Relationships among Feed Intake, Blood Metabolites, Follicle Size and Progesterone Concentration in Ewes Exhibiting or Not Exhibiting Estrus after Estrous Synchronization in the Tropics

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ABSTRACT

The aim of this study was to determine the relationships among feed intake, blood metabolites, follicle size, and progesterone (P_4) concentration in ewes that did or did not exhibit estrus after estrous synchronization. Sixteen cycling, Merino ewes were synchronized for 12 days with a controlled internal drug release (CIDR) devices (300 mg P_4). Ewes were classified by estrous behavior as exhibited estrus (n=12) or did not exhibit estrus (n=4). Blood samples were collected at 0, 24, 48, and 72 h after CIDR removal to measure glucose, blood urea nitrogen (BUN), and P_4 concentrations. Follicular size was examined by transrectal ultrasonography on the day of CIDR removal. Ewes exhibiting estrus had greater concentrations of glucose at 24 and 48 h after CIDR withdrawal than ewes that did not exhibit estrus. Ewes exhibiting estrus had decreased concentrations of P_4 at 24 and 48 h after CIDR withdrawal compared with ewes that did not exhibit estrus. At the time of CIDR withdrawal, the diameter of largest follicle was observed greater in ewes exhibiting estrus than that in ewes not exhibiting estrus. By 48 h after CIDR removal, 91.7% of ewes that displayed estrous behavior had exhibited estrus. In all ewes, a significant positive relationship was observed between feed intake and glucose concentration. Follicle diameter was positively correlated with glucose and P_4 concentrations but was negatively correlated with time to onset of estrus in ewes exhibiting estrus. No relationship was detected between BUN and other parameters in all ewes. In conclusion, increased estrous activity in ewes synchronized to estrus may result from increased size of follicle, low P_4 levels, and sufficient production of glucose during the follicular development. Thus, these data highlight the importance of follicle diameter, glucose concentration, and time to onset of estrus in ewes exhibiting estrus under tropical conditions.

Keywords: Blood metabolites; estrous synchronization; ewes; follicle; tropical conditions
1. Introduction

Sheep have probably been present in Thailand for several hundred years (Falvey, 1980). Nevertheless, sheep was not a popular animal in comparison to other livestock animals, such as poultry, swine, cattle, and goat (Supakorn et al., 2013). In northern Thailand, the numbers of sheep based on data from the Department of Livestock Development, Thailand in 2014 were 4,708 heads. Moreover, the demand of mutton slightly increased every year (Supakorn et al., 2013).

The optimization of the reproductive management is necessary for improvement and development of animal production in sheep under tropical conditions (Contreras-Solis et al., 2009). Modern sheep husbandry is improving the efficiency of extensive production and is controlling the reproductive process for intensive production (Lindsay, 1991; Ozyurtlu et al., 2010). For this reason, exogenous hormones are administered in order to increase the reproductive performance of ewes. A progesterone-releasing controlled internal drug releasing (CIDR) treatment is usually preferred for the control of estrous synchronization in sheep (Kaya et al., 2013). The efficacy of estrous synchronization in small ruminants (sheep and goats) depends on many factors, including estrous synchronization protocols, age of puberty, body condition, feed intake, blood metabolites, hormonal response, and follicular development (Kusina et al., 2001; Ali, 2007; Ying et al., 2011; Khanthusaeng et al., 2012; Naqvi et al., 2012). However, few studies have investigated the relationships among feed intake, blood metabolites, hormonal response, and follicular development. We hypothesized that differences between ewes exhibiting or not exhibiting estrus after estrous synchronization could be partly explained by differences in feed intake, blood metabolites, hormonal response as well as follicular development. Therefore, the objective of the present study was to determine the relationships among feed intake, blood metabolites, follicle size, and progesterone ($P_4$) concentration in ewes that did or did not exhibit estrus after estrous synchronization.

2. Materials and methods

2.1. Experimental site

This experiment was conducted at Goat and Sheep farm, Department of Animal and Aquatic Science, Faculty of Agriculture, Chiang Mai University situated at longitude 98° 55’ 54.3” E, latitude 18° 45’ 40.3” N and an altitude of 312 m above sea level. The climate was tropical with distinct differences between dry (Oct–April) and wet (May–September). Average annual rainfall based on data from the Northern Meteorological Center, Thai Meteorological Department, Thailand for the period 2013 to 2014 is 1263 mm. The ambient temperature and relative humidity during the experiment (September 2013 to August 2014) ranged from 21.6 to 32.4 °C and 51.5 to 89.1%, respectively.

2.2. Animals and management

Sixteen mature crossbred ewes (Merino to Thai native; 12 to 24 months of age; 28 to 30 kg of body weight) were used for this study. Ewes were kept in individual pens and water was available to ewes ad libitum. All ewes received pangola hay (91.7% dry matter; 6.4% crude protein; 33.7% crude fiber; 1.9% ether extract; 12.7% ash) ad libitum and concentrate (89.0% dry matter; 14.6% crude protein; 5.5% crude fiber; 6.2% ether extract; 7.3% ash) at 1.5% of body weight. All ewes received two meals of equal allotments of feed at 0900 and 1700 h and the refusals were removed and weighed each time.

2.3. Estrous synchronization and estrous detection

Estrous cycle was synchronized in all ewes with a controlled internal drug release devices (Eazi-Breed CIDR, 300 mg $P_4$; Pfizer Animal Health, New Zealand), for 12 days. All animals were observed for estrus twice daily (at 0800 and 1800 h) within 24 to 72 h after CIDR removal. Observation for estrus commenced 24 h after CIDR
withdrawal. Estrus was detected twice daily for a minimum of 30 min per observation with teaser rams for 3 days. Commencement of estrus was defined as the time when the ewe first stood to be mounted by the ram (Ozyurtlu et al., 2010). Ewes were classified by estrous behavior as exhibited estrus (n=12) or did not exhibit estrus (n=4).

2.4. Ovarian ultrasonography and blood sampling

Transrectal ultrasonography was performed with a 7.5 MHz linear-array transducer (TOSHIBA JustVision 2000, Japan). Ewes were examined ultrasonographically on the day of CIDR removal to estimate the diameter of largest follicle. At each scan, the diameter of largest follicle was measured and recorded on follicular maps, which allowed identification for subsequent analyses. Jugular vein blood samples (10 ml) were collected into venipuncture at 0, 24, 48, and 72 h after CIDR removal to measure glucose, blood urea nitrogen (BUN), and P₄ concentrations.

2.5. Glucose, BUN, and P₄ assays

Plasma samples were obtained in a gray-top potassium oxalate/sodium fluoride tube and sent to the laboratory for glucose analysis by the hexokinase method (Aekplakorn et al., 2007). Blood urea nitrogen was determined by enzyme kinetic method. Serum P₄ concentrations were measured in duplicate by competitive enzyme-linked immunosorbent assay (competitive ELISA; adapted from Brown et al., 2005). Using duplicate 10 μl aliquots, the assay sensitivity was 0.201 ng/ml. Inter- and intra-assay coefficients of variation were 14.3 and 10.3%, respectively.

2.6. Statistical analyses

Data are presented as mean±SEM. Feed intake, glucose, BUN, and P₄ concentrations were analyzed with ANOVA with the general linear model (GLM) procedure of SAS (SAS Institute, Cary, NC, USA). Differences between means were evaluated by Student t test (Steel et al., 1997). Differences with P≤0.05 were considered significant, and those with 0.05<P<0.10 were considered a tendency. Simple linear correlations between specific variables were determined by using PROC CORR of SAS (SAS Institute, Cary, NC, USA) as previously described (Moonmanee et al., 2013).

3. Results and discussion

3.1. Feed intake, glucose, and BUN concentrations

Feed intake, plasma glucose, and BUN concentrations of ewes that exhibited estrus and those that did not exhibit estrus are illustrated in Fig. 1. At 0, 24, and 48 h after CIDR removal, total feed intake did not differ (P>0.05) in ewes exhibiting and not exhibiting estrus. However, total feed intake was higher in ewes exhibiting estrus than that in ewes not exhibiting estrus at 72 h (977.9±23.7 vs. 885.7±28.2 g DM/d, respectively) after CIDR withdrawal (Fig. 1a). Ewes exhibiting estrus had higher (P<0.05) concentrations of glucose at 24 and 48 h after CIDR removal compared with ewes not exhibiting estrus (55.1±2.2 vs. 47.0±2.1 mg/dl and 55.4±3.7 vs. 44.3±2.2 mg/dl for 24 and 48 h after CIDR withdrawal, respectively; Fig. 1c). The concentrations of BUN did not differ (P>0.05) between ewes exhibiting and not exhibiting estrus throughout the experimental period (Fig. 1c). The result of this study demonstrated that different pattern of feed intake after estrous synchronization has important consequences for subsequent estrous activity, possibly mediated by differences in glucose concentrations. The control of feed intake and regulation of energy balance are influenced by a number of factors (Scaramuzzi et al., 2006). In fact, a regulator of body energy content is apparently interfaced with a controller of feed intake that maintains a balance of energy input and output (Scaramuzzi et al., 2006). Furthermore, a novel observation of a previous study was indicated that restriction of dietary energy intake resulted in less body weight gain, delay, and suppression of estrus following synchronization (Koyuncu and Canbolat, 2009). In the present study, different feed intake, along with changed increasing glucose concentration, affected ovarian and estrous activities in synchronized ewes. In general, glucose
consumed by ovarian follicles can be used for energy production (Sutton-McDowell et al., 2010). Moreover, Ying et al. (2011) suggested that short-term different dietary intake, along with changed circulating glucose, insulin, and glucagon levels, affected folliculogenesis and intrafollicular environment.

Fig. 1. (a) Total feed intake, (b) glucose, and (c) BUN concentrations (±SEM) at 0, 24, 48, and 72 h after CIDR removal in ewes that exhibited estrus (dotted line; n=12) and those that did not exhibit estrus (solid line; n=4). Asterisks denote significant (*, P<0.05) differences between groups.

3.2. Largest follicle

The diameter of largest follicle at the time of CIDR removal was examined, but the diameter of ovulatory follicle prior to ovulation was not determined. Data for diameter of largest follicle is shown in Fig. 2. At the time of CIDR withdrawal in ewe exhibiting estrus, the size of largest follicle was 4.3±0.15 mm, significantly greater (P<0.05) than that for ewes not exhibiting estrus (3.4 ± 0.27 mm), and similar to previously reported that the size of preovulatory follicles at the time of sponge removal (on day 12) was 4.3±0.20 mm (Leyva et al., 1998). The time to onset of estrus in ewes exhibiting estrus in our study (37.0 ± 3.9 h) was similar to that in a previous study (Ozyurtlu et al., 2010). This implies that the largest follicle of ewes exhibiting estrus proceeded to ovulation while the largest follicle of ewe not exhibiting estrus became atretic. Furthermore, in ewes synchronized with medroxyprogesterone acetate-sponge for 12 days, the ovulatory follicles were heterogeneous in their growth patterns prior to ovulation. The fact that growth of some of these ovulatory follicles was extended while others grew very rapidly may have implications with regard to oocyte quality at the time of ovulation (Leyva et al., 1998). In the cattle model, cows that possessed a larger dominant follicle (DF) at CIDR withdrawal had a short interval to the onset of estrus than those that had a smaller DF (Utt et al., 2003).

Fig. 2. Mean (±SEM) diameter of largest follicle at the time of CIDR removal in ewes that exhibited estrus (solid bar; n=12) and those that did not exhibit estrus (open bar; n=4). Asterisks denote significant (*, P<0.05) differences between groups.
3.3. Serum $P_4$ concentrations

Data for serum $P_4$ concentrations of ewes that exhibited estrus and those that did not exhibit estrus is illustrated in Fig. 3. Ewes exhibiting estrus had decreased concentrations of $P_4$ at 24 and 48 h after CIDR withdrawal compared ($P<0.05$) with ewes that did not exhibit estrus (Fig. 3). At the time of CIDR withdrawal in ewes not exhibiting estrus, $P_4$ concentrations averaged 4.3 ± 0.7 ng/ml, but had declined to 1.8 ± 0.4 ng/ml by 24 h later, and to 1.3 ± 0.2 ng/ml by 48 h (Fig. 3). At the time of CIDR withdrawal in ewes exhibiting estrus, $P_4$ concentrations averaged 5.7 ± 0.8 ng/ml. At 24 h after CIDR withdrawal, $P_4$ concentration averaged 0.7 ± 0.4 ng/ml and declined to 0.5 ± 0.2 ng/ml at 48 h after CIDR withdrawal (Fig. 3). Similarly, a previous study demonstrated that $P_4$ concentrations at the time of CIDR removal averaged 5.1 ± 0.3 ng/ml and declined to 0.4 ± 0.04 by 20 h (Van Cleeff et al., 1998). Furthermore, the estrogenic status of the follicle seemed to be examined primarily by the concentration of $P_4$ circulating at the time of CIDR removal (Van Cleeff et al., 1998), which was higher for ewes exhibiting estrus compared with ewes not exhibiting estrus (5.7 ± 0.8 vs. 4.3 ± 0.7 ng/ml). In fact, $P_4$ exerts a negative feedback effect on the hypothalamus and pituitary to regulate gonadotrophin release, mainly directed towards luteinizing hormone (LH) release (Leyva et al., 1998). In ewes not exhibiting estrus, one possible explanation for this fact decreased levels of serum $P_4$ after CIDR removal were not adequate to suppress the hypothalamus-pituitary axis, resulting in estrous response (Leyva et al., 1998; Khanthusaeng et al., 2012). In addition, the estrous response in this study was 75.0% using CIDR treatment which is lower than what observed by Hashemi et al. (2006), who demonstrated a 93.3% estrous response when using CIDR and equine chorionic gonadotropin. In ewes exhibiting estrus, the time to onset of estrus was 37.0 ± 3.9 h after CIDR withdrawal, which is in agreement with Ozyurtlu et al. (2010).

3.4. Correlation coefficients between specific variables of ewes exhibiting or not exhibiting estrus

In ewes not exhibiting estrus, there was a positive correlation ($P<0.05$) between total feed intake and plasma concentrations of glucose (Table 1). In addition, follicle size tended to be positively correlated ($P=0.09$) with plasma concentrations of glucose (Table 1). No relationship was observed ($P>0.05$) between BUN and other parameters (Table 1). In ewe exhibiting estrus, total feed intake was positively correlated ($P<0.05$) with glucose concentration but tended to be negatively correlated ($P=0.08$) with time to onset of estrus (Table 2). Plasma concentrations of glucose tended to be positively correlated ($P=0.07$) with $P_4$ concentrations (Table 2). Moreover, follicle size was negatively correlated ($P<0.01$) with time to onset of estrus but was positively correlated ($P<0.05$) with glucose and $P_4$ concentrations (Table 2). However, no relationship was detected ($P>0.05$) between BUN and other parameters (Table 2). This result was in agreement with the observations made Catunda et al. (2013), who suggested that a proper nutrient intake in ewes resulted increased glucose concentration. Moreover, a previous study reported that a positive correlation between glucose concentrations and metabolic hormones (insulin and leptin) in sheep (Tokuda et
al., 2002). In fact, energy status (glucose) is generally considered to be a major nutritional factor that influences folliculogenesis and reproductive processes in ewes (Scaramuzzi et al., 2006; Catunda et al., 2013). Similar result was reviewed by Scaramuzzi et al. (2006), who reported that glucose infused for 3 or 5 days stimulated folliculogenesis by increasing the number of large follicles. These observations can be interpreted to suggest that follicle size at the time of CIDR removal is closely correlated with glucose, $P_4$ concentrations as well as estrous response in ewes exhibiting estrus. Furthermore, Somchit et al. (2007) suggested that follicular fluid glucose concentrations are closely correlated with plasma glucose concentrations. This implies that glucose concentrations became increased as follicle size increased probably because their energy requirement. In cattle, it has been shown that follicle size has a positive relationship with peak concentrations of estradiol, but only among cows that exhibiting estrus (Perry et al., 2014).

### Table 1
Correlation coefficients among variables evaluated for ewes that did not exhibit estrus ($n=4$).

<table>
<thead>
<tr>
<th>Items</th>
<th>Follicle size</th>
<th>Total feed intake</th>
<th>Glucose</th>
<th>BUN</th>
<th>$P_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total feed intake</td>
<td>$r = 0.719$</td>
<td>-</td>
<td>$r = 0.945$</td>
<td>$r = 0.807$</td>
<td>$r = 0.088$</td>
</tr>
<tr>
<td>$P &gt; 0.05$</td>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.05$</td>
<td>$P &gt; 0.05$</td>
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</tr>
<tr>
<td>Glucose</td>
<td>$r = 0.903$</td>
<td>$r = 0.945$</td>
<td>-</td>
<td>$r = 0.763$</td>
<td>$r = 0.059$</td>
</tr>
<tr>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.05$</td>
<td>-</td>
<td>$P &lt; 0.05$</td>
<td>$P &gt; 0.05$</td>
<td></td>
</tr>
<tr>
<td>BUN</td>
<td>$r = 0.502$</td>
<td>$r = 0.807$</td>
<td>$r = 0.763$</td>
<td>-</td>
<td>$r = 0.600$</td>
</tr>
<tr>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.05$</td>
<td>$P &gt; 0.05$</td>
<td></td>
</tr>
<tr>
<td>$P_4$</td>
<td>$r = -0.354$</td>
<td>$r = 0.088$</td>
<td>$r = -0.059$</td>
<td>$r = 0.600$</td>
<td>-</td>
</tr>
<tr>
<td>$P &gt; 0.05$</td>
<td>$P &lt; 0.05$</td>
<td>$P &gt; 0.05$</td>
<td>$P &gt; 0.05$</td>
<td>$P &gt; 0.05$</td>
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</tr>
</tbody>
</table>

### Table 2
Correlation coefficients among variables evaluated for ewes that exhibited estrus ($n=12$).

<table>
<thead>
<tr>
<th>Items</th>
<th>Follicle size</th>
<th>Total feed intake</th>
<th>Glucose</th>
<th>BUN</th>
<th>$P_4$</th>
<th>Onset of estrus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total feed intake</td>
<td>$r = 0.258$</td>
<td>-</td>
<td>$r = 0.602$</td>
<td>$r = 0.462$</td>
<td>$r = -0.073$</td>
<td>$r = -0.518$</td>
</tr>
<tr>
<td>$P &gt; 0.05$</td>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.05$</td>
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<tr>
<td>Glucose</td>
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<td>$r = 0.602$</td>
<td>-</td>
<td>$r = 0.120$</td>
<td>$r = 0.542$</td>
<td>$r = 0.448$</td>
</tr>
<tr>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.05$</td>
<td>-</td>
<td>$P &lt; 0.05$</td>
<td>$P &gt; 0.05$</td>
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<tr>
<td>BUN</td>
<td>$r = -0.102$</td>
<td>$r = 0.462$</td>
<td>$r = 0.120$</td>
<td>-</td>
<td>$r = -0.159$</td>
<td>$r = -0.203$</td>
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<td>$P &lt; 0.05$</td>
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</tr>
<tr>
<td>$P_4$</td>
<td>$r = 0.605$</td>
<td>$r = -0.073$</td>
<td>$r = 0.542$</td>
<td>$r = -0.159$</td>
<td>-</td>
<td></td>
</tr>
<tr>
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<td>$P &gt; 0.05$</td>
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<td>$r = -0.448$</td>
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<td>$r = -0.122$</td>
<td>-</td>
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### 4. Conclusions

Our present results support the hypothesis that differences between ewes exhibiting or not exhibiting estrus after estrous synchronization could be partly explained by differences in feed intake, blood metabolites, hormonal response, and follicular development. Moreover, increased estrous activity in ewes synchronized to estrus may result from increased size of follicle, low $P_4$ levels, and sufficient production of glucose during the follicular development. Thus, these data highlight the importance of follicle diameter, glucose concentration, and time to onset of estrus in ewes exhibiting estrus under tropical conditions.

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