Estrogen Level in Female Local Rabbit Fed Commercial Cod Liver Oil Supplementation

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Abstract. This study aimed to determine the estrogen level in female local rabbit after being fed commercial supplementation by cod liver oil. 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 3% (P1), 4.5% (P2), and 6% (P3) of cod liver oil. Each treatment consisted of ten rabbits as replication and treatment was given to rabbits from aged 4 to 6 months. The parameters observed were the estrogen level. The results showed that supplementation of cod liver oil with different grade on commercial feed had significant effect (P<0.05) to rabbit estrogen level. It can be concluded that supplementation of cod liver oil on commercial feed up to 4.5% can increase the estrogen level of female local rabbit.

1. Introduction
Nutrition is one of the environmental factors that influence hormonal profile and consequently, reproductive and productive performance of animals [1-3]. Fish oil is a source of long chain polyunsaturated fatty acids (PUFA). PUFA can not be synthesized by the body so it is an essential fatty acid from the point of nutrition. PUFA is indispensable for various physiological processes within the animal body so that n-3 α-linolenic acid PUFAs such as eicosapentanoic acid (EPA) and docosahexenoic acid (DHA) need to be provided in feed [4]. PUFAs are one of fatty acid that have two or more double bonds and exist as cis- or trans-isoform following by structure of double bonds. The follicles, oocytes, and spermatozoa contain large amount of PUFAs [5]. Fatty acid play an important role in the regulation of normal reproductive function [6].

Many studies that are involved with effect of fish oil by die-tary on male and female reproductive were reported. Cod liver oil supplementation up to 4.5% could increase testosterone levels and the quality of causative spermatooza of local rabbit epididymis [7]. Fish oil diet positively affect testes developments and spermatogenesis in the goat [8]. In cows fed a diet supplemented with LNA (n-3), there was an increase in oestradiol during the follicular phase [9]. Supplementation of n-3 PUFA rich fish oil significantly increased the number of preovulatory follicles and ovulation rate in goat [10]. Dietary PUFAs are known to mediate a broad range of actions in reproductive tissues including effect on membrane fluidity, intra-cellular cell-signalling cascades and susceptibility to oxidative injury [4]. Change in the composition of dietary fatty acids not only modifies fatty acid composition in the blood plasma but also of the reproductive tissues including, follicular fluid, cumulus cells and the oocytes [11-
which can directly influence the competence of oocytes for further development and/or fertility [15,16]. The reproductive function will occur after cattle experience puberty which is regulated by the endocrine glands and the hormones produced. Hormones that play a role in reproducing females include GnRH, FSH, LH, estrogen and progesterone. Estrogen is a hormone that causes estrus in female animals. The low estrogen hormone can cause no estrus symptoms so there is no occurrence of fertilization. The absence of fertility results in a decrease in livestock productivity. Based on that, then conducted research that aims to determine estrogen level in female local rabbit after being fed commercial supplementation by cod liver oil.

2. Materials and Methods

2.1. Animal
The animal used is 40 female local rabbits aged 4 months with an average body weight of 2000.60 to 2100.15 g. Rabbits were obtained from Riang Gede Village, Tabanan Regency, Bali. Animals were kept in enclosures of individual battery systems. 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%.

2.2. Administration of feed
Forty mature female local rabbits divided into four feeding treatments, i.e. commercial feed without supplementation by cod liver oil (P0) as control, commercial feed supplemented by 3% (P1), 4.5% (P2), and 6% (P3) of cod liver oil. Each treatment consisted of ten rabbits as replication and the treatment was given to rabbit from aged 4 to 6 months.

2.3. Collection of blood samples
After administration of feed then blood sample collected by auricularia vein into PET vacuum tube without additives. The sample was centrifuged at 1000 rpm for 10 min and the supernatant decanted.

2.4. Hormone analysis
The hormones were estimated using the standard protocols of Rabbit E ELISA kits. E ELISA kits is obtained Gamma Scientific Biolab, Malang, Indonesia.

2.5. Data Analysis
The data obtained was to analyse statistically using one-way ANOVA. If, the data that was obtained real distinction so that will be continued by Duncan’s Multiple Range Test (DMRT) toward 5% significant level.

3. Result and discussion
The results of statistical analysis of estrogen level of female local rabbits after being fed commercial supplementation by cod liver oil can be seen in Table 1.

Table 1. Estrogen level in female local rabbits after being fed commercial supplementation by cod liver oil.

| Parameter                  | Treatment | P0    | P1    | P2    | P3    |
|----------------------------|-----------|-------|-------|-------|-------|
| Serum level of estrogen (ng/mL) |           | 10.30a| 10.32a| 12.87b| 12.85b|

The values followed by different letters in the same column show significantly different results (P<0.05). P0=0% (control), P1=3%, P2=4.5%, P3=6%.
Based on the observations listed in Table 1, it appears that local rabbits fed commercial supplementation of cod liver oil showed significantly different results (P<0.05) in the mean of estrogen level. Further tests also showed significantly different results (P<0.05) between feed treatments. The rabbits treated with P1 were not significantly different from the group P0 (control), the rabbits treated with P2 and P3 were significantly different from the group P0 (control) while the group P2 and P3 were not significantly different. The highest estrogen level are found in group P2. This indicated that the supplementation of cod liver oil on P2 treatment is 4.5% level most influential to the increase of estrogen level.

Estrogen are a group of steroid compounds, named for their importance in the estrous cycle and functioning as the primary female sex hormone. It is also a main reproductive hormone affecting growth, development, maturation and functioning of reproductive tract as well as the sexual differentiation and the behavior [17]. Animal body naturally produce three main forms of estrogen, which are estradiol17β (E2), Estrone (E1) and Estriol (E3). Estrone and estriol were firstly identified in the urine of pregnant women and this was followed by the identification of E2 in the follicular fluid of sow by Edward Adelbert Doisy between 1929-1936 [18]. In the ovary, E2 is the most physiologically active type of estrogen produced by granulosa cells of pre-ovulatory follicles through the aromatization of thecal androgen by the granulose cells of growing follicles. Estrogen also acts as an intra-gonadal factor and has negative and positive feedback influences on the hypothalamic-pituitary axis to regulate gonadotrophin secretion. It has been known for many years that estrogen has a direct influence on folliculo genesis. Oestradiol-17β (E2) and its analogues have both proliferative and differentiative effects on somatic cells of follicles [19].

Nutrition is one of the environmental factors that influence hormonal profile and consequently, reproductive and productive performance of animals [1-3]. Nutritional factors influence hypothalamic-pituitary function and therefore gonadotrophin profiles, directly through effects of nutrients or metabolic hormones such as insulin acting on target organs or through changes in sensitivity of these organs to oestradiol, progesterone and other hormonal feedback mechanism [20, 21]. In this study showed 4.5% fish oil supplementation (P2 group) was able to increase estrogen levels of rabbits. High estrogen levels indicate the rabbit is estrus and ready to mate. Other groups showed high estrogen levels and were ready to mate but the P2 rabbits were the most obvious results. Other research results that are in similar with the results of this study. In cows fed a diet supplemented with LNA (n-3), there was an increase in oestradiol during the follicular phase [9]. Once follicles reach an ovulatory size, they secrete estrogens in increasing amounts and rabbits show sexual receptivity for a period of time. When those follicles degenerate, secretions of estrogen decline and females rabbits become non receptive [22]. Likewise, results of others have shown that receptive rabbits had more large follicles and a higher concentration of estradiol in the follicular fluid than those of non receptive rabbits [23].

Shortening the unproductive period before the first litter would automatically increase productivity. Does generally reach puberty when they have grown to 70 to 75 percent of their mature weight. However, it is usually preferable to wait until they reach 80 percent of their mature weight before breeding them. These relative weights should not be considered absolute thresholds for all rabbits, but rather limits applicable to the population as a whole. Sexual behaviour (acceptance of mating) appears long before the ability to ovulate and bear a litter. Such behaviour should not be regarded by the breeder as a sign of puberty, but as prepuberty play [24].

In general, female rabbits reach puberty (i.e., the onset of sexual receptivity and ovulation) at around 14 weeks of age [25], although the age at first mating depends upon the breed of rabbit [26]. In the Californian and New Zealand breeds, the females are sexually receptive and achieve high reproductive performance (ovulation and in litter size) by 17-20 weeks of age [25, 27]. Different feeding systems (ad libitum vs restrictive) will affect reproductive performance in rabbits [25], and some researchers recommended that breeding begin when the female rabbit reaches about 75% of its adult weight [27]. Better litter size was observed in rabbits with higher body weights (i.e., fed ad libitum) at first insemination [25].

Female rabbits are classified as induced or reflex ovulators because ovulation takes place after mating hence, rabbits do not have a regular estrous cycle as in other domestic species (e.g., cattle). Rabbits display periods of sexual receptivity and non receptivity. Investigators have reported that the
period of receptivity lasted about 7 to 10 days in the absence of mating. This period was followed by 1 to 2 days of non receptivity [26]. Sexual receptivity, or the willingness of the female rabbit to allow mating [28], involved the adoption of a lordosis posture [29]. In female rabbits, lordosis is usually displayed in response to male pelvic thrusting [29,30]. In females that are willing to accept mating, it is more likely to observe an enlarged reddish-purple vulva as a result of high concentrations of estrogens; however, some females accepted mating when the vulva was small and pale [29].

4. Conclusion
Supplementation of cod liver oil on commercial feed up to 4.5% to capable increase the estrogen level in female local rabbits.

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