

Original Article

Blood metabolite concentration, milk yield, resumption of ovarian activity and conception in grazing dual purpose cows supplemented with concentrate during the post-partum period

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Abstract

Cattle grazing on natural pasture face seasonal variation in pasture availability and nutritive value. Improving the nutrition of cows by providing supplementary feeds during periods of pasture or nutrient deficit is necessary for improved productivity. This study was conducted to determine the effect of feed supplementation during the post-partum period on the metabolic status and reproductive performance of 40 grazing Sanga and Friesian × Sanga cows. Twenty out of a total of 40 cows (10 Sanga and 10 Friesian × Sanga cows) were supplemented with 2.5 kg of concentrate per day for 16 weeks after calving. Parameters measured include daily milk yield, concentration of blood metabolites, resumption of ovarian activity and conception. Supplemented cows had higher partial milk yield than their non-supplemented counterparts (2.07 vs. 1.60 kg/day; $P < 0.001$). Sanga cows had lower milk yield than the Friesian × Sanga crossbreds (1.61 vs. 2.05 kg/day; $P < 0.01$). Supplemented cows had higher mean total protein (86.7 vs. 81.3 g/L; $P = 0.007$) and globulin (53.0 vs. 47.7; $P = 0.014$) concentrations than non-supplemented cows. Sanga cows had higher glucose ($P = 0.027$), total protein ($P < 0.001$) and globulin ($P < 0.001$) but lower triglyceride ($P = 0.003$) and progesterone ($P = 0.023$) concentrations than Friesian × Sanga cows. Supplemented cows had less proportion not cycling (20 vs. 55%; $P = 0.022$) and less days open (95.8 vs. 106 days; $P = 0.032$) than non-supplemented cows. The Friesian × Sanga cows had higher progesterone concentrations at first progesterone rise (3.34 vs. 1.32 ng/mL; $P = 0.032$) and shorter interval from calving to conception (96.7 vs. 106 days; $P = 0.042$). The results from this study indicate the beneficial effects of feed supplementation in terms of increased milk yield, better metabolic status and reproductive performance.

Keywords: Blood metabolites, cows, conception, milk yield, ovarian activity.

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Introduction

Cattle production continues to be a major contributor of meat and milk and source of employment in most African countries. In Ghana, most cattle are grazed extensively on natural pasture without being provided feed supplements. A major challenge in this system of over-dependence on natural grazing land is the seasonal variation in pasture availability and nutritive value (Hills *et al.* 2015). The protein and energy contents of most natural and tropical grasses reduce drastically during the dry season limiting forage intake and animal performance due to

ineffective microbial protein synthesis (Detmann *et al.* 2014). There is therefore the need to improve nutrition of cows by providing supplementary feeds during periods of pasture or nutrient deficit to increase dry matter intake and improve productivity as has been suggested in a review by Hills *et al.* (2015).

Good nutrition influences fertility in cattle through the supply of vital nutrients required for development of gametes and synthesis of metabolites in the blood [luteinizing hormone (LH) insulin, insulin-like growth factor – 1 (IGF-I), leptin, nonesterified fatty acids (NEFA), β -hydroxybutyrate (BHB), glucose,

protein] associated with ovulation and pregnancy (Robinson *et al.* 2006; Konigsson *et al.* 2008; Samadi *et al.* 2013). However, inadequate nutrition during the post-partum period can increase the incidence of anoestrus in cows (Roche 2006; Peter *et al.* 2009) and decrease the profitability in cattle production enterprises by increasing days open, calving intervals, the number of service per conception and cost of veterinary services (Roche 2006; Kafi & Mirzaei 2010).

The use of the Sanga and Friesian \times Sanga crossbred cows in the extensive grazing system of production in the coastal savannah zone of Ghana for meat and milk production is popular. However, these breeds suffer nutritional deficiencies resulting in poor growth, low milk yield and less than optimum reproductive performance (Sottie *et al.* 2009; Obese *et al.* 2010). Thus, the provision of appropriate supplementary feed to complement nutritional needs would be required to enhance productivity of these breeds in the production system. It has been demonstrated in a number of studies that feed supplementation improves growth, milk yield and reproductive performance in cattle grazing tropical and subtropical forages (Msangi *et al.* 2004; Karikari *et al.* 2008; Filho *et al.* 2014; Selemani & Eik 2016; Almeida 2017; da Silva *et al.* 2017). Apart from a few studies (Teye *et al.* 2010; Obese *et al.* 2018), information is limited on the effect of feed supplementation on growth, milk yield, metabolic status and reproductive performance of Sanga cattle and its crossbred Friesian \times Sanga in the extensive system of production in the coastal savannah zone in Ghana.

This study therefore, evaluated the effects of feed supplementation on milk yield, metabolic status, resumption of ovarian activity and conception in grazing Sanga and Friesian \times Sanga cows during the post-partum period.

Materials and methods

Location of study

The study was conducted at the Animal Research Institute's Katamanso Station located in the Accra Plains of Ghana on latitude 05° 44' N and longitude 00° 08' W. The area is about 63 m above sea level.

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.* 2018). The study received approval from the In-house Committee for Research of the Animal Research Institute.

Management of animals

Multiparous cows in their second to fifth lactation were used in the study. They calved between January and May (mostly in the dry season). At the start of the experiment, the Sanga cows ($n = 20$) had a mean (\pm SEM) bodyweight (BW) of 289.6 ± 4.9 kg and body condition score (BCS) of 7.1 ± 0.28 (scale 1 – 9; Nicholson & Butterworth 1986). The Friesian \times Sanga cows ($n = 20$) had an average BW of 291.2 ± 9.8 and BCS of 6.9 ± 0.26 . Ten cows of each breed were supplemented before grazing, while the other 10 which served as controls were not supplemented. The two herds were housed separately in open kraals and were also grazed separately, but on plots within the same field of natural pasture with similar nutritive value. Grazing period was from 08.00 h to 16.00 h daily. Water was provided twice daily and cows were milked once daily in the morning between 05.00 h to 06.30 h. Partial milking was practiced: calves were separated from their dams in the evening and brought to suckle for a few minutes to stimulate milk let-down before milking. Milk was 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 6 months of age. Cows and their calves were treated against ecto-parasites, mainly ticks, fleas and mange mites using a pour-on acaricide (Flumethrin 1% m/v) once a month during the dry season and fortnightly in the wet season. Treatment against endo-parasites was done using an anti-helminth, Albendazole (10%) once a month during the dry season and

fortnightly in the wet season. They were treated against diseases as the need arose and vaccinated against contagious bovine pleuropneumonia once a year. Body weight (BW) and body condition score (BCS) of cows were determined weekly. The BCS of cows was determined using a 9-point score (1 = very thin to 9 = obese; Nicholson & Butterworth 1986). Cows were monitored for oestrus by visual observation two times per day by three trained technicians while at pasture. Resumption of post-partum ovarian activity and conception were determined by measuring the progesterone concentrations in plasma samples from cows from week 1 to week 16 post-partum.

Feeding of animals

The cows were grazed on natural pasture comprising a mixture of grasses and broad-leaved plants including *Digitaria insularis*, *Sporobolus pyramidalis*, *Brachiaria deflexa*, *Milletia thonningii*, *Griffonia simplicifolia*, *Grewia carpinifolia*, *Stylosanthes hamata* and *Stylosanthes guineensis*. The supplementary diet was a concentrate with composition presented in Table 1. It was fed at 2.5 kg daily to each cow in the supplemented groups before grazing for a period of 16 weeks. The chemical composition of pasture grazed by cows and the concentrate fed to cows are presented in Table 2. The *in vitro* dry matter digestibility of the forage and the supplement were 62.4 and 76.3%, respectively.

Blood sampling

Blood samples were collected from cows once every week, from week 1 to 16 post-partum after morning milking at 08.30 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 (glucose, total protein, albumin, triglyceride and urea). Blood samples for determination of glucose concentration were collected into evacuated tubes containing fluoride oxalate. All samples collected were then placed on ice immediately and transported to the laboratory where plasma was separated by centrifugation at 1800g for 15 min at 4°C.

Table 1. Composition of concentrate fed to Sanga and Friesian × Sanga cows

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 ^a	0.5
TOTAL %	100

^aThe premix provided the following per kg of concentrate: Vitamin A 30,000 IU, Vitamin D 35,000 IU, Vitamin K 33.75 mg, Vitamin B₂ 10 mg, Se 0.375 mg, Mn 150 mg, Iodate 5 mg, Zn 125 mg, Cu 15 mg, Choline Chloride 300 mg and Antioxidant 62.5 mg.

Table 2. Chemical composition of forage (basal diet) and supplement (concentrate) fed to Sanga and Friesian × Sanga cows

Fraction (% DM)	Forage	Supplement
Dry matter (%)	90.2	88.0
Crude protein	5.40	16.0
Neutral detergent fibre (NDF)	77.5	49.7
Acid detergent fibre (ADF)	49.0	13.8
Hemicellulose	28.5	35.9
Cellulose	31.9	3.97
Lignin	11.0	5.00
Silica	3.71	3.71
Digestible Energy (MJ/kg DM)	11.2	13.9

The plasma samples were stored at −20°C, until assayed for progesterone and blood nutritional metabolites.

Blood nutritional 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 Analyser (Nanshan, China). Plasma glucose was measured based on the method of Trinder (1969). The total protein and albumin concentrations in the plasma were determined based on the methods of Doumas *et al.* (1981), and Doumas & Biggs (1972), respectively, while globulin concentration was computed as

the difference between the total protein and albumin concentrations (Mapekula *et al.* 2011). Urea determination was based on the method by Sampson *et al.* (1980).

Progesterone assay

Progesterone concentrations were determined in the plasma from weeks 1 to 16 using a commercial ELISA Kit (DiaMetra, S.r.l, Italy). Cows were classified as having resumed ovarian activity when plasma progesterone concentration of ≥ 1 ng/mL was recorded in a plasma sample. Cows were classified as non-cycling if progesterone concentration remained below 1 ng/mL throughout the study period. The progesterone assay had a sensitivity of 0.05 ng/mL.

Statistical analyses

The effects feed supplementation had on BW, BCS, milk yield and plasma concentration of metabolites (progesterone, glucose, total protein, albumin, globulin, triglycerides and urea) in the Sanga and Friesian \times Sanga cows were determined using repeated measures analysis of variance procedure of GenStat Release 12th Edition (VSN International, 2009). The model included the fixed effects of breed (Sanga vs. Friesian \times Sanga), dietary regime (Supplementation vs. Non-supplementation) and time, as well as interactions. The chi-square test was used to determine the association between resumption of ovarian activity and breed or dietary regime. Values reported are least square means \pm standard error of means (SEM), unless otherwise stated. Mean values were considered to be statistically significant when $P < 0.05$ and considered a tendency when $P < 0.10$ but > 0.05 .

Results

Body weight, body condition score and milk yield

The body weight (BW) and body condition score (BCS) were similar in the supplemented and non-supplemented cows (Table 3), however, partial milk

yield was higher in supplemented than non-supplemented cows (2.07 vs. 1.60 kg/day; $P < 0.001$). Friesian \times Sanga cows had higher partial milk yield than Sanga cows (2.05 vs. 1.61 kg/day; $P = 0.001$). Dietary regime by breed interaction was significant for milk yield ($P = 0.007$; Table 3).

Blood metabolite concentrations

Supplemented cows had higher total protein (86.7 vs. 81.3 g/L; $P = 0.007$) and globulin (53.0 vs. 47.7; $P = 0.014$) concentrations than non-supplemented cows (Table 4).

Sanga cows had higher plasma glucose (4.43 vs. 4.09 mmol/L; $P = 0.027$), total protein (84.0 vs. 73.1 g/L; $P < 0.001$) and globulin (47.5 vs. 40.01 g/L; $P < 0.001$), but lower triglyceride (0.25 vs. 0.27 mmol/L; $P = 0.003$) and progesterone (3.79 vs. 0.58 ng/mL; $P = 0.023$, Fig. 1) concentrations than Friesian \times Sanga cows during the period of study (Table 4). Dietary regime by breed interaction was significant for only total protein ($P = 0.031$).

Resumption of ovarian activity and conception

The interval from calving to resumption of ovarian activity, progesterone concentration at first progesterone rise, proportion of non-cycling cows, interval from calving to conception and number of cows that conceived are outlined in Table 5. Feed supplementation did not significantly ($P > 0.05$) affect the interval from calving to resumption of ovarian activity, progesterone concentration at first progesterone rise and the proportion of cows that conceived. A lower proportion of supplemented cows, however, did not resume ovarian activity by the end of the study period compared to non-supplemented cows (20 vs. 55%; $P = 0.022$). Also, the interval from calving to conception (days open) was shorter in supplemented than non-supplemented cows (95.8 vs. 106 days; $P = 0.032$). Friesian \times Sanga cows had higher plasma progesterone concentrations at first progesterone rise (3.34 vs. 1.32 ng/mL; $P = 0.032$) and shorter interval from calving to conception (96.7 vs. 106 days; $P = 0.042$) than Sanga cows. The interval from calving to resumption of ovarian activity, and

Table 3. Body weight, body condition score and milk yield of Sanga and Friesian \times Sanga cows during the post-partum period

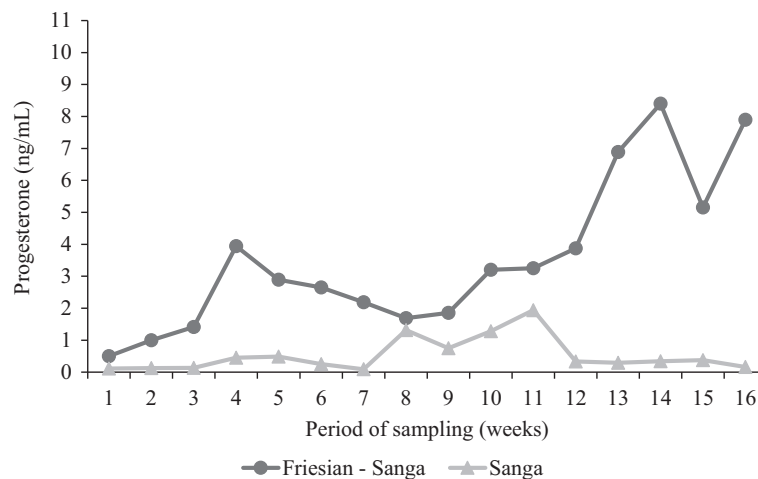
Parameter	Dietary Regime ^a			Breed ^b			<i>P</i> value ^c						
	Sup	Non-Sup	SEM	Sanga	F \times S	SEM	Dietary Regime	Breed	Time	B \times D	B \times T	D \times T	B \times D \times T
BW (kg)	296.4	283.7	6.67	285.8	294.2	6.67	0.185	0.379	<0.001	0.260	<0.011	<0.001	0.437
BCS	7.14	6.91	0.138	7.50	6.84	0.138	0.245	<0.079	<0.008	0.072	<0.001	0.736	0.018
Milk yield (kg/day)	2.07	1.60	0.088	1.61	2.05	0.088	<0.001	0.001	<0.001	0.007	<.001	<0.001	<0.001

^aSup = Supplemented, Non-sup = Non-supplemented. ^bS = Sanga, F \times S = Friesian-Sanga. ^cB \times D = Breed \times Dietary Regime interaction, B \times T = Breed \times Time interaction, D \times T = Dietary Regime \times Time interaction, B \times D \times T = Breed \times Dietary Regime \times Time interaction.

Table 4. Blood metabolites concentrations of Sanga and Friesian \times Sanga cows during the post-partum period

	Dietary Regime ^a			Breed ^b			<i>P</i> value ^c						
	Sup	Non-sup	SEM	S	F \times S	SEM	Dietary Regime	Breed	Time	B \times D	B \times T	D \times T	B \times D \times T
Glucose (mmol/L)	4.14	4.37	0.104	4.43	4.09	0.104	0.120	0.027	<0.001	0.866	<0.001	0.075	0.014
Total protein (g/L)	86.7	81.3	1.34	84.0	73.1	1.34	0.007	<0.001	<0.001	0.031	<0.001	0.054	0.273
Albumin (g/L)	33.7	33.6	0.502	34.0	33.3	0.502	0.902	0.347	<0.001	0.289	<0.001	0.544	0.129
Globulin (g/L)	53.0	47.7	1.47	47.5	40.1	1.47	0.014	<0.001	<0.001	0.108	0.001	0.030	0.508
Triglycerides (mmol/L)	0.26	0.26	0.005	0.25	0.27	0.005	0.870	0.003	<0.001	0.247	0.003	0.702	0.080
Urea (mmol/L)	5.05	5.08	0.08	5.09	5.04	0.08	0.773	0.642	<0.001	0.065	0.530	0.370	0.018
Progesterone (ng/mL)	3.32	1.05	0.952	0.58	3.79	0.952	0.101	0.023	0.030	0.105	0.011	0.373	0.384

^aSup = Supplemented, Non-sup = Non-supplemented. ^bS = Sanga, F \times S = Friesian-Sanga. ^cB \times D = Breed \times Dietary Regime interaction, B \times T = Breed \times Time interaction, D \times T = Dietary Regime \times Time interaction, B \times D \times T = Breed \times Dietary Regime \times Time interaction.

**Fig 1** Changes in plasma concentration of progesterone in Sanga and Friesian \times Sanga cows during the post-partum period.

the proportion of non-cycling cows or those that conceived were similar ($P > 0.05$) in the two breeds.

Discussion

Inadequate nutrition is a major challenge to productivity in most cattle raised in the tropics. The imbalance of nutrients leads to poor growth and loss in body condition, low milk yield and poor reproductive performance (Drackley & Cardoso 2014).

The higher milk yield in supplemented than non-supplemented cows (Table 3) is an indication of the benefit of feed supplementation in improving milk yield of cows similar to results in some studies (Kari-kari *et al.* 2008; Coffie *et al.* 2013; Obese *et al.* 2018). The higher milk production of Friesian × Sanga cows compared to the Sanga may be due to channelling more of their dietary energy for milk production. Generally, exotic and crossbred cows tend to have higher milk yield and lactation length than their indigenous counterparts (Endris 2017).

Blood metabolite profiles play important roles in assessing the nutritional, physiological and health status of cattle (Ndlovu *et al.* 2007). The higher total protein concentration in supplemented than non-supplemented cows (Table 4) may probably be due to improved amino acid absorption arising from increased microbial protein synthesis from additional nitrogen provided by the feed supplement. The higher globulin concentration in the

supplemented than non-supplemented cows suggest that feed supplementation may provide an additional advantage by improving the immune status of the cows. Glucose concentration in the blood reflects energy status in an animal and higher glucose concentration in the Sanga (4.43 mmol/L) than the Friesian × Sanga (4.09 mmol/L) cows may indicate adequate energy supply to the Sanga cows (Boonprong *et al.* 2007) probably due to their lower energy requirement. The values observed for the two breeds were within the normal physiological range of 2.2–5.6 mmol/L reported for cattle (The Merck Veterinary Manual, 2010). The total protein concentrations obtained suggest better protein status for the Sanga cows. The values observed for both the Sanga (84 g/L) and Friesian × Sanga (73.1 g/L) were within the normal physiological range of 67–87 g/L reported for cows (Otto *et al.* 2000; The Merck Veterinary Manual, 2010). Circulating concentrations of globulin provides an indication of an animal's health status and its response in fighting infections and diseases (Kapale *et al.* 2008). The higher globulin concentrations in the Sanga compared to the Friesian × Sanga (47.5 vs. 40.5 g/L) cows explains the better ability of Sanga cows to resist diseases and their high adaptability to the environment than the Friesian × Sanga crossbred cows. Also, breed differences could account for the higher triglyceride and progesterone concentration in Friesian × Sanga crossbred than the Sanga.

Table 5. Resumption of ovarian activity and conception in Sanga and Friesian × Sanga Cows (Mean ± SEM)

Parameter	Dietary Regime ^a		Breed		P value	
	Sup	Non-sup	Sanga	Friesian × Sanga	Dietary Regime	Breed
Interval from calving to resumption of ovarian activity (days)	53.5 ± 6.27	63.0 ± 8.33	56.6 ± 4.67	56.0 ± 8.65	0.378	0.953
Progesterone concentration at first progesterone rise (ng/ml)	3.70 ± 1.27	2.28 ± 0.50	1.32 ± 0.092	3.34 ± 1.39	0.312	0.032
Non-cycling ^b	4/20	11/20	9/20	6/20	0.022	0.327
Calving to conception interval	95.8 ± 2.96	106 ± 2.83	106 ± 2.38	96.7 ± 2.96	0.032	0.042
Number conceived	11/20	7/20	7/20	11/20	0.204	0.204

^aSup = Supplemented, Non-sup = Non-supplemented. ^bNon-cycling at the end of progesterone monitoring period (>112 days post-partum).

Adequate nutrition and maintenance of good body condition during the early post-partum period is necessary for early resumption of ovarian function (Drackley & Cardoso 2014). Inadequate nutrition in the early post-partum period results in negative energy balance which inhibit or delays ovulation by inhibiting luteinizing hormone pulsatility. The inhibition of LH pulse frequency as well as suppression of blood concentrations of glucose, insulin and insulin-like growth factor-1 lead to low oestradiol concentration which prevents the induction of gonadotrophin surge necessary for ovulation to occur (Peter *et al.* 2009; Walsh *et al.* 2011; Drackley & Cardoso 2014; Soca *et al.* 2014). Better nutritional status enhanced the resumption of ovarian activity in cows evidenced by the lower proportion (20%) of supplemented cows resuming ovarian activity than the non-supplemented cows (50%). Furthermore the improved nutritional status and the apparently shorter interval from calving to resumption of ovarian activity in the supplemented than non-supplemented cows may have contributed to the shortening of their interval from calving to conception (Table 5). This may have been mediated by higher plasma concentrations of protein and globulin in the supplemented, than the non-supplemented cows as improved nutritional status enhances metabolic status and conception in cattle (Robinson *et al.* 2006; Almeida 2017). Also, the interval from calving to resumption of ovarian activity is a major determinant of calving to conception and calving intervals. Maintenance of yearly calving intervals to maximize profit is dependent on conception within 85 days after calving (Crowe *et al.* 2014; Nuraddis & Ahmed 2017). Results from the present study indicate that the 96 days calving to conception interval in supplemented cows was closer to the ideal value of 85 days than the 105.8 days obtained for the non-supplemented cows. This emphasizes the need to supplement cows in the extensive system of production practiced in Ghana in order to enhance the onset of ovarian cyclicity and conception after calving.

The shorter calving to conception interval in the Friesian × Sanga than Sanga cows may suggest the ability of the Friesian × Sanga to conceive more easily once it has resumed ovarian activity as indicated by its higher progesterone concentration at

first progesterone rise than the Sanga (5.17 ng/mL vs. 1.25 ng/mL; Table 5). Other studies have also indicated a better reproductive performance of crossbreds over purebred cows (Karikari 1990; Heins *et al.* 2008). Karikari (1990), obtained lower intervals from calving to resumption of ovarian activity (82.7 days vs. 181.0 days) and shorter calving interval (368.0 vs. 511.6 days) in Friesian × N'Dama crossbreds than N'dama cows at the Dairy/Beef Cattle Research Station in the humid forest zone of Ghana. Also Heins *et al.* (2008) reported that F₁ Holstein × Jersey cows had fewer days open (127 days vs. 150 days) and a greater percentage of cows pregnant by 150 days post-partum (75% vs. 59%) than pure Holstein cows.

Conclusion

Feed supplementation improved milk yield, total protein and globulin status, and reduced cyclicity problems and days open in supplemented than non-supplemented cows during the post-partum period suggesting the beneficial effects of feed supplementation in the extensive system of grazing in the coastal savannah zone in Ghana. A more beneficial effect of feed supplementation in Friesian × Sanga cows was demonstrated through higher milk yield, earlier resumption of ovarian activity and fewer days open.

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Conflict of interest

The authors declare that there is no conflict of interest with any financial organization.

Ethical Statement

The study received approval from the In-house Committee for Research of the Animal Research Institute of the Council for Scientific and Industrial Research, Ghana, and was conducted in accordance with national and international guidelines on handling animals.

Contributions

FYO AND LKA designed the study. FYO, LKA AND PT were responsible for data collection, analysis and writing of the manuscript.

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