

Tropical and Subtropical Agroecosystems

E-ISSN: 1870-0462

ccastro@uady.mx

Universidad Autónoma de Yucatán

México

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Tropical and Subtropical Agroecosystems, vol. 11, núm. 1, 2009, pp. 225-232

Universidad Autónoma de Yucatán

Mérida, Yucatán, México

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SHORT NOTE [NOTA CORTA]

*Tropical and  
Subtropical  
Agroecosystems*

INFLUENCE OF PREGNANCY AND LACTATION ON GLUCOSE  
METABOLISM OF NUBIAN GOATS

[INFLUENCIA DE LA GESTACIÓN Y DE LA LACTANCIA SOBRE EL  
METABOLISMO DE GLUCOSA EN CABRAS NUBIAS]

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SUMMARY

Two *in vivo* metabolic challenges were conducted to assess the changes in glucose metabolism during three intervals prepartum (-6, -4, -2 weeks) and three postpartum (+2, +4, +6 weeks) in six multiparous pregnant Nubian goats. Challenges consisted of intravenous administration of 1) glucose (62.5 g/goat) and 2) L-epinephrine (0.7 µg/kg body weight). Blood samples were collected via jugular cannula from 30 min pre-injection (basal concentrations) to four hours post-injection. Response variables for glucose challenge were glucose concentration at zero time ( $t_0$ ) glucose disappearance rate ( $t_{1/2}$ ), insulin and NEFA concentrations; for the epinephrine challenge glucose, NEFA and insulin integrated responses were determined through the four hours of sampling. Data were analyzed according to a repeated-measures design. Dry matter intakes ( $1.8 \pm 0.07$  kg/d) were not different throughout the study ( $P > 0.1$ ). Average milk production ( $649 \pm 69$  g/d) was not different among periods ( $P > 0.1$ ). Basal glucose and insulin concentrations were not different ( $P > 0.1$ ) between pregnancy and lactation, with means ( $\pm$  standard error) of  $77.9 \pm 3.7$  mg/dl, and  $0.264 \pm 0.034$  ng/dl, respectively. Basal NEFA concentrations were greater ( $P < 0.001$ ) during weeks 2 and 4 postpartum ( $0.40 \pm 0.2$  and  $0.57 \pm 0.3$  mmol/l) and lowest during prepartum periods. Responses to glucose challenge did not differ throughout periods evaluated, with means for  $t_0$  of  $289 \pm 17$  mg/dl ( $P > 0.1$ ) and for  $t_{1/2}$  of  $31 \pm 15$  min ( $P > 0.1$ ). Insulin responses were similar for all periods ( $63.3 \pm 8.2$  ngml<sup>-1</sup>min) ( $P > 0.1$ ). The epinephrine challenge resulted in similar changes in glucose and insulin integrated responses throughout the periods evaluated ( $P > 0.1$ ), with corresponding means for glucose of  $3886.5 \pm 318$  mgml<sup>-1</sup>min, and  $21.6 \pm 7.7$  ngml<sup>-1</sup>min, but elicited a significant ( $P < 0.001$ ) increase in plasma NEFA concentrations during weeks 2 and 4 postpartum ( $1.24 \pm 0.6$  and  $1.40 \pm 0.3$  mmol.l<sup>-1</sup>min), but not during the prepartum periods. Metabolic

responses of dairy goats to exogenous glucose and epinephrine were markedly influenced by physiological stage, mainly with onset of lactation.

**Key words:** *Energy metabolism, dairy goat, metabolic challenge, glucose, insulin, NEFA.*

INTRODUCTION

Pregnancy and lactation represent two important physiological states in which different tissues undergo an extensive series of metabolic adaptations to support the main tissues involved, fetus during pregnancy and mammary gland during lactation. If tissue adaptations are not sufficient to allow for the nutrient needs of those tissues, then animal performance is affected and animal health compromised. Much evidence for metabolic adaptations occurring to support pregnancy and lactation in ruminants has been based on variations observed in circulating concentrations of metabolites and hormones. It is difficult to determine whether chronic changes in blood concentrations represent alterations in the production or utilization of a metabolite or a hormone.

The effect of physiological state on tissue metabolism and the regulation of metabolism are better characterized in non-ruminants, especially the rat. However, peculiarities of the ruminant's digestive system leading to marked differences in absorbed nutrients make it difficult to extrapolate from knowledge that has been achieved in monogastric species, particularly in the area of regulation of energy metabolism. The dairy goat is no exception. There is a lack of information about the manner in which these animals manage its nutrient requirements during the last third of pregnancy and the initiation of lactation. Some studies have shown glucose concentration changes during pregnancy and lactation, varying from 60 mg/dl at 42 to 56 days of pregnancy to about 46 mg/ml at 112 to 126 days (Khan and Ludri, 2002).

Others have reported values of plasma glucose in pregnant goats of 63 mg/dl vs. 72 mg/dl in non-pregnant goats (Sandabe *et al.*, 2004). These examples demonstrate that physiological state in goats plays a major role in regulating energy metabolism.

In an effort to elucidate the changes in glucose and energy utilization by the dairy goat during two physiological stages, pregnancy and lactation, the objective of this project was to assess the changes in energy homeostasis over the interval of six weeks prepartum to six weeks postpartum using two *in vivo* metabolic challenges. These were 1) a single injection of glucose, to evaluate glucose tolerance and changes in plasma concentrations of insulin and non-esterified fatty acids (NEFA); and 2) an injection of L-epinephrine, to assess changes in plasma concentrations of glucose, insulin and NEFA.

## MATERIALS AND METHODS

Six Nubian multiparous pregnant goats, with an average body weight of  $54.5 \pm 5.8$  kg,  $37 \pm 9$  months of age, and entering their third pregnancy, were dried off at forty-five days from estimated date of parturition and housed individually in stalls with free access to feed and water. All animals were in their third pregnancy. During the dry period they were fed *ad libitum* an extruded ration based on alfalfa hay, sorghum stover, ground corn, soybean meal, sugarcane molasses and mineral premix with 94% DM and 15% CP, and for the lactation period with 95% DM and 15.3% CP.

Animals were weighed at the beginning of the experimental period (six weeks prepartum), at parturition and at six weeks postpartum. During lactation, animals were housed with their offspring and milked once a day at 06:00 h. At approximately 15:00 daily, kids were separated from their mother and placed in rearing pens where they remained until next morning when goats returned from the milking parlor.

A separate group of two non-pregnant, non-lactating goats, with similar age and body weight and with two finished previous lactations, were used as a control group in order to observe the response to the metabolic challenges in this physiological state. These animals were managed and fed as for the pregnant animals.

### Metabolic challenges

The response to two metabolic challenges was compared in two physiological states, late pregnancy and early lactation. Three periods were evaluated in each state being 6, 4, and 2 weeks prepartum, and 2, 4, and 6 weeks postpartum. Timing for the prepartum challenges was calculated according to estimated date of parturition. The two metabolic challenges consisted of: 1) a single intravenous (IV) injection of 250 ml of a

25% glucose solution (62.5 g glucose total), and 2) a single IV injection of L-epinephrine (0.7 µg/kg of body weight). Challenges were administered on two successive days at each time period in a sequence of glucose (day 1) and epinephrine (day 2). To facilitate injections and blood sampling, an indwelling catheter (Subclavicat<sup>TM</sup>) was placed in a jugular vein of each goat on day 1, and maintained viable with a 1% EDTA solution. A total of 10 blood samples were withdrawn at approximately 08:00 h during each challenge, starting at -30, -15 and 0 minutes before the metabolic challenge, and subsequently at 5, 10, 30, 60, 90, 120 and 240 minutes after injection, removing 5 ml of blood at each sampling time, placed in tubes containing 0.5 ml of a 1% EDTA solution, and immediately centrifuged at 5000 rpm for 20 min; the plasma was harvested and frozen until subsequent analysis.

The criteria of response to the two metabolic challenges, were: plasma concentrations of glucose (glucose-oxidase method (HYCEL<sup>TM</sup> kit No. 70478) expressed as mg/dl, NEFA (enzymatic commercial method (RANDOX<sup>TM</sup> NEFA kit FA115), values expressed as mmol/l, and insulin, assayed using the double antibody radioimmunoassay method developed by the Instituto Nacional de Nutrición-Salvador Zubirán, México, D.F., using iodinated human <sup>125</sup>I-Insulin. Results are expressed as ng/ml.

**Glucose challenge:** The plasma glucose response was evaluated by comparing the disappearance rate, expressed as  $t_{1/2}$  in minutes, and the concentration at zero time in mg/dl ( $t_0$ ) for all periods. To calculate  $t_{1/2}$ , regression of log-transformed post-injection values (adjusted for basal concentration) against time of sampling was calculated and the slope ( $k$ ) was used according to Twardock (1977) in the formula:  $t_{1/2} = \ln 2/k$ . The antilog of the 'y' intercept of the regression line was used to estimate the "instantaneous" concentration at zero time. Plasma concentrations of NEFA and insulin for each goat for each period were plotted against time, and the areas under the curve above or below the basal value were determined as criteria of responses (Easy Plot<sup>TM</sup>,) and expressed as  $\text{mmol} \cdot \text{l}^{-1} \cdot \text{min}$  and  $\text{ngml}^{-1} \cdot \text{min}$ , respectively.

**Epinephrine challenge:** The areas of graphically represented responses of glucose, NEFA and insulin to exogenous epinephrine were also used as criteria to evaluate this metabolic challenge, and were determined following the same procedure as for NEFA and insulin in the glucose challenge. For glucose the integrated response was expressed as  $\text{mgdl}^{-1} \cdot \text{min}$ .

Individual values from the six animals for all the parameters were evaluated for each challenge during all periods. Data were subjected to regression analysis using the GLM and DUNCAN procedures of

Statistical Analysis Systems Institute Inc. (SAS Institute Inc., 2001).

## RESULTS AND DISCUSSION

Body weight changes, dry matter intake and milk production are presented in Table 1. Goats lost significant ( $P < 0.01$ ) body weight from parturition ( $51.0 \pm 7.9$  kg) to six weeks postpartum ( $43.8 \pm 6.9$  kg), but no differences were found in daily dry matter intake or daily milk production ( $P > 0.1$ ), with averages of  $1.8 \pm 0.07$  kg/d, and  $1.25 \pm 0.15$  kg/d, respectively.

Recorded weight loss demonstrates the higher demand for nutrients imposed by the mammary gland with the initiation of lactation which dry matter intake could not compensate (remained unchanged). Milk production reported does not consider milk consumed by the respective kids, and only represents production obtained during the once-a-day mechanical milking.

Average kids per goat was  $2.33 \pm 0.7$  with an average body weight at birth of  $2.86 \pm 0.3$  kg for female kids and  $3.2 \pm 0.2$  for male kids, and at 41 days of age of  $8.1 \pm 0.3$  kg for female kids and  $8.8 \pm 1.0$  for male. Glucose and insulin basal concentrations were the same throughout the periods evaluated ( $P > 0.1$ ) with an average of  $77.9 \pm 30$  mg/dl and  $0.26 \pm 0.27$  ng/ml, for plasma glucose and insulin, respectively (Table 2).

NEFA basal concentrations were similar during the prepartum periods varying from  $0.13 \pm 0.1$  to  $0.20 \pm 0.2$  mmol/l, but during lactation concentrations increased significantly ( $P < 0.001$ ) during weeks 2 and 4 postpartum ( $0.40 \pm 0.2$  and  $0.57 \pm 0.3$  mmol/l, respectively). The increase in NEFA concentration coincides with the weight loss animals experienced during lactation, which indicates that they were in negative energy balance, although it could not be estimated due to suckling from their kids. It also may be an indicative of an increase in lipid mobilization from adipose tissue or a reduction of its rate of utilization by other tissues, but probably the principal effect is due to the former due to the energy demand imposed by milk production which could not be covered by animal intake.

### Response to glucose challenge

Response of the goats to the exogenous 62.5 g glucose challenge is shown in Table 3 and Figure 1. The peak glucose increment after injection ( $t_0$ ) did not vary between physiological stages, with a mean of  $290 \pm 17$  mg/dl. Assuming instantaneous mixing of exogenous glucose, this suggests that volume of distribution was the same among the animals and between periods.

The disappearance rate of the glucose load, expressed as  $t_{1/2}$  in minutes, was similar ( $P > 0.1$ ) during pregnancy and lactation, with a mean of  $9.4 \pm 1.2$  min. In other species it has been observed that rate of utilization of glucose increases significantly with the initiation of lactation, which probably happens in goats too, but due to the animal variation observed in the present study, this could not be verified.

Figure 1 depicts the temporal patterns of plasma glucose, insulin and NEFA responses to glucose challenge throughout the 240 min after glucose administration.

Insulin concentration was increased above basal concentrations following glucose challenge but increase was not different between periods ( $P > 0.1$ ) with a mean of  $63.3 \pm 8.2$  ngml<sup>-1</sup>min (Table 3). Higher numerical values were observed at weeks 2 and 4 postpartum, which were similar to non-pregnant, non-lactating goats.

Although tissue sensitivity to insulin could differ at different physiological periods, variation among animals did not allow detecting differences (Figure 1).

NEFA concentrations were significantly increased with glucose administration between pregnancy and lactation ( $P < 0.001$ ). The average of these concentrations during prepartum periods was  $0.21$  mmol.l<sup>-1</sup>min while during the postpartum periods the average was  $0.71$  mmol.l<sup>-1</sup>min. Response was contrary to what was expected, since an increase in plasma glucose should have inhibited lipid mobilization as in the non-pregnant, non-lactating animals (Table 3).

Probably, the glucose load was rapidly removed by mammary gland during lactation not permitting other tissues to utilize it. In dairy cattle, mammary gland removes from 60 to 80% of circulating glucose (Weekes, 1980) and in ewes up to 60% (Bergman and Hogue, 1967); this information is not available in the dairy goat, but probably a similar glucose removal occurred in the dairy goat not permitting insulin to respond in a rapid manner. This needs further verification.

### Response to epinephrine challenge

Epinephrine administration elicited an increase in plasma concentrations of glucose and insulin (Table 4) during all periods evaluated, but changes were not significant ( $P > 0.1$ ). Figure 2 depicts the temporal pattern of response to the epinephrine challenge of plasma concentrations of glucose, insulin and NEFA. It was evident that epinephrine increased plasma glucose from basal concentrations, which was probably due to the glycogenolytic effect of this

hormone in the liver; however, it is not known the liver concentration of glycogen in the goat which could explain this response. In dairy cattle, it is documented that liver reserves of glycogen are lowest during early lactation (Baird, 1979). On the other hand, the hyperglycemic response observed could be the result of reduced glucose utilization for lactose synthesis due to decreased blood flow to the mammary gland.

Low blood glucose concentrations have been reported in dairy goats around parturition which rose after 20 days into lactation (Khan and Ludri, 2002; Dunshea *et al.*, 1989).

NEFA concentrations were significantly increased after epinephrine injection ( $P < 0.001$ ). Figure 2 depicts the temporal pattern of response to the epinephrine challenge of plasma concentrations of glucose, insulin and NEFA. During pregnancy, epinephrine increased NEFA concentrations above basal but increases were not as dramatic as those during lactation (Table 4), which were highest at 4 weeks postpartum ( $1.40 \pm 0.3$  mmol.l<sup>-1</sup>min), followed at 2 weeks postpartum ( $1.24 \pm 0.6$  mmol.l<sup>-1</sup>min).

It is well established that epinephrine increases rate of lipolysis in ruminant adipose tissue (Bauman, 1976; Metz and van den Bergh, 1972), but the present study demonstrates that the magnitude of this response varies according to physiological stage, and clearly lactation imposes different controls. Mobilization of fatty acids from adipose tissue coincides with the loss in body weight of the animals at six weeks postpartum (Table 1) and it is closely related to a probable negative energy balance experienced by the animals, which as mentioned earlier, could not be estimated.

In the non-pregnant, non-lactating goats, NEFA concentration was not different from basal values, but only a small change was observed (Table 4). Clearly, the adipocytes appear to be more responsive to epinephrine during lactation, probably due to changes in receptors number or affinity for epinephrine.

## CONCLUSION

Greater responses to the metabolic challenges were evident during lactation than during pregnancy, demonstrating that nutrient demand for milk production imposes important metabolic adaptations in liver and adipose tissue to prevent alterations in the energy homeostasis of the lactating goat. Feeding strategies for goats under different production systems should be finely developed to meet their nutritional needs during the last third of pregnancy and the first third of lactation.

## ACKNOWLEDGEMENTS

Research was partially funded under CONACYT grant No. I0002-67849, and was part of the MSc Thesis of the first author.

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Table 1. Body weight, dry matter intake and milk production of Nubian goats at different periods relative to parturition

Periods <sup>1</sup>	Body weight	Dry matter intake	Milk production
Prepartum	.....	kg	.....
-6	54.5 <sup>a</sup> ± 5.8	1.48 ± 0.4	-
-4	-	1.78 ± 0.4	-
-2	-	1.87 ± 0.3	-
Postpartum	51.0 <sup>a</sup> ± 7.9	-	-
+2	-	1.71 ± 0.4	1.30 ± 0.8
+4	-	1.79 ± 0.5	1.26 ± 0.6
+6	43.8 <sup>b</sup> ± 6.9	2.00 ± 0.3	1.22 ± 0.5
<u>Non-pregnant, non-lactating</u>			
Animal No. 11	53.9	-	-
Animal No. 614	54.9	-	-

<sup>1</sup> Weeks relative to parturition; values represent the mean ± standard deviation.<sup>a,b</sup> Means within the same column with dissimilar superscripts are significantly different (P<0.01).Table 2. Basal glucose, insulin and NEFA plasma concentrations of Nubian goats at different physiological stages<sup>1, 2</sup>

Periods	Weeks relative to parturition	N	Glucose	Insulin	NEFA
			mg/dl	ng/ml	mmol/l
Prepartum	-6	3	93.2 ± 35.7	0.22 ± 0.1	0.13 <sup>c</sup> ± 0.1
	-4	6	70.3 ± 23.9	0.25 ± 0.2	0.20 <sup>bc</sup> ± 0.2
	-2	6	70.1 ± 22.1	0.24 ± 0.2	0.17 <sup>bc</sup> ± 0.1
Posrpartum	+2	6	88.4 ± 42.3	0.33 ± 0.4	0.40 <sup>ab</sup> ± 0.2
	+4	6	71.2 ± 23.6	0.25 ± 0.4	0.57 <sup>a</sup> ± 0.3
	+6	6	82.2 ± 29.4	0.28 ± 0.3	0.17 <sup>bc</sup> ± 0.1
<u>Non-pregnant, non-lactating</u> <sup>3</sup>					
Animal No. 11			79.1 ± 13	0.33 ± 0.1	0.61 ± 0.1
Animal No. 614			73.1 ± 6	0.31 ± 0.1	0.40 ± 0.2

<sup>1</sup> Values represent means ± standard deviation.<sup>2</sup> Basal values represent pooled values of the average of three pre-injection blood samples from each of the two metabolic challenges.<sup>3</sup> Received the two metabolic challenges on two occasions during the prepartum periods. Values represent the average of the two occasions.<sup>a,b,c</sup> Means within the same column with dissimilar superscripts are different (P<0.001)

Table 3. Response in glucose concentrations of glucose, insulin and NEFA to glucose challenge in Nubian goats at different periods around parturition

Period	Weeks relative to parturition	Glucose <sup>1</sup>		Integrated response <sup>2</sup>	
		t <sub>0</sub>	t <sub>1/2</sub>	Insulin	NEFA
		mg/dl	min	ngml <sup>-1</sup> min	mmol.l <sup>-1</sup> min
Prepartum	-6	370 ± 204	10 ± 15	55 ± 75	0.20 <sup>b</sup> ± 0.2
	-4	314 ± 126	6 ± 5	94 ± 19	0.16 <sup>b</sup> ± 0.1
	-2	269 ± 67	4 ± 2	76 ± 26	0.27 <sup>b</sup> ± 0.2
Postpartum	+2	256 ± 77	8 ± 7	84 ± 33	0.69 <sup>ab</sup> ± 0.5
	+4	290 ± 87	9 ± 7	73 ± 29	1.01 <sup>a</sup> ± 0.7
	+6	279 ± 77	16 ± 11	63 ± 39	0.43 <sup>b</sup> ± 0.2
<u>Non-pregnant, non-lactating<sup>3</sup></u>					
Animal No. 11		212 ± 8	11 ± 3	225 ± 156	-2.3
Animal No. 614		229 ± 62	18 ± 19	98 ± 56	-1.5

<sup>1</sup> Glucose dose injected via jugular catheter was 62.5 g/kg body weight throughout all periods evaluated. Values represent the mean ± standard deviation

<sup>2</sup> Response computed as area under the curve as described in Materials and Methods

<sup>3</sup> Received the two metabolic challenges on two occasions during the prepartum periods. Values represent the average of the two occasions

<sup>a b c</sup> Means within the same column with dissimilar superscripts are different (P<0.001)

Table 4. Response in glucose concentrations of glucose, insulin and NEFA to epinephrine challenge in Nubian goats at different periods around parturition<sup>1</sup>

Period	Weeks relative to parturition	Integrated response <sup>2</sup>		
		Glucose	Insulin	NEFA
		mgdl <sup>-1</sup> min	ngml <sup>-1</sup> min	mmol.l <sup>-1</sup> min
Prepartum	-6	3404 ± 1364	13 ± 11	0.45 <sup>c</sup> ± 0.1
	-4	4178 ± 1333	10 ± 9	0.54 <sup>c</sup> ± 0.3
	-2	4271 ± 1324	10 ± 9	0.64 <sup>c</sup> ± 0.2
Postpartum	+2	3559 ± 2516	20 ± 11	1.24 <sup>ab</sup> ± 0.6
	+4	3361 ± 1291	11 ± 10	1.40 <sup>a</sup> ± 0.3
	+6	4305 ± 2845	19 ± 5	0.72 <sup>b</sup> ± 0.3
<u>Non-pregnant, non-lactating<sup>3</sup></u>				
Animal No. 11		4814 ± 906	31.6 ± 15	-0.24
Animal No. 614		4920 ± 165	39.8 ± 1	0.07

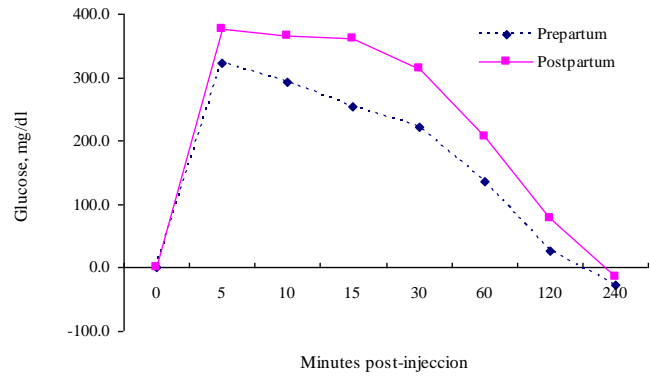
<sup>1</sup> L-epinephrine dose (Pisa<sup>TM</sup>) injected via jugular catheter was 0.7 µg/kg body weight throughout all periods evaluated. Values represent the mean ± standard deviation

<sup>2</sup> Response computed as area under the curve as described in Materials and Methods

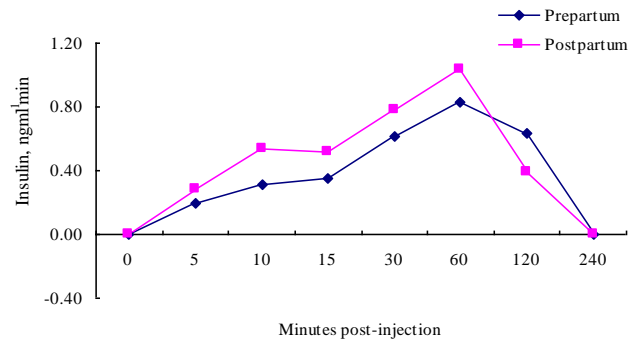
<sup>3</sup> Received the two metabolic challenges on two occasions during the prepartum periods. Values represent the average of the two occasions

<sup>a b c</sup> Means within the same column with dissimilar superscripts are different (P<0.001)

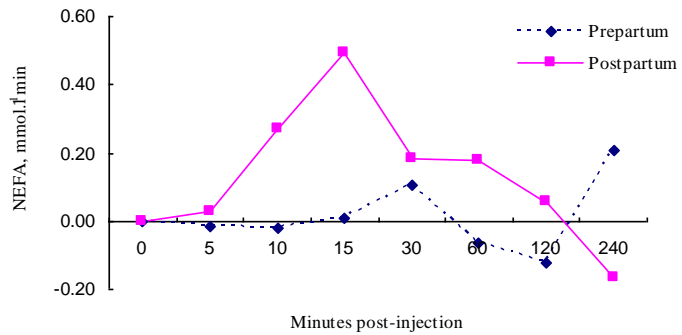
Figure 1. Plasma glucose (a), insulin (b) and (c) NEFA responses to glucose challenge in Nubian goats at two physiological states. Number of animals and basal concentrations (zero time value) as in Table 2.



a)



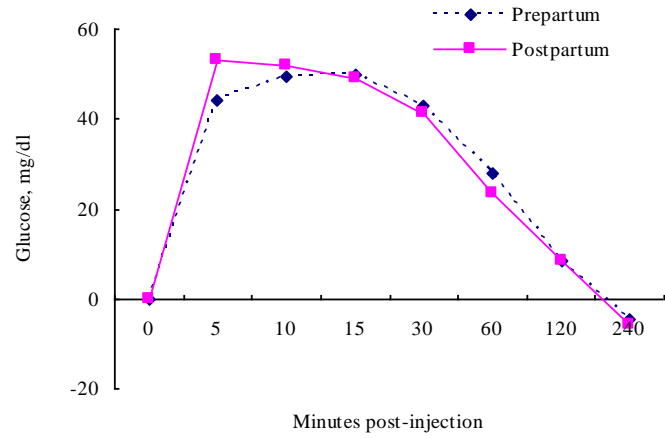
b)



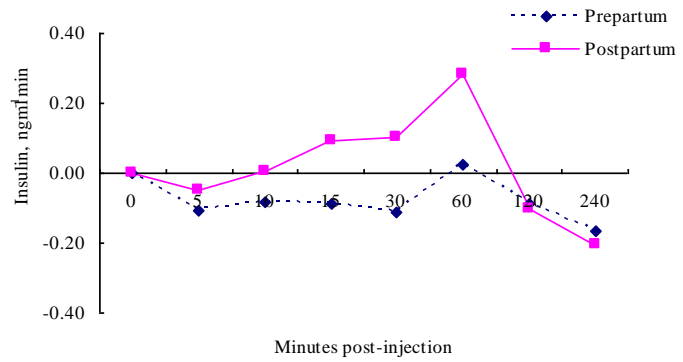
c)



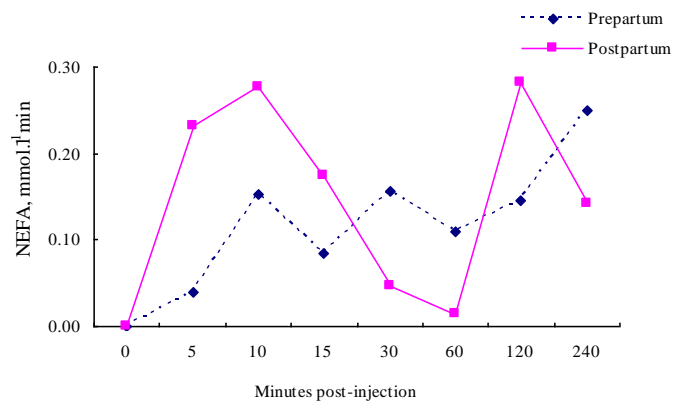
Figure 2. Plasma glucose (a), insulin (b) and (c) NEFA responses to the epinephrine challenge in Nubian goats at two physiological states. Number of animals and basal concentrations (zero time value) as in Table 2.



a)



b)



c)