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SHORT TERM CHANGES IN PLASMA HORMONES, METABOLITES, MILK YIELD AND PHYSIOLOGICAL RESPONSES IN EPINEPHRINE ADMINISTRATED COWS

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ABSTRACT

This investigation summarizes the effect of epinephrine administration on milk production and plasma hormones. The epinephrine and nor-epinephrine hormone are released during stress and may have adverse effect on milk yield of lactating animals. Lactating cows were administered epinephrine intravenously (@0.5 ug/kg body wt./ day) for a period of five days. Blood samples were collected before and after the administration of epinephrine. Plasma metabolites and hormones were analyzed. Epinephrine treatment significantly increased plasma epinephrine and nor-epinephrine ($P<0.01$) levels concomitant to decline in milk yield ($P<0.01$) in all the cows on day 1 of injections and the effect on milk yield continued during the treatment period. Plasma insulin and glucose level increased ($P<0.05$) after the treatment, however plasma cortisol, thyroid hormone and NEFA level was not influenced by treatment. Further, there was no effect of treatment on milk composition (fat, protein, lactose, solid not fat and somatic cell count), respiration rate, pulse rate, rectal temperature and skin temperature. It was concluded that elevated plasma epinephrine and norepinephrine level inhibit milk secretion ($P<0.05$) in cows, however milk composition, physiological responses and plasma cortisol levels remain unaffected.

***Keywords**:* catecholamines, milk yield, plasma metabolites, physiological responses, cows

Number of Tables: 3	**Number of Figures: 3**	**Number of References: 25**

INTRODUCTION

Catecholamine (epinephrine and norepinephrine) and their metabolitesare used for the evaluation of several types of behaviors, neuro-endocrine disorders and physiological and pathological stress situations as a neurotransmitter or as hormone (Connolly *et al*., 1991). The concentrations of E and NE in cattle have been measured during fasting (Frohli and Blum, 1988), heat exposure (Johnson and Vanjonack, 1976; Katti et al., 1991) and during chronic pain in animals(Ley *et al*., 1992, 1996).The rise in epinephrine level exerts potent stimulatory effects on glucose production principally by enhancing hepatic glycogenolysis, although rise in circulating nor-epinephrine hormone has minimal effects (McGuinness*et al*., 1997). Cortisol increases supply of gluconeogenic precursors reaching the liver and augments hepatic glycogen stores despite concomitant elevations in the other counter regulatory hormones(Fujiwara *et al*., 1996). To date, however, what role elevated circulating catecholamine play in driving the metabolic and physiological responses to epinephrine infusion has not been examined in lactating cows raised in tropical climates? In the present study, effect of exogenous administration of epinephrine on plasma hormones, milk yield and physiological responses was determined in crossbred cows.

MATERIALS AND METHODS

Six lactating crossbred cows having body weight av. 425.5, parity 2.83, in mid lactation (170 d) was managed in a separate shelter shed having brick floor. The animals were offered *ad lib*. green fodder berseem (*trifoliumalexandrium*) and water as per their choice. Concentrate mixture was fed based on milk yield (@2kg/kg milk yield) during a.m., noon and p.m. milking and the milk yields were recorded. The crude protein and total digestible nutrients of the diet was 20% and 70%, respectively. Epinephrine bitartrate dissolved in normal saline was injected intravenously in jugular vein @0.5µg/kg body weight/d for a period of 5 days. Blood samples were collected in tubes containing EDTA and sodium metabisulphite through jugular canula before (0) and after treatment at 30, 60 and 90 minute interval. Aliquots of milk samples from individual animal were analyzed for fat, protein, lactose, solid not fat (Milcotester) and somatic cell count (Singh and Ludri, 2001). Physiological responses viz., respiration rate (RT), skin temperature (ST), rectal temperature (RR) and pulse rate (PR) was recorded. Plasma epinephrine (E), norepinephrine (NE), cortisol (CORT), insulin (INS) and thyroid hormones was measured using radio immune assay methods (Singh and Ludri, 1999). The inter assay and intra assay coefficient of variation for CORT, T_3, T_4, INS, E and NE was 8.3 % and 7.2%, 6 % and 7.9%, 9.2 % and 10.5 % , 7.9 and 12.5, 9.9 and 13.5 and 10.1 and 12 %, respectively. Plasma glucose was estimated by kit and the NEFA by Shipe and Fountain (1980) method. Statistical analysis of data was carried out by two way ANOVA with interaction. Mean values were compared with Duncans Multiple Range

Test and the correlation among various parameters were found out.

RESULTS AND DISCUSSION

Administration of epinephrine significantly decreased (P<0.01) milk yield on day 1 of injection and the inhibitory effect on milk yield continued during the treatment period (Table 1). Milk yield varied (P<0.01) between day and between animals. The effect of epinephrine treatment on milk yield was variable (P<0.05) and the milk yield declined by 3.2, 22.14, 15.15, 18.01 and 18.79 % on day 1, 2, 3, 4 and 5 of treatment respectively. However restoration of milk yield of cows on day- 1 after the cessation of treatment indicated that high epinephrine and nor-epinephrine levels impair milk secretion in cows (Table 1). The negative correlation of milk yield (<0.01; r=-0.295) with epinephrine and nor-epinephrine levels further confirms this fact. Further non-significant changes in milk fat, SNF, lactose, SCC and protein indicated that both the hormones did not affect milk synthesis in mammary gland (Table1).Administration of epinephrine significantly (P<0.05) increased plasma E and NE levels(Figure 1, 2) concomitant to decline in milk yield during the treatment period, but cortisol, T_3 and T_4 concentration was not affected. Plasma E (0.10 to 0.25g/ml) and NE level (0.07-0.28 ng/ml) was significantly less (P<0.01)on day 1before administration of epinephrine and the concentration increased (P<0.01) after 30, 60 and 90 minute of treatment. The significant changes in E and NE was also reflected in significant interaction between day x interval (P<0.01). The values of plasma E and NE level was significantly (P<0.01) higher on day 4 and 5 in comparison to day 1 and 2 of treatment. Plasma insulin concentration was significantly low (P<0.01) before injection and increased significantly (P<0.01) at 30, 60 and 90 minute post injection. Plasma glucose level was less before treatment and increased significantly (P<0.01) in samples collected at 30, 60 and 90 minute intervals, however plasma NEFA concentration varied non-significantly (Table 2).

ST of cows varied significantly (P<0.01) during different days of experiment. Skin temperature varied non-significantly between interval and day x interval. RTrangednon- significantly between 99.90±0.82 to 101.80 ±0.12°F during the experiment (Table 3). RRand PR averaged 27.16±0.83 to 32.66±0.76 breaths/ minute and 66.33±0.87 to 70.16±0.59 beats/minute and was non-significant. Correlation between plasma insulin and NE level was significant (P<0.01; r=0.314).

In this investigation, administration of epinephrine neither increased respiration rate, rectal temperature, pulse rate nor plasma NEFA concentrations indicating thereby that short term injection of epinephrine was not detrimental to the health of cows. The decline in the milk yield was due to less supply of nutrients to the mammary gland which impaired milk synthesis (Bruckmaier et al, 1997).The significant reduction in the milk yield of cows concomitant to higher catecholamine level suggest that elevated E and NE level was inhibitory to milk secretion (Bernabe and Ricordel, 1985). This fact was further evident from restoration of milk yield on day 1post treatment. The result of the experiment cannot be compared as few studies have measured catecholamines in farm animals because of the high cost of assays, practical difficulties in collection and measurement of catecholamines and short half -life (1-2 min) in plasma (Hjemdahl, 1993). The non-significant changes in milk composition further indicated that synthesis of milk constituents in the epithelial cell of mammary gland was not influenced by epinephrine treatment. Somatic cell counts which are affected by various factors in cows, buffaloes and goats ((Marcus and Dale, 1994; Singh and Ludri, 2001; Shailja and Singh, 2004) remain unaffected by epinephrine treatment. The epinephrine treatment caused 3-4 fold increase in plasma epinephrine and 7-9 fold increase in nor-epinephrine levels which influenced milk yield, plasma glucose and insulin levels in the present study. A similar rise of this magnitude was reported earlier in cows exposed to high ambient temperature and humidity (35 °C and 95 % RH), (Naik et al., 2012). The significantly higher level of NE than the E level could be attributed tothe fact that baseline level of nor -adrenaline are consistently higher than that of adrenaline in bovine studies leading to in a complementary increase in both adrenaline and noradrenalin levels (Lefcourt and Elasser, 1995). The study in Holstein dairy cattle during normal lactation by Katti et al. (1991) revealed an E: NE ratio of 0.21 and a ratio of 0.494 in buffaloes. Norepinephrine concentrations rise (4.50 nmol/l) immediately after birth, whereas post-partum epinephrine levels did not show an increase (Rausch et *al*., 1989). The increase in glucose level following E treatment was due to the hepatic glycogenolysis than gluconeogenesis as later is more responsiveto high levels of nor-epinephrine (Connolly*et al*., 1991; Stevenson,1991). The increase in blood glucose level is mediated by alpha 2-adrenergic receptors by reducing the rate of glucose phosphorylation (Hunt et al., 2002) .We did not observed significant rise in plasma NEFA level due to greater variability but response of plasma NEFA and glycerol to epinephrine challenge (0.1, 0.2, 0.4, 0.8, 1.2 and 1.6 micrograms/kg body wt., iv.) markedly increases (McGuinness*et al*., 1997).NEFA concentration is influenced by stress (Uetake*et al*., 2006) and NEFA is frequently used to evaluate the energy status of animals (Kida, 2002; Macrae*et al*., 2006). Acute stress results in activation of the sympathetic-adrenal medullary axis and the hypothalamic–pituitary–adrenal cortex axis resulting in a more gradual rise in ACTH and cortisol concentrations that peak by 10–20 min (Ley *et al*., 1992). This enhances fuel availability due to NEFA mobilization due to the activation of lipase by both hormones (Cunningham and Klein, 2007). Contrary to this plasma cortisol level was not significantly influenced in this study. The non-significant changes in T_3, T_4 and cortisol level further confirms that metabolism was not significantly influenced by the short term epinephrine treatment (Table 2, Figure 3).The sympathetic responses such as increased heart rate, respiratory rate, body temperature and secretion of epinephrine have been used as physiological indicators of stress (Broom &Johnson 1993). However there was no effect of epinephrine treatment on physiological responses in this study, except skin temperature which varied due to day-to day variations in ambient temperature.

CONCLUSION

Administration of epinephrine i.v @0.5µg/kg b.wt/day for a period of 5 days significantly decreased milk yield concomitant to rise in plasma epinephrine, nor-epinephrine, insulin and glucose concentration. However, plasma cortisol, NEFA, T_3, $T_4$and physiological responses were not influenced by the treatment suggesting there by that catecholamines have inhibitory role in milk secretion of crossbred cows.

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Table 1: *Overall mean (±SE) milk yield and milk composition changes in epinephrine administered crossbred cows.*

Attributes	Before Treatment	During Treatment	After Treatment	Significance
Milk Yield (kg)	10.52^{a}±0.77	8.62^{b} ±1.05	10.62^{a} ±0.76	P<0.01
Fat %	3.60 ±0.50	3.74±0.5	3.83±0.15	NS
Protein %	3.23±0.21	3.21±0.26	3.40±0.20	NS
Lactose %	4.28±0.19	4.31±0.24	4.24±0.26	NS
SNF %	9.32±0.50	9.26±0.169	9.72±0.20	NS
SCC (x 10^5 cells/ml)	1.99±0.138	1.22±0.141	1.21±0.22	NS

Values with different superscripts a, b differ (P<0.05) in a row

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Table 2: *Mean (± SE)) plasma glucose and NEFA levels before and after administration of epinephrine in lactating KF cows .*

Day	Before Treatment	After treatment (minute)		
	0	30	60	90
Glucose (mg/dl)				
1	53.21±0.49[a]	53.61±1.65[a]	56.78±1.40[a]	58.30±1.58[b]
2	52.48±0.80[a]	52.29±1.82[a]	55.15±1.01[a]	61.00±1.75[b]
3	49.60±1.46[a]	58.81±2.15[b]	57.69±2.35[b]	59.03±1.54[b]
4	50.48±1.00[a]	54.53±0.68[a]	59.03±0.80[b]	58.20±0.80[b]
5	50.44±1.55[a]	52.98±0.99[a]	53.06±1.84[a]	55.35±1.29[b]
NEFA (uMol/ml)				
1	0.30±0.02	0.38±0.05	0.41±0.03	0.45±0.03
2	0.40±0.03	0.41±0.03	0.34±0.07	0.42±0.09
3	0.40±0.06	0.35±0.04	0.15±0.01	0.20±0.04
4	0.18±0.04	0.32±0.08	0.32±0.06	0.23±0.06
5	0.21±0.04	0.40±0.06	0.41±0.05	0.31±0.05
Triiodothyroniine (ng/ml)				
1	1.11±0.20	0.81±0.11	1.01±0.21	1.11±0.31
2	1.50±0.21	1.02±0.10	1.21±0.22	1.20±0.12
3	1.30±0.20	1.02±0.21	1.52±0.14	1.51±0.20
4	1.51±0.11	1.03±0.11	1.04±0.22	1.01±0.22
5	098±0.10	1.04±0.10	1.05±0.21	1.02±0.31
Thyroxine (ng/ml)				
1	33.01±4.20	39.10±6.10	34.00±5.10	27.10±3.10
2	31.10±5.00	32.00±5.21	37.01±3.21	38.20±6.00
3	35.12±3.00	33.01±4.31	32.30±5.13	40.40±5.20
4	34.23±6.10	27.20±3.02	38.10±2.04	35.10±2.20
5	36.15±2.10	36.00±1.63	39.20±1.14	36.40±2.10
Insulin (uU/ml)				
1	13.00±0.70[ax]	16.01±3.00[ay]	19.02±2.00[by]	21.03±2.01[by]
2	15.01±1.00[ax]	19.00±1.50[ax]	21.10±0.81[bx]	24.01±0.60[by]
3	18.00±1.00[ay]	21.03±1.20[ay]	21.02±1.41[ay]	19.03±1.03[ax]
4	20.03±0.50[ay]	21.00±0.60ay	18.00±1.50[ay]	21.02±1.50[ay]
5	20.01±2.80[ay]	22.02±0.80[ay]	24.00±0.70[ay]	24.00±0.40[ay]

Value with different superscript a, b differ (P<0.05) in a row

Table 3: *Mean (± SE)) physiological responses before and after administration of epinephrine in lactating crossbred cows*

Day	Before Treatment	After treatment (minutes)		
	0	30	60	90
Skin temperature (oF)				
1	96.10±0.69^{x}	98.20±0.47^{x}	98.10±0.85^{x}	97.10±0.63^{x}
2	93.20±0.42^{y}	94.30±0.49^{x}	95.30±0.74^{y}	100.30±0.26^{y}
3	93.10±0.49^{y}	99.10±0.30^{y}	93.10±0.63^{y}	93.20±0.54^{z}
4	98.40±0.82^{x}	100.20±0.12^{y}	96.20±1.10^{y}	98.50±1.00^{x}
5	95.10±0.13^{x}	94.10±0.32^{x}	95.30±0.12^{y}	96.20±0.41^{z1}
Rectal temperature (oF)				
1	99.80±0.32	100.10±0.31	100.50±0.53	101.00±0.12
2	99.60±0.36	100.20±0.29	100.01±0.29	100.30±0.26
3	98.90±0.49	99.60±0.30	100.10±0.39	100.51±0.54
4	98.10±0.82	100.10±0.20	100.40±0.13	100.40±1.10
5	100.10±0.24	100.00±0.24	100.50±0.15	100.40±0.16
Pulse rate/min.				
1	67.00±1.87	69.66±1.12	68.00±1.54	68.16±1.06
2	67.83±1.34	70.16±1.21	68.00±2.09	69.00±0.78
3	67.83±1.11	68.50±0.99	70.16±0.59	70.00±0.54
4	68.50±1.62	69.00±1.22	68.00±1.05	68.33±1.28
5	67.33±1.21	68.66±1.38	68.32±1.09	66.33±0.87
Respiration rate /min.				
1	28.83±1.23	29.66±1.64	31.16±1.23	28.00±2.44
2	30.83±1.23	32.16±1.67	32.66±0.76	28.66±1.14
3	30.50±0.69	29.00±0.74	27.16±0.83	31.66±1.23
4	30.33±1.66	29.50±1.34	30.83±1.06	32.00±1.24
5	27.33±1.44	28.33±1.09	29.66±0.87	29.16±0.43

Value with different superscript x, y differ (P<0.05) in a column

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Fig 1: *Plasma epinephrine levels during different days of epinephrine administration in lactating KF cows*

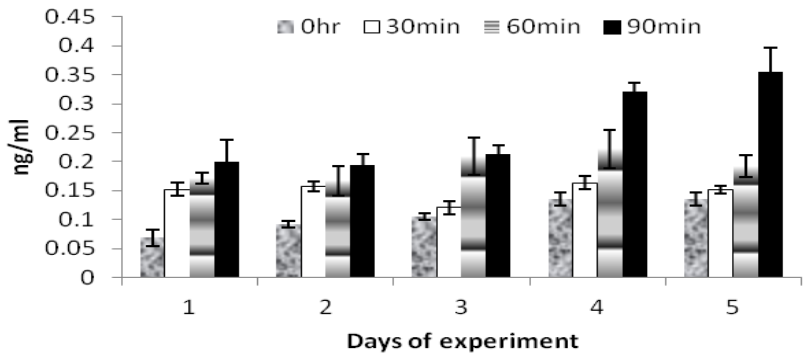


Fig 2: *Plasma norepinephrine levels during different days of epinephrine administration in lactating KF cows*

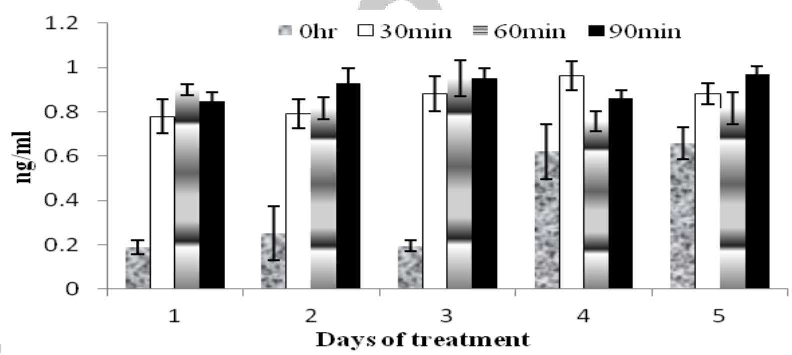
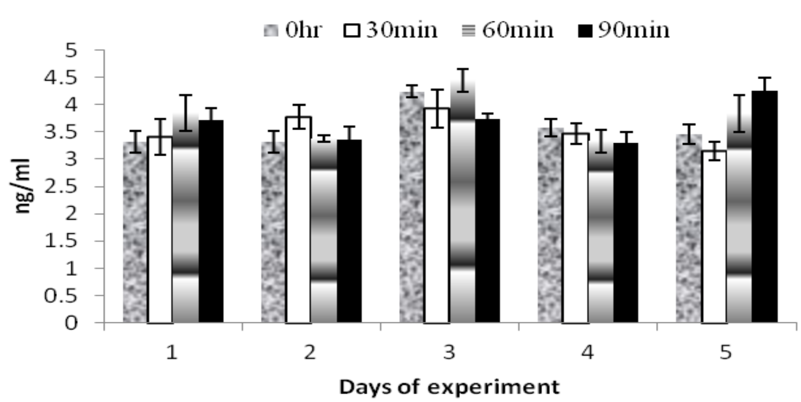


Fig 3: *Plasma cortisol levels during different days of epinephrine administration in lactating KF cows*



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