Online Journal of Animal and Feed Research Volume 9, Issue 1: 13-19; January 25, 2019



## IMPACT OF BODY CONDITION, MILK YIELD AND BLOOD METABOLITE CONCENTRATIONS ON THE RESUMPTION OF OVARIAN ACTIVITY IN CATTLE

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Supporting Information

ABSTRACT: Body condition of cows, level of milk production and concentrations of blood metabolites can influence the resumption of ovarian activity in cattle. This study investigated the relationships among body weight (BW), body condition score (BCS), milk yield, blood metabolite [Glucose, total protein, albumin, globulin, urea, triglyceride, non-esterified fatty acids (NEFA) and Beta-hydroxybutyrate (BHB)] concentrations and resumption of postpartum ovarian cyclicity in 20 Sanga and 20 Friesian × Sanga cows. Cows were classified as having resumed ovarian activity when they had plasma progesterone concentration of ≥1 ng/mL. Based on the resumption of ovarian activity, cows were also classified into three ovarian cyclicity groups namely; early resumption of ovarian cyclicity (< 56 days postpartum), late resumption of ovarian cyclicity (57-112 days postpartum) and non-resumption of ovarian cyclicity (by 112 days postpartum). BW. BCS and milk yield did not affect (P<0.05) cycling status in Sanga cows. Friesian × Sanga that resumed ovarian cyclicity early (2.54 L/day) or late (2.01 L/day) had higher milk yield (P<0.05) than their counterparts that did not resumed ovarian cyclicity during the period of study (1.61 L/day). However, BW and BCS were similar (P>0.05) in the three ovarian cyclicity groups in the Friesian × Sanga cows. Sanga cows that resumed ovarian cyclicity early had lower total protein concentration than those that resumed ovarian cyclicity late (76.8 versus 89.3 g/L; P<0.05). Globulin concentration was also lower (P<0.05) in cows that resumed cyclicity early (42.6 g/L) than late (54.2 g/L) and non-cycling (49.7g/L) Sanga cows. There were significant (P<0.05) positive and negative correlations among some of the plasma metabolites determined. In conclusion, earlier resumption of ovarian cyclicity was associated with higher milk yield in Friesian × Sanga cows and lower concentrations of total protein and globulin in Sanga cows. Improved feeding and management strategies are recommended for the achievement of reproductive success in cows.



Keywords: Body Weight, Cow, Nutritional Status, Ovulation, Relationships, Supplement

#### INTRODUCTION

The changes in health, nutritional and physiological status of most cows during the transition period adversely affects their performance. The period of late pregnancy is marked by increased growth and development of the foetus which consequently increase the nutritional needs of pregnant cows. Additionally, increased dietary intake fails to keep pace with rising milk production during early lactation leading to a state of negative energy balance (Esposito et al., 2014). Cows mobilize their lipid reserves during this period as an additional source of energy for maintenance and milk production resulting in body condition loss and increase in blood concentrations of non-esterified fatty acids (NEFA) and ketone bodies such as beta-hydroxybutyrate (BHB) (Denicke, 2018). Negative energy balance (NEB) affect reproductive performance adversely by inhibiting the release of luteinizing hormone, oestrogen and progesterone and the growth factor I (IGF-I) concentrations during NEB in early lactation could delay ovulation in cows (Konigsson et al., 2008; Soca et al., 2014).

Cows in the extensive system of production in Ghana, depending mostly on natural herbage without feed supplementation face declining performance in terms of growth, milk production and reproductive performance as a consequence of seasonal variation in pasture availability and important nutrients such as energy and protein (Obese et al., 2018). Feeding and management is important for metabolic adaptation and achievement of reproductive success in cows during the transition period (Drackley and Cardoso, 2014; Lucy et al., 2014). There is, however, a dearth of information on relationships among body weight, body condition score, concentrations of blood metabolites and resumption of postpartum ovarian activity in dual-purpose cows in the extensive system of cattle production in the Accra plains of Ghana.

This study therefore, evaluated the relationships among body weight (BW), body condition score (BCS), milk yield, blood metabolite concentrations and resumption of postpartum ovarian activity in Sanga and Friesian × Sanga

crossbred cows provided feed supplement in the extensive system of cattle production in the Accra plains of Ghana. The associations among plasma concentrations of blood metabolites were also investigated.

#### MATERIALS AND METHODS

#### Location of study

The study was conducted at the Animal Research Institute's Katamanso Station located in the Accra Plains on latitude 05° 44' N and longitude 00° 08' W. The vegetation is grassland with sparsely distributed shrubs. The area has a bimodal rainfall pattern with the major wet season occurring from April to July and a minor season from September to November. The remaining months constitute the dry period. Annual rainfall and temperatures range between 600-1000 mm and 21°C to 33°C respectively and relative humidity ranges from 69 to 94% (Obese et al., 2015). The study received approval from the In-house Committee for Research of the Animal Research Institute.

#### Management of animals

Forty multiparous made up of 20 Sanga and 20 Friesian × Sanga cows in their second to fifth lactation were used in a study which lasted for 16 weeks during the postpartum period. They calved between January and February (mostly in the dry season). At the start of the experiment, the Sanga cows (n=20) had a mean ( $\pm$  SEM), BW of 289.6  $\pm$  4.9 kg and BCS (BCS) of 7.1 $\pm$ 0.28 (scale 1 – 9; Nicholson and Butterworth, 1986). The Friesian × Sanga cows (n=20) had an average BW of 291.2 $\pm$ 9.8 and BCS of 6.9 $\pm$ 0.26. The two herds were housed separately in open kraals and also grazed separately, but on plots within the same field of natural pasture with similar nutritive value. The natural pasture grazed comprised a mixture of grasses and broad-leaved plants such as *Panicum insularis*, *Sporobolus pyramidali, Brachiaria deflexa, Milletia thonningii, Griffonia simplicifolia, Grewia carpinifolia, Stylosanthes hamata and Stylosanthes guaineesis*. Grazing period was from 08.00 h to 16.00 h daily. Cows received a supplementary diet (Table 1) before grazing. The supplement had a crude protein of 16% and digestible energy of 13.9 MJ/Kg DM. Each cow received 2.5 kg of the supplement for a period of 16 weeks. Water was provided in morning and evening. Cows were milked once daily in the morning between 05.00 h to 06.30 h.

Partial milking was practiced with milk collected from two quarters of the udder, and the other two quarters were reserved for the calves. Mating was natural with service bulls running freely with females all year round. Calves were weaned at about six months of age. Cows and their calves were treated against ecto-parasites, and endo-parasites once a month during the dry season and fortnightly in the wet season. They were also treated against diseases as the need arose and vaccinated against contagious bovine pleuropneumonia once a year as has been reported in earlier studies (Obese et al., 2018). Cows were weighed weekly and the BCS of cows determined weekly using a 9-point score (1= very thin to 9 = obese; Nicholson and Butterworth, 1986).

#### Blood sampling

Blood samples were collected from cows once every week, from week 1 to 16 postpartum after morning milking at 06.30 h by jugular venipuncture into a 7.5-mL EDTA-coated vacutainer tubes (BD Vacutainer Systems, Plymouth, UK) for analysis of metabolic hormone (progesterone) and blood nutritional metabolites (total protein, albumin, triglyceride, and urea, NEFA and BHB). Blood samples for determining concentrations of glucose was collected into evacuated tubes containing fluoride oxalate. All samples collected were then placed on ice immediately after collection and transported to the laboratory where plasma was separated by centrifugation at 1800 × g for 15 min at 4°C. The plasma samples were stored at -20°C, until assayed for the blood metabolites.

#### Blood metabolite analyses

The concentrations of glucose, total protein, albumin, triglyceride and urea were determined in the plasma at weeks 1, 3, 5, 7, 9, 11, 13 and 15 using the Mindray BA-88A Semi-Auto Chemistry Analyzer (Nanshan, China). Globulin concentration was computed as the difference between the total protein and albumin concentrations. The concentration of NEFA in the plasma was determined by enzymatic calorimetric techniques using an assay kit (Diasys Diagnostic Systems, Germany) while plasma BHB concentration was measured using a BHB assay kit (Randox Laboratories, UK). The BHB and NEFA concentrations in the plasma were determined at weeks 1, 3, 5, 7 and 9.

Resumption of postpartum ovarian activity and conception were determined by measuring the progesterone concentrations in plasma samples from cows from week 1 to week 16 postpartum (the end of the study period). Plasma progesterone concentrations were determined using a commercial ELISA Kit (DiaMetra, S.r.I, Italy). Cows were classified as having resumed ovarian activity when plasma progesterone concentration of  $\geq 1$  ng/mL was recorded in plasma samples (Tamadon et al., 2011). Cows were classified as not cycling if progesterone concentration remained below 1 ng/mL throughout the study period. Based on the resumption of ovarian activity, cows were also classified into three ovarian cyclicity groups namely; early resumption of ovarian cyclicity ( $\leq$  56 days postpartum), late

resumption of ovarian cyclicity (57-112 days postpartum) and non-resumption of ovarian cyclicity (>112 days postpartum). The progesterone assay had a sensitivity of 0.05 ng/mL.

#### Statistical analyses

The effects of BW, BCS, milk yield and plasma concentrations of metabolites (glucose, total protein, albumin, globulin, triglyceride, urea, NEFA and BHB) on resumption of ovarian activity in in Sanga and Friesian × Sanga cows were analysed using repeated measures analysis of variance procedure of GenStat Release 12<sup>th</sup> Edition (GenStat, 2009). Pearson's partial correlation coefficients were calculated to describe linear relationships among the concentrations of plasma metabolites using the IBM SPSS v.22.0 (2013). Values reported are least square means and SEM, unless otherwise stated. Mean values were considered to be statistically significantly different when P<0.05 and considered a tendency when P<0.10 but >0.05.

Table 1 - Composition of supplement (concentrate)						
Ingredient	Composition (%)					
Maize	40.0					
Wheat Bran	42.0					
Soya bean Meal	10.0					
Dicalcium Phosphate	2.0					
Oyster Shell meal	5.0					
Salt	0.5					
Premix*	0.5					
TOTAL	100					
* The premix provided the following per kg of concentrate: Vit A= 30,000 IU, Vit D = 35,000 IU, Vit K = 33.75 mg, Vit mg, Mn = 150 mg, Jodate 5 = mg, Zn = 125 mg, Cu = 15 mg, Chaline Chloride = 300 mg and Antioxidant 62.5 mg	B = 210 mg, Se = 0.375					

#### **RESULTS AND DISCUSSION**

## Body condition score (BCS) and milk yield

The effect of BW, BCS and partial milk yield on the resumption of ovarian cyclicity in the two breeds of cows are presented in Table 2. BCS at calving or loss of BCS during the early postpartum period could inhibit resumption of ovarian cycles and increase calving to conception intervals in cows (Roche et al., 2009; Soca et al., 2014). In the present study, BW or BCS did not affect (P>0.05) cycling status in Sanga or Friesian × Sanga cows. The overall mean BCS of range of 7.18 -7.25 for the Sanga and 6.73 - 7.06 for the Friesian× Sanga indicates there were in moderate to good condition on the scale of 1-9 (1=thin, 9= obese; Nicholson and Butterworth, 1986). Higher nutrient demands for milk production especially during early lactation could induce negative energy balance in cows leading to delays in ovulation and abnormal resumption of ovarian cycles (Esposito et al., 2014). Friesian × Sanga cows that resumed ovarian activity early were probably able to meet their nutrient requirements and partitioned it into milk production accounting for their higher (P<0.05) milk yield than their counterparts that did not resume ovulation (2.54 versus 1.61 L/day; Figure 1). Also, nutritional status is known to influence the secretion of hormones such as growth hormone involved in milk production and other metabolic hormones including luteinizing hormone, insulin and insulinlike growth factor-I which are associated with resumption of ovarian activity in cows (Diskin et al., 2003; Peter et al., 2009). The results in the present study is at variance with the report of Obese et al. (2015) who observed no significant differences in milk yield among early, late or non-cycling Friesian × Sanga cows which grazed extensively on natural pasture without any feed supplementation. Variation in the nutritional status of cows may account for the observed differences.

Table 2 - Effect of body weight,	body condition score an	id partial milk yield	on resumption of	ovarian cyclicity in
Sanga and Friesian × Sanga cow	during the postpartum	period		

Sanga						Fr	iesian x Sar			
Parameter	ERO	LRO	NRO	SEM	P-value	ERO	LRO	NRO	SEM	P-value
	(n= 6)	(n=5)	(n=9)			(n=7)	(n=7)	(n=)		
BW (kg)	273.42	294.30	289.4	9.92	0.269	288.1	298.2	296.8	15.54	0.868
BCS	7.18	7.25	7.18	0.036	0.973	7.06	6.73	6.73	0.043	0.642
Milk yield (L/day)	1.69	1.67	1.53	0.022	0.659	2.54ª	2.01 <sup>ab</sup>	1.61 <sup>b</sup>	0.039	0.011

n = number of cows; ER0= Early resumption of ovarian activity ( $\leq$ 56 days); LR0=Late resumption of ovarian activity (57-112 days); Non-resumption of ovarian activity (by 112 days); BCS = Body condition score; Means in the same row within each breed with different superscripts (a, b) are significantly different (P<0.05)



Figure 1 - Partial Milk yield in Sanga and Friesian × cows with early, late or non-resumption of ovarian cyclicity during the postpartum period.

### Blood metabolite concentrations

The blood metabolite concentrations in cows that resumed ovarian cyclicity early, late or failed to resume ovarian cyclicity by 112 days postpartum is shown in Table 3. Blood glucose, albumin, triglyceride, urea, NEFA and BHB concentrations were similar (P>0.05) in Sanga cows suggesting no relationship of these metabolites with the resumption of ovarian activity. Also, the non-significant differences (P>0.05) in the concentrations of all plasma metabolites determined in the Friesian × Sanga cows indicates no correlation of these metabolites with resumption of ovulation in this breed.

Glucose is a major blood metabolite defining the energy metabolism in cattle. The overall mean glucose concentrations in the two breeds fell within the normal physiological range of 2.2-5.6 mmol/L for cattle (The Merck Veterinary Manual, 2010) suggesting adequate energy supply to the cows. Sanga cows resumed ovarian cyclicity early had lower total protein concentrations in the blood than those that cycled late (76.8 versus 89.3 g/L; P<0.05). Ahmad et al. (2004) also reported of lower total protein concentrations in cycling than non-cycling cows. However, higher concentrations of total protein in cycling than non-cycling cows have been reported in other studies (EL-Azab et al., 1993; Saleh et al., 2011). The variations could be due to differences in breed and level of nutrition. Excessive intake of protein in the diet coupled with inadequate supply of energy can delay resumption of ovarian activity and affect conception in cows by increasing urea nitrogen concentrations in the blood thus interfering with uterine function (Bisinotto et al., 2012; Bindari et al., 2013). The overall mean total protein concentration in Sanga (83.1 g/L) and Friesian × Sanga (84.3 g/L) were within the normal range of 67 - 85 g/L reported for cows (Otto et al., 2000; Merck Veterinary Manual, 2010). This suggests both breeds of cows were in good protein status. The overall mean albumin concentrations obtained in the present study were 34.2 g/L and 33.4 g/L for the Sanga and Friesian × Sanga breed respectively. These were within the normal range of 25.0-38.0 g/L reported for cows (The Merck Veterinary Manual 2010) and suggest that the cows used in this study were not malnourished since albumin concentration in the blood significantly correlate with nutritional status in cattle (Coppo, 2004).

Circulating concentrations of globulin usually give an indication of an animal's immune state and its response to fighting diseases and infections (Kapale et al., 2008). The significantly (P<0.05) lower globulin concentration in early (42.6 g/L) than late (54.2 g/L) and non-cycling (49.7 g/L) cows (Table 3) is contrary to the expectation that cows with lower globulin concentrations should rather experience delayed resumption of ovarian activity. Cows with periparturient diseases (endometritis or mastitis) have been found to have delayed resumption of ovarian activity and conception (LeBlanc et al., 2002; Williams et al., 2007). However, cows in this study were not physically observed to have mastitis or endometritis. Lipid metabolism parameters include blood triglyceride and total cholesterol levels. The overall mean triglyceride concentrations of 0.27 and 0.25 for Sanga and Friesian × Sanga cows were within the normal physiological range of 0.1 - 0.3 mmol/L and suggested the preserved liver function within fat metabolism (Šamanc, 2009).

Urea levels in the blood serve as indicator of protein utilization and the similar concentrations in the cycling and non-cycling groups in both breeds could imply efficient utilization of protein in both breeds of cows. The overall mean values of 5.30 and 5.05 mmol/L obtained for the Sanga and Friesian × Sanga cows were within the normal physiological range of 3.6-8.9 reported for cows (The Merck Veterinary Manual, 2010). Blood levels of NEFA and BHB are indicators for energy metabolism. Moreover, higher NEFA concentrations have been associated with anovulatory postpartum anoestrus in cows (Butler, 2003, Montagner et al., 2016). However, blood NEFA concentrations in the two breeds were not associated with resumption of ovarian cyclicity in the present study.

# Table 3 – Plasma concentrations of metabolites in Sanga and Friesian × Sanga cows with early, late or non-resumption of ovarian cyclicity during the postpartum period.

	Sanga					D	Friesian x Sanga					D
Parameter	ERO (n=6)	LRO (n=5)	NRO (n = 9)	SEM	Overall Mean	value	ERO (n=7)	LRO (n=7)	NRO (n=6)	SEM	Overall Mean	value
Glucose (mmol/L)	4.42	4.44	4.42	0.082	4.43	0.989	3.97	4.14	4.16	0.065	4.09	0.506
Total Protein (g/dL)	76.8 <sup>b</sup>	89.3ª	83.1 <sup>ab</sup>	2.60	83.1	0.024	83.0	86.0	83.9	1.73	84.3	0.722
Albumin (g/L)	34.2	35.0	33.5	0.67	34.2	0.505	32.9	34.2	33.0	0.607	33.4	0.492
Globulin (g/L)	42.6 <sup>b</sup>	54.2ª	49.7ª	2.16	48.8	0.018	50.2	51.6	50.8	1.67	50.9	0.962
Triglyceride (mmol/L)	0.26	0.27	0.27	0.009	0.27	0.326	0.24	0.25	0.26	0.925	0.25	0.289
Urea (mmol/L)	5.18	4.96	5.76	0.148	5.30	0.563	5.10	4.83	5.21	0.081	5.05	0.226
BHB (mmol/L)	0.29	0.38	0.38	0.020	0.35	0.226	0.40	0.46	0.37	0.021	0.41	0.340
NEFA (mmol/L)	0.17	0.17	0.20	0.010	0.18	0.447	0.17	0.18	0.21	0.011	0.19	0.655

N= number of cows; ERO= Early resumption of ovarian activity ( $\leq$ 56 days), LRO=Late resumption of ovarian activity (57-112 days), Non-resumption of ovarian activity (by 112 days); Means in the same row within each breed with different superscripts (a, b) are significantly different (P<0.05)

### Correlation among blood metabolites

The partial correlation coefficients among concentrations of the plasma metabolites, glucose, total protein, albumin, globulin, triglycerides, urea, BHB, NEFA and urea are presented in Table 4. There were negative and significant relationships between glucose and total protein (r = -0.255; P<0.01), globulin (r = -0.200; P<0.01) and triglyceride (r = -0.2550; P<0.01) suggesting these metabolites are affected in the opposite direction in the nutritional management of the cows during the postpartum period. Total protein was significant and positively correlated with albumin (r = 0.371; P<0.01), globulin (r = 0.763; P<0.01) and triglyceride (r = 0.485; P<0.01) an indication that variations in plasma total protein concentration invariably influence plasma concentrations of albumin, globulin and triglyceride during the postpartum period. Obese et al. (2015b) in an earlier study observed a significant positive relationship between plasma total protein and globulin concentration in Friesian × Sanga cows grazing extensively on natural pasture without feed supplementation in the Accra plains of Ghana. On the other hand significant and negatively correlation between albumin and globulin (r = -0.317; P<0.01) suggest the opposite influence of nutritional management on the concentrations of these metabolites. The significant and positive relationships between albumin and triglyceride (r = 0.473; P<0.01), albumin and BHB (r = 0.218; P<0.01), albumin and NEFA (r = 0.183; P<0.05) may indicate similar influence of nutritional management on the concentrations of these metabolites during the period of study. This also applies to the relationship between globulin and triglyceride (r = 0.166; P<0.01). However, triglyceride concentration was significant and negatively correlated with urea (r = -0.119; P<0.05) and NEFA (r = -0.188; P<0.05) suggesting opposite influence of nutritional management on these two metabolites.

Table 4 – Partial correlation coefficients among plasma concentrations of glucose, total protein, albumin, globulin, triglycerides, urea, BHB and NEFA in Sanga and Friesian × Sanga Cows (n=40) during weeks 1,3,5,7, 9. 11, 13 and 15 postpartum									
Variables	TP	Albumin	Globulin	Trig.	Urea	BHB	NEFA		
Glucose TP	-0.255**	-0.088 0.371**	-0.200** 0.763**	-0.225** 0.485**	0.034 -0.075	0.079 -0.042	0.014 -0.101		
Albumin			-0.317**	0.473**	-0.044	0.218**	0.183*		
Globulin				0.166**	-0.046	-0.098	-0.142		
Trig.					-0.119*	-0.085	-0.188*		
Urea						0.029	0.021		
ВНВ							0.096		

TP = Total Protein; Trig = Triglycerides; BHB = Betahydrobutyrate; NEFA = Non-esterified fatty acid; \*Significant at P<0.05; \*\*Significant at P<0.01; Correlation among all metabolites except NEFA and BHB = 306; Correlation among NEFA, BHB and other metabolites = 196

## CONCLUSION

Earlier resumption of ovarian activity was associated with higher milk yield in Friesian × Sanga cows and lower concentrations of total protein and globulin in Sanga cows. Positive or negative relationships were observed among the concentrations of some of the metabolites measured. Improved feeding and management practices are recommended for the achievement of reproductive success in cows. Further studies should be carried out to evaluate the quality of feed supplements and the time of initiation of supplementation in such extensive systems of cattle production.

## DECLARATIONS

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### Authors' contribution

FYO designed the trial, supervised the data collection and laboratory work and contributed to the interpretation of the results and drafting of manuscript. LKA participated in the design of the study, interpretation of data and drafting of manuscript. KD contributed to data collection, laboratory and Statistical analysis.

### Acknowledgement

Julius Benyuo and Doreen Owusu Ntumy of the CSIR – Animal Research Institute are acknowledged for collection and processing of blood samples for analyses.

### **Competing interest**

The authors declare that they have no competing interests.

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