International Journal of

Dairy Science

ISSN 1811-9743

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS International Journal of Dairy Science

ISSN 1811-9743
DOI: 10.3923/ijds.2016.28.34

Research Article
Impact of High Rumen Undegraded Protein (HRUP) Supplementation to Blood Urea Nitrogen and Reproduction Performance in Early Lactation Dairy Cows

Rochijan, Budi Prasetyo Widyobroto and Ismaya

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

Abstract
The objective of this study was to determine the impact of High Rumen Undegraded Protein (HRUP) supplementation to blood urea nitrogen and reproduction performance in early lactation dairy cows. Twelve early lactation Friesian Holstein cows were divided into two groups. Six cows were used as a control group and other 6 cows as HRUP 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. Drink water was given by *ad libitum*. The observed variable were feed intake, performance reproduction (postpartum mating, service per conception, days open) and Blood Urea Nitrogen (BUN). The results indicated that postpartum mating (91.83±31.16 vs. 81.33±19.83 day), service per conception (1.50±0.55 vs. 1.17±0.41 time) and days open (142.33±66.87 vs. 97.33±41.52 day) between controls and HRUP group were not significant ($p>0.05$). The average BUN value between controls (15.58±1.99 mg dL^{-1}) and HRUP group (18.09±1.50 mg dL^{-1}) were significant ($p<0.05$). The conclusion is HRUP supplementation in early lactation dairy cows would be impact on BUN value, but no impact on reproduction performance of dairy cows.

Key words: Rumen undegraded protein, reproduction performance, blood urea nitrogen, dairy cows

Received: September 25, 2015 Accepted: October 23, 2015 Published: December 15, 2015

Citation: Rochijan, Budi Prasetyo Widyobroto and Ismaya, 2016. Impact of high rumen undegraded protein (HRUP) supplementation to blood urea nitrogen and reproduction performance in early lactation dairy cows. Int. J. Dairy Sci., 11: 28-34.

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 main produce of dairy cows are milk, calve and by product (faeces). There is a critical period on dairy cows performance especially for those with high milk production. It happens in the first lactation period, which is the first quarter of lactation. It is also called the first three semesters (month) of lactation (Rochijan *et al*., 2014). The efforts to increase milk production or productivity cows are by increasing genetic quality and by feed management. If the feed intake provided is insufficient, either in terms of quantity or quality, then the cow will utilize the nutrients available within its body by mobilizing the stored energy within their body tissues. As the consequence of this mobilization, the cow will lose so much weight, which will affect the cow production and reproduction performance (Mundingsari, 2006; Rochijan *et al*., 2014). Baumann *et al*. (2004) also explained the low production or reproduction response in postpartum cows is associated with low forage digestibility or low intake of Amino Acids (AA), which cause a mobilization of adipose and muscle tissue.

Most previous studies, cows were grazing on various types of pasture and also supplemented with concentrates such as degraded and undegraded protein. Increasing Rumen Undegraded Protein (RUP), or replacing Rumen Degraded Protein (RDP) sources with RUP sources, in concentrates has not given consistent effect on milk production or composition (Santos *et al*., 1998; Bargo *et al*., 2003). In several studies RDP sources such as soybean meal, sunflower meal, urea or rapeseed meal have been replaced with RUP sources such as animal protein blend, Fish Meal (FM), maize gluten meal, expeller soybean meal, blood meal, feather meal or heat-treated rapeseed meal (Bargo *et al*., 2003). Positive responses to RUP supplementation, above that observed with energy, are most likely in early lactation, high yielding, multiparous cows, when high amounts of concentrate grain are fed (Hongerholt and Muller, 1998; Schor and Gagliostro, 2001). Evaluations of the levels of RUP with friesian holstein cows with high or low genetic merit did not show interactions between the RUP level and genetic potential, but demonstrated beneficial effects on production and reproduction performance (Westwood *et al*., 2000).

Feed management plays important role because in a short time its effect on productivity cows (Agus, 1997). Daily feed consumption depends on feed palatability level, body weight, production, reproduction, health and factors of the cow itself. Dairy cows consume feed (DM) about 3% of their body weight (Perry, 1984). Dairy cows with high milk production consume more feed than those with low production under normal conditions (Veerkamp *et al*., 2003).

This study was conducted to determine the impact of High Rumen Undegraded Protein (HRUP) supplementation to blood urea nitrogen and reproduction performance in early lactation dairy cows. Hopefully, it helps dairy farmers, dairy industry and related government in their effort to solve problem of low quality of concentrate, also low performance reproduction in early lactation dairy cows and the development of feed processing technology cow.

MATERIALS AND METHODS

This experiment was conducted in Dairy Cow Units, Department of Agriculture, Daerah Istimewa Yogyakarta. 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, Universitas Gadjah Mada.

Animals and diets: This experiment used twelve early lactation holstein friesian cows were divided into two groups, permanent enclosure models stanchion barn with cement floors which feed and drink water. Six cows were used as a control group and other 6 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 (Table 1). Rumen undegraded protein for control group was 27.47% and HRUP group was 32.78%. 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, absorbent paper, spectrophotometer merk microlab, timer and distilled water.

Data and sample collection

Data of reproduction performance: Data collection was performed by jotting reproduction performance on each friesian holstein dairy cows. Reproduction performance includes postpartum mating, service per conception and days open were collected. Postpartum mating refers to the time from when a cow calves until the first time she is bred. Service per conception refers to the average number of time the cow mates to that cow were pregnant. Days open refers to the time from when a cow calves until when she conceives.

Sampling and analysis of blood urea nitrogen: At 7 and 90 day of the experiment, blood samples from each individual animal were collected aseptically via caudalis artery (3-4 h after the morning feeding) into 3 mL vacutainer K3 Ethylene Diamine Tetraacetic Acid (EDTA) tubes. The tubes were rolled gently several times to ensure enough anticoagulant mixing 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 plasma is stored at a temperature of -20°C freezer until blood urea nitrogen analysis. After all collected, plasma concentration of urea nitrogen were determined using an auto-analyzer.

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

RESULTS AND DISCUSSION

Chemical composition of feed ingredients 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. The control group getting concentrate containing Dry Matter (DM) 88.24%, Crude Protein (CP) 18.55%, Total Digestible Nutrient (TDN) 56.94% and Rumen Undegraded Protein (RUP) 27.47%, while the HRUP group getting concentrate containing Dry Matter (DM) 89.06%, Crude Protein (CP) 18.83%, Total Digestible Nutrient (TDN) 58.91% and Rumen Undegraded Protein (RUP) 32.78%. Content ingredients and nutrient compositions concentrates formulation of experimental diet was presented in Table 1.

Feed intake: Table 2 showed the average intake of dairy cows during the experiment. 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, 6.67 kg DM/head/day, respectively. This was similar to the report 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. The high protein intake was caused by concentrates in ration has high protein content.

Table 1: Ingredients and nutrient compositions concentrate formulation of experimental diet

	Group	
	Control	HRUP
Concentrates formulation	(% DM)	
Ingredients		
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	100.0
	Group	
	Control (%)	HRUP (%)
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 and TDN: Total digestible nutrient, [a]: Results of the analysis in sacco degradation and formulas Widyobroto (1997), [b]: Results of the formula Hartadi *et al.* (2005)

Table 2: Feed and nutrient intake of dairy cows receiving control and HRUP

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

DM: Dry matter, OM: Organic matter, CP: Crude protein, TDN: Total digestible nutrient

Dry Matter Intake (DMI) has special importance to meet nutrient requirement of fresh cows to maintain their health and production. Le Floc'h *et al*. (2004) and Sejrsen *et al*. (2006) that reported low DMI and deficiency in nutrient supply, specially protein and asam amino acids has led to immunosuppression and incidence of metabolic disorders on body cows. Thus, diets that have higher levels of crude protein and RUP are effective in maintaining of production and Body Condition Score (BCS) (NRC., 2001).

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.

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 HRUP 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 research 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).

Table 3: Average of reproduction performance in dairy cows

	Group	
Parameters	Control	HRUP
Postpartum mating (day)^{ns}	91.83±31.16	81.33±19.83
Service per conception (time)^{ns}	1.50±0.55	1.17±0.41
Days open (day)^{ns}	142.33±66.87	97.33±41.52

ns: Non significant (p>0.05)

Performance reproduction: Success of reproduction performance is mainly determined by days open, the longer days open means the longer calving interval which affects the lowering amount of milk production throughout the cow life. The cow pregnancy detection was carried out or checked by artificial inseminator from Department of Agriculture, Daerah Istimewa Yogyakarta by performing a detection of pregnancy. The results overall of reproduction performance was presented in Table 3.

Postpartum mating (PPM): The result shows that the shortest average PPM found in the HRUP group is 81.33±19.83 days and control group is 91.83±31.16 days. Based on the PPM data obtained, the HRUP group still in normal range of the first time cows mating after parturition. It’s higher than, Aboozar *et al*. (2012) results about HRUP value 57.50±7.30. This range confirms, Salisbury and Vandemark (1993) opinion that first mating is the best performed within 60-90 days after parturition cows. The lower of PPM value, it will be more efficient the reproduction performance of dairy cows. The difference in PPM on each cow is caused by differences in the occurrence of estrous as the result of milk production difference which will cause weight loss. Therefore the time to achieve the normal body weight for reproduction will be varies. These variations are caused by differing negative energy, which triggers reproductive problems such as sub estrous and silent heat or quiet ovulation, as stated by Peters and Ball (1995), that very low nutrition quality and quantity are the main causes of anestrous and the prolonging of estrous period after parturition. Berry *et al*. (2003) adds that the PPM normal results of optimal (60-90 days) and this could be attributed to different factors. Most researchers suggest that the reason for the delay in interval to first service is greater negative energy balance in modern dairy cows. Negative energy balance delays the continuation of ovarian activity.

Service per conception (S/C): The result shows that the shortest average S/C found in the HRUP group is 1.17±0.41 times and control group is 1.50±0.55 times. It’s lower than, Aboozar *et al*. (2012) research about HRUP value 1.76±0.34. The average estrous cycle in each group could be calculated because there were dairy cows with service per conception score 1, therefore upon these dairy cows the estrous cycle could not be determined. This long interval is due to silent estrous caused by negative nutrient imbalance. This is parallel with Perry (1984) opinion, who said that lack of energy after giving birth will extend estrous interval and will delay ovulation. Yifat *et al*. (2009) stated that appropriate and in time heat detection and insemination could be attributed to lower or higher number of service of per conception. The findings of the present study on services per conception suggested comparatively better insemination services at the herds during the period of the study, because S/C value is normal and noting >2.00.

Days Open (DO): The result shows that the shortest average S/C found in the HRUP group is 97.33±41.52 days and control group is 142.33±66.87 days. It's higher than Aboozar *et al*. (2012) study about HRUP value 90.38±1.53. The control group has long days open, this not in line with Antiyatmi (2009) that the optimal days open or the safest time to bring a cow into mating again is between 60-90 days after parturition, if it is too long, production during the next lactation period will decrease between 75-110 days. The long days open period is caused by the decrease of PPM and by the high service per conception. The long days open period will extend calving interval, which will decrease the number of calves produced throughout the cow lifetime. This in line with the opinion of Foley *et al*. (1972) that the long days open will lower the cows productivity during its lifetime.

Blood Urea Nitrogen (BUN): The average BUN value between controls and HRUP were significant ($p<0.05$). The average BUN value of each group is 15.58±1.99 vs. 18.09±1.50 mg dL^{-1}. BUN concentrations are presented in Table 4. The BUN value of the control group fed with CP content of 18.55% and RUP 27.47% lower than the BUN group HRUP with CP feed containing 18.83 and 32.78% RUP. The BUN values will decrease in animals offered the high synchrony index diets. The results disagreed with Sinclair *et al*. (2000), who found that plasma urea levels were unaffected by a synchronous treatments in temperate fed basal diet. Urea is a small molecule that equilibrates between the reproductive tract and plasma (Duby and Trischler, 1986). Deleterious effects of high blood urea have been demonstrated on sperm viability (Duby and Trischler, 1986) and embryo survival both *in vitro* (Dasgupta *et al*., 1971) and *in vivo* (Saitoh and Takahashi, 1977). In addition, feeding high rumen degradable protein diets altered uterine pH in heifers (Elrod and Butler, 1993), hormonal patterns during the estrous cycle and early pregnancy (Berardinelli *et al*., 2001) and thus have a direct effect on gametes (Canfield *et al*., 1990) and embryo survival (Berardinelli *et al*., 2001). Recently, in this experiment it was found that beef cattle fed a diet containing a higher synchrony index had a lower BUN, this would indicate that a synchronous diet increased N-utilization efficiency. Synchronizing the rate of dietary energy and nitrogen release is a possible way to avoid excess blood urea nitrogen and excessively high levels of plasma ammonia, leading to improved reproductive efficiency. However, Ferguson *et al*. (1993) reported that a blood urea nitrogen concentrations exceeding 20 mg% was associated with a reduced conception rate in lactating cows. Since the BUN concentration in this experiment was lower than 20 mg%, BUN would not affect the fertility in dairy cows.

Table 4: Average concentration of blood urea nitrogen

	Group	
Sampling	Control	HRUP
Blood I (first week of the study)	15.60±2.43	18.30±2.09
Blood 2 (the last week of the study)	15.55±3.94	17.88±1.79
Average*	15.58±1.99	18.09±1.50

*Significant ($p<0.05$)

CONCLUSION

The conclusion is HRUP supplementation to early lactation dairy cows would be impact on BUN value, but no impact on reproduction performance of dairy cows.

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).

REFERENCES

Aboozar, M., H. Amanlou, A.M. Aghazadeh, K.N. Adl, M. Moeini and T. Tanha, 2012. Impacts of different levels of RUP on performance and reproduction of Holstein fresh cows. J. Anim. Vet. Adv., 11: 1338-1345.

Agus, A., 1997. Effect of the type concentrate as an energy source on milk production and composition in high yielding dairy cows. Bulletin of Animal Science, Faculty of Animal Science, Gadjah Mada University, 21: 45-54.

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

Antiyatmi, 2009. Reproductive performance of friesian holstein dairy cows of three age group at Pasir Salam animal husbandry Sukabumi West Java. Faculty of Animal Science. S.Pt. Undergraduate Thesis, Gadjah Mada University, Indonesia.

Bargo, F., L.D. Muller, E.S. Kolver and J.E. Delahoy, 2003. Invited review: Production and digestion of supplemented dairy cows on pasture. J. Dairy Sci., 86: 1-42.

Baumann, T.A., G.P. Lardy, J.S. Caton and V.L. Anderson, 2004. Effect of energy source and ruminally degradable protein addition on performance of lactating beef cows and digestion characteristics of steers. J. Anim. Sci., 82: 2667-2678.

Berardinelli, J.G., J. Weng, P.J. Burfening and R. Adair, 2001. Effect of excess degradable intake protein on early embryonic development, ovarian steroids and blood urea nitrogen on days 2, 3, 4 and 5 of the estrous cycle in mature ewes. J. Anim. Sci., 79: 193-199.

Berry, D.P., F. Buckley, P. Dillon, R.D. Evans, M. Rath and R.F. Veerkamp, 2003. Genetic relationships among body condition score, body weight, milk yield and fertility in dairy cows. J. Dairy Sci., 86: 2193-2204.

Canfield, R.W., C.J. Sniffen and W.R. Butler, 1990. Effects of excess degradable protein on postpartum reproduction and energy balance in dairy cattle. J. Dairy Sci., 73: 2342-2349.

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.

Dasgupta, R.R., A.B. Kar and M.D. Dhar, 1971. Spermicidal activity of urea. Indian J. Exp. Biol., 9: 414-415.

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.

Duby, R.T. and J.P. Trischler, 1986. Effect of dietary protein on reproduction in the ewe and dairy cow. Proceedings of the New England Dairy Feed Conference, (NEDFC'86), Concord, NH., pp: 339-339.

Elrod, C.C. and W.R. Butler, 1993. Reduction of fertility and alteration of uterine pH in heifers fed excess ruminally degradable protein. J. Anim. Sci., 71: 694-701.

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.

Ferguson, J.D., D.T. Galligan, T. Blanchard and M. Reeves, 1993. Serum urea nitrogen and conception rate: the usefulness of test information. J. Dairy Sci., 76: 3742-3746.

Foley, R.C., D.L. Bath, F.N. Dickinson, H.A.A. Tucker and R.D. Appleman, 1972. Dairy Cattle: Principles, Practices, Problems, Profits. Lea and Febiger, Philadelphia, PA., ISBN-13: 9780812103090, Pages: 693.

Hartadi, H., S. Reksohadiprodjo and A.D. Tillman, 2005. Tables of Feed Composition for Indonesia. Gadjah Mada University Press, Yogyakarta.

Hongerholt, D.D. and L.D. Muller, 1998. Supplementation of rumen-undegradable protein to the diets of early lactation Holstein cows on grass pasture. J. Dairy Sci., 81: 2204-2214.

Le Floc'h, N., D. Melchior and C. Obled, 2004. Modifications of protein and amino acid metabolism during inflammation and immune system activation. Livest. Prod. Sci., 87: 37-45.

Miller, W.J., 1979. Dairy Cattle Feeding and Nutrition. Academic Press, New York, ISBN-13: 978012395895, Pages: 428.

Mundingsari, R.D., 2006. The effect of substitution energy and protein concentrates supplement on milk production and some parameters reproduction of early lactation Friesian Holstein Crossbred cows. MP. Thesis, Gadjah Mada University, Indonesia.

NRC., 2001. Nutrient Requirements of Dairy Cattle. 7th Rev. Edn., National Academy Press, Washington, DC., Pages: 333.

Perry, T.W., 1984. Animal Life-Cycle Feeding and Nutrition. Academic Press Inc., San Diego, ISBN-13: 978-0125520607, Pages: 319.

Peters, A.K. and P.J.H. Ball, 1995. Reproduction in Cattle. Butterworth and Co., Sevenoaks.

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. [Milk production and composition of Friesian Holstein Crossbred cows with 3% outdate milk powder supplementation in early lactation period]. Proceedings of Seminar Nasional Sinergi Pangan, Pakan, dan Energi Terbarukan (SPRINT 2014), October 21-23, 2014, Yogyakarta, pp: 325-331.

Saitoh, M. and S. Takahashi, 1977. Embryonic loss and progesterone metabolism in rats fed a high energy diet. J. Nutr., 107: 230-241.

Salisbury, G.W. and A.L. Vandemark, 1993. Physiology Reproduction and Artificial Insemination in Cattle. Gadjah Mada University Press, Yogyakarta.

Santos, F.A.P., J.E.P. Santos, C.B. Theurer and J.T. Huber, 1998. Effects of rumen-undegradable protein on dairy cow performance: A 12-year literature review. J. Dairy Sci., 81: 3182-3213.

Schor, A. and G.A. Gagliostro, 2001. Undegradable protein supplementation to early-lactation dairy cows in grazing conditions. J. Dairy Sci., 84: 1597-1606.

Sejrsen, K., T. Hvelplundand and M.O. Nielson, 2006. Ruminant Physiology: Digestion, Metabolism and Impact of Nutrition on Gene Expression, Immunology and Stress. Wageningen Academic Publishers, Wageningen, The Netherlands, ISBN-13: 9789076998640, Pages: 600.

Sinclair, K.D., L.A. Sinclair and J.J. Robinson, 2000. Nitrogen metabolism and fertility in cattle: I. Adaptive changes in intake and metabolism to diets differing in their rate of energy and nitrogen release in the rumen. J. Anim. Sci., 78: 2659-2669.

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

Veerkamp, R.F., B. Beerda and T. van der Lende, 2003. Effects of genetic selection for milk yield on energy balance, levels of hormones and metabolites in lactating cattle and possible links to reduced fertility. Livestock Prod. Sci., 83: 257-275.

Westwood, C.T., I.J. Lean, J.K. Garvin and P.C. Wynn, 2000. Effects of genetic merit and varying dietary protein degradability on lactating dairy cows. J. Dairy Sci., 83: 2926-2940.

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 Seminar Nasional II Ilmu Nutrisi dan Makanan Ternak, July 15-16, 1997, Bogor, pp: 33-34.

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]. Faculty of Animal Science, Gadjah Mada University, Yogyakarta.

Yifat, D., B. Kelay, M. Bekana, F. Lobago, H. Gustafsson and H. Kindahl, 2009. Study on reproductive performance of crossbred dairy cattle under smallholder conditions in and around Zeway, Ethiopia. Livest. Res. Rural Dev., Vol. 21.

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.