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METABOLIC PROFILES IN CROSSBRED DAIRY COWS UNDER THE EFFECTS OF CONVENTIONAL AND EXTENSIVE FEEDING SYSTEMS IN ASSIUT GOVERNORATE

(with 6 Tables and 2 Figures)

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صورة التمثيل الغذائي في الأبقار الحلابة الخليط تحت تأثير نظام التغذية التقليدي والمكثف في محافظة أسيوط

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لدراسة صورة التمثيل الغذائي في الأبقار الخليط حول فترة الولادة وأثناء منتصف الحليب، تم اختيار عدد ثمانون بقرة خليط فريزيان مع محلي بالتساوي من محطات استثمارية تعتمد على الغذاء المكثف ومن قطعان صغيرة تابعة لبعض المربين تعتمد على نظام التغذية التقليدي . تم أخذ عينات دم من هذه الأبقار قبل الولادة بشهر ثم بعد الولادة بشهر وأربعة أشهر لقياس البروتين الكلي والألبيومين والجلوبيولين والجلوكوز والبيتاهيدروكسي بيوترات والكالسيوم. وقد أظهرت النتائج أن المتوسط المشترك للثلاث مراحل في النظام المكثف كان أعلى من النظام التقليدي في كل من البروتين الكلي والألبيومين والجلوكوز ولكنه كان أقل في كل من الجلوبيولين والبيتاهيدروكسي بيوترات بينما لم يختلف النظامان في قيم الكالسيوم. وبالنسبة إلى التداخلات داخل كل نظام فقد أظهر تحليل التباين وجود اختلافات جوهرية بين المراحل المختلفة داخل النظام المكثف ليشمل كل العناصر بينما كان هناك اختلافات في كل من البروتين الكلي والألبيومين والجلوبيولين والبيتاهيدروكسيبيوترات فقط بين المراحل المختلفة داخل النظام التقليدي . هذا وقد تم وضع مصادر هذه الاختلافات بين المراحل المختلفة داخل كل نظام وأيضا بين كل مرحلة ومثيلتها في النظام الآخر في جداول وتم تمثيلها في منحنيات بعد اختبارها بطريقة مقارنة الأزواج المتعدد . وقد استنتجت الدراسة أيضا وجود ارتباط إيجابي بين الجلوكوز وكل من البروتين الكلي والجلوبيولين والكالسيوم كما كان هناك ارتباط سلبي بين البيتاهيدروكسي بيوترات وكل المتغيرات الأخرى . وعلى الرغم من أن متوسطات قيم العناصر التي تم قياسها كانت داخل القيم المرجعية لهذه الحيوانات إلا إن نسبة من الأبقار كانت تحتوي على بعض المؤشرات خارج هذه القيم المرجعية مما يشير إلى وجود مشاكل غذائية خلال هذه الفترات وخاصة مخاطر الكيتوزيز تحت الإكلينيكي والتي كانت أكثر وضوحا في الأبقار التي تعتمد على النظام التقليدي .

SUMMARY

Lactation and transition from non-lactation to lactation imposes severe stress on health and production capacities of dairy cows. To study the metabolic profiles in crossbred cows during peripartum period and mid-lactation, a total of eighty crossbred Friesian x native cows were selected equally from investment stations which depend on extensive feeding system (ES) and from small flocks belonged to private owners which depend on random feeding (conventional system, CS). Blood was sampled from each cow at one month before in addition to one and four months after calving for estimation of total protein, albumin, globulin, glucose, β- hydroxybutyrate (β-HB) and calcium. Between systems, pooled least-square means of the three periods of ES cows was higher for total protein, albumin, A/G ratio and glucose and lower for globulin and β-HB than CS cows but calcium did not differ. Within each system, F-test revealed significant variation between periods in all metabolites in ES, but CS showed significant variations in total protein, albumin, globulin and β-HB only. Sources of these variations either between periods within each system or between each period and the corresponding one in the other system were tabulated and presented in figures after they were tested by pair-wise multiple comparison procedures. Results showed also positive correlation between glucose and each of total protein, globulin and calcium and negative correlation between β-HB and the all estimated metabolites. Although blood metabolite averages were within the reference ranges given for these animals, there was a variable proportion of cows were outside these ranges, indicating some possible nutritional constraints, especially risks of subclinical ketosis, which were more prominent in CS cows.

Key words: *Metabolic profile, Crossbred cattle, Feeding.*

INTRODUCTION

The term "metabolic profile" was early introduced by Payne *et al.* (1970) to describe a system of serological monitoring of dairy herds as an aid in the assessment of their nutritional and metabolic status. Thereafter, the metabolic profile was used for economic evaluation of many health issues (Carpenter and Norman, 1983), as an early warning indicator of subclinical diseases and as a diagnostic procedure for many metabolic diseases in dairy cattle (Blowey, 1992). The basic metabolic profile in high-grade dairy cows had been fully established and used as part of a multidisciplinary approach for dairy herds in temperate countries (Blowey, 1992 and Ingvartsen & Andersen, 2000).

The transition period from non-lactation to lactation imposes severe stress on health and production capacities of dairy cows, and the dairy farmers must receive high yield with the least economic coasts (Grant and Albright, 1995). The last decade had seen a considerable increase in basic and applied research, and nutritional physiology and metabolism of the transition dairy cow had been the subject of numerous reviews (e.g., Bell, 1995; Grummer, 1995; Drackley, 1999; D'Mello, 2000 and Drackley *et al.*, 2001). Nutritional disturbances at the peripartum and lactation periods quickly lead to metabolic problems such as ketosis, fatty liver, paresis, abomasal displacement, retained placenta, mastitis and lameness in addition to decreased productive and reproductive performances (Whitaker, *et al.* 1995 & 1999; Ingvartsen & Andersen, 2000; Radostits, *et al.* 2000 and Rabelo, *et al.* 2003).

Exotic breeds of cattle were introduced to the third world to improve the efficacy of local cattle breeds, however, they were faced by the specific local environment and management. In tropical or subtropical regions, the environment is complexly interacting directly and or indirectly with the animal health (Saleh, 1996 and Payne and Wilson, 1999). Evaluation of the effectiveness of the metabolic profile technique for identifying constraints on productivity in small herds in environments less favorable for milk production were studied on local breeds and their crosses in subtropical and tropical countries (Parra *et al.*, 1999 and Whitaker *et al.*, 1999). In Upper Egypt, Amer *et al.* (1977), Salem (1980), Sayed (1991) and Saleh (1996) had investigated the effects of local environment and levels of production on some metabolic profiles in dairy cattle.

Up till now the effects of nutritional strategies on the animal health have not yet been fully established in tropical dairy cattle or their crosses with temperate breeds. The aim of the present work was designed to illustrate the effect of nutrition system on the metabolic profile as a strategy of health monitoring during the transition period of late gestation and early lactation of crossbred dairy cows in Assiut Governorate as a representative area of subtropical conditions.

MATERIALS AND METHODS

Animals:

A total of eighty crossbred (Friesian x native) pluriparous cows at late stage of pregnancy (average one month before calving) were selected randomly from flocks located in periurban areas at Assiut City. These cows were classified equally (40 cows each) according to their management and nutrition into extensive fed and conventional fed cows. The extensive fed group (ES) belonged to the recent investment stations while the conventional fed group were allocated sporadically in small flocks belonged to private owners.

Management and Feeding:

Quantitatively, extensive feeding system (ES) consisted mainly of manufactured concentrate mixture plus green Barseem (Trifolium alexandrinum) at a rate of 4-5 and 40-50 kg/day respectively pre-calving which increased by about 25% post-calving. The concentrate mixture consisted physically of 37% wheat bran, 35% maize, 20% decorticated cotton seed meal, 5% rice polish and 2% limestone and 1% NaCl. Conventional feeding system was composed mainly of wheat bran and or white corn at a rate of 2-3 kg/day plus 30-40 kg green Barseem/day pre-calving which was also slightly increased post-calving. The qualitative constituents of these ingredients are presented in table 1. The average milk yield of ES cows varying from 15-18 kg/cow/day, while in CS cows, the average was low and varying from 9-12 kg/cow/day.

Table 1: Chemical composition (%) of the commonly used feeds.*

Ingredients**	Conc. mix	Barseem	Wheat bran	White corn
DM %	89.1	19.0	89.5	88.4
CP %	18.3	13.1	18.3	9.0
EE %	05.8	03.2	4.7	4.6
CF %	12.6	32.3	11.7	3.3
NFE %	54.9	42.1	57.8	81.7
Ash %	08.4	09.3	7.7	1.4

*According to Abdel-Raheem (1998) and Kobeisy, *et al.* (2001). **DM: Dry matter, CP: Crude protein, EE: Ether extract, CF: Crude fiber, NFE: Nitrogen free extract.

Sampling and biochemical investigations:

Blood was collected by jugular vein puncture from each cow by using vacuumed tubes (Terumo Co., Terumo Europe N. V., Belgium) approximately one month before calving, then at 1 and 4 months after calving, for serum collection. Blood serum was analyzed for total proteins, albumin, glucose, β- hydroxybutyrate (β-HB) and calcium after the methods described by Henry *et al.* (1974). Globulin was calculated mathematically (Thomas, 2000).

Statistical analysis:

Firstly, linear model Analysis of Variance (ANOVA) was performed on pooled data of each system using a software program (SPSS, Ver.10) according to Borenstein *et al.* (1997). The Least Squares Means (LSM) were compared with comparison-wise standard error rate after significant F-tests. The fixed factors included the effect of feeding system (between systems) and the effect of period (within each system) representing one month before and after calving in addition to mid-lactation. The interactions between the three stages within each system were included in the model using pair-wise multiple comparison procedures (Duncan's new multiple range test). Pearson Product Moment Correlation (PPMC) was performed on the arranged all-raw data of serum metabolites regardless any effect, and represented by its degree of correlation and significance level.

RESULTS

The ANOVA of pooled data revealed significant differences in the pooled LSM between the two systems for all parameters except calcium (Tab. 2). F value was significant for the values of total protein, albumin, globulin, A/G ratio, glucose, β-HB and calcium within extensive (ES) feeding systems under the effect of different productive periods. In conventional system, F value revealed significant variations in total protein, albumin, globulin and β-HB, but there were non-significant variations in A/G ratio, glucose and calcium.

Table 3 shows the LSM of the metabolic profiles of CS and ES fed cows during periparturient and mid-lactation periods. The LSM interactions of blood serum metabolites between the two systems during the three categories are expressed by the P value (presented in table 4 and illustrated in figure 1). There were significant variations in all metabolites between the two systems at the prepartum period except for total protein. In the postpartum period, there was also significant variation between the two systems in albumin, globulin A/G and β-HB, while at mid-lactation period the two systems were differed in total protein, albumin, A/G and β-HB.

Figure 1 elucidates the source of variations in the estimated F value within each system by using Duncan's new multiple range test during late gestation, early lactation and mid-lactation. In both ES and CS cows, the mean values of blood serum total protein, albumin and globulin were decreased postpartum and then they restored their value or increased in mid-lactation period in ES but not in CS cows. In these trends the A/G ratio showed highly significant variations between periods.

Table 2: Pooled least-square means (LSM), standard error of means (SEM), F value within and P value between conventional and extensive fed cows[a] under the homeorrhetic effects periparturient and mid-lactation.

Metabolite	Unit	Conventional system (CS)			Extensive system (ES)			Pvalue[b]
		LSM	SEM	F[c]	LSM	SEM	F	
T.P	gm/dl	7.01	0.056	7.652***	7.40	0.059	29.31***	>0.001
Albumin	gm/dl	2.58	0.033	6.990**	3.10	0.039	46.36***	>0.001
Globulin	gm/dl	4.43	0.044	4.023*	4.27	0.043	4.419*	0.008
A/G	Ratio	0.58	0.008	3.000[ns]	0.73	0.010	10.07***	>0.001
Glucose	mg/dl	52.6	0.47	1.043[ns]	54.3	0.47	11.152***	0.011
β-HB	mmol/l	0.84	0.02	151.5***	0.72	0.02	135.8***	>0.001
Ca	mg/dl	8.89	0.071	1.261[ns]	8.98	0.068	18.179***	0.461

[a]: Based on 120 samples representing 3 functional stages from 40 cows of each system.
[b]: Level of significance between the two LSM. [c]: F critical at 119 (2 between and 117 within) degree of freedom (*df*) =3.0737, 4.7912, 7.3323 at confidence levels =/<0.05, 0.01 and 0.001 respectively.

Table 3: Metabolic profiles (LSM ±SD) of conventional and extensive fed crossbred dairy cattle during periparturient and mid-lactation.

		One month before calving		One month after calving		Four months after calving	
		CS	ES	CS	ES	CS	ES
T.protein	gm/dl	7.3 ±0.08	7.5 ±0.08	6.8 ±0.08	6.9 ±0.10	6.9 ±0.11	7.8 ±0.09
Albumin	gm/dl	2.7 ±0.053	3.1 ±0.048	2.4 ±0.054	2.8 ±0.049	2.6 ±0.056	3.5 ±0.049
Globulin	gm/dl	4.6 ±0.066	4.4 ±0.070	4.4 ±0.063	4.1 ±0.076	4.3 ±0.092	4.3 ±0.070
A/G	Ratio	0.59 ±0.01	0.71 ±0.01	0.55 ±0.01	0.68 ±0.01	0.61 ±0.01	0.81 ±0.01
Glucose	mg/dl	53.6 ±0.69	57.2 ±0.69	52.1 ±0.98	52.4 ±0.74	52.2 ±0.74	53.4 ±0.84
β-HB	mmol/l	0.57 ±0.01	0.49 ±0.01	1.04 ±0.03	0.87 ±0.02	0.91 ±0.02	0.79 ±0.02
Ca	mg/dl	8.89 ±0.11	9.24 ±0.08	8.76 ±0.13	8.46 ±0.12	9.04 ±0.13	9.23 ±0.11

Table 4: Levels of significance interaction (*P value*) between conventional and intensive fed cows during periparturient and mid-lactation periods.

	One month before calving	One month after calving	Four months after Calving
T.P	0.072	0.489	>0.001
Albumin	>0.001	>0.001	>0.001
Globulin	0.045	0.003	1.00
A/G	>0.001	>0.001	>0.001
Glucose	>0.001	0.777	0.306
β-HB	>0.001	>0.001	>0.001
Ca	0.014	0.093	0.258

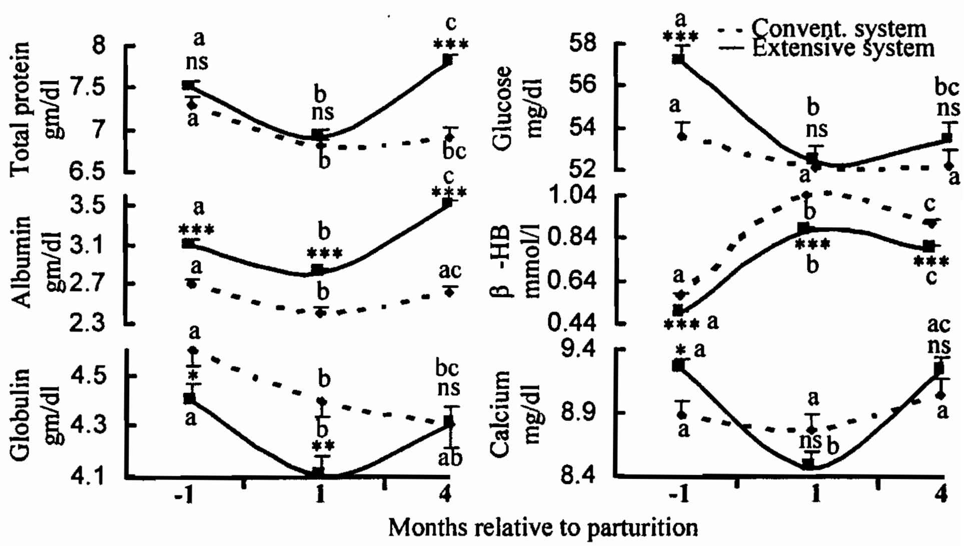

Fig. 1: Patterns of metabolic profile in peri-parturient and mid-lactating crossbred dairy cows under conventional and extensive feeding. ns: non significant, *,** and *** are the significance level at P=/<0.05, 0.01 and 0.001 respectively between the two systems at the different periods. The superscript a, b and c letters indicate the differences between periods within each treatment. The unlike superscript litters are significantly differing at P=/<0.05.

The mean value of blood serum glucose was reduced in postpartum and mid-lactation if compared by the prepartum period in ES cows, while it was not changed in CS cows. The contrast was observed in the mean values of β-HB which increased in postpartum period in both systems and then it slightly declined (but significant) in mid-lactation. The interaction of blood serum calcium between the productive periods was less severe and non-significant except during postpartum period in ES cows where it significantly reduced.

Table 5: Pearson Product Moment Correlation (R) and levels of significance (P) of the studied blood serum metabolites.

		Albumin	Globulin	Glucose	β-HB	Calcium
Total protein	R	-0.0553	0.702***	0.558***	-0.208**	0.114
	P	0.393	<0.001	<0.001	0.0011	0.078
Albumin	R		0.651***	0.0596	-0.129*	0.127*
	P		<0.001	0.358	0.0466	0.0487
Globulin	R			0.456***	-0.255***	0.121
	P			<0.001	<0.001	0.0540
Glucose	R				-0.207	0.126*
	P				0.0013	0.0496
β-HB	R					-0.151*
	P					0.0197

Table 6: Numbers and % (from 40 cows) of blood serum metabolite values outside the critical threshold during different periods in both systems.

Metabolite	Unit	critical threshold*		Numbers and % of metabolite values outside the critical threshold						
		Before calving	After calving		One month before calving		One month after calving		Four months after calving	
					CS	ES	CS	ES	CS	ES
Albumin	gm/dl	<3.00	<3.00	No	22	16	35	12	31	9
				%	*55*	*40*	*87.5*	*30*	*77.5*	*22.5*
Globulin	gm/dl	>5.00	>5.00	No	12	9	7	6	6	6
				%	*30*	*22.5*	*17.5*	*15*	*15*	*15*
Glucose	mg/dl	<50.0	<50.0	No	10	8	14	10	13	9
				%	*25*	*20*	*35*	*25*	*32.5*	*22.5*
β-HB	mmol/l	>0.06	>1.00	No	17	8	34	11	14	10
				%	*42.5*	*20*	*85*	*27.5*	*35*	*25*
Ca	mg/dl	<8.00	<8.00	No	5	3	5	5	4	3
				%	*12.5*	*7.5*	*12.5*	*12.5*	*10*	*7.5*

*According to Whitaker *et al.* 1995 & 1999.

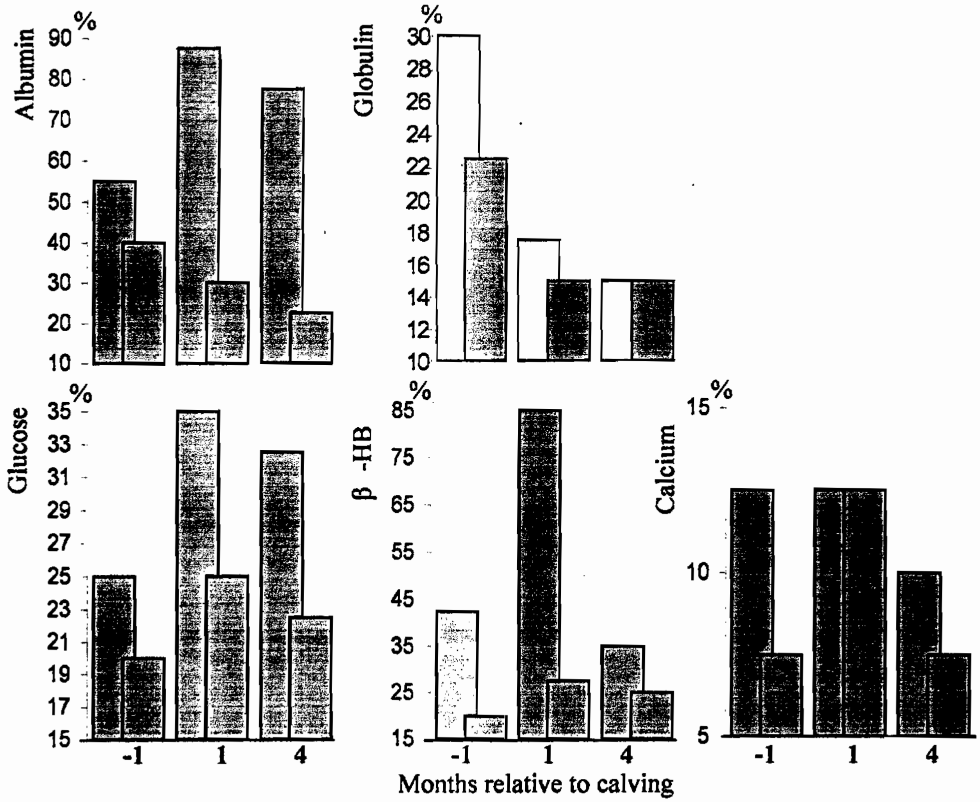

Fig. 2: Percent of blood serum metabolite values outside the critical threshold (see table 6) in periparturient and mid-lactating cows under conventional (open bars) and extensive (solid bars) systems. Total N =40 in each category.

The correlation coefficient revealed significant negative correlation between β-HB and the other estimated metabolites. A positive correlation was observed between glucose and each of total protein, globulin and calcium (Table 5). Table 6 and Fig 2 show the percent of blood serum metabolite values beyond the critical values (Whitaker *et al.*, 1995 & 1999). The results revealed that CS cows had a relatively higher percent of serum metabolites outside the threshold values during periparturient and mid-lactation periods than ES cows especially in albumin, glucose and β-HB.

DISCUSSION

The mean values of blood serum metabolites in conventional and extensive feeding systems either pooled (for the coalesced three studied productive stages) or separately (for each stage) were within the normal reference ranges cited for dairy cattle (Kaneko *et al.* 1997 D'Mello, 2000 and Thomas, 2000). In general, the effect of feeding system on the pooled data was highly significant and clarified that ES cows had higher metabolite values, which is coincided with their feed intake and reflecting the nutritional status of these cows. This result agree with the recognized previous reports (Clement, *et al.* 1991; Kida, 2002 and Holtenius, *et al.* 2003) except for calcium, which did not differ between the two systems, perhaps as a result of satisfaction of this mineral which offered to CS cows in the green fodder. Similar results was also obtained in high and low yielding dairy cows despite greater losses of this mineral in milk (Aeberhard, *et al.* 2001).

In ES cows, albumin values showed 6.9, 6.7 and 14.8 % increase while globulin was decreased by 2.2, 3.5 and 0 % during pre, postpartum and mid-lactation periods respectively than CS cows. All these differences were significant except the 0 value. The variation in blood serum albumin is related directly to protein intake (Thomas, 2000), so that the higher values of albumin in ES cows were a reflection to the nutritional status and protein % in the ration. The liver catabolizes the glucogenic precursors of these proteins under the gluconeogenic pathway via tricarboxylic acid cycle, oxaloacetate and pyruvate carboxylase (Drackley *et al.* 2001). These ways can provide the body by about 15-25 % of its need from glucose especially at the early lactation period (Reynolds *et al.* 2003) which may be interpretive for protein reduction during this period in both systems at the postpartum period. The higher globulin in CS cows might be resulted from the management differences between the two systems. The CS cows were not well managed and might be stressed by subclinical or chronic infectious diseases in addition to internal and external parasites (which were ignored in this study). Furthermore, recent studies showed that the nutritional stress especially during peripartum period enhance cytokines production which stimulate the creation of acute phase proteins (Ingvartsen and Andersen, 2000) and in turn higher globulin (positive acute phase responder) and lower albumin (negative acute phase responder) levels were produced.

Although it is subject to fairly precise homeostatic control, glucose is an essential metabolite for milk production and is commonly used as an indicator of energy status in late pregnant or lactating animals (Drackley *et al.*, 2001). In this study, the allover mean value of blood serum glucose was higher in ES than CS cows (P=0.011), which indicates a relatively higher energy status in ES cows which appeared only at prepartum period. In this concept, Holtenius *et al.* (2003) resulted that intensive fed cows may have had a higher gastrointestinal uptake of glucose precursors, like propionate and glucogenic amino acids, and thus a higher hepatic rate of glucose synthesis. Within each system, F value indicates a significant interaction between periods in ES (decreased at postpartum period by 8.4%) but not changed significantly in CS cows. During early lactation, the requirements for glucose is greater than input (Reynolds *et al.,* 2003). The homeostatic regulation in this period included a dramatic increase of gluconeogenesis in the liver and presumably coordinated glycogen mobilization in addition to a marked decrease of insulin and moderate degree of insulin resistance in adipose tissue and mussels (Aeberhard *et al.* 2001 and Drackley *et al.,* 2001). In despite, glucose requirements increase 2-3 folds from prepartum to postpartum in dairy cows resulting in a postpartal negative energy balance (Knight, 2001, Holtenius *et al.* 2003 and Reynolds *et al.* 2003). So that the reduction of glucose levels during early lactation period in ES cows was expected. On the other hand, dairy cows can experience a severe energy constraint and have an ability to adapt their output to the feed available (Whitaker *et al.*, 1999; Holcomb *et al.*, 2001 and Rabelo *et al.,* 2003). This can be applied in particular to situations where energy supply was inadequate, as presented in CS cows in this study. In these cows there was a relatively lower mean values of glucose levels than ES cows, in addition to the absence of periodical variations in this metabolite. It seems that these cows had adapted their homeostatic response for input output regulation and decrease their buffering capacity in utilization of energy yielding substrates as reported by Aeberhard, *et al.* (2001) and Rabelo *et al.* (2003). This was correctly reflected by the differences between the two systems in their milk production in this study and may interpretative to the positive correlation between glucose and other metabolites.

In contrast to glucose, β-HB was higher in CS than ES cows (14.3% in pooled values), and postpartum than prepartum (45.2 and 42.7% in CS and ES respectively). The differences between and within systems in the mean values of blood serum β-HB are in consistency with the reports cited by Bell (1995), Grummer (1995) and Radostits *et al.* (2000). These authors emphasized that β-HB is closely related to energy status in animals where there is a high metabolic demand for glucose and can be used as a mean of identifying the presence of risk of metabolic diseases. Ingvartsen and Andersen (2000) and Drackley *et al.* (2001) found that high β-HB values implied to short of feed rather than a particular nutrient deficiency, and indicate a dietary energy constrain in addition to increased body tissue mobilization. Recent evidences showed that changes in net liver release of β-HB after calving could be attributed both to changes in portal-drained viscera release of n-butyrate and acetoacetate as well as changes in liver removal of NEFA (Reynolds *et al.,* 2003). The present study showed negative correlation between β-HB and all estimated metabolites, which confirm the previous reports which informed that this metabolite is a good indicator for nutritional balance during peripartum and lactation stresses (Reynolds *et al.,* 2003). The negative correlation between β-HB and calcium might due probably to increased loss of base in the urine to compensate for acidosis (Radostits *et al.*, 2000 and Roche *et al.,* 2003a,b).

Although blood metabolite averages were within the reference ranges given for cattle (Whitaker *et al.,* 1995, 1999 and Radostits *et al.*, 2000), there was a variable proportion of cows in both systems and at different physiological periods were outside these ranges, indicating some possible nutritional constraints. The incidences of these deviating values (low levels of albumin and glucose and higher levels of β-HB) were higher in CS cows, indicating that these animals were undergoing a higher risk of metabolic problems especially ketosis which was more clear at the early lactation period. Similar findings were also reported in tropical and subtropical breeds (Parra *et al.,* 1999 and Whitaker *et al.,* 1999) and in European organic and conventional farms (Hardeng and Edge, 2001). Fat cow syndrome, fat necrosis, bovine lipomatosis and ketosis which are a common sequel of these metabolic stresses in high yielding cows, was also reported in undernourished local and foreign breeds reared under Upper Egypt conditions (El-Sepaie *et al.,* 1985; El-Sepaie and Hofmann, 1992; Dakka *et al.,* 1992; Hofmann and El-Sepaie, 1995 and Ali and El Sepai, 2002).

In conclusion, the metabolic profile testing provides useful information on the presence of nutritional constraints and other diseases during periparturient and lactation periods. As in high yielding dairy cattle in temperate countries, the transition period from late gestation to early pregnancy imposes severe risk on crossbred dairy cattle reared in tropical countries especially in those reared under traditional random feeding. To ensure that important constraints are not overlooked and that others are not erroneously assumed to be present, metabolite testing should be used as a part of multidisciplinary crossbred dairy cow development programs.

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