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Estradiol Concentration and Estrous Behaviour in Tropical Heifers Supplemented with Undegradable Protein

Novia Dimar Dwitarizki, Ismaya, Budi Prasetyo Widyobroto, Sigit Bintara and Tety Hartatik
Faculty of Animal Science, Universitas Gadjah Mada, Fauna Street 3, Bulaksumur,
55281 Yogyakarta, Indonesia

Abstract: This research was aimed to examine the effect of Rumen Undegradable Protein (RUP) supplementation on estradiol profile and estrous behaviour in heifers maintained in tropical climate. A total of 36 Brahman Cross (BX) beef heifers were divided into 2 groups, control group (ration without supplemented RUP) and treatment group (ration with supplemented RUP). The heifers were inseminated on third days after ovaries checking with USG and synchronized with $PGF_{2\alpha}$. Blood sampling was conducted to measure the estradiol concentration in 5 points: during the estrous (0); 5; 17; 19 and 21 days. Estrous behaviour were recorded 4 times a day. Results showed no significant effect ($p>0.05$) of feeding RUP between control and treatment group to estradiol concentration (130.96±40.96; 134.75±39.00; 137.31±47.24; 133.48±36.90; 143.89±55.83 pg mL^{-1} vs. 126.59±34.86; 124.93±38.82; 123.20±42.39; 126.11±46.45; 126.25±32.66 pg mL^{-1}) and estrous behaviour but there was a significant correlation ($p<0.01$) between estradiol concentration to estrous behaviour. In conclusion, the level of estradiol concentration and estrous behaviour in heifers were not effected by undegradable protein supplementation.

Key words: Estradiol, estrous, heifers, tropical, rumen undegradable protein

INTRODUCTION

Beef cattle population is the biggest large livestock population in tropical area in Indonesia which reached 14.73 mln. head in 2014 with the 68.14% of females beef cattle. This is due to the adaptation of beef cattle (*Bos indicus*) to high temperature and humidity in rough and harsh tropical environment. Beefcattle (*Bos indicus*) has a tendency to exhibit estrous during the night and in a short duration. This specificity greatly effected the efficiency of the Artificial Insemination (AI) program in beef cattle managed in tropical areas which required an appropriate time of estrous detection (Baruselli *et al*., 2004).

Detection of estrous in the right time is crucial in beef cattle husbandry. The mistaken in detection of estrous and poor reproductive performance are related to loss of profit due to the extended calving intervals, a waste of maintenance cost and minimized the total of calf production as the consequence of shorter productive times (Roelofs *et al*., 2010). Poor reproductive performance in beef cattle can impact on theunbalance between calf stock and slaughtered animals.

Many factors effected the cattle reproductive performance such as nutrients contained in given feed. Proteinis one of the important nutrients influencing reproductive function. Protein consumed by ruminants was first used for the ruminal microbes. The protection of high-quality protein source with high degradation in the rumen needs to be done, so that most of the protein in feed material in the form of amino acids can be absorbed in the intestine and utilized by livestock.

Supplementation of RUP is generally performed in dairy cows to meet the protein requirements for an increase in milk production but the current study tried to do RUP supplementation in beef cattle. According to Kane *et al*. (2004) RUP supplementation may effected reproductive performance in beef heifers through the change in pituitary and ovarian function. In beef heifers, the optimum level of RUP supplementation seemed to improve FSH secretion and pituitary expression.

In mammalian species, estrogen is produced in ovary when FSH binds to its receptors within granulosa cells of the developing follicle andmainly secreted as estradiol-17β (Rozell and Okrainetz, 2009). Estrogen affects the FSH secretionand synthesis through positive and negative feedback to the pituitary and hypothalamus (Greco and Stabenfeldt, 2007). Estrous signs and behaviour are associated with increasing levels of estrogen followed by ovulation (Squires, 2010). The concentrations of estrogen in the bloodstream provided valuable information about their reproductive status. This

Corresponding Author: Ismaya, Faculty of Animal Science, Universitas Gadjah Mada, Fauna Street 3, Bulaksumur, 55281 Yogyakarta, Indonesia

description prompted the researchers to examine the effect of RUP supplementation on estradiolhormone profiles and estrous symptoms and behaviourin beef heifers. We hypothesized that supplementation of RUP would increase the estradiol concentration and will further increase the detection of estrous to overcome the problem in estrous detection in beef cattle managed under tropical conditions.

MATERIALS AND METHODS

The present study was conducted on brahman cross heifers maintained at pandanaran arta perkasa company, Klaten, Central Java, Indonesia. Hormones sample analysis performed in the laboratory of Physiology, Faculty of Veterinary Medicine, Universitas Gadjah Mada while the feed proximate analysis carried out in the Animal Nutrition Laboratory, Faculty of Animal Science, Universitas Gadjah Mada. The research lasted for 9 months, from June 2015-February 2016. The experimental procedures used in this study in accordance with the ethical guidelines and regulations set forth and approved by Universitas Gadjah Mada animal care and use committee.

Animals, management experimental design: A total of 36 BX heifers aged 29.08±7.20 month (about 2.5 year) and average liveweight of 399.67±45.19 kg (BCS = 3.18±0.32) (1-5 point scale of body condition scoring system) were randomly assigned to 1 of 2 treatments, control and treatment group. The control group (diet without RUP supplementation) were divided into 3 flocks, each flock consists of 6 Cattle. The treatment group (dietwith supplementing RUP) were also divided into 3 flocks each flock also consists of 6 Cattle. Samples were taken by taking 2 Cattle in each flock randomly both in control and treatment group in order to obtain samples of 6 Cattle from the control group and 6 Cattle from the treatment group.

The cows were managed in tropical climate. The temperature and humidity observation of the environment in this study showed the temperature in the morning at 05:00 am ranges from 22-23°C with humidity of 80-90%. Temperature and humidity during the day at 01:00 pm reach 34-35°C with humidity ranges from 40-50% while in the afternoon at 05:00 pm the temperature ranges 29°C humidity 62%. There was a decrease in temperature and increase in humidity in the evening at 09:00 pm about 25-26°C with humidity of 70-81%.

The six flocks were housed at a time in the 6 paddock during the period of the study. The floor of the paddock was covered with cement and the roof made from asbestos material with monitor roofing type. At one corner of the paddock, there was provision of drinking water trough with the running tap water made from cement and the feeding trough also made from cement. The paddock hedge was surrounded by metal pipes. The cows were weighed with T-scale NTW (Tscale Electronics Mfg, Kunshan, China) digital scales with capacity of 1 ton and accuracy of 1 kg and the feed was balanced with Acis AP-30X (Libra Emas Permata Company, Jakarta, Indonesia) digital scales with capacity of 30 and accuracy of 0.002 kg.

Body Condition Score (BCS) assessment was based on a standard assessment for beef cow using 1-5 point scale and conducted on the beginning and the end of the research. All heifers were fed concentrates (from PT. Widodo Makmur Perkasa) ad libitum water and rice straw with the proportion of rice straw:concentrate of 40:60. The feeding based on the DM 3% of body weight and CP 13% of DM. The feed consisted of control diet (without supplementation RUP) and treatment diet (by supplementing RUP) with the total RUP difference between the control and treatment ration of 8%. This research used chemically treated soybean meal created by a patent chemical process using formaldehyde to increase its resistance to ruminal degradation (ruminally protected soybean meal as RUP source). Adaptation of livestock to feed were carried out for 3 weeks prior to data collection. The ingredients and nutrient composition of the experimental diets can be seen in Table 1 and 2.

Table 1: Ingredients of control (without RUP supplementation) and treatment diet (with RUP supplementation)

	Diets	
Ingredients (%)	Control diet	Treatment diet
Cassava pomace	009.40	009.45
Maize grain	009.10	009.14
Maize cobs	006.07	006.09
Wheat bran	003.64	003.66
Sugarcane molasses	001.82	001.83
Kopra meal	007.94	007.93
Palm kernel meal	042.49	042.41
Soybean meal	018.74	000.00
Protected soybean meal	000.00	018.70
Urea slow release	000.49	000.49
Premix	000.30	000.30
Total	100.00	100.00

Table 2: Nutrient composition of control (without RUP supplementation) and treatment diet (with RUP supplementation)

	Diets	
Nutrient composition (%)	Control diet	Treatment diet
Dry Matter (DM)[a]	91.69	91.59
Organic Matter (OM)[a]	89.62	89.67
Crude Protein (CP)[a]	18.09	18.12
Ether Extracts (EE)[a]	03.70	03.61
Crude Fiber (CF)[a]	14.12	14.13
Non-Nitrogen Extract (NNE)[a]	53.70	53.81
Total Digestible Nutrients (TDN)[b]	51.73	51.88
Rumen Degradable Protein (RDP)[c]	57.54	48.89
Rumen Undegradable Protein (RUP)[c]	42.15	50.81

[a]Based on analysis of Animal Nutrition Laboratory, Faculty of Animal Science, Universitas Gadjah Mada; [b]Based on formula of Hartadi *et al.* (2005); [c]Based on in sacco degradation analysis and formula of Widyobroto

Synchronization, blood sampling and estradiol analysis: After checking ovaries with Ultrasonography (USG) heifers were assigned to $PGF_{2\alpha}$ treatment administrated at the beginning of the synchronization protocol after palpation. Heifers were synchronized using single injection of $PGF_{2\alpha}$ intramuscularly (25 mg of Dinoprost tromethamine, Lutalyse™, Pfizer Manufacturing, Belgium); the estrous heifers were inseminated using Wagyu sperm straws in 3 days after $PGF_{2\alpha}$ injection. The day of estrous was marked as day 0. Six heifers from the control group and 6 heifers from the treatment group blood samples were taken in the fifth period of time in the estrous cycle, i.e., when estrous (day 0) 5, 17, 19 and 21 days after estrous, thus obtained 60 samples for estradiol hormone analysis.

Blood samples were collected from the caudal vein. Samples werestored at 4°C for 12-24 h and centrifugedfor 20 min at 2,200 rpm to obtain the serum, the serum was collected and stored a -20°C until assayed (Barnwell *et al*., 2015). Estradiol concentration in serum was determined by Enzyme Linked Immuno Sorbent Assay (ELISA) DRG Estradiol ELISA (DRG Instruments Gmbh, Germany).

Estrous symptoms and behaviour observation: Data were collected from 12 BX heifers for recording estrous behaviour. The observers recorded the symptoms and behavioural signs of estrous4 times a day (at 05:00 a.m., 01:00 p.m., 05:00 p.m. and 09:00 p.m. for 60 mins each time) since the cows initiate to exhibit estrous, include restlessness, mounted but not standing, mounting on other cows, standing to be mounted, vulvar mucus discharge, and tumefaction and reddening of vulvar mucus membrane. This is consistent with Landaeta-Hernandez who reported the detection of estrousin the morning, at noon in the afternoon and at night. The estrous detection time was divided into four periods within 24 h.

Statistical analyses: Data of nutrient intake, body weight and BCS, estradiol concentration and estrous behaviour were analyzed by independent sample t-test. The relationship between estradiol and estrous behaviour analyzed by Bivariate correlations (pearson) using SPSS Version 17.0 statistics (Astuti, 2007).

RESULTS AND DISCUSSION

Nutrient consumption: The effect of RUP supplementation on nutrient intake was similar between control diet and treatment diet (Table 3). There were no significant effect on DMI, OM, CP, EE, CF, NFE and TDN consumption between treatment (RUP supplementation) and control (no RUP supplementation) group.

Table 3: Mean±SD nutrients intake in Brahman Cross heifers fed rations without RUP supplementation (control group) and with RUP supplementation (treatment group)

Variables	Control group	Treatment group
Consumption (kg DM/head/d)		
Concentrate	6.750±1.04[a]	06.86±0.95[a]
Rice straw	4.400±0.74[a]	04.39±0.75[a]
DM	11.15±1.22[a]	11.25±1.18[a]
OM	10.49±1.14[a]	10.59±1.10[a]
CP	01.55±0.16[a]	01.57±0.15[a]
EE	00.34±0.04[a]	00.33±0.03[a]
CF	02.71±0.31[a]	02.72±0.31[a]
NFE	05.90±0.70[a]	05.97±0.66[a]
RUP	00.67±0.07[a]	00.80±0.07[b]
RDP	00.87±0.09[a]	00.76±0.07[b]
TDN	06.20±0.00[a]	06.26±0.58[a]
Requirement (kg DM/head/d)[X]		
DM	9.80	9.80
CP	0.73	0.73
RUP	0.05	0.05
RDP	0.64	0.64
TDN	4.90	4.90
Balance (kg DM/head/d)		
DM	+1.35	+1.45
CP	+0.82	+0.84
RUP	+0.62	+0.75
RDP	+0.23	+0.12
TDN	+1.30	+1.36

[a,b]Different superscript letters in the same row indicate significant differences ($p<0.05$); [X] based on NRC

Table 4: Mean±SD body weight, ADG and BCS in Brahman Cross heifers fed rations without RUP supplementation (control group) and with RUP supplementation (treatment group)

Variables	Control group	Treatment group
Initial body weight (kg)	410.17±53.38[a]	387.50±49.86[a]
Final body weight (kg)	455.33±60.72[a]	446.17±42.19[a]
ADG (kg)	000.65±0.18[a]	000.84±0.18[a]
Initial BCS	003.25±0.42[a]	003.17±0.26[a]
Final BCS	003.42±0.38[a]	003.83±0.26[b]

[a,b]Different superscript letters in the same row indicate significant differences ($p<0.05$)

The DM, OM, CP, EE, CF, NFE and TDN consumption in both groups had about the same value between the control and treatment group but the RUP intake was higher ($p<0.05$) in treatment than control group (0.80±0.07 kg DM/head/day vs. 0.67±0.07 kg DM/head/day) and RDP consumption was higher ($p<0.05$) in control than treatment group (0.87±0.09 kg vs. 0.76±0.07 kg DM/head/day). It because both groups received rations with relatively similar in feed nutrient content and only different in RUP and RDP content with 8% in different between groups.

Body weight and body condition score change: The comparison of initial and final body weight, Average Daily Gain (ADG) as well as initial and final BCS was shown in Table 4. There were no significant difference between the control and the treatment group in initial body weight, final body weight, ADG and the initial BCS. The control group had a relatively greater in initial BCS than the

Table 5: Estradiol serum concentration in Brahman Cross heifers fed rations without RUP supplementation (control group) and with RUP supplementation (treatment group)

		Estradiol concentration (pg mL^{-1})				
Groups	Sample	0	5	17	19	21
Control	K1	124.93	184.66	193.77	188.81	193.77
	K2	204.84	181.27	190.92	167.71	228.06
	K3	097.12	100.22	097.30	100.97	104.39
	K4	144.88	127.03	144.61	128.45	137.82
	K5	092.72	096.94	083.59	099.12	083.90
	K6	121.29	118.40	113.67	115.80	115.37
Average (mean±SD)		130.96± 040.96^{a}	134.75± 039.00^{a}	137.31± 047.24^{a}	133.48± 036.90^{a}	143.89± 055.83^{a}
Treatment	T1	178.28	156.31	159.23	181.94	162.81
	T2	101.53	112.00	103.24	093.24	106.74
	T3	096.94	087.39	098.39	086.10	102.86
	T4	163.11	188.11	192.34	188.81	172.75
	T5	110.76	105.17	098.75	105.95	099.12
	T6	108.93	100.60	087.23	100.60	113.25
Average (mean±SD)		126.59± 034.86^{a}	124.93± 038.82^{a}	123.20± 042.39^{a}	126.11± 046.45^{a}	126.25± 032.66^{a}

$^{a, b}$Different superscript letters in the same colomn indicate significant differences ($p<0.05$)

Table 6: The comparison of estrous symptoms and behaviour recorded in Brahman Cross heifers fed rations without RUP supplementation (control group) and with RUP supplementation (treatment group)

	Number of symptoms observed/animal/estrous (mean±SD)		
Estrous symptoms and behaviour	Control group	Treatment group	Frequency (%)
Restlessness	5.50±3.56^{a}	6.00±3.22^{a}	71.88
Mounted but not standing	1.33±1.51^{a}	0.33±0.82^{a}	10.42
Mounting on other cows	0.33±0.82^{a}	0.83±0.75^{a}	07.29
Standing to be mounted	3.67±1.63^{a}	3.33±1.75^{a}	43.75
Mucus discharge	7.67±0.52^{a}	6.83±0.98^{a}	90.63
Tumefaction and reddening of vulvar mucus membrane	7.17±0.75^{a}	7.67±0.52^{a}	92.71

$^{a, b}$Different superscript letters in the same row indicate significant differences ($p<0.05$)

Table 7: Relationship between estradiol concentration and estrous appearance

Trait	Pearson correlation	p-values
Estrous symptoms and behaviour to		
Estradiol concentration day 0	0.934**	<0.01
Estradiol concentration day 5	0.895**	<0.01
Estradiol concentration day 17	0.904**	<0.01
Estradiol concentration day 19	0.873**	<0.01
Estradiol concentration day 21	0.888**	<0.01

**Correlation is significant at the 0.01 level (2-tailed)

treatment group (3.25±0.42 vs. 3.17±0.26) but in final BCS, the treatment group had a greater ($p<0.05$) BCS than the control group, 3.83±0.26 and 3.42±0.38, respectively.

Estradiol serum concentration: The comparison of estradiol serum concentration between control and treatment group can be seen in Table 5. Statistical analysis showed no significant differenceson estradiol serum concentration betweencontrol group (no supplementation, 670±70 g RUP/heifer/day) andtreatment group (with supplementation, 800±70 g RUP/heifer/day) on day 0, 5, 17, 19 and 21.

Estrous symptoms and behaviour: Brahman Cross heifers initiated onset of estrous in 2 days after Lutalyse ($PGF_{2\alpha}$) injection. Results for estrous symptoms and behaviour observation was shown in Table 6. Number of estrous symptoms observed/animal/estrous did not significantly differ ($p>0.05$) between heifers in control and in treatment group. Tumefaction and reddening of vulvar mucus membrane had greatest frequency (92.71%) during the observation while mounting on other cows had smallest frequency (7.29%) during observation. Standing to be mounted only observed in 43.75% of the heifers in estrous during the observation. It suggested that all the heifers both in the control group and the treatment group are in a phase of estrous after synchronizing with $PGF_{2\alpha}$ 3 days before but there were unsignificant differences in the appearance of estrous in control and treatment group due to the unsignificant difference in concentration of estradiol between control and treatment group.

Relationship between estradiol serum concentration and estrous symptoms and behaviour: Bivariate correlation coefficients between estrous behaviour (Table 6) and estradiol serum concentration in different time are presented in Table 7. Based on Table 7, it can be seen that estrous symptoms and behaviour and estradiol serum concentration on 0, 5, 17, 19 and 21 days were found significantly and positively correlated ($p<0.01$). It explained that the increased in estradiol concentrations along with the increased in estrous symptoms and behaviour.

These results of non-significantly differences in DMI between control and treatment group were supported by Jahani-Moghadam *et al.* (2015) they presented that the different ratio of RDP: RUP in different feed sources had unsignificant effect in DM in take in dairy cows. This results was also supported by Jabbar *et al.* (2013) study, they indicated that the variation of RUP percentage in feed either in 30, 40, 50 or 60% RUP of total CP had no effect in DM, CP, NDF and ADF in take in early lactating buffaloes. Diets with iso-caloric and isonitrogenous in composition had similar DM, CP, ADF and NDF in take, though the diets contained different degradability of protein.

According to Chen *et al.* (2011) the DM and OM intake were higher ($p<0.05$) in cattle fed 25 and 35% RUP in diet than in cattle fed 15% RUP in diet but there was no significant difference between 25 and 35% RUP in DM and OM intake. Milad *et al.* (2010) also presented that the treatment of diet, protein and RUP source had no effect ($p>0.05$) on the DM and OM consumption.

Our finding showed no significant difference between treatment in final body weight but our finding showed

higher BCS ($p<0.05$) at the end of study in treatment group. These results were similar with Aboozar *et al.* (2012) who reported an unsignificant difference between the experimental diet (6.65 RUP; 7.72 RUP; 8.79% RUP) in body weight changes and Aboozar and Niazi (2013) who reported higher ($p<0.05$) BCS using greater amount of RUP in diet. The selection of highly digestible RUP source manipulated the supply of metabolizable protein and amino acid balance.

Robinson *et al.* (2004) also reported that body condition score was greater ($p<0.05$) in dairy cows supplemented with RUP. Van *et al.* (2007) suggested that glucogenic nutrients such as RUP supplements, lead to increase the BCS by increasing DM intake, therefore decreased body tissue mobilization. Glucogenic diets, compared with lipogenic diets, resulted in energy deposition in the body while BCS is an indicator of energy balance.

Energy balance is the difference between available nutrients from feed intake and body reserves and the nutrients requirements for maintenances. A reduced in energy balance increase the duration of postpartum anestrous and cause delay puberty. Itindicated by low levels of blood glucose which increases the negative feedback by estradiol on GnRH release and decreases LH secretion. The negative energy balance also decreased the level of IGF-1 together with decreased LH and contributes to decrease follicular growth and maturation, reduced the follicles size and increased turnover of the dominant follicle (Squires, 2010).

Tamminga (2006) stated that the negative energy balance due to high RDP in diets may happened because the energy involved in detoxification and excretion of excess ammonia. An insufficient energy and an imbalance between glucogenic, lipogenic and aminogenic nutrients in early lactation initiate the adverseeffects of a negative energy balance on fertility. The end products of the degradation of an excess of aminogenic energy such as ammonia and urea may also affected directly on fertility in the preovulatory stage.

Based on Table 5, it can be seen that each group has a concentration of estradiol with a large standard deviation in each sampling point. It because the estradiol concentration has considerable variation between individuals and species, phase of estrous cycle, to various hormonal treatment.

Estradiol-17β follicular fluid concentration in large follicle has thegreatest concentration compare with in small and medium follicle and the estradiol-17β serum concentration was strongly correlated with the follicular fluid along with the increasing diameter of the follicular (Kor, 2014; Aller *et al.*, 2013) stated that the effect of FSH treatment in follicular fluid concentrations of estradiol in beef cows showed that the estradiol concentration with no FSH treatment was higher than with FSH treatment (155.6±51.1 vs. 51.7±4.5 pg mL^{-1}). Different hormonal treatment was performed by Bleach *et al.* (2001) they showed that the average concentration of estradiol during synchronization with $PGF_{2\alpha}$ on day-6 relative to LH surge ranges from 1.5 pg mL^{-1} and the estradiol concentrations reach the highest range insome hours before the LH surge, about 13.5 pg mL^{-1}.

The results from Stevenson *et al.* (2012) study showed that dairy cows treated with $PGF_{2\alpha}$ 3 days prior to GnRH had similar concentrations of estradiol to the cows treated with double $PGF_{2\alpha}$ injection interval of 14 days (1.7±0.1 vs. 1.8±0. 1 pg mL^{-1}).

Scully *et al.* (2014) found that the concentration of circulating estradiol was significantly increase between day 2 and 3 and between day 7 and 8 in beef heifersduring the emergence of the follicular waves. There was a significant increase in the concentration of estradiol 2 days before ovulation up to 5 pg mL^{-1} but it ranges about 1.5 pg mL^{-1} during ovulation.

Mondal *et al.* (2006) gave different results, they demonstrated that during the peri-estrous period, the highest peak concentration of estradiol was range 26 pg mL^{-1} and the highest peak concentration of total estrogen was range 55 pg mL^{-1}. It occurred at 15 h before the onset of estrous in mithun cattle.

Other factor that may effected the estrogen concentrations was the pregnant status after insemination. It was evident from the drop in estrogen at day 21 which indicated the cows were not returned to estrous. This statement was consistent with Youngquist (2007) they stated that when a cow was not able to return to estrous at around 18-24 days after breeding, it suggested that conception has occurred. The most general cause of failure of cows to have normal estrous cycles is a pregnancy because bovine embryos indicated their presence around 15-17 days after ovulation. The maternal estrous cycle was suspended and the corpus luteum was maintained until parturition.

CONCLUSION

Our study foundno significant differences on estradiol serum concentration between group without RUP supplementation (control group) and group with RUP supplementation (treatment group) on 0, 5, 17, 19 and 21 days post-estrous. This is similar with the results of Kane *et al.* (2004) that no significant differences were observed in serum estradiol-17β following 30-32 days of RUP supplementation between treatments for low

(115 g RUP) mid (216 g RUP) and high (321 g RUP) RUP supplementation (2.0; 1.6; 1.7±0.4 pg mL^{-1}, respectively). Diskin *et al.* (2003) stated that the effect of nutrients on ovarian function include the systemic effects in the synthesis and release of GnRH at hypohalamus level, the control of FSH, LH and GH synthesis and secretion at anterior pituitary level and the regulation in follicle growth, steroid synthesis and the binding of growth factors to its binding proteins at ovarian level.

Brahman cross heifers treated with lutalyse ($PGF_{2\alpha}$) intiated to exhibit estrous in second day after injection. Perry *et al.* (2014) showed that the peak estradiol concentration range 10 until 11 pg mL^{-1} in interval 27 until 48 h after $PGF_{2\alpha}$ injection in cows that exhibited estrous.

In bos indicus cattle, standing to be mounted was only expressed in less duration, about 5.15±2.05 and ended in 20.17±2.32 h before ovulation time. It make the detection of this behaviour in estrous cows was uncertain (Layek *et al.*, 2011). Negussie *et al.* (2002) found that approximately one out of eight of the cows in estrous did not mount other cows, so it make more difficulties in finding cows in standing to be mounted. Layek *et al.* (2011) observed that the estrous signs like reddening of vulva, tumefaction of vulva, mucus discharge and uterine tone were exclusively found during the estrous period and their degree of expression was also more intense in case of bos indicus cattle with intensity of 84.48; 84.34; 70.17; and 91.36%, respectively. According to Lyimo *et al.* (2000) standing to be mounted by another cow was observed in <50% of the estrous cows whereas, this behaviour has been the primary sign to determine the appropriate time for artificial insemination.

The behavioural characteristics of estrous in cows was also effected by the changes in environment, like temperature and humidity which differentiated the frequent of estrous behaviour during the day and the night (Galina and Orihuela, 2007).

Landaeta-Hernandez *et al.* (2002) stated that heat stress also affected the expression of estrous in the tropically adapted Brahman cows. The potential interactions between type of estrous (synchronized or spontaneous estrous) and THI can exert a significant influences on estrousbehavioural characteristics in Brahman cows.

Lyimo *et al.* (2000) found that estradiol concentration was highly correlated with estrous behaviour. The concentration of estradiol reached its highest level at the same time as the highest score of estrousbehaviour. The increasing of estradiol concentrations will in turn stimulate the estrous behaviour and LH secretion to cause ovulation.

Mondal *et al.* (2006) showed that the plasma estradiol concentrations in mithun cows increased from 6 days before estrous and reached a maximum level on the day of estrous (day 0) with range 18 pg mL^{-1} and then decreased to a basal level on day 4 of the cycle with range 4 pg mL^{-1} and exhibiting minor fluctuation. Estrous behaviour was significantly and positively correlated with maximum estradiol peak. The significant and positive correlation between total estrogen during the peri-estrous period and behavioural estrous obviouslyimplied that other form of estrogen were also crucial for exhibiting estrous symptoms and behaviour.

Based on this study, it can be concluded that the level of estradiol concentration and estrous symptoms and behaviour in beef heifers were not effected byundegradable protein supplementation. The positive and significant correlation between estrous symptoms and behavioral and estradiol serum concentrations on 0, 5, 17, 19 and 21 days showed that the increasing in estradiol concentration along with the increased in estrous symptoms and behavioral.

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