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Estrous and pregnancy rate responses of postpartum Bali cattle to concentrate supplementation with different protein levels of rice-straw as basal ration

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Abstract. The aim of the research is to determine the effect of concentrate with different protein level rice straw as basal ration on the percentage of pregnancy rate of Bali cattle maintained on traditional farming system. The research was divided into two experiments. First experiment: a number of 46 cows divided into 4 treatment groups. Each group respectively protein level 0%, 10%, 12%, and 14%. Experiment 2: a number of 324 Bali cows postpartum got concentrate supplementation with different protein levels were divided into three groups, each got a protein level of 10%; 12%, and 14%. The rice straw diet given ad-libitum and concentrate as much as 1 kg per head per day for two months. Livestock that appears estrous after feeding directly mated naturally and partially performed artificial insemination. Pregnancy examinations carried rectal palpation and partly validated with ultrasound. The results showed that estrous respon in the first experiment not significantly affected by supplementation of concentrate with protein level for 10–14%. In contrast, estrous intensity was obviously visible in group treatment with 14% protein level. The supplementation of protein level in concentrate increased natural estrous 66.05% and natural mating generated 91.65% of pregnancy rate. However, 43.95% cows were artificially inseminated generating 81.63% of pregnancy rate. It's sonclude that supplementation of concentrate for feed Bali cows with rice straw as basal in the dry season caused increasing the percentage of postpartum estrous and highest of pregnancy rate.

1. Introduction

Bali cows are considered to have high fertility rate, one calf per year. High reproductibility of Bali cows are characterized by the ovary activities and rebreed in less than two months of postpartum [1]. This condition is possible to be achieved in effective provision of nutrients in terms of quality and quantity. The optimum capability of Bali cows reproduction is a crucial factor due to time efficiency, energy, and cost. One of the constraints in the Bali cows reproduction postpartum is the signs of silent estrous and therefore, calving interval becomes longer and increase the number of inpregnant cows.



The signs of estrous is affected by the physical, age, breed, temperature, environment, body weight (BW), and nutrition [2]. Lack of nutrient supplementation for postpartum cow is one of primary factors of reproductive disorder and it is able to inhibit the occurrence of estrous. The farm condition causes uncontrolled cattle, primarily due to feed management resulting in reproductive issues and slow estrous (postpartum anestrous) in Bali cows. Various efforts to accelerate estrous due the lack of nutrition; are possible to be implemented in various methods. One of methods is by supplying good-quality feed. Concentrate as feed amplifier may improve the digestibility because concentrates composed of easily digested materials for cattle. After giving birth, Bali cows require feed with high protein content and energy for the growth and recovery of reproductive organs. One condition of the farmers during the summer is retard of estrous postpartum. This is due to the cows were only given rice straw as basal feed, while they were still weaning and in the process of return of the anatomical structure of the reproductive organs. At the time, the uptake energy was more used than intake energy. It is resulting in a negative energy balance in new postpartum and lactation cows, and will be anestrous postpartum. There is associated with in calculated negative energy balance a key factor driving poor fertility [3]. The better nutritional levels are expected to accelerate the onset of estrous in Bali cows.

The efforts to improve reproductive efficiency have been investigated by several researchers. The aspect of reproduction showed that the first breeding postpartum was achieved after 127.6 days, with Service per Conception (S/C) of 1.2 and 75% pregnancy rate in cows by urea multi nutrients molasses block (UMMB), while the first breeding of the postpartum cows were not provided UMMB reached 159.8 days, 1.7 S/C and 69.2% pregnancy rate [4]. Astawa et al [5] reported that supplementation of vitamins and minerals in rice straw and commercial feed can increases digestibility coefficients of dry matter and organic matter, partial VFA, ammonia concentration, and production of methane. However, there was no effect on digestibility coefficients of crude protein, crude fiber and rumen pH. Supplementation of 0.2% vitamins-minerals can improve feed utilization in Bali cows.

Research about the impact of level of proteins supplementation on pregnancy rate postpartum in farming level is still limited. Therefore, this study aims to investigate the effect of concentrate addition in feed of Bali cows postpartum with different protein levels to accelerate the presence of estrous and pregnancy rate maintained in farming level.

2. Materials and methods

The study was conducted from January to March 2017, in Lompo Tengah and Mattirowali village, Tante Rijaja district, Barru, South Sulawesi. The study is divided into two stages; (1) the effect of concentrate supplementation in different protein levels on characteristics of estrous and (2) on pregnancy rate.

2.1. Experiment 1: Estrous characteristics of young Bali cows

The materials of the research consisted of fourty Bali cows aged 2 years maintained intensively. Bali cows were fed by concentrate formulation containing rice bran, corn bran, coconut cake, urea, mineral, salt and cattlemix with different protein levels. A total of 46 of Bali cows aged 2 years were randomly divided into 4 groups; control treatment used 10 cows and the other treatments used 12 Bali cows and different protein level respectively. Feed adaptation period was performed for 3 weeks with the same feed composition. Treatments were divided into four groups. The group 0 was given 0% crude protein (CP) or control groups, the other groups were given 10%, 12%, and 14% crude protein respectively. The contents of feedstuffs are presented in table 1. The experiment was performed experimentally based on Completely Randomized Design (CRD) consisting of 4 treatments and 10 replications. Therefore total units of the research are 40 Bali cows. The treatment was arranged in different protein levels as below:

- P0 = Rice straw only
- P1 = Rice straw + 1 kg Concentrate (Crude Protein 10%)
- P2 = Rice straw + 1 kg Concentrate (Crude Protein 12%)

P3 = Rice straw + 1 kg Concentrate (Crude Protein 14%)

Table 1. The feed ration of treatment with different protein levels.

Ration Materials	Composition Feed Materials and Protein Content (%)		
	Ration A	Ration B	Ration C
Rice Bran (10.1)*	85 (8.6)	80 (8.1)	83 (8.4)
Corn Bran (8.7)	5.5 (0.5)	4.5 (0.4)	6 (0.5)
Coconut cake (21)	4.5 (0.9)	10 (2.1)	4.5 (0.9)
Urea (27)	0 (0)	0.5 (1.4)	1.5 (4.2)
Mineral (0)	2 (0)	2 (0)	2 (0)
NaCl (0)	3 (0)	3 (0)	3 (0)
Totals	100	100	100
Protein content (%)	(10 %)	(12%)	(14%)

*Protein content in feed materials

2.2. Experiment 2: Effect of feed improvements to the presence of natural estrous and pregnancy rate

This experiment was carried out in collaboration with breeders who have a Bali cow that has just given birth for more than two months. The number of cattle treated with concentrate supplementation was 324 cows and 26 were controls. A total of 324 of Bali cows age 3–7 years, anestrous post partus, were randomly into 3 groups supplemented the different protein level respectively. The group A (97 cows) were given 10% crude protein, the group B (65 cows) and C (132 cows) were given 12 % and 14 % crude protein. Feed adaptation period was performed for 4–8 weeks with the same feed composition (table 1). Each treatment was given concentrate before feeding ration of basal rice straw or grazing. Water was supplied by *ad libitum*. There is a problem in collecting data, there are a small number of farmers who do not pay attention to the estrus of their cows, because of their business and other reasons. This results in some cows not being mated either in natural mating or mating with artificial insemination (table 4). The variables measured in this research were:

2.2.1. *Estrous speed (Day)*. Speed of estrous was calculated starting from feeding up to the first onset of estrous that showed in days.

2.2.2. *Estrous intensity (%)*. Estrous intensity was determined in score 0 to 3; score 0 (anestrous), score 1 (estrous less clear), score 2 (moderate estrous intensity) and score 3 (estrous with obvious intensity) [6, 7]. The percentage of estrous intensity was calculated using the formula:

$$\% \text{ Estrous Intensity} = \frac{\text{Total of Estrous Cows}}{\text{Total of Sample Cows}} \times 100 \%$$

2.2.3. *Service per Conception (S/C)* is number of artificial insemination (AI) required to produce a pregnancy;

$$\text{Service/Conception (S/C)} = \frac{\text{Total of inseminations were performed}}{\text{Total of pregnant cows were detected}}$$

Conception Rate (CR) or pregnancy rate is the percentage of pregnancy of inseminated cows. Pregnancy status in cows was clarified by rectal palpation at the age of two months of gestation. The percentage of pregnancy was calculated using the formula:

$$\% \text{ Conception Rate} = \frac{\text{Number of Pregnant Cows}}{\text{Total of All Cows}} \times 100 \%$$

3. Result and discussion

3.1. Experiment 1: Estrous characteristics of Bali cows

3.1.1. *Estrous speed.* Speed of estrous (day) is an interval from the start of feeding and the onset of estrous. Speed of estrous in every treatment is presented in table 2. Based on analysis of variance, different protein level treatment i.e. R1, R2, and R3 had not significantly affected ($P > 0.01$) estrous speed. However, Bali cows treated by different protein level i.e. R1, R2, and R3 caused the indication of estrous compared to R0. It indicates that concentrates with different protein level of 10-14% in Bali cows led to changes in reproductive status. One of the factors that affect the cattle reproduction is the availability of sufficient protein as nutrient source, thus it can affect the estrous speed of Bali cattle reproduction functional. Relationship between amount of crude protein and the estrous postpartum has been explained by the Sasser et al [8] that the extension of postpartum estrous occurs in cows fed with crude protein deficiency (0.32 kg/head/day) compared to the cows fed with enough crude protein (0.96 kg/head/day). Crude protein requirement in the diet for normal reproductive are 13–20%. Kartadisastra [9] argued that the need for cow protein will increase along with body weight gain.

Table 2. Speed of estrous with different levels of protein concentrates.

Treatments	Estrous Speed (Day)
R0	0 ± 0 ^a
R1	35 ± 8.1 ^b
R2	29 ± 5.4 ^b
R3	31 ± 7.2 ^b

Superscript in the same column indicated highly significant different ($P < 0.01$).

Hafez and Hafez [10] and Yendraliza et al [11] explained that the development of reproductive organs greatly depends on the ability of endocrine function in producing reproductive hormones. Therefore, nutrient availability affects functional body comprehensively. Protein as nutrient source affects brain function as the central stimulus into reproductive hormone releasing factor. In line with Diskin et al [12], stated that the effect of nutrients directly influence on GnRH on gonadotrophin secretion in the hypothalamus or pituitary. According to Son et al [13] and Romano et al [14], the cattle supplied with intake with energy and protein sufficiency led to fast growing and normal estrous symptoms in cattle.

3.1.2. *Estrous intensity.* Estrous symptoms and estrous intensity of the cows observed in this study, appeared significantly different among treatment groups (table 3).

Table 3. Estrous intensity with different protein levels

Treatments	Σ Cows	Estrous Intensity (%)			
		0	1	2	3
R0	10	10 (100) ^a	0 (0.00) ^a	0 (0.00) ^a	0 (0.00) ^a
R1	12	0 (0.00) ^b	5 (41.66) ^b	7 (58.33) ^b	0 (0.00) ^a
R2	12	0 (0.00) ^b	4 (33.33) ^b	6 (50.00) ^b	2 (16.66) ^b
R3	12	0 (0.00) ^b	0 (0.00) ^a	4 (33.33) ^c	8 (66.66) ^c

Score 3 (Estrous with obvious intensity); Score 2 (Estrous moderate intensity); Score 1 (Estrous intensity is less clear); Score 0 (Anestrous). Superscript in the same column indicates significantly different ($P < 0.05$).

The results showed that R0 (control) there was no intensity of estrous of all cows because there was no signs of estrous in this treatment. Third level of estrous intensity in feed treatment with estrous was clear (score 3) i.e; 0%, 16.66% and 66, 66% respectively for the treatment of R1, R2 and R3. The intensity of estrous was very clear on R3 treatment. It was observed due to the secretion of FSH concentrations. Therefore, the folliculogenesis progressed well. A number of research showed that livestock provided with sufficient feed of energy and protein led to rapid growth and the cattle showed indications of normal estrous [15, 16].

In the observations of estrous, Bali cows in R3 with CP 14% showed clear signs of estrous in vulva including transparent sticky mucus, red color, swollen, restlessness, decreasing appetite, frequent trumpeted, silent behavior in same-sex mating in female cattle. This is in accordance with the opinion of Partodihardjo [7], stating that characteristic of estrous is becoming restless cattle, decreased appetite, swollen vulva, mucus and vulva become red. In addition, Suharto [17] reported that the cattle provided good quality ration showed higher estrous intensity, and improves the clarity of estrous (swelling of the labia of the vulva, vaginal temperature, cervical mucus pH, color and abundance mucosa vagina mucus).

The results of this study also showed that the level of moderate estrous intensity (score 2) in the range of 58.33%, 50% and 33.33% respectively for R1, R2 and R3. While the level of less clear estrous intensity in range of 41.66%, 33.33% and 0% respectively for R1, R2 and R3. The low intensity of estrous variously was indicated by red vulva without swelling and mucus with less viscosity as well as cattle behavior looks nervous but refuses to be ridden by other cattle. This is in accordance with the opinion of Kune and Solihati [18] that estrous intensity of light or moderate due to individual factors that may be related to hormonal patterns, especially estrogen levels play a role in stimulating estrous.

3.2. Experiment 2: Effect of feed improvements to the presence of natural estrous and pregnancy rate

Estrous response treated feed concentrate supplementation generate natural estrous indication about 66.05% of 324 breeding cattle on 29–35 days after feeding. The number of cows without concentrate supplementation was participated in this research as much as 26 cows were not appeared estrus until more 6 months. The data of feed improvement effect or concentrate supplementation with different levels of protein basic ration of hay to Bali cattle reproductive efficiency were presented in table 4.

Cows supplied by concentrate supplementation generates 93.45% of pregnancy rate, which pregnancy rate of natural mating was 91.95% and pregnancy rate of artificial insemination was 81.63%. These data indicated that the pregnancy rate of the cows supplied feed concentrate supplementation naturally generate estrous and generally mate with natural mating. The cows can not natural mating have been mated using artificial insemination. The problems of natural mating are the farmers must have and maintain superior bull. In breeding is the weakness of the bull that causes defect on the dwarfishness of physical morphology. Meanwhile, if the calves resulted from Artificial insemination have a better morphological appearance due to the use of superior bull.

Table 4. Effect of repair concentrate feed with the different protein levels on the presence of natural estrous, and pregnancy rate mated by natural mating.

Ration type (Number of cows)	Natural estrous	An-estrous (Number of cows)	Natural Mating (Number of cows)	Rate Pregnancy of natural mating	AI Mating	Rate Pregnancy from AI	Sub Total of pregnant cow (natural mating + AI)
A (N=97)	72/97 (74.22%)	25/97 (25.77%)	60	57/60 (95.00 %)	12	11/12 (91.67%)	57+11=68 (68/97=70.1%)
B (N=95)	68/95 (71.57%)	27/95 (28.42%)	46	42/46 (91.30 %)	26	20/26 (76.92%)	42+20=62 (62/95=65.26%)
C (N=132)	74/132 (56.06%)	58/132 (43.93%)	68	61/132 (89.70%)	11	9/11 (81.81%)	61+9=70 (70/132=53.03%)

Total	214/324	110	174	160/174	49	40/49	160+40= 200
(N= 324)	(66.05%)			(91.95 %)		(81.63%)	(200/324=61.72%)

A: Protein Content of 10%; B: Protein Content of 12%; Protein content of 14%. The value in parenthesis is percentage.

The issues of the implementation of artificial insemination are the inadequate infrastructure in farm, such as expensive liquid nitrogen low fund for inseminator (only Rp 10.000, - each cow), and distant location. Estrous reports from the farmer are sometimes late and the farmers have difficulties in recognizing estrous ϕ in their cows. S/C was only two successful insemination.

Issues in unsuccessfully pregnant cows experiencing natural estrous without mating are caused by lack of attention from the farmers or the farmer distant location to the bull pen and no report to the inseminator. Cows which were not pregnant that experienced a natural estrous, but were not mated may be caused by lack of attention of farmers or location of farmers was far the bulls location and there was not report to the officer of artificial insemination. Another factor is inadequate nutrition in feed quantity supplemented to cows after giving birth. The quantity problem of feed given was still not maintenance the cows after getting "negative energy balance" at which time the cow gave birth, it expend more energy than the energy intake that coming from the feed. Anestrous condition is no indications of estrous often occurs in heifer having mature enough and inactive ovaries [19, 20], as well as delayed puberty. Generally, the last state that was found in heifer delayed puberty in the mature age in this case, the ovaries are still expected to grow normally again with feed improvement [19, 20]. The impact of imbalance energy in birth processing and breastfeeding the body experienced catabolism to change body energy maintenance. The lack of energy caused due to reproductive problems for a long time and high protein feed supplementation is still unable to handle this negative energy balance. Protein is one of the necessary nutrients for maintenance, growth and production [21]. Cow protein needs will further increase as the increase in body weight [22]. The production process begins with a microbial protein hydrolysis process the entire protein in feed by rumen microbes. The process of hydrolysis of proteins into amino acids followed by a process of deamination to form ammonia, and ammonia released and will be used for the production of microbial protein along with other sources of energy. Providing protein would lead to the production of GnRH from the hypothalamus and gonadotropin hormone (FSH and LH) from the pituitary gland to increase and improve ovarian response to stimulation LH. The increase in the rate of secretion of FSH and LH will lead to the growth of follicles that have an impact on the granulosa cells of preovulation follicles produce estrogen and ultimately increase reproductive efficiency [23, 24].

The role of nutrition on reproductive performance has been widely discussed by several researchers [21-23]. Effect of feed on reproduction can be divided into three parts: (1). Long-term influence, affect the cattle from birth, puberty to adulthood, (2). The influence of the medium-term effect on the annual reproductive cycle in adult female animals, and (3). short-term effect during the periods before and after mating. The third has been done on sheep, where feeding supplement before and after mating period ("flushing" method) can increase the level of ovulation and fertility of cows.

Some research indicated that energy restriction in cattle rations may cause anestrous on the cattle with estrous normal cycle [21–23]. Lactating cows provided with limited energy feed produced sub functional corpus luteum during estrous cycles before anestrous [25, 26]. Energy level reduction in cow ration may alter follicle development [26], lowering the diameter of preovulation follicle and inhibit the follicle growth with the largest diameter during the estrous cycle [24–26] and lower concentrations of "insulin-like growth factor "(IGF-1) [24,27,28]. IGF-1 stimulates cell proliferation and cell differentiation in vitro [25, 26]. In conclusion, supplementation of concentrate in rice straw basal ration with 10-14% protein level (R1, R2, R3) induced estrous response, whereas the control treatment (R0) did not show estrous response. The intensity of estrous in treatment R3 with a protein level of 14% showed a clear estrous intensity compared to R1 and R2 with 10-12% protein level showed less intensity of estrous clearly. Supplementation of concentrate in rice straw basal ration with 10-14% protein level (R1, R2, R3) raised natural estrous response was 66.05% with pregnancy rate form natural mating was 91.95% and artificial insemination was 81.63% with meant total of pregnancy was 61.72 %.

4. Conclusions

The supplementation of concentrate for feed Bali cows with rice straw as basal in the dry season caused increasing the percentage of postpartum estrous and highest of pregnancy rate compared to without concentrate supplementation.

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