ISSN 1819-1878

Asian Journal of Animal Sciences

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

OPEN ACCESS Asian Journal of Animal Sciences

ISSN 1819-1878
DOI: 10.3923/ajas.2016.175.181

Research Article

Effect of High Rumen Undegraded Protein (HRUP) Supplementation on Estrous Response and Progesterone Hormone Profile in Dairy Cows Raised Under Indonesia Tropical Environmental Conditions

Rochijan, Budi Prasetyo Widyobroto and Ismaya

Department of Animal Production, Faculty of Animal Science, Gadjah Mada University, Yogyakarta, Indonesia

Abstract

This study was designed to determine the effect of High Rumen Undegraded Protein (HRUP) supplementation on estrous response and profile progesterone hormone in early lactation dairy cows under Indonesia tropical environment. The experiment was conducted with twelve early lactation Friesian Holstein cows divided into two groups. Six cows were used as a control group and other six cows as HURP group. Both control and HRUP group obtained basal diet with forage to concentrate ratio 60:40 (DM basis) but different concentrate composition for each groups. Rumen undegraded protein for control group was 27.47% and HRUP group was 32.78%. Feeding twice daily, morning and afternoon. Water was given by *ad libitum*. The observed variable were feed intake, estrous response and concentration of progesterone hormone. Data obtained were examined by t-test analysis, except estrous response analyzed descriptively. The result showed that 100% estrous response value was normal and the comparison of progesterone hormone on 1st, 5th, 10th, 15th, 17th, 21st and 26th day between control and HURP group was 0.13±0.02 vs. 0.13±0.04, 0.26±0.24 vs. 0.20±0.08, 0.13±0.03 vs. 1.72±2.23, 0.10±0.04 vs. 1.72±3.77, 0.09±0.02 vs. 3.66±3.76, 0.09±0.01 vs. 0.66±0.80 and 0.12±0.03 vs. 1.20±1.08 ng mL^{-1}. It was concluded that HRUP supplementation on early lactation dairy cows could not increase the concentration of profile progesterone hormone. However estrous response value as well as estrous indication and behavior was normal.

Key words: Rumen undegraded protein, estrous response, progesterone hormone, dairy cows, tropic

Received: November 25, 2015 Accepted: February 23, 2016 Published: April 15, 2016

Citation: Rochijan, Budi Prasetyo Widyobroto and Ismaya, 2016. Effect of High Rumen Undegraded Protein (HRUP) supplementation on estrous response and progesterone hormone profile in dairy cows raised under indonesia tropical environmental conditions. Asian J. Anim. Sci., 10: 175-181.

Corresponding Author: Rochijan, Department of Animal Production, Faculty of Animal Science, Gadjah Mada University, Yogyakarta, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The increasing of productivity on dairy cows is an effort should be made to improve the population in order to fulfill the need of cow protein that improving, especially milk. Livestock productivity in general involves various aspects, such as the production of calves, meat, eggs and milk. From the aspect of reproduction, improved reproducibility is a very necessary thing for the breeding, because this condition is a series that should not be broken in order to improve the reproductive efficiency, starting from cows born, puberty, mating and maternity. The efforts have been improved the reproductive efficiency, the role of feed is very high for subsistence in productivity during lactation cow. For lactating cows in the three semester especially early lactation, many cows require large DM intake to maintain milk production and initiate repair the reproductive tract (Rochijan *et al*., 2014). Therefore, the need for dairy cow must meet their needs and produce normally.

The process in reproduction related to mechanism of hormonal system, between the hypothalamic hormones gonadotropin releasing hormone (GnRH), Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH), ovarian hormones (estrogen and progesterone) and uterine hormones (prostaglandin) (Hafez, 2000). Hormone ovary which has a big role on reproduction are estrogen and progesterone. Hartantyo (1995) report that the concentration of blood plasma progesterone can determine the state of the animal in a state of infertility, normal, estrous and pregnant so it can use to detection of estrous, pregnancy examinations and knowing other pathological conditions and Gunn *et al*. (2014) also said that feeding increased dietary Crude Protein (CP) abundant in Rumen Undegraded Protein (RUP) fraction impacted reproductive parameters prior to ovulation that would be indicative of improved fertility in cattle (impact in ovarian follicular growth patterns and affected follicular estradiol production and circulating concentrations of progesterone in the subsequent estrous cycle). Diets high in CP support high milk yield, but are also associated with lower reproductive performance. High protein can result in elevated plasma urea concentrations that affect the uterine environment and fertility. Nutritional interactions resulting in poor fertility of high producing dairy cows include the antecedent effects of negative energy balance and effects of high dietary protein (Litoi, 2014).

Indonesia is a tropical country and the climate is fairly even all year round. The climate and weather of Indonesia is characterized by two tropical seasons, which vary with the equatorial air circulation (the Walker circulation) and the meridian air circulation (the Hardley circulation), so that weather usually has high temperatures and humidity (Thayeb, 2015). The weather in Indonesia is characterized by high temperature and humidity, which ranged between 27.7-34.6°C and humidity ranged between 55.8 and 86.8% (BPS., 2010; Hernawan, 2014) and temperature humanity index value in Indonesia is 78-80. Productivity of dairy cows in tropical environment, which are generally thought to result from the lower lack of management that is not in accordance with the demands of livestock to produce optimally. The efforts have been made to improve the performance of dairy cows to increase the quality and quantity of rations and understanding the to fulfill the nutrients of rumen microbes and host cow, such as feeding the Rumen Undegraded Protein (RUP) which can prevent protein degradation in the rumen and can be used directly by the animal host by absorption in the small intestine. So from that the results of this study will influence the data obtained with feed high rumen undegraded protein on estrous response and profile progesterone hormone of early lactation dairy cows.

MATERIALS AND METHODS

Time and place of experiment: This experiment was conducted in Dairy Cow Units, Department of Agriculture, Daerah Istimewa Yogyakarta. Weather in Indonesia is generally hot (23-34°C) and humid (73-87% relative humidity) (Thayeb, 2015). Analysis of samples was conducted in Laboratory of Dairy Science and Milk Industry, Faculty of Animal Science and Laboratory of Pathology Clinic, Faculty of Veterinary Medicine, Gadjah Mada Universities.

Animals and diets: The experiment was conducted with twelve early lactation Friesian Holstein cows divided into two groups. Cows housed in pens models stanchion barn, equipped with a feed and drink water. Six cows were used as a control group and other six cows as HURP group. The diets used for control and HRUP group were forages and concentrate with a ratio of 60:40 (DM basis), but different concentrate composition for each groups (Table 1). Feeding twice daily, morning and afternoon. The cow feeds after parturition. Drink water was given by *ad libitum*. The equipment used in blood sampling and analysis was vacutainer needle merk BD Vacutainer, 3 mL venoject tube merk Vaculab, holder vacutainer merk Brand, cotton, centrifuge merk Eppendorf Centrifuge 5417C, 1.5 mL eppendrof tube merk Brand, refrigerators merk sharp, freezers merk Modena, microtiter plate reader, micropipette,

Table 1: Concentrate formulation ingredients and nutrient compositions of experimental diet

	Group	
Concentrates formulation	Control	HRUP
Ingredients	**----------DM (%)----------**	
G-Pro	8.0	3.0
Soy bean meal-HCHO	-	9.0
Kapok seed meal	6.0	6.0
Copra meal	14.0	12.0
Corn gluten feed	10.7	10.0
Wheat pollard	16.7	14.0
Palm kernel meal	13.4	13.0
Coffee husk	14.3	11.0
Corn tumpi	3.3	7.0
Cassava waste	-	4.0
Molasses	8.3	6.0
Mineral mix	5.3	5.0
Total	100.0	100.0
Analyzed compositions	**----------(%)----------**	
DM	88.24	89.06
OM	87.93	88.37
CP	18.55	18.83
CF	16.81	16.06
EE	5.10	4.70
RUP[a]	27.47	32.78
RDP[a]	63.55	60.84
TDN[b]	56.94	58.91

DM: Dry matter, OM: Organic matter, CP: Crude protein, CF: Crude fibre, EE: Ether extract, RUP: Rumen undegraded protein, RDP: Rumen degraded protein, TDN: Total digestible nutrient, HRUP: High rumen undrgraded protein, [a]Results of the analysis in sacco degradation and formulas Widyobroto *et al.* (1997) and [b]Results of the formula of Hartadi *et al.* (2005)

absorbent paper, spectrophotometer merk Microlab, timer, distilled water and a set of progesterone ELISA analysis.

Estrous response indication and behavior: This observation, in the form of changes in behavior and reproductive organs that occur during estrus include: behavior and quietly climbed up the other fellow cows, cattle restless, mooing cow-bellow, decreased appetite and changes in the condition of the vulva include: the presence of the vulva red (red, swollen, warm), clear mucus, thick and hang (Putro, 2008).

Methods blood sampling and hormone analysis: Blood sampling performed on cows (control and HRUP) whose have as many as six head estrous through artery caudals of 3 mL vacutainer K3 Ethyline Diamine Tetraacetic Acid (EDTA) tube, with methods of Delany *et al.* (2010) and Zetina-Cordoba *et al.* (2012). Blood samples were centrifuge for 15 min at 3000 rpm and the plasma was taken using a micropipette and then transferred into 1 mL eppendorf tube. After that, the sample is stored at a temperature of -20°C freezer until ELISA analysis.

Measurement of kit progesterone hormone using Enzyme-Linked Immunosorbent Assay (ELISA) (DRG., 2007). A total of 25 mL of solution, 25 mL of standards, controls 25, 25 and 200 mL sample of enzyme conjugate was added to each microtiter plate wells using a micro pipette and wait for 10 sec until fully mixed. Afterwards, sample incubated for 60 min without a cover plate at room temperature. The next process is done washing 3 times with the addition of a solution of 400 mL wash solution for each microtiter plate wells using ELISA tool. Furthermore, the residual droplets attached to the microtiter plate removed. A total of 200 mL substrate solution to each wells were incubated for 15 min at room temperature. Stop solution was then added 100 mL at any pitting. Determination of Optical Density (OD) enzyme conjugate is done after 10 min of adding stop solution, using a microtiter plate reader at a wavelength of 450±10 nm.

Statistical analysis: The data obtained were examined by t-test analysis using Statistical Program for Social Science or SPSS version 16.0 (Supardi, 2013) except estrous response analyzed descriptively.

RESULTS AND DISCUSSION

Intake of forage and concentrates: Chemical composition of feed ingredients concentrates consist of G-Pro, soy bean meal-HCHO, kapok seed meal, copra meal, corn gluten feed, wheat pollard, palm kernel meal, coffee husk, corn tumpi, cassava waste, molasses and mineral mix. Content ingredients and nutrient compositions concentrates formulation of experimental diet was presented in Table 1.

The average intake of dairy cows during the experiment was presented in Table 2. The results showed that intake of DM, OM, CP, CF and the TDN of cow given HRUP supplementation and those of control were not different, which were DM 11.64, 10.15, 1.50, 3.10, 6.63 vs. 11.54, 10.10, 1.50, 3.05 and 6.67 kg DM/head/day, respectively. This was similar to the study of Widyobroto *et al.* (2001) and Widyobroto (2013) which found that the increase of RUP in the ration was not influenced by the intake of DM. The intake of OM was improved with decreasing RUP in the current study. These observations were in accordance with Kiran and Mutsvangwa (2007) who found that increasing dietary RDP from 60-70% of CP increased OM intake (ranging from 1.2-1.4 kg day^{-1}) in growing lambs. It has been reported that dietary RDP can promote intake by enhancing gastrointestinal motility (Egan and Moir, 1965).

There is many information on the effects of Forages:Concentrates (F:C) ratio on DMI, growth performance and production in housing-feeding cows. In this study the

Table 2: Nutrient intake of dairy cows receiving control and HRUP

	Ration	
Nutrient	Control	HRUP
Forages		
DM (kg DM/head/day)	7.15	7.09
OM (kg DM/head/day)	6.21	6.16
CP (kg DM/head/day)	0.67	0.66
CF (kg DM/head/day)	2.35	2.34
TDN (kg DM/head/day)	4.09	4.05
Concentrate		
DM (kg DM/head/day)	4.49	4.45
OM (kg DM/head/day)	3.95	3.93
CP (kg DM/head/day)	0.83	0.84
CF (kg DM/head/day)	0.75	0.71
TDN (kg DM/head/day)	2.53	2.62
Total intake		
DM (kg DM/head/day)	11.64	11.54
OM (kg DM/head/day)	10.15	10.10
CP (kg DM/head/day)	1.50	1.50
CF (kg DM/head/day)	3.10	3.05
TDN (kg DM/head/day)	6.63	6.67

DM: Dry matter, OM: Organic matter, CP: Crude protein, CF: Crude fibre, TDN: Total digestible nutrient and HRUP: High rumen undrgraded protein

ratio of F:C is 60:40, with the difference RUP value of the control group 27.47% and the HRUP group 32.78%, still showed a normal cow performance. Cantalapiedra-Hijar *et al*. (2009) observed DMI was not affected with the percentage of concentrate increasing in the diet from 30-70% in goats. Aguerre *et al*. (2011) found that increasing F:C ratios (47:53, 54:46, 61:39 and 68:32) in the diet had no effect on DMI of Holstein cows. A similar result was observed by Agle *et al*. (2010), who reported no change in DMI of lactating dairy cows fed diets contained 52 and 72% concentrate feeds. In contrast, Desnoyers *et al*. (2008) reported that DMI of dairy goats was increased (2.69-2.88 kg day^{-1}) with increasing the percentage of concentrate in the diet from 30-60%. Similarly, Murphy *et al*. (2000) noted that cows fed 30:70 diets of F:C ratio had a significantly higher DMI than cows fed 50:50 diets.

Dietary fibre content (CF) had no influence on OM intake in this trial. Commonly, an increase in dietary CF can be achieved by increasing the level of concentrates. Therefore, the results of the current study are comparable with those obtained by Cantalapiedra-Hijar *et al*. (2009) and Ramos *et al*. (2009) who found no effect of dietary concentrate:forage ratios (30:70 and 70:30) on OM intake in both goats and sheep fed above maintenance. The differences in CF of the diets were mainly due to the variance in CF concentrations. However, Allen (2000) noted that no effect of CF ranging from 25-40% was found on DM intake in dairy cows, although feed intake generally decreases with increasing CF. The CF in concentrate was high (coffee husks and corn tumpi), hence the need/requirement of fiber still can be fulfilled. This means that the intake of forages was low, the negative effect in the digestive process was not happened since the concentrates given still have high structural carbohydrates. This condition could be used as a reference by farmers, especially in the dry season where the forages were difficult to get and relatively expensive. Miller (1979) reported that the energy could affect the efficiency of the ration used, excess energy in ration and also caused decrease ration efficiency used and tended to be accumulated in the body fat. One of the disadvantage was the excess of the amino acids is increased further and these need to be deamination and excreted, with consequent reduction in the energy value of the diet and increased pollution.

The high protein intake was caused by concentrates in ration has high protein content. There were not just low energy and high protein content in the ration caused low protein efficiency but the ratio of energy:protein should be considered to get better protein efficiency. Thus, the condition should be considers for protein protection to avoid rumen degradation. Protein and energy intake from control and treated rations were more than enough to fulfill the need of maintenance and production. This wasn't similar to the results of research by Encinias *et al*. (2005) that there was no difference in intake of DM on dairy cows fed Brome grass hay (9.6% CP) which is supplemented by undegraded protein. However, most studies suggests that increasing energy intake will increase both content and production of protein in milk and milk production is improved by increasing CP intake (De Peters and Cant, 1992).

Estrous response indication and behavior: The observation result of estrous response indication and behavior of dairy cows supplementation with HRUP show symptoms similar to those reported by Siregar *et al*. (2004) which showed symptoms of vulvar mucus and hang white, red and swollen vulva, as well as behavioral changes such as aggressive, bellowing-bellow and decreased appetite. Symptoms and estrous behavior cows in this study also supported by reported by Toelihere (2003) which mean the strain in Indonesia showed the same symptoms of estrous with cows in general, transparent white mucus from the vagina, vulva change into warm, red and wet and the cow became agitated, bellowing-bellow and decreased appetite. The cows wich used in the study showed that aggressive changes, trumpeted-bellow, decreased appetite and cows that secrete mucus indicates the cow estrous.

Dairy cows showed symptoms of quiet estrous (silent heat), where cows are asymptomatic clinically estrous but the ovaries ovulation and decreased appetite. Cows that showed decreased appetite and less aggressive, is likely to be

caused due to estrogen levels under the threshold value that could not stimulate the clinical estrous (Karmita *et al*., 2001). Dairy cows that have estrogen levels above a threshold value able to displaying symptoms and estrous behavior. The threshold value of each individual animal is different, according to the physiological conditions. The presence of inhibition of LH receptors in the corpus luteum, resulting in LH does not activate the enzyme adenylate cyclase that functions in the reaction step for the sustainability of the life of the corpus luteum. The enzyme is not active cause lysis of the corpus luteum and is unable to secrete the hormone progesterone. The process also followed by the secretion of Follicle Stimulating Hormone (FSH) causing estrous (Kaltenbach and Dunn, 2001).

Changes in the external genitalia dairy cows observed when estrous was the vulva look wet because the mucus which come from vagina and vulva were look reddish and swollen. Toelihere (2003) reported that when the vulva was touched by the hands will relatively feel warmer. Changes in the vulva and vaginal mucosa anestrous in dairy cows did not look slimy or dry and pale and no swelling. It agrees with report of Putro (2008) that the symptoms when the cows were in anestrous condition could be visible from the outside was vulvar not mucus or dry and pale and no swelling.

Progesterone hormone profile: Based on the statistical analysis shows that cow which is feeding with a HRUP indicated no significant effect on the concentration of progesterone hormone (Table 3). The average concentration of progesterone hormone in the control group 1, 5, 10, 15, 17, 21 and 26 days was 0.13±0.02, 0.26±0.24, 0.13±0.03, 0.10±0.04, 0.09±0.02, 0.09±0.01, respectively and 0.12±0.03 ng mL^{-1} with a range of 0.06-0.54 ng mL^{-1}. The treatment group was 0.13±0.04, 0.20±0.08, 1.72±2.23, 1.72±3.77, 3.66±3.76, 0.66±0.80 and 1.20±1.08 ng mL^{-1} with a range of 0.10-7.66 ng mL^{-1}.

The ovary's activity after giving birth is a very important consideration in order to improve reproductive performance. Developing and functioning of reproductive organs after birth is depend on the level of FSH and LH from the anterior pituitary is controlled by GnRH secreted by the hypothalamus. Hana (2004) reporting that the range of the progesterone hormone during estrous between 0.83±0.14-1.04±0.11 ng mL^{-1}. The similar report by Valdez *et al*. (2005) mention that the new progesterone hormone levels could be detect since four day after estrous. The low levels of the progesterone hormone during the estrous were due to a follicular phase and high levels of estrogen (Hafez, 2000). High progesterone concentrations probably decrease LH pulsatility and the early decrease of estradiol allows an early rebound of FSH concentrations, promoting the emergence of the second follicular wave. Thereafter, the emergence of the subsequent wave is also advanced (Pang *et al*., 2010). This is in agreement with pharmacological studies performed in the cow (Adams *et al*., 1992).

Changes in the concentration of the progesterone hormone could be seen in Fig. 1. It could be shown that feeding with a HRUP capable of affecting the function of the corpus luteum to produce high amount of progesterone. The highest concentration of progesterone (3.12-7.66 ng mL^{-1}) occurred on the 17th day of estrous cycle. Although, progesterone concentration indicating no significant differences between the two conditions, but the average treatment on 17th day was higher than the controls variable (3.66 vs. 0.09 ng mL^{-1}). These results were contrary with the research by Astuti (2000) that a cow will show the highest concentrations of progesterone on 16th day after estrous.

The concentration of progesterone in Fig. 1 shows that the HRUP group began to increase graph in 5th day. Furthermore, in 10th, 15th-17th day, the concentration of progesterone hormone appeared to increase significantly and decreased in 17th-21st day. On 21st day, the concentration of

Table 3: Average concentration of progesterone hormone profile in day of estrous cycle

	Group (ng mL^{-1})	
Day of estrous cycle	Control	HRUP
1	0.13±0.02	0.13±0.04
5	0.26±0.24	0.20±0.08
10	0.13±0.03	1.72±2.23
15	0.10±0.04	1.72±3.77
17	0.09±0.02	3.66±3.76
21	0.09±0.01	0.66±0.80
26	0.12±0.03	1.20±1.08

HRUP: High rumen undrgraded protein

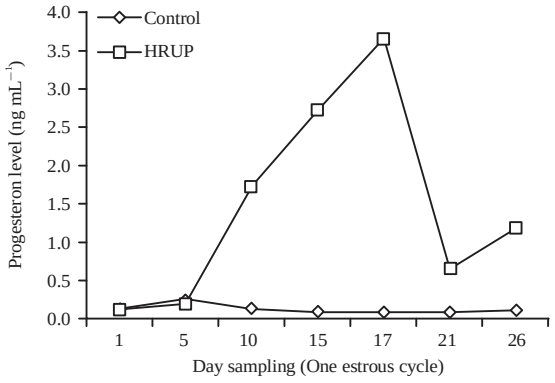

Fig. 1: Curves concentration of progesterone hormone profile in day of estrous cycle

the progesterone hormone is relatively low due to the corpus luteum regression and estrous imminent. Hafez (2000) reported that the corpus luteum regresses from 16th day of the estrous cycle, thus resulting in lower concentrations of the progesterone hormone. In the control group also began to increase in the 5th day, but in the next day decreased until 21st day. This indicates that the cows in the control group did not undergo fertilization.

CONCLUSIONS

It was concluded that HRUP supplementation on early lactation dairy cows could not increase the concentration of profile progesterone hormone. However estrous response value as well as estrous indication and behavior was normal.

ACKNOWLEDGMENTS

Authors are thankful to the Dairy Cow Units, Department of Agriculture, Daerah Istimewa Yogyakarta for providing experimental facilities, materials and technical support throughout this experiment. Thanks are also extended to Dirjen Dikti Ministry of Education and Culture, Indonesia for Beasiswa Unggulan (Superior Scholarship).

REFERENCE

Adams, G.P., R.L. Matteri and O.J. Ginther, 1992. Effect of progesterone on ovarian follicles, emergence of follicular waves and circulating follicle-stimulating hormone in heifers. J. Reprod. Fertil., 96: 627-640.

Agle, M., A.N. Hristov, S. Zaman, C. Schneider, P.M. Ndegwa and V.K. Vaddella, 2010. Effect of dietary concentrate on rumen fermentation, digestibility and nitrogen losses in dairy cows. J. Dairy Sci., 93: 4211-4222.

Aguerre, M.J., M.A. Wattiaux, J.M. Powell, G.A. Broderick and C. Arndt, 2011. Effect of forage-to-concentrate ratio in dairy cow diets on emission of methane, carbon dioxide and ammonia, lactation performance and manure excretion. J. Dairy Sci., 94: 3081-3093.

Allen, M.S., 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci., 83: 1598-1624.

Astuti, P., 2000. [The comparison of progesterone content which was contained within various samples (blood, milk and urine) by using RIA]. Buletin Peternakan, 24: 51-56, (In Indonesian).

BPS., 2010. [Statistics Indonesia 2010]. Badan Pusat Statistik (BPS), Jakarta, Indonesia, (In Indonesian).

Cantalapiedra-Hijar, G., D.R. Yanez-Ruiz, A.I. Martin-Garcia and E. Molina-Alcaide, 2009. Effects of forage: Concentrate ratio and forage type on apparent digestibility, ruminal fermentation and microbial growth in goats. J. Anim. Sci., 87: 622-631.

DRG., 2007. Progesterone ELISA (EIA-1561). Division of DRG International Inc., Frauenbergstr, Germany, pp: 3-5.

De Peters, E.J. and J.P. Cant, 1992. Nutritional factors influencing the nitrogen composition of bovine milk: A review. J. Dairy Sci., 75: 2043-2070.

Delany, K.K., K.L. Macmillan, C. Grainger, P.C. Thomson, D. Blache, K.R. Nicholas and M.J. Auldist, 2010. Blood plasma concentrations of metabolic hormones and glucose during extended lactation in grazing cows or cows fed a total mixed ration. J. Dairy Sci., 93: 5913-5920.

Desnoyers, M., C. Duvaux-Ponter, K. Rigalma, S. Roussel, O. Martin and S. Giger-Reverdin, 2008. Effect of concentrate percentage on ruminal pH and time-budget in dairy goats. J. Anim. Sci., 2: 1802-1808.

Egan, A.R. and R.J. Moir, 1965. Nutritional status and intake regulation in sheep. I. Effects of duodenally infused single doses of casein, urea and propionate upon voluntary intake of a low-protein roughage by sheep. Aust. J. Agric. Res., 16: 437-449.

Encinias, A.M., G.P. Lardy, J.L. Leupp, H.B. Encinias, L.P. Reynolds and J.S. Caton, 2005. Efficacy of using a combination of rendered protein products as an undegradable intake protein supplement for lactating, winter-calving, beef cows fed bromegrass hay. J. Anim. Sci., 83: 187-195.

Gunn, P.J., R. Lemenager and A. Bridges, 2014. Excess rumen undegradable protein alters parameters of reproductive function in beef cows. Animal Industry Report No. ASL R2852. http://lib.dr.iastate.edu/ans_air/vol660/iss1/17/.

Hafez, E.S.E., 2000. Anatomy of Male Reproduction. In: Reproduction in Farm Animals, Hafez, E.S.E. and B. Hafez (Eds.). 7th Edn., Chapter 1, Lippincott William and Wilkins, USA., ISBN: 9780683305777.

Hana, A., 2004. [The concentration normal of progesterone on estrous import cows and estrous local cows which live in low urea]. J. Sain Vet., 22: 57-59, (In Indonesian).

Hartadi, H., S. Reksohadiprodjo and A.D. Tillman, 2005. [Tables of Feed Composition for Indonesia]. Gadjah Mada University Press, Yogyakarta, (In Indonesian).

Hartantyo, S., 1995. Calculation of percent progesterone in skim milk fraction when centrifugation temperature and butterfat of whole milk are known. Bull. FKH-UGM, 14: 1-6.

Hernawan, A.E., 2014. Effect of banana peel application in ration on hematological level, nitrogen retention and body weight gain of heat exposed broiler chicken. Sci. Pap.: Ser. D: Anim. Sci., 42: 101-107.

Kaltenbach, C.C. and T.G. Dunn, 2001. Endocrinology of Reproduction. In: Reproduction in Farm Animals, Hafez, E.S.E. (Ed.). 3rd Edn., Lea and Febiger, Philadelphia, pp: 241-255.

Karmita, M., R.R. Noor and A. Farajallah, 2001. [Purity test of Bali cattle by using isoelectric focusing methods]. Media Peternakan, 24: 94-100, (In Indonesian).

Kiran, D. and T. Mutsvangwa, 2007. Effects of barley grain processing and dietary ruminally degradable protein on urea nitrogen recycling and nitrogen metabolism in growing lambs. J. Anim. Sci., 85: 3391-3399.

Litoi, G., 2014. Research on feeding dairy cows in puerperal period. Sci. Pap.: Ser. D: Anim. Sci., 57: 157-160.

Miller, W.J., 1979. Dairy Cattle Feeding and Nutrition. Academic Press, New York, ISBN-3: 978012395895, Pages: 428.

Murphy, M., M. Akerlind and K. Holtenius, 2000. Rumen fermentation in lactating cows selected for milk fat content fed two forage to concentrate ratios with hay or silage. J. Dairy Sci., 83: 756-764.

Pang, X.S., Z.Y. Wang, T.G. Zhu, D.Z. Yin, Y.L. Zhang, L. Meng and F. Wang, 2010. Concentrations of progesterone and estradiol in peripheral plasma during the estrous cycle and after ovariectomy in Huanghuai goats of high or poor prolificacy. Asian-Australasian J. Anim. Sci., 23: 188-196.

Putro, P.P., 2008. [The dynamics of dominant follicular and corpus luteum development after estrous synchronization in Friesian Holstein Crossbreed]. Ph.D. Thesis, Gadjah Mada University, Yogyakarta, (In Indonesian).

Ramos, S., M.L. Tejido, M.E. Martinez, M.J. Ranilla and M.D. Carro, 2009. Microbial protein synthesis, ruminal digestion, microbial populations and nitrogen balance in sheep fed diets varying in forage-to-concentrate ratio and type of forage. J. Anim. Sci., 87: 2924-2934.

Rochijan, B. Rustamadji and Kustono, 2014. Effect of 3% outdated milk powder supplementation in commercial concentrates on reproduction performance dairy cattle. Proceedings of the 16th Asian-Australasian Association of Animal Production Societies Congress, November 10-14, 2014, Yogyakarta, pp: 2260-2263.

Siregar, T.N., N. Areuby, G. Riady and Amiruddin, 2004. [The influence of giving PMSG to the response of ovary and embryo quality in prepruberty local goats]. Media Kedokteran Hewan, 20: 108-112, (In Indonesian).

Supardi, U.S., 2013. Statistical Applications in Research. Change Publication, Jakarta.

Thayeb, T.M.H., 2015. Profile of Indonesia. Embassy of the Republic of Indonesia, November 24, 2015.

Toelihere, M.R., 2003. Increasing the success rate and adoption of artificial insemination for genetic improvement of Bali cattle. Proceedings of the Workshop on Strategies to Improve Bali Cattle in Eastern Indonesia, February 4-7, 2002, Bali, Indonesia, pp: 29-33.

Valdez, K.E., S.P. Cuneo, P.J. Gorden and A.M. Turzillo, 2005. The role of thecal androgen production in the regulation of estradiol biosynthesis by dominant bovine follicles during the first follicular wave. J. Anim. Sci., 83: 597-603.

Widyobroto, B.P., 1997. [Effect of formaldehide treatment to soybean meal on protein degradation in the rumen and digestibility of undegraded protein in the intestine]. Proceedings of the Seminar Nasional II Ilmu Nutrisi dan Makanan Ternak, July 15-16, 1997, Bogor, pp: 33-34, (In Indonesian).

Widyobroto, B.P., S.P.S. Budhi and A. Agus, 2001. Effect of undegraded levels on rumen fermentation parameters kinetic and microbial protein synthesis in friesian holstein grade cows. Journal of the Indonesian Tropical Animal Agriculture, Faculty of Animal Science, Diponegoro University, Special Edition, pp: 211-221.

Widyobroto, B.P., 2013. [Implemented dairy cattle ration formulation based on intestine protein degradation in Indonesia]. Pidato Pengukuhan Guru Besar Pada Fakultas Peternakan, Universitas Gadjah Mada, Yogyakarta, (In Indonesian).

Zetina-Cordoba, P., M.E. Ortega-Cerrilla, M.T.S. Torres-Esqueda, J.G. Herrera-Haro, E. Ortega-Jimenez, J.L. Reta-Mendiola and J. Vilaboa-Arroniz, 2012. Reproductive response of ewes fed with Taiwan grass hay (*Pennisetum purpureum* Schum.) supplemented with Duckweed (*Lemna* sp. and *Spirodela* sp.). Asian-Australasian J. Anim. Sci., 25: 1117-1123.