

# CORRELATIONS AMONG CONCENTRATIONS OF SOME METABOLIC HORMONES AND NUTRITIONALLY-RELATED METABOLITES IN BEEF COWS

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**ABSTRACT:** A study was conducted to investigate correlations among some metabolic hormones and nutritionally-related metabolites in plasma samples from sixteen multiparous Sanga cows raised extensively on natural pasture during early lactation. Blood was sampled from cows once every two weeks, from week 1 to 9 postpartum. The samples were processed for plasma and concentrations of the metabolic hormones and nutritionally-related metabolites were measured. Insulin-like growth factor-I (IGF-I) was positively correlated with insulin (0.377;  $P < 0.001$ ) and glucose (0.249;  $P < 0.05$ ), but negatively correlated with urea (-0.241;  $P < 0.05$ ). Insulin was positively correlated with glucose (0.440;  $P < 0.05$ ), total protein (0.262;  $P < 0.05$ ), and albumin (0.242;  $P < 0.05$ ), but negatively related with cholesterol (-0.279;  $P < 0.05$ ). Leptin was correlated positively with total protein (0.338;  $P < 0.001$ ) and albumin (0.351;  $P < 0.001$ ). There was a positive correlation between glucose and total protein (0.410;  $P < 0.001$ ) or albumin (0.425;  $P < 0.001$ ), but the correlation with urea was negative (-0.291;  $P < 0.01$ ). Total protein was positively correlated with albumin (0.682;  $P < 0.001$ ), but negatively correlated with cholesterol (-0.561;  $P < 0.01$ ). Furthermore, albumin was negatively correlated with creatinine (-0.294;  $P < 0.01$ ), while cholesterol was positively correlated with urea (0.253;  $P < 0.05$ ), and creatinine (0.294;  $P < 0.01$ ). The positive relationships among the nutritionally-related metabolites and metabolic hormones suggests that the effect of alterations in energy balance and (or) protein balance on postpartum ovarian function could be mediated through changes in the secretory patterns of these metabolic hormones.

**Key words:** Body Condition Score, IGF-I, Ovarian Activity, Postpartum Period

## INTRODUCTION

A number of metabolic hormones and nutritionally-related metabolites in the blood are involved in energy/protein homeostasis and reproductive function, especially the resumption of ovarian cyclicity in cattle during the postpartum period (Wettemann et al., 2003). The metabolic hormones include growth hormone (GH), thyroid hormones (thyroxine and triiodothyronine), insulin, insulin-like growth factor-I (IGF-I) and leptin. The nutritionally-related metabolites include glucose, cholesterol, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), total protein, albumin, globulin, blood urea nitrogen and creatinine (Diskin et al., 2003). The changes in circulating concentrations of metabolic hormones and nutritionally-related metabolites are important signals of the metabolic state of the animal especially on the adequacy of nutrient supply in relation to nutrient utilization (Lindsay et al., 1993).

Positive correlations have been reported among IGF-I, insulin, and body condition score (BCS) in heifers and beef cows (Bishop et al., 1994; Vizcarra et al., 1998). IGF-I and insulin are physiologically linked and both increase with BCS. However, the regulation of each hormone individually may vary according to metabolic status, and the direction of change in body weight (Leon et al., 2004). Plasma concentrations of leptin were also positively correlated with insulin and glucose, but negatively correlated with concentrations of GH and NEFA in dairy cows (Block et al., 2001). Presently information is lacking on the relationships among metabolic hormones and nutritionally related metabolites in beef cows raised in pasture-based systems in the coastal savannah zone of Ghana.

The main objective of this study was to investigate the correlations among the concentrations of some metabolic hormones and nutritionally related metabolites in the plasma from Sanga cows. This should provide information on the nutritional and metabolic status to guide management decisions towards improved animal productivity.

## MATERIALS AND METHODS

### Location of study, animals and design



The study was conducted at the Animal Research Institute's Katamanso station located at Lat 05° 44' N and Long 00° 08' W in the Accra Plains of Ghana. The area has a bimodal rainfall pattern with the major wet season occurring from April to July and the minor season from September to November. The remaining months constitute the dry period. Annual rainfall and temperatures range between 600-1000 mm and 20°C to 34°C respectively. Sixteen multiparous Sanga were housed in an open kraal and grazed daily from 05.00 h to 10.00 h and 13.00 h to 16.00 h mainly on natural pastures comprising *Panicum maximum*, *Sporobolus pyramidalis*, *Vertiveria fulvibarbis*, *Griffonia simplicifolia*, *Baphia nitida* and *Milletia thonongii*. Water was provided twice daily; morning and evening. Cows were milked twice a day; morning and evening during the rainy season and once a day during the dry season. Mating was natural, with service bulls running freely with females all year round. Calves were weaned naturally (between 6 and 9 months of age). The animals were treated against ecto-parasites 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. Cows were treated against diseases as the need arose and vaccinated against Contagious Bovine Pleuropneumonia once a year. Cows and their calves were weighed monthly, using a scale. Body condition score of cows was determined weekly, using the 9-point score (1= very thin and 9 = obese; Nicholson and Butterworth, 1986).

Blood samples were collected from cows once every two weeks, from week 1 to 9 postpartum at 08:00 h by jugular venipuncture into a 10 mL heparinised vacutainer tube (BD Vacutainer Systems, Plymouth, UK). They were then placed on ice immediately after collection, and plasma was separated by centrifugation at 1800×g for 15 min at 4°C. Plasma was stored at -20°C, until assayed for IGF-I, insulin, leptin, glucose, cholesterol, total protein, albumin, globulin, urea and creatinine at week 1, 3, 5, 7, 9 postpartum. Plasma concentration of IGF-I was measured in duplicate by the chloramine-T radioimmunoassay (RIA) method described by Gluckman et al. (1983). Interference by binding proteins was minimized by acid-ethanol cryoprecipitation method validated for ruminants by Breier et al. (1991). Insulin concentration in plasma was measured by double-antibody RIA as described by Tindal et al. (1978) while, leptin concentration in plasma was measured in duplicate by double-antibody RIA method of Blache et al. (2000). The concentrations of the nutritionally-related metabolites were measured using the VITROS 950 Chemistry Systems Auto-analyser (Ortho Clinical Diagnostics, UK). Glucose was measured based on the method by Trinder (1969) and cholesterol was determined based on the enzymatic method by Allain et al. (1974). Total protein concentration was determined based on the biuret reaction (Dumas et al., 1981). Albumin concentration was determined based on the method by Dumas and Biggs (1972), while globulin concentration in the plasma was computed as a difference between total protein and albumin concentration (Mapekula et al., 2011). Urea determination was based on the method by Sampson et al. (1980), while creatinine determination was based on the method by Ambrose (1983).

#### Data analysis

The effect of week of observation on the concentrations of insulin, IGF-I, leptin, glucose, cholesterol, total protein, albumin, globulin, urea and creatinine during the postpartum period was analyzed using the analysis of variance procedure in SPSS v.16.0 (SPSS, 2007). The Pearson's partial correlation coefficients were calculated to describe linear relationships among the concentrations of metabolic hormones and nutritionally-related metabolites from week 1 to 9 postpartum using SPSS v.16.0 (SPSS, 2007).

### RESULTS AND DISCUSSION

The concentration of insulin increased ( $P<0.05$ ), while that of urea decreased as lactation progressed (Table 1). This might be due to an improvement in the energy balance status of cows during this period. During early lactation, the energy requirements for milk production and maintenance of a cow exceed the available energy from feed intake leading to a state of negative energy balance (Jorritsma et al., 2003). The energy balance status of cows, however improves as lactation progresses (Lucy, 2000).

**Table 1 - Concentration of metabolic hormones and nutritionally-related metabolites during week 1 and 9 postpartum in Sanga cows**

Metabolite	Postpartum period (weeks)					P-value
	1	3	5	7	9	
IGF-I (ng/mL)	18.0	15.5	16.9	21.4	21.6	0.442
Insulin (µU/mL)	3.51 <sup>b</sup>	4.10 <sup>ab</sup>	3.65 <sup>ab</sup>	4.18 <sup>ab</sup>	4.30 <sup>a</sup>	0.021
Leptin (ng/mL)	1.13	1.14	1.11	1.08	1.11	0.754
Glucose (mmol/L)	3.52	3.62	3.78	3.78	3.62	0.066
Cholesterol (mmol/L)	2.37	2.66	2.66	2.69	2.37	0.220
Total protein (g/L)	84.0	81.9	83.3	84.0	83.5	0.397
Albumin (g/L)	29.6	28.7	28.9	29.8	30.4	0.244
Globulin (g/L)	54.3	53.1	54.0	53.6	53.1	0.758
Urea (mmol/L)	7.49 <sup>a</sup>	6.78 <sup>ab</sup>	6.56 <sup>ab</sup>	6.07 <sup>b</sup>	5.99 <sup>b</sup>	0.020
Creatinine (µmol/L)	96.5	96.6	97.2	98.1	98.6	0.989

a,b: Means within a row with different superscripts differ significantly ( $P<0.05$ )



Partial correlation coefficients for plasma concentrations of IGF-I, insulin, leptin, glucose, total protein, albumin, total cholesterol, urea and creatinine during week 1 to 9 postpartum are shown in Table 2. IGF-I concentration was positively correlated with insulin ( $r = 0.377$ ,  $P < 0.001$ ) and glucose ( $r = 0.249$ ,  $P < 0.05$ ), but negatively correlated with urea ( $r = -0.241$ ,  $P < 0.05$ ). This suggests that energy status or energy intake may regulate plasma concentrations of IGF-I, insulin and glucose in the same direction, whilst regulating IGF-I and urea concentrations in different directions. Circulating IGF-I, insulin and glucose concentrations have been found to influence ovarian function in cattle through the stimulation of gonadotrophin releasing hormone (GnRH) and luteinizing hormone (LH) secretion (Diskin et al., 2003; Wettemann et al., 2003). Ciccioli et al. (2003) observed a positive correlation between plasma IGF-I, insulin and glucose concentrations in Angus x Hereford primiparous cows. Also, plasma IGF-I was positively related with glucose and negatively correlated with urea in Holstein-Friesian cows (Zulu et al., 2002; Obese et al., 2009).

Insulin was positively correlated with glucose ( $r = 0.440$ ,  $P < 0.05$ ) total protein ( $r = 0.262$ ,  $P < 0.05$ ), and albumin ( $r = 0.242$ ,  $P < 0.05$ ), but negatively correlated with total cholesterol ( $r = -0.279$ ,  $P < 0.05$ ). Insulin regulates the use of glucose, and therefore glucose uptake by cells depends on the hormone insulin (Wettemann et al., 2003). The release of GnRH from the hypothalamus is stimulated by the combined action of insulin and glucose (Arias et al., 1992). In early lactation, when cows are in negative energy balance, they may develop ketosis and experience depressed insulin and glucose levels, with elevated ketones, free fatty acids, and cholesterol in the blood (Schwalm and Schultz, 1976).

There is a positive relationship between nutrient intake and concentration of leptin in plasma of cattle, as increased plane of nutrition is associated with increased circulating leptin concentrations (Delavaud et al., 2002; Leon et al., 2004). The significant positive relationships between leptin and total protein ( $r = 0.338$ ,  $P < 0.001$ ), and albumin ( $r = 0.351$ ,  $P < 0.001$ ) in the present study suggest that nutrient intake will regulate leptin, total protein and albumin in the same direction.

Glucose is utilized by all animal cells to produce energy (Richards et al., 1995). In the present study, glucose concentration was significant and positively correlated with total protein ( $r = 0.410$ ,  $P < 0.001$ ) and albumin ( $r = 0.425$ ,  $P < 0.001$ ), but negatively correlated to urea ( $r = -0.291$ ,  $P < 0.01$ ). The concentrations of glucose, total protein and albumin remained relatively constant during the postpartum period, while that of urea declined as lactation progressed (Table 1).

**Table 2 - Pearson's partial correlation coefficient (r) among some metabolic hormones and nutritionally-related metabolites during weeks 1 to 9 postpartum in Sanga cows**

Variable	IGF-I	Insulin	Leptin	Glucose	Total protein	Albumin	Total Cholesterol	Urea	Creatinine
IGF-I	-	0.377***	0.030	0.249*	0.064	0.219	-0.046	-0.241*	0.027
Insulin		-	0.066	0.440	0.062*	0.242*	-0.279*	-0.188	-0.212
Leptin			-	0.207	0.338***	0.351***	-0.141	-0.080	0.036
Glucose				-	0.410***	0.425***	0.190	-0.291**	0.054
Total Protein					-	0.682***	-0.561***	-0.202	-0.014
Albumin						-	-0.279	-0.014	-0.294**
Total Cholesterol							-	0.253*	0.294**
Urea								-	0.054
Creatinine									-

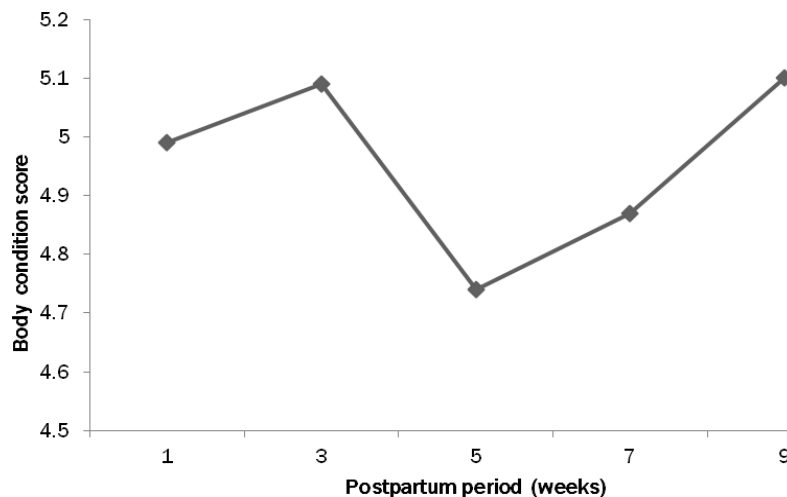
\* $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

Total protein was positively correlated with albumin ( $r = 0.682$ ,  $P < 0.001$ ) and negatively correlated with total cholesterol ( $r = -0.561$ ,  $P < 0.001$ ). Total protein reflects the total amount of proteins in blood plasma needed for the transport of lipids, hormones, vitamins, metals and enzymes. Albumin, a portion of total protein, is the main protein of blood plasma which serves as an early nutritional indicator of protein status (Agenas et al., 2006). Since albumin is the main protein of blood plasma, an increase in albumin concentration results in an increase in total protein. The poor nutritional status of cows in this study, evidenced by the low average BCS of 4.7 to 5.1 (Fig.1) on a 9-point score of Nicholson and Butterworth (1986), coupled with the possible increased demand for energy or glucose may lead to negative energy balance. Increased lipolysis due to low glucose concentrations to meet energy requirements will result in increased blood levels of low density lipoproteins (LDL). The elevated levels of plasma LDL concentrations in turn will increase the rate of synthesis of cholesterol, leading to high cholesterol concentrations (Meyer and Harvey 1998; Trail et al., 2004).

Albumin was negatively correlated to creatinine concentrations ( $r = -0.294$ ,  $p < 0.01$ ). Creatinine concentration is influenced by factors including an animal's diet and muscle mass (Otto et al., 2000). Protein breakdown of cows in this study was due to their poor nutritional status and this increased the plasma creatinine levels relative to albumin concentration.

Total cholesterol was positively correlated with urea ( $r = 0.253$ ,  $P < 0.05$ ), and creatinine ( $r = 0.294$ ,  $P < 0.01$ ). Due to poor nutritional status and BCS of the experimental cows, relatively less fat was available to be metabolized to provide energy. Thus more protein was metabolized to meet the energy requirements and this elevated the urea and creatinine concentrations. Also, during periods of energy restriction, the shortfall in energy may be met by the catabolism of body protein, resulting in increased urea concentrations (Greenwood et al., 2002).





**Figure 1 - Average body condition score of cows during week 1 to 9 postpartum in Sanga cows**

## CONCLUSION

There were positive or negative relationships among the concentrations of some of the metabolites measured. The positive relationships between changes in some energy and protein- related metabolites and metabolic hormones supports the assertion that the effect of alterations in energy balance and (or) protein balance on postpartum ovarian function could be mediated through changes in the secretory patterns of these metabolic hormones.

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