

Effect of protein and energy levels on faecal progesterone concentration in Sabi ewes during oestrus cycle

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Abstract

The study investigated the reliability of using faecal progesterone as an indicator of ovarian activity in Sabi ewes and how this is affected by level of feeding. Three diets were formulated to simulate feed availability in communal areas of Zimbabwe, a below maintenance diet, a maintenance diet and an above maintenance diet. Faecal and blood samples were collected from 18 ewes (six in each treatment) once every two days for two oestrus cycles in order to determine progesterone levels. Diet had a significant effect on the plasma progesterone concentration ($P < 0.05$) and a non significant effect on faecal progesterone concentration ($P > 0.05$). On all diets, faecal progesterone concentrations were higher than plasma progesterone concentration. The pattern of faecal and plasma progesterone concentration were positively correlated in ewes on a maintenance diet ($r = 0.920$, $P < 0.05$) than on a below maintenance diet and an above maintenance diet ($r = 0.6881$, $P > 0.05$ and $r = 0.50$, $P > 0.05$). Response-relationship between faecal and plasma progesterone concentration profiles was non-linear ($R\text{-square} = 0.25$, $P > 0.05$) for ewes on an above maintenance diet and linear for a below maintenance diet and a maintenance diet ($R\text{-square} = 0.46$, $P < 0.05$ and $R\text{-square} = 0.84$, $P < 0.05$). Faecal progesterone can be used as an alternative method of monitoring the ovarian activity in ruminant animals on a maintenance level of feeding.

Keywords: Faecal progesterone, diet, ovarian activity, plasma progesterone, Sabi ewes

1.0 Introduction

Small ruminant production by resource-poor farmers in the communal areas of Zimbabwe is limited by low reproductive rates. Inadequate nutrition is one of the major limiting factor and main contributor to poor reproduction (Nyamukanza *et al.*, 2010, Clatworthy, 1998). Currently, the resource-poor farmers are striving

to increase the number of livestock they keep in order to uplift their life styles. There is need for a cost effective method for monitoring the reproductive performance especially ovarian function by small holder farming communities where basic facilities for collection, processing and storage from research animals are not available.

Progesterone is a steroid hormone secreted mainly by cells of the *corpus luteum*, placenta and adrenalin gland. In sheep the length of a normal oestrus cycle is 17 days, the *corpus luteum* attains full secretory activity between the sixth and eighth day and continues to secrete progesterone at a constant rate until day 15. Thereafter secretion declines until the next oestrus (Viñoles *et al*, 1999, Cunningham *et al.*, 1975). This decline is associated with the abrupt termination of the functionality of the *corpus luteum* that occurs at the end of the luteal phase (Oakley *et al*, 2009, Gordon, 1997). The regression of the *corpus luteum* occurs only if fertilisation does not occur. If fertilization occurs after ovulation the *corpus luteum* does not regress and the concentration of progesterone in the blood remains elevated through out pregnancy (Skinner *et al* 2001, Gordon 1997). In ewes progesterone secreted by the *corpus luteum* has many functions before and after fertilization. Exogenous progesterone administration improved embryonic survival in animals at risk of abortion (Hussein 2003, Parr *et al.*, 1987). Serum progesterone has also been used to check the effectiveness of ovulation induction. Luteinizing hormone stimulates glandular production of progesterone in the uterus, which then facilitates implantation of the embryo and maintenance of pregnancy. Progesterone influences udder development (Hamudikuwanda., 1998) by complete development of the alveoli of the mammary gland.

Quantity and quality of natural grazing varies with season (Nyamukanza *et al.*, 2010). This affects productive and reproductive performance of livestock (Clatworthy, 1998). Nutritional factors can influence the hypothalamus-pituitary function and therefore gonadotrophin profiles, directly through effects of nutrients or metabolic hormones such as insulin acting on targets organs or through changes in sensitivity of these organs to oestradiol, progesterone and other hormonal feedback (Rhind, 1992).

In cows, ovarian activity can be monitored by measuring concentration of circulating steroid hormones in blood and faeces (Masunda *et al.*, 1998). Rabieea *et al.* (2001) showed that it is possible to measure progesterone concentration in milk and faeces and relate levels to ovarian function in ewes but only to lactating dairy cows. Urine samples can also be collected to monitor ovarian activity (Marai *et al.*, 2006) but animals must be in metabolic cages. Progesterone can be measured in faeces irregardless of the animal's physiological status. Most studies concentrated on faecal progesterone measurement in relation to ovarian activity and pregnancy (Desauliners *et al.*, 1989; Masunda *et al.*, 1988). There are no reports on the level of feeding on oestrus induced fluctuations of faecal progesterone concentration. This information is useful for timing sample collection and predicting the reproductive status of animals under range conditions where availability and quality of feeds is highly variable with seasons.

It is imperative to find effective and appropriate techniques to monitor ovarian function of small ruminant animals using basic equipment. This will provide information on possible means of manipulating reproduction and improving reproduction rates. The study aims to find whether faecal progesterone can be used as a reliable indicator of ovarian activity in ewes and investigate the effect of protein and energy levels of feeding on faecal progesterone.

2.0 Materials and methods

Study Site

The study was carried in an animal house located at the department of Animal Science, University of Zimbabwe.

Animals and Management

Eighteen non-pregnant, non lactating Sabi ewes which were known to have shown regular oestrus cycle prior to the experiment were used in this study. Two weeks prior to the start of the experiment oestrus was synchronised in the ewes by administering two injections (11 days apart) of a synthetic prostaglandin, Estrumate®. Five millilitres were injected subcutaneously into each animal. Synchronization of oestrus was done to enable the simultaneous collection of blood and faecal samples at different stages of oestrus cycle. The animals were put in metabolism crates designed to separate faeces and urine and allow each animal to be fed individually. Fresh water was always available. The animals were fed *ad-libitum* fresh diets every morning after the removal of the refusal from the

previous meal. Animals were weighed weekly and feed intake recorded daily. Faecal and feed samples collected were then used to calculate the digestibility of the treatment diet at the end of the trial. Digestibility was calculated as the difference between the DM (dry matter) percentage of feed and faecal samples divided by feed sample DM. Weight gain of the animals was also calculated.

Treatments

Three experimental diets which simulated seasonal changes in feed availability and quality (protein and energy) in communal areas were fed as shown in Table 1. Sheep concentrate is high energy and protein formulated animal feed. Treatment 1 contained 90% Katambora hay and 10% sheep concentrate, which supplied 0.5 times energy and protein required for maintenance of ewes. It simulated feed available during peak dry season (early July to late September). Treatment 2 supplied a maintenance level of feed, which simulated availability during the start of the rainy season (early October to late November and then late April to late June). The diet contained 35% sheep concentrate and 65% Katambora hay. Treatment 3 diet contained 12.5% Katambora hay and 87.5% sheep concentrate which supplied 1.5 times the protein and energy required by the ewe for maintenance. It resembled feed availability in communal areas during peak of the rain season (late November to late March).

Table 1. Physical and Chemical composition of diets

	<i>Treatments</i>		
	1	2	3
Ingredients			
Katambora Hay (%)	90.0	65.0	12.5
Sheep concentrate (%)	10.0	35.0	87.5
Chemical composition			
DM (%)	89.0	89.0	90.0
CP (%DM)	5.44	7.54	11.2
CF(%DM)	34.1	27.5	13.9
EE (%DM)	5.45	5.08	4.28
ME (MJ/kg DM)	9.01	10.7	14.0

Experimental Design

A completely randomised design (CRD) was used. The animals were stratified into weight groups and then from each group there were randomly allocated to the treatment groups such that each group had six animals.

Sample collection and Processing

Blood and faecal samples were collected from each ewe once every two days for two oestrous cycles. Sampling was done between 0800 and 1000hrs. The first collection of samples was done two days after the administration of the second estrumate injection. During collection, ten millilitres of blood was collected by jugular vein puncture and placed into tubes containing EDTA (anticoagulant factor). The samples were immediately chilled in iced water at 4°C. The blood samples were centrifuged at 2000g for 15 minutes within 1 hour of collection. The separated plasma was stored at -20°C subsequent progesterone assays. Fresh faeces, which dropped into the

collection trays during sampling time, for each ewe, were collected and dried in an oven at 60°C for 48 hours. Thereafter, they were ground with a Wiley grinding mill passing through a 3mm screen size. The samples were then stored at room temperature for subsequent progesterone analysis.

Progesterone assays were extracted from faecal samples using a protocol described by Desauliners *et al.* (1989) with a few modifications. A sample of 200-250mg of dried and ground faeces was weighed and put in a test tube. Distilled water (2ml) was added and the mixture was extracted three times using 3ml diethyl ether at each extraction. During extraction, the mixture was vortexed for 3 minutes. The extracted sample was put in a freezer overnight at -20°C to allow the separation of the organic and aqueous phases. The organic phase was decanted into 10ml glass tubes and left to evaporate under

a fume chamber. The residue was dissolved in 1ml of ethanol and then diluted a further 100-times using an assay buffer (0.1M sodium phosphate dibasic, 0.9% sodium chloride, 0.1% sodium azide and 0.1% gelatine) of Ph 7.0 before radio immunoassay were performed.

During the assay for progesterone, a direct solid phase radio-immuno assay method using antibody coated tubes and I^{25} Progestrone (coat-a-Count Kits, Diagnostic Products, Corporation, Los Angeles, USA) were used to determine the plasma and faecal progesterone concentration.

Statistical analyses

Pearson's correlation coefficient between plasma progesterone and faecal progesterone concentrations throughout the oestrus cycle was computed using PROC CORR procedure of the Statistical analysis system. A t- test for the difference in live weight gain was computed. A PROC GLM procedure was used to calculate the mean, standard error and maximum and minimum progesterone concentrations in plasma and faecal samples. Faeces progesterone concentration was regressed against plasma concentration. Co-variation analysis for difference in initial mean live weight was also considered. Results of the regression analysis were used to formulate equations which explain the cause response relationship between plasma and faecal progesterone concentration.

3.0 Results

Chemical composition of the diets

DM digestibilities of the three treatment diets showed significant differences ($P < 0.05$). Treatment 1, 2 and 3 had 43, 57, and 72% DM digestibility, respectively. As expected, the diets differed in CF, CP, and ME contents (see Table 1). The highest percentage of CF was observed in treatment 1 and lowest in treatment 3. There was no significant differences in the fat content ($P > 0.05$) as shown in Table 1.

Feed intake and live weight gain

There was a significant difference in DM intake ($P < 0.01$) of the treatment diets. Animals that were on below maintenance diet (treatment 1) consumed an average of 0.65kgDM/day. This low average intake resulted in a corresponding low intake of the feed nutrients in the diets (i.e. CP, CF, ME, and Fat) as shown in Table 2. Animals on a maintenance diet (treatment 2) and an above maintenance diet (treatment 3) consumed an average of 1.75kgDM and 1.53kgDM of feed per day, respectively. This consequently increases the intake of CP and ME per day. The intake of fats was not significantly different among the three treatments (see Table 2).

Table 2: Feed intake and live weight gain in association with DM digestibility of the treatment diets.

	Treatments		
	1	2	3
DM Digestibility (%)	43 ^a	57 ^b	72 ^c
Nutrient intake			
CPI (g/day)	35.5 ^a	87.2 ^b	172
ME (MJ/kg DM/day)	58.8 ^a	123 ^b	214 ^c
CFI (g/day)	223 ^a	318 ^b	212 ^c
Fat (g/day)	35.6 ^a	58.8 ^a	41.8 ^a

NB* ME is calculated as 0.15x TDN (AFRC technical committee on response to nutrients 1993)

^{a,b} Row means with different superscripts differ (P <0.05)

Table 3 summarizes the effect of three treatments on live weight. Treatment diets showed a significant effect on live weight gain (P<0.01). Ewes on treatment 1 showed a significant difference in the initial and final weight which resulted in ewes losing an average of 75g/day whilst those on treatment 2 maintained their weight throughout the trial with non-significant live weight gain of 15g/day. A continuous significant change in live weight in ewes on treatment 3 was observed and the ewes gained weight at a rate of 150g/day.

Table 3. Change in live weight of ewes during the trial

	Treatment		
	1	2	3
Initial mean weight (kg)	33.5 ^a	34.2 ^a	33.4 ^a
	(0.84)	(0.64)	(0.96)
Mean live weight per week (kg)			
1	30.1 ^b	35.1 ^a	34.0 ^{ab}
2	27.3 ^b	35.0 ^a	36.0 ^{ab}
3	26.5 ^b	35.3 ^a	38.0 ^{ab}
4	25.4 ^b	34.3 ^a	36.0 ^{ab}
5	25.2 ^b	32.8 ^a	39.3 ^{ab}
Final mean live weight (kg)	24.4 ^b	35.3 ^b	41.1 ^b
Live weight gain (g/day)	-75 ^a	10 ^b	160 ^c

^{a,b} Row means with different superscripts differ (P <0.05)

* () Standard errors

Effect of diet and day on progesterone concentration profiles

Plasma progesterone concentration

Diet and day had a significant effect on plasma progesterone concentration profile (diet, $P < 0.05$ and day, $P < 0.05$) as illustrated in Figure 1. There was no significant effect of day and diet during post ovulatory period (Day 1 to 4, $P > 0.05$). From day 6 to 18, a significant effect was observed with highest progesterone concentration profiles in treatment 3 and lowest in treatment 1. The last two days of the oestrus cycle showed no significant effect of day and diet on plasma progesterone concentration profile. The trend of the profile remained the same across the treatments. There was no change in progesterone concentration in ewes on treatment 1 ($P > 0.01$) during the oestrus cycle. The difference in the concentration across treatments was highly significant between day 6 and day 18 ($P < 0.01$)

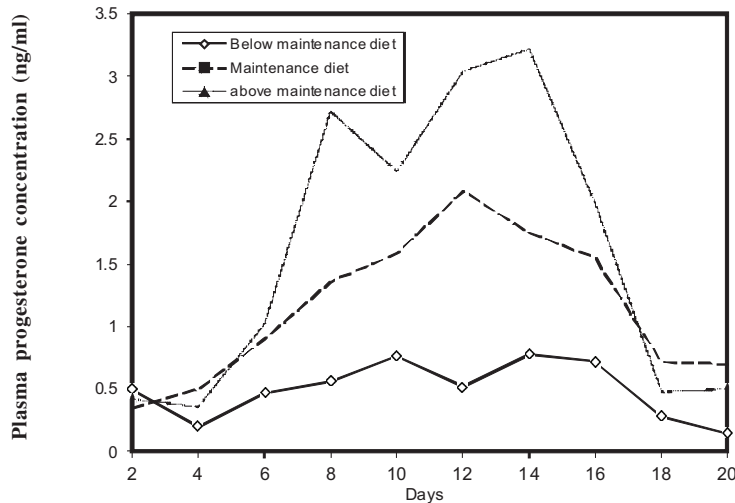


Figure1: Mean concentration of progesterone in plasma of ewes exposed to different levels of feeding during the oestrous cycle

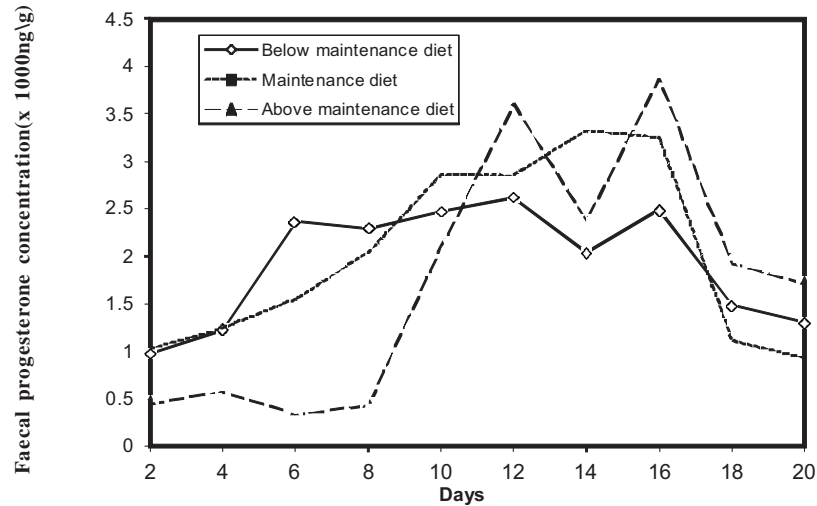
Faecal progesterone concentration

A significant effect of day on faecal progesterone concentration ($P < 0.05$) was observed especially after day 4 of the oestrus cycle as shown in Figure 2. However, the effect of diet could not be separated ($P > 0.05$). There was no significant effect of day on faecal progesterone concentration in ewes on treatment 3 during post ovulatory period

(day 1 to day 8). There after, an elevation in progesterone concentration was observed until day 12. A sharp decline in the concentration was evident for ewes on treatment 1 and 3 on the 14th day of oestrus cycle. Faecal progesterone concentration of ewes on treatment 1 and 2 were observed to be following the same trend with higher concentrations from day 1 to day 11 as compared to those on treatment 3. From day 4 to day 8, ewes on treatment 1 had the highest concentration as compared to their counter-parts in treatment 2 and 3.

Figure 2: Mean faecal progesterone concentration of ewes exposed to different levels of feeding during the oestrous cycle

Plasma and faecal progesterone concentration



Figures 3, 4 and 5 show the trend of plasma and faecal progesterone concentration in ewes exposed to treatments 1, 2 and 3, respectively. The concentration of faecal and plasma progesterone in ewes on treatment 2 were correlated ($P < 0.05$; $r = 0.92$) and a similar relationship was found in ewes on treatment 1 ($P < 0.05$; $r = 0.68$). There was no correlation between faecal and plasma progesterone concentration in ewes on treatment 3 ($r = 0.50$, $P > 0.05$).

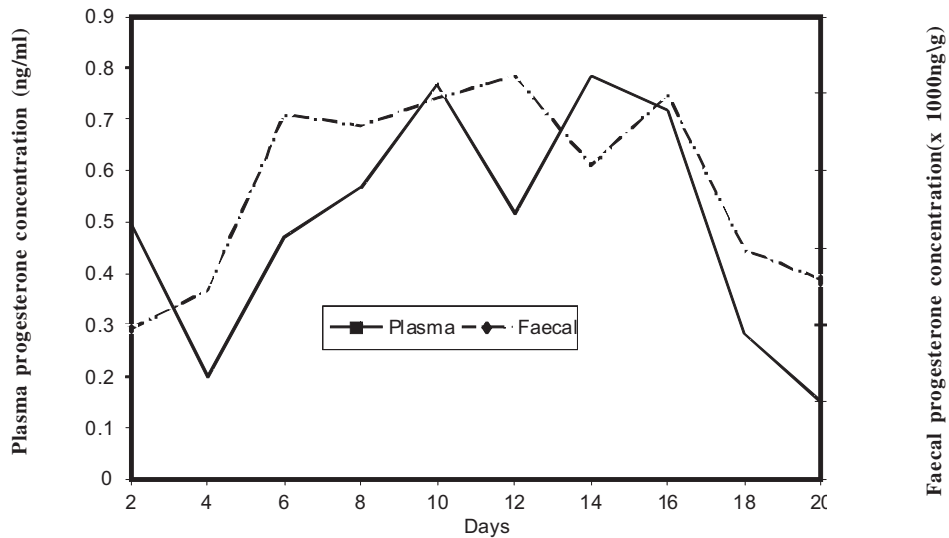


Figure 3: Average concentration of progesterone in plasma and faeces of ewes fed a below maintenance diet

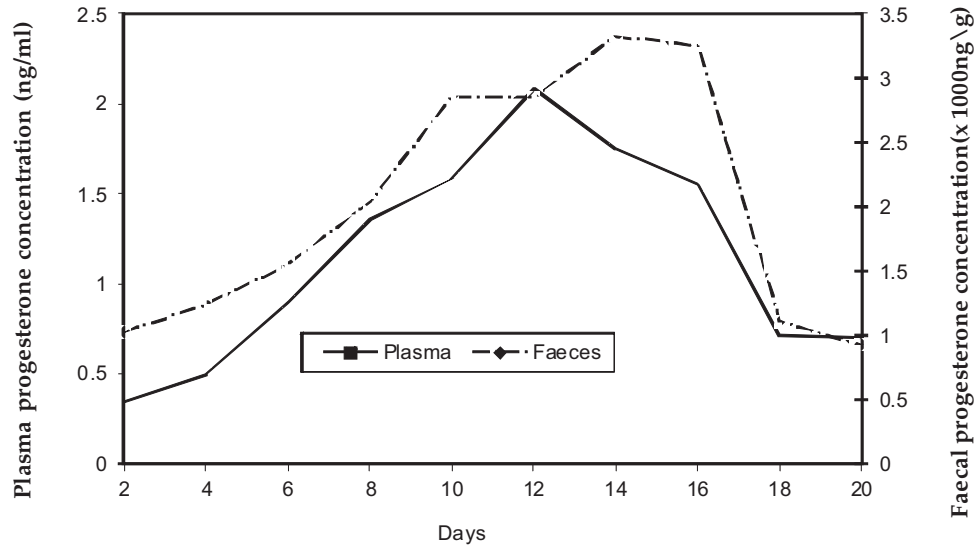


Figure 4: Mean concentration of progesterone in plasma and faeces in sabi ewes fed a maintenance diet

Equations showing plasma and faecal progesterone concentration cause response are shown below,

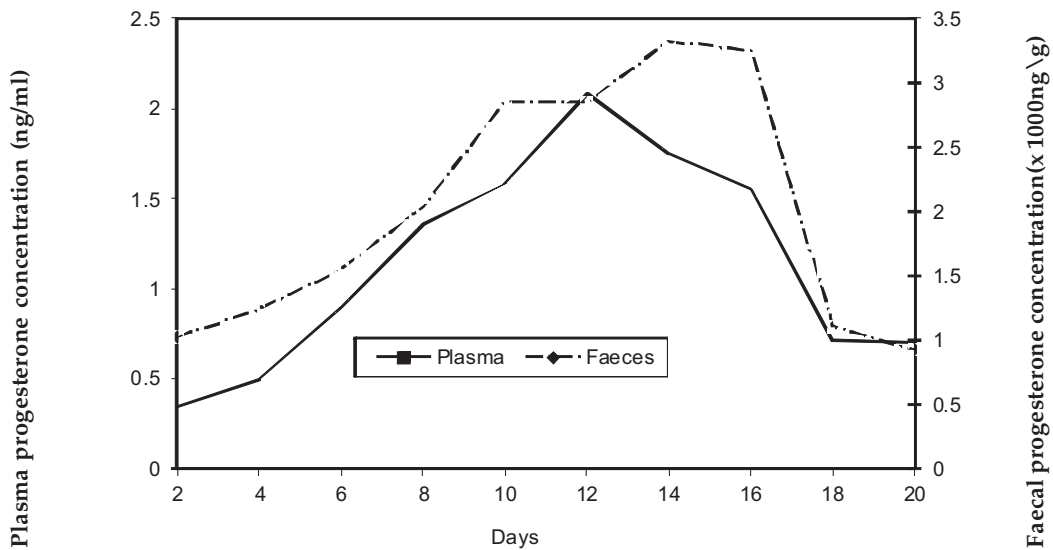
For treatment 1 $Y=0.02+0.26X$ (R-square=0.46, $P<0.05$)

For treatment 2 $Y=0.02+0.57X$ (R-square=0.85, $P<0.05$)

For treatment 3 $Y=0.82+0.45X$ (R-square=0.25, $P>0.05$)

Where: Y= plasma progesterone concentration (ng/ml)

X=faecal progesterone concentration (x1000ng/g)



The regression coefficient (0.56) and R-square (0.85) for ewes on treatment 2 were greater than those on treatments 1 and 3 as indicated in the equations above.

Figure 5: Average concentration of progesterone in plasma and faeces of sabi ewes fed a diet above maintenance

4.0 Discussion

The pattern of plasma progesterone levels observed during the oestrus cycle was similar to that previously reported for ewes (Rossi *et al.*, 2007, Viñoles *et al.*, 1999,). The profile, however, shows that diet had an effect on the ovulation rates reflected by the difference in plasma progesterone concentration in ewes exposed to different treatment diets. Ewes subjected to chronic underfeeding show suppressed oestrous.

The faecal progesterone concentrations obtained in this study are within the range reported for Holstein heifers (Desauliniers *et al.*, 1989, Larter *et al.* , 1994). Catabolised progesterone in the liver is passed through bile into the digestive tract and end up in faeces as conjugated steroids. The concentration of progesterone is affected by the level of dietary fibre (O'Callaghan *et al.*, 2000, Wasser *et al.*, 1994). The authors noted that dietary fibre increase gastro-intestinal (GI) transit time, while increasing faecal bulk and total faecal progesterone. This is attributed to the reduced rate of passage and could be an explanation for the profile of faecal progesterone concentrations observed in ewes on treatment 1 (day 4-8). The reduced rate of passage allowed the accumulation of progesterone in the GIT

which then tend to elevate the concentrations in faeces. Earlier observations by Wasser *et al.* (1993) proposed that relatively high circulating basal concentrations of progesterone result in a persistently high baseline excretion rate in faeces regardless of dynamic changes in plasma progesterone concentration. This explains why a significant and non-significant change in progesterone concentration in faeces and faeces and plasma, respectively was detected at the same time in ewes on treatment 1.

There was a long lag-time (of about 4 days) between progesterone secretion in the blood and excretion in faeces (excretion lag-time), thus resulting in the progesterone secretion profiles being unreliable for informing the physiological events occurring in the ovaries. This could be attributed to low CF percentage and high digestibility of the diet which shortens the storage time of the faeces in the gastro-intestinal tract (GIT). In ewes on treatment 2, the lag time was almost zero with a correlation close to one ($r=0.92$, $P<0.05$). This proves the rationale behind the use of faecal progesterone as an alternative method of monitoring the activity in animals on a maintenance diet. This observation was, however, not applicable for ewes on treatment 1 and 2.

The amount of variation in plasma progesterone that can be accounted for the collection of faeces for progesterone was highest in ewes on treatment 2 (R-square=0.85) and lowest in ewes on treatment 3 (R-square=0.25). Treatment 3 showed that the relationship of

plasma and faecal concentration do not fit in a linear model distribution ($P>0.05$). Treatment 1 and 2 fit very well in the model ($p<0.05$).

Conclusion

There are two limitations associated with the use of faecal progesterone for monitoring physiological events in the ovaries of ruminant animals, especially in communal areas. Firstly, the time-course of progesterone excretion in faeces relative to biological events in the ovaries for animals during late November to late March (above maintenance level of feed) is long and secondly, faecal progesterone concentration mis-inform the ovarian activities in animals during the period from early July to late September (below maintenance feed level). The concentration of progesterone in the faeces exaggerates the ovarian activities in the ruminant animals.

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