

The Effect of Flushing Feeding on the Development of Female Rabbit`S Reproductive Organs

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Abstract— The aim of this study was to determine the effect of feed flushing on the development of the ovaries and reproductive tract in female rabbits. The research was conducted from January 20 to February 13, 2024 in Sleman and the Livestock Reproduction Laboratory of Mercu Buana Yogyakarta University. This study used a completely randomized design with a one-way pattern using 15 rabbits. The flushing feed ingredients were rice vinegar, fermented banana heart, fermented bamboo shoots, fermented catfish, hereinafter referred to as MOL, and egg yolk. There were 5 treatments, those were, P1: Basal feed + 100% MOL; P2: Basal feed + 75% MOL + 25% egg yolk; P3: Basal feed + 50% MOL + 50% egg yolk; P4: Basal feed + MOL 25% + egg yolk 75% and P5: Basal feed + 100% egg yolk. The variables observed were ovarian morphometry, follicle count, morphometry of the reproductive tract of female rabbits, and the onset of estrus. Data were analyzed using analysis of variance (ANOVA), followed by Duncan's New Multiple Range Test (DMRT). The results showed that P4 had a significant effect (P < 0.05) on the length and volume of the ovaries. P5 had a significant effect (P < 0.05) on the ovarian weight and the number of follicles, livestock in heat were found in P2, P3 and P5 except in P1 and P4. In P1 and P2, the measurements of the length, weight and volume of the fallopian tubes were not significantly different (P>0.05), but were significantly different (P<0.05) when compared to P3, P4, and P5. The Uterine length was significantly different (P < 0.05) in each treatment. The weight of uterus was not significantly different (P>0.05) between P4 and P5, but was significantly different (P<0.05) from P1, P2, and P3. The volume measurements was not significantly different (P > 0.05) between P3 and P5, but were significantly different (P < 0.05) from P1, P2, and P4. The cervical length and weight were not significantly different (P>0.05) between P1 and P2, but were significantly different (P < 0.05) with P3, P4, and P5. The volume measurements were not significantly different (P>0.05) between P2 and P5, but were significantly different (P<0.05) with P1, P3, and P4. It was concluded that flushing feed with a egg yolk level up to 100% enhanced the development of reproductive organs and tracts, as well as accelerated the onset of heat.

Keywords—Flushing, feed, ovary, reproductive tract, rabbit

I. INTRODUCTION

Background

The reproduction aspect plays an important role in population growth. An important part of rabbit reproduction is the breeder's expertise in predicting estrus and the right timing for mating the rabbits. In terms of the animal husbandry, there are still many rabbit breeders who have limited knowledge, even to the point of breeding. Thus, the quality of rabbits produced is fairly poor and can have a negative impact on the future [1].

The low level of knowledge among breeders results in a low level of reproductive efficiency, which will affect the increase in the rabbit population. One of the causes of the low level of reproductive efficiency is the late age of puberty which results in a slow first breeding age and a long inter-breeding interval. [2]. The development of reproductive organs will enhance good livestock reproduction. One of the factors to improve livestock reproduction is the quality and quantity of good feed, including protein. Protein is a precursor of reproductive hormones. During the reproductive phase, high-protein feed is needed, which can be met by providing flushing feed. Flushing feed is the provision of extra protein and energy above the basal feed. Flushing is a method to improve the body condition of livestock through feed enhancement so that the livestock are ready to carry out the reproduction process. Improving the body condition of the breeding livestock before mating can optimize the reproductive process of livestock, thereby reducing repeated mating [3].

II. MATERIALS AND METHODS

The study was conducted from January to February 2024 in Sleman, Yogyakarta. A total of 15 female New Zealand White rabbits aged 4-5 months were used in the study. The flushing feed material was MOL consisting of rice vinegar, fermented banana heart, fermented bamboo shoots and fermented catfish, as well as egg yolk. The necessary tools included rabbit cages and equipment, tools for making fermented feed, scales, and documentation tools. The method used was an experimental method, with a completely randomized design with a one way pattern. There were 5 treatments, those were P1 = basal feed +100% MOL + 0% egg yolk; P2 = basal feed + 75% MOL + 25% egg yolk; P3 = basal feed + 50% MOL + 50% egg yolk; P4 = basal feed + 25% MOL + 75% egg yolk and P5 = basal feed + 0% MOL + 100% egg yolk, each treatment was repeated 3 times. The research variables included ovarian development consisting of length, weight, ovarian volume, number of follicles, morphometry of the fallopian tubes, uterus, cervix and ovaries, as well as observation of lambing/estrus. Supporting data in the form of feed consumption CP (Crude Protein) and DM (Dry Matter). The data obtained would subsequently be analyzed using ANOVA followed by the DMRT test.



III. RESULTS AND DISCUSSION

Feed Consumption

Based on the results of CP and DM consumption research (Table 1), it showed that the total CP and DM feed in each treatment did not differ significantly (P>0.05). This was because the CP and DM of the ration were relatively the same. The application of flushing feed technology will help improve feed quality, which can affect the quality and quantity of feed consumed by rabbits [4]. The flushing feed contains nutrients for reproductive development such as zinc, vitamin A, magnesium, and cholesterol. Providing rabbit feed that meets their needs as well as proper management of animal husbandry will result in good rabbit growth. Livestock growth is influenced by feed consumption; the higher the level of feed consumption, the higher the nutrients that enter the livestock's body, thereby increasing the growth rate of the livestock [5].

TABLE 1. Average CP and DM Cons	sumption for each treatment

Treatment	CP Consumption (ns)	DM Consumption (ns)		
P1	28.22	51.25		
P2	27.89	51.29		
P3	27.67	51.32		
P4	27.45	51.35		
P5	27.12	51.39		
M	· (•)			

Note: ns = *non-significant*

Based on the results of research on flushing feeding, there were rabbits in heat. Treatment P5 with 100% egg yolk flushing dose showed a higher estrus level in rabbits, as the egg yolk contains nutrients that trigger reproduction. In addition, the rabbits also experienced a faster weight gain and an increase in body immunity. Flushing is done before mating in order to maintain the breeding livestock's body weight. Even if flushing continues during pregnancy, it can prevent weight loss after giving birth, allowing the breeding livestock to mate again more quickly and reducing the interval between birth [6]. The application of flushing technology greatly support addressing reproductive issue such as delayed puberty, delayed estrus, delayed post-estrus partum, difficulty in giving birth and other reproductive problems [7].

Macroscopic View of Female Rabbit Ovary

The results showed that there were differences in the color and shape of the ovaries among the treatments. P1 ovary was elongated and appeared empty, whereas P2, P3, and P4 were shaped like mulberry fruits and appeared full. In accordance with the statement [8] the shape of the ovary varies according to the species. In each treatment, the right ovary appears bigger than the left ovary.

Based on the macroscopic observations of ovarian color, there were differences between P1, P3, and P5. The ovaries in P1 and P4 appeared pinkish and whitish, while in P2 and P3 appeared brownish-red, and in P5 appeared brownish-red or grayish with some yellowish color. The ovaries have a smooth surface at the time before regular ovulation, the color is gray to pink. During adolescence, the surface of the ovaries becomes uneven due to the formation of many new follicles as well as mature follicles, in addition to the presence of the corpus luteum and corpus albicans.



Figure 1. Female rabbit ovary macroscopically. Description: P1) 100% MOL, P2) 75% MOL and 25% egg yolk, P3) 50% MOL and 50% egg yolk, P4) 25% MOL and 75% egg yolk, P5) 100% egg yolk

Ovarian Morphometry of Female Rabbits



Based on the research results, the weight, length and volume of the rabbit ovaries under the flushing feed treatment are fully detailed in the following table.

The rabbit's ovary are divided into two parts, right and left. Morphometrically, it can be seen in Table 2 that the right ovary of the female rabbits is longer compared to the left ovary. It can be seen that the average weight and length of the ovary in P1 is smaller than P2, P3, P4 and P5. The morphometry of the ovaries of P3 and P5 female rabbits are longer compared the ovaries in P1, P2, and P4. After being statistically analyzed, the developmental characteristics of the female rabbit ovaries (weight, length and volume) showed a significant effect on the different flushing feed dosage statuses (P < 0.05). Flushing feed can meet the nutritional needs of rabbits so that it can increase reproductive hormones, facilitating estrus, and increasing the number of ovums released from the ovaries. Flushing can also induce estrus (ovulation).

TABLE 2. Average Ovarian Morphometry of Female Rabbits						
Treatment	Length of Ovary (mm)		Ovary Weight		Ovary Volume (ml)	
	Right	Left	Right	Left	Right	Left
P1	11.73 ^a ±3,44	10.73 ^a ±2,78	0.07 ^a ±0,02	0.06 ^a ±0,02	0.1ª±	0.1ª±
P2	13.33 ^a ±4,57	13.33 ^b ±3,80	0.11 ^a ±0,05	$0.10^{a}\pm0.06$	0.13 ^b ±	$0.1^{a}\pm$
P3	13.83 ^a ±1,08	11.56 ^a ±1,15	0.09 ^a ±0,02	$0.09^{a}\pm0.02$	0.1 ^a ±	$0.1^{a}\pm$
P4	14.05 ^{ab} ±1,05	10.73 ^a ±0,70	$0.09^{a}\pm0,01$	$0.08^{a}\pm0.01$	0.15°±	$0.1^{a}\pm$
P5	13.5 ^b ±1,85	12.4 ^b ±0.46	0.43 ^b ±0,42	$0.10^{a}\pm0,10$	0.13 ^b ±	$0.2^{a}\pm$
Means*	13.198	11.75	0.15	0.08	0.12	0.6

Note :*) Different superscripts on rows in the same column indicate significant differences (P<0.05)

Table 2 shows that the length of the right ovary in P1 (11.73a \pm 3.44) and the left ovary (10.73a \pm 2.78), the weight of the right ovary (0.07a \pm 0.02), and the left ovary (0.06a \pm 0.02) and had the average value smaller than P2, P3, P4 and P5. In P2, there had a mixed dose of egg yolk, and in P3 had a dose of 1: 1 between egg yolk and MOL, resulting in more nutrients. P4 used a higher dose of egg yolk and there was a longer rabbit ovary morphometry, while in P5 had a heavier morphometry than in P1.

Based on the results of the analysis, a higher treatment of egg yolk significantly (P <0.05) affected the length of the rabbit's ovaries. Ovarian length at the P1 treatment level was significantly different (P <0.05) from P4 and P5 with the higher doses of egg yolk. The most effective level for dosing egg yolk and MOL was P4 as much as 75% and 25%.

The provision of egg yolk doses and MOL doses significantly differed (P <0.050) in the length of rabbit ovaries among each treatment. Longer ovaries were found in P4, with an average of 14.05 cm, while the P1 rabbits had an average of 11.73 cm. Based on the analysis of variance, the provision of egg yolk had a significant effect (P < 0.05) on the weight of the rabbit ovaries. After conducting further DMRT tests, it was shown that P5 was significantly different from P1, P2, P3 and P4. The most effective level for ovarian weight was P5, which consisted of 100% egg yolk without any MOL dosage. In P5, the egg contained several hormone precursor substances, which increased reproductive hormones such as Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH). The FSH hormone will stimulate the growth and development of follicles so that the weight of the ovaries increases [8]. Based on the analysis of variance, treatments with a higher level of egg yolk had a significant effect (P < 0.05) on the volume of rabbit ovaries. The results of the DMRT further test in Table 2 show a significant difference in the effect of the increased egg yolk level.

The treatment of giving 75% egg yolk and 25% MOL in P4 gave a very significant effect on the volume of rabbit ovaries compared to P1 100% MOL without egg yolk, a higher dose of

egg yolk compared to the dose of catfish had also affected the ovarian volume. This is because egg yolks contain complete nutritional profile needed by reproductive hormones, those are cholesterol, zinc, vitamin A, and magnesium, besides egg yolks also have a high cholesterol content which is not found in MOL [9].

Cholesterol functions to produce hormones, coat nerve cells so that they can transmit stimuli appropriately, and form the outer membrane of body cells. Cholesterol is the most important fat component for the body [10]. Cholesterol is a precursor to steroid hormones in the body including estradiol, progesterone, testosterone, and vitamin D [11]. Estrogen and progesterone hormones can be produced in balanced amounts. To convert cholesterol into steroid hormones, the body requires vitamin A and thyroid hormone. Therefore, consuming foods rich in vitamin A can also be a way to increase progesterone hormone [12].

Zinc deficiency can lead to decreased secretion of GnRH from the hypothalamus, resulting in reduced concentrations of FSH and LH hormones, thereby halting the ovulation process. This can encourage ovulation and send signals to the ovaries to produce more progesterone if pregnancy occurs. Magnesium is important to support the body's process of producing cristols and progesterone [13]. The main reproductive hormones in reproduction include FSH and LH. FSH will stimulate follicular growth and development while LH will cause ovulation [8] [14].

This indicates that the weight, length and volume of rabbit ovaries are influenced by adequate feed nutrition. In line with the statement [15] [16] that nutrition is one of the important factors affecting the growth and development of ovarian morphometry. When feed consumption decreases, it will result in a decrease in ovarian weight, number of follicles and ovarian dysfunction [17].

Number of Female Rabbit Follicles



The results of the calculation of the number of follicles from various treatments of feed flushing ovarian follicular development of female rabbits are presented in Table 3.

TABLE 3. Average number of follicles				
Treatment	Number of Follicles			
Treatment	Right	Left		
P1	$7^a \pm 2$	$5^{a}\pm1$		
P2**	9 ^a ±5	9 ^a ±5		
P3**	$10^{a} \pm 4$	$8^a \pm 3$		
P4	9 ^b ±2	$10^{b}\pm1$		
P5**	$10^{\circ} \pm 5$	12° ±5		
Means*)	9	8.8		

Note :*) Different superscripts on rows in the same column indicate significant differences (P<0.05) **heat breeding

Based on the results of the analysis of variance, it is known that flushing feed with different levels of egg yolk had a significant effect (P < 0.05) on the number of follicles for each ovary. The highest number of follicles in P5 which contains 100% egg yolk. This is because the nutritional content of egg yolk is much more complete when compared to the nutrient sources from MOL [18]. Egg yolks contain high but controlled cholesterol, which is the key to hormonal balance in the body, including progesterone. In eggs, there is a zinc content that helps the pituitary gland in producing follicle-stimulating hormone or FSH. [19] states that ovaries with many follicles will give a high FSH picture. This hormone is a hormone that stimulates follicular growth and maturation of oocytes within

the follicle through increased proliferation of follicular cells and the conversion of androgenic steroids into estrogens. In P1, there were no rabbits in heat. Whereas in P2, P3, and P5, there were animals in heat. Symptoms of heat indicates that the follicles are mature enough to produce eggs (ovulation) [8].

In the ovaries and follicles, there is a preovulatory phase. During this phase, there is the estradiol hormone which can induce estrus in livestock {20]. Furthermore, [21] it is stated that in the Graafian phase, the follicles grow under the influence of FSH (Follicle Stimulating Hormone) and produce estradiol, so that follicles develop and are filled with follicular fluid. Estradiol increases the blood supply to the genital tract and enhances follicular development in the ovaries.

Morphology of the Female Rabbit Reproductive Tract

The results of macroscopic observations of the reproductive organs of non-estrus female rabbits can be seen in Figure 2. Meanwhile, the reproductive organs of estrus female rabbits in this study can be seen in Figure 3.

Macroscopically in Figure 2, the reproductive organs tracts of non-estrus female rabbits have similarities in color, as both of the fallopian tubes, uterus, and cervix are white, as well as the shape of both fallopian tubes, uterus, and cervix have similarities; in P1 and P2, the fallopian tubes were slightly thick and convoluted, while the uterus and cervix in both treatments were thinner and smaller.



Figure 2. Reproductive tract of female rabbits in treatment P1, P4 that are not in estrus



Figure 3. Reproductive tract of female rabbits treated with P2, P3, and P5 in estrus

Macroscopically based on Figure 3. There are differences in the color and shape aspects of the fallopian tubes between

treatments P2, P3, and P5. The fallopian tubes in the P2 and P3 treatments had a reddish white color, while fallopian tubes in

the P5 treatment had a dark red color (+5). In terms of the shape, the fallopian tubes in P2 were thinner and more convoluted, in P3 they were slightly thicker, while in P5 had a thicker compared to the other treatments. Fallopian tubes or oviducts is a small, winding channels, connecting the ovaries with the uterine cornua. The fallopian tube is reddish-white in color and has a pliable consistency [22].

The estrus phase is basically influenced by the hormonal system of the livestock body. The hormonal system that affects estrus is centered on gonadotropin hormones from the anterior pituitary and ovarian hormones, namely FSH and estrogen. Changes in FSH and estrogen in the blood cause physiological changes in the livestock's body, manifested in physical changes such as vulvar swelling or red vulva. Changes also occur in the mucus cell type composition of the vagina [23].

Morphometry of the Female Rabbit's Reproductive Organs

Morphometric measurements (length, weight, and volume) of the reproductive tract of female rabbits P1, P2, P3, P4, and P5 can be seen in Table 4.

TABLE 4. Morphometry of The Female Rabbit Reproductive Tract							
Reproductive tract	Measurement parameters	Treatment					
		P1	P2	P3	P4	P5	
Fallopian tubes	Length (cm)	15 ^a ±1	12.7 ^a ±1.15	19.7 ^d ±2.7	17.65 bc ±2.35	15.3 ^b ±1.15	
	Weight (g)	2.2 ^a ±0,15	2.9 ^a ±1.23	5.26 ^b ±3.51	7.79 ^{cd} ±1.47	7.66°±2.70	
	Volume (ml)	2.7 ^a ±1,5	3 ª ±1.73	6.7 ° ±5.5	5.5 ^b ±4.5	7 ^d ±3	
Uterus	Lenght (cm)	4 ^a ±1	4.3 ° ±0.58	6.3 ^d ±1.15	4.5 cd ±1.5	4.17 ^b ±1.04	
	Weight (g)	2.52 ° ±0.30	3.86 ° ±0.62	4.5 ^d ±2.8	3.5 ^b ±0.60	3.43 ^b ±0.91	
	Volume (ml)	4.6° ±2.9	5.6 ^d ±3.05	2.6 ª ±0.58	3.5 ^b ±0.5	2ª±1	
Cervix	Length (cm)	1 ^a ±	1 ^a ±	1.5 ° ±0.5	1.4 ^b ±0.1	2.8 ^d ±0.9	
	Weight (g)	0.6 ^a ±0.31	0.5 ^a ±0.11	1.68 ^{cd} ±0.61	1.16°±0.3	1.15 ^b ±0.34	
	Volume (ml)	1.3 ° ±0.58	1.1 ^b ±0.1	$1.3^{d} \pm 0.58$	1ª±	1 ^b ±	

Note :*) *Different superscripts in the same row indicate a significant difference* (P < 0.05)

Based on the results of the analysis, the provision of flushing feed had a significant effect (P < 0.05), on the development of female rabbit's reproductive organs. This is due to the protein content in the flushing feed. Flushing feed is a way to improve the physical condition of livestock so that they are ready to carry out the reproductive process. Improving the body condition of breeding livestock before mating can optimize the reproductive process of the livestock, so as to reduce repeated mating [24].

The flushing feed treatment had no significant effect (P>0.05) on the morphometry of the fallopian tube length P1 vs P2, but had a significant effect (P<0.05) on P3, P4, and P5. The same was observed for the weight and volume of the fallopian tubes. However, the measurements of weight and volume showed an increasing trend from treatment P1 to treatment P5.

The provision of feed flushing had a significant effect on the morphometry of uterine length (P < 0.05) but did not affect the weight and volume of the uterus. The DMRT test results showed no effect (P>0.05) between P1 and P2, but significantly different (P<0.05) when compared to P3, P4, and P5. Meanwhile, the volume in the cervix statistically showed no significant difference (P>0.05) in P2 with P5, but significantly different (P<0.05) compared to treatments P1, P3, and P4.

IV. CONCLUSIONS AND SUGGESTIONS

Conclusions

Treatment with 100% egg yolk level added with MOL is the best treatment that can enhance the development of the ovaries and reproductive tracts of female rabbits as well as induce estrus.

Suggestions

It is recommended to use egg yolk combined with MOL as a feed flushing for 10 days.

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