked decrease in platelet counts in P3 rabbits may be caused by DHA and EPA is an antithrombotic and anti-aggregation agent. According to reference in [41] as an antithrombotic and anti-aggregation agent, DHA was able to compete with AA, so as to increase AA by reducing TXA2 production. The decrease in TXA2 results in a decrease in platelet aggregation. In this study, the average platelet count in control rabbits and feed treatment ranged from 525-530x10^9/µl. The results of this study are still within the normal range of 250-600x10^9/µl [29] and 353-821x10^9/µl [32].

An increase in the count of RBC will be followed by an increase in haemoglobin levels and an increase in PCV. Rabbit P3 produces the highest haemoglobin level and PCV. This shows that rabbit P3 requires additional blood capacity to carry oxygen to meet the needs of greater muscle mass because rabbit P3 produces the highest growth rate. In this study, the mean haemoglobin level in control rabbits and feed treatment ranged from 10.55-10.60 g/dl. The results of this study are still in the normal range of 9.6-14.6 g/dl [42], 9.5-13.7 g/dl [43], 9.3-19.3 g/dl [33], 8.0-17.0 g/dl [34], and 13.2-13.4 g/dl [43]. In this study the average haematocrit value in control rabbits and feed treatment ranged from 32.37-32.46%. The results of this study are still within the normal range of 29.7-45.8% [42], 30-35% [33], 31.0-51.0% [34], and 40.0-40.2% [43]. Normal range of values for haemoglobin indicated that the vital physiological relationship of haemoglobin with oxygen in the transport of gases (oxygen and carbon dioxide) to and from the tissues of the body has been maintained and was normal [44]. Reference in [45] reported that haematological traits especially Packed Cell Volume (PCV) and Haemoglobin (Hb) were correlated with the nutritional status of the animal. According to reference in [18] Packed Cell Volume is involved in the transport of oxygen and absorbed nutrients.

3.2. Spermatozoa Concentration

The average spermatozoa concentration of local rabbits given commercial feed supplemented with cod liver oil showed significantly different results (P<0.05) with control rabbits. Further tests also showed significantly different results (P<0.05) between feed treatments (Table 1). Group R1 was not significantly different from group R0 (control), while group R2 and R3 were significantly different from
group R0 (control). This shows that at the level of 3% it can increase and at the level of 4.5% increase the concentration of rabbit spermatozoa. The R3 group produced the highest average spermatozoa concentration of 340.33x10⁶/ml, followed by the R2 group that was 320.37x10⁶/ml and the R1 group was 310.41x10⁶/ml. The average concentration of spermatozoa in groups R1, R2, R3 were 0.08%, 3.19% and 8.86% higher than that of group R0 (control).

High concentrations of spermatozoa indicate a high level of fertility and are useful in supporting more rabbits AI programs [46]. The increased concentration of spermatozoa is probably caused by increased activity in the seminiferous tubules and epididymis. Increased activity in the epididymis and seminiferous tubules is controlled by testosterone through the hypothalamic-pituitary axis. Reference in [47, 22] stated that supplementation of n-3 PUFA in fish oil had a direct effect on the hypothalamic-pituitary-testicular axis. The results of this study that support this statement include fish oil supplementation at the level of 2% [48] and 1.5% [49] can increase the concentration of rabbit spermatozoa. Reference in [50] stated that 5% tuna fish supplementation in feed can increase the concentration of broiler chicken spermatozoa. In this study, R3 rabbits produced the highest average spermatozoa concentration of 340.33x10⁶/ml. The results of the study reported that the concentration of rabbit spermatozoa varied. The range of spermatozoa concentrations of rabbits is 150-500x10⁶/ml [51,52], 278x10⁶/ml [53]. Different types of rabbits, the concentration of spermatozoa produced was also different, including Rex rabbits 415.10x10⁶/ml, NZW 416.72x10⁶/ml, California 454x10⁶/ml and Baldi Red 423x10⁶/ml [54, 55]. This difference is caused by several factors, namely feed, frequency of sampling, age, ejaculation sequence and ambient temperature [56].

4. Conclusion
Supplementation with cod liver oil to the extent of 4.5% in commercial feed able to maintain normal haematological values and can increase the concentration of caudal epididymis spermatozoa of local rabbits.

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