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Effect of Supplementation with Malate on Some Metabolites, Reproductive Performance and Milk Constituents in Early Lactating Egyptian Buffaloes

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Abstract: The objective of this study was to evaluate the effects of malate on some metabolites, reproductive parameters, calves performance and milk constituents in early lactating Egyptian buffaloes. Ten multiparous pregnant Egyptian buffaloes were divided into two equal groups in a randomized design; the control group (no supplementation) and the treated group (supplemented with 40 g/head/day as initial malate supplementation, one month pre-partum then 85 g/head/day from day of birth till day 60 post-partum (pp). Blood samples were collected on day of birth and each two weeks until two months pp. and analyzed for glucose, T3 and T4. Results indicated that there was an increase in the concentrations of serum glucose in the treated group compared with the control one at days 30, 45 and 60 pp. Malate increased serum T3 at day 60 pp and T4 at day of birth and at days 45 and 60 pp. There was an increase ($P<0.01$) in serum progesterone level on the 3rd day after the 1st ovulation, on the 3rd and the 7th days after estrus and services, respectively, as well as at the 21th day after conception in the treated buffaloes. Malate-treated group showed decreased number of days to first ovulation and to first detected estrus and there was a higher conception rate compared with the control one. Furthermore, treated group showed a decrease in days open and number of services/conception. There was an increase ($P<0.05$) in milk protein, lactose and solid not fat percents due to malate supplementation; meanwhile the increase in milk fat percent was non-significant. Also, there was an increase ($P<0.05$) in weaning weight and ($P<0.01$) average daily gain of calves from treated dams compared with those from the control one. It was concluded that malate could be used as a feed supplement at late pregnancy and early lactation to prevent acidosis and to improve productive and reproductive performance as well as calves performance.

Key words: Malate · Metabolites · Milk · Reproduction · Buffaloes

INTRODUCTION

Buffaloes are well known as a source for higher grade milk, meat and drought power, but their productive potential has not yet been fully utilized. Reproductive efficiency has a significant influence on economic returns from buffaloes. Long sexual inactivity after calving resulting in a long intercalving period that has an adverse effect on both reproductive and productive potentials [1]. Nutritionists now consider that the transition period (the period between the last 2-3 weeks of gestation and first 2 weeks of lactation) is the key phase in lactation cycle. During this period dams go through a highly demanding phase associated with important physiological, metabolic and nutritional changes [2]. In transition period nutrition and management programs directly affect the incidence of post-calving disorders, milk production, dam and calf health, fertility and ultimately, the overall productivity of the dairy farm. Nutritionists have to program high energy diets to properly feed the animals, but these extreme diets can lead to severe disorders, particularly rumen acidosis [3]. Malic acid is a four-carbon dicarboxylic acid that is an intermediate in the succinate – propionate pathway of ruminal bacteria [4]. *In vitro*, malate lead to increased concentrations of propionate and total volatile fatty acids [5 -7] feed digestibility [8] and decreased methane production [7] and lactate concentration [9, 7]. Some studies reported that malate supplementation increased milk performance [10, 11]. Carboxylic acid salts activate the transformation of lactic acid into propionic acid through the *Selenomonas rumnantium* bacteria by using the succinate-propionate pathway [12], which prevents pH reduction in the rumen. Propionic acid is an essential

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substratum for glucose and lactose synthesis, by this way malate increases the available energy for growth and milk production [5]. Kung *et al.* [13] found that the addition of malate increased persistence in milk production. Supplementation of dairy cows with malate increased milk production with subtle increase of milk fat contents during first 63 days of lactation and improved energy availability as higher blood glucose and insulin, lower blood beta-hydroxybutyric acid and non-estrified fatty acids and lower urine ketones [14]. Furthermore, the data on the effects of malate supplementation in the early lactation period on milk constituents, blood metabolites and reproductive performance are limited.

The aim of this work was to evaluate the effects of malate supplementation on blood metabolic profiles, reproductive performance and milk constituents in early lactating Egyptian buffaloes and performance of their calves.

MATERIALS AND METHODS

The present experiment was conducted during the period from June, 2007 to December, 2007 at the experimental buffaloes farm of the Animal Reproduction Institute (ARRI) EL-Ahram, Giza.

Animals Management: The experiment was carried out on ten, 4-7 years-old pregnant (during the last month of pregnancy) multiparous Egyptian buffaloes weighing 600–650 kgs. Buffaloes were housed in an open free-stall barn under natural light and temperature. All animals were fed on balanced ration formulated to meet established nutrient requirements of pregnant and lactating dairy animals and water *ad-libitium* [15]. Animals were classified according to age, parity, body weight and according to their treatment into two equal groups. Group: I (control group): buffaloes were fed basal diet and received no treatment. Group II (treated group), buffaloes were fed basal diet plus the treatment which was initiated approximately four weeks before calving by 40 g/head /day of malate=carboxylic acid salt {Rumalato: Norel and Nature,Norel–Misr Suaz Gulf P.O.B.157Suaz} and after that by 85 g/head/day from the day of calving until two months in milk.

Blood Sampling and Hormonal Assay: Blood samples were collected from all animals before treatment for hormonal assay and at day of birth and there after every two weeks for estimation of glucose, T3 and T4 levels. Ten ml of blood was collected from the jugular vein before feeding and allowed to coagulate and serum was harvested and kept frozen at – 20°C. Meanwhile, blood samples were collected from all animals for progesterone assay twice a week starting from day 15 post-calving till conception, to detect ovarian-activity (1^{st} ovulation and first detected estrus) and to confirm conception. Serum samples for hormonal assay were analyzed by ELISA technique (DRG Instruments GnbH, Germany. Division of DKG–international Inc. Fravenberg Str.18, D. 325039 Marburg). Samples were analyzed for T3[16],T4[17] and progesterone [18] using solid phase ELISA. The minimum detectable concentrations were 0.2ng/ml for T3, 0.4 ug/dl for T4 and 0.054ng/ml for progesterone.

Meanwhile serum glucose was determined by enzymatic method [19].

Milk Sampling: Milk samples were taken every two weeks until 60 days post-partum and were analyzed using infrared milk analyzer (Bentley-150), to estimate percentages of fat, protein, lactose, urea,total solid (TS) and solid not fat (SNF).

Reproductive Measures and Calves Performance: Beginning from day 15 post-calving, buffaloes of the two groups were tesed by three vasectomized buffalo-bulls twice daily (7.00 am and 16.00 pm for 30 min) for estrous detection. Estrus was confirmed by changes in serum progesterone (P_4) level, when a buffaloe had a P_4 level of < 1ng /ml with a subsequent increase of = 1ng /ml over the next consequent three samples [20]. Once a buffaloe was recorded in estrus (40 days post-partum) it was bred by a fertile bull and day of service was recorded. Pregnancy diagnosis was carried out by ultrasound scanner (200 pies Medica co. - Netherlands, Holland) about one month post-mating. Days to the first ovulation were recorded (which was assumed to have occurred 3 - 4 days before an increase in serum P_4 = 1ng /ml). Also, days to the first detected estrus and conception (days open) and conception rates were recorded. Number of services per conception was recorded for each pregnant buffalo. Borned calves were sexed, weighed and numbered at the day of birth (birth weight, BW) and were kept with their dams for suckling until weaning at 90 days of age. Calves were weighed again at weaning (weaning weight, WW). The growth rate (g /day) of both male and female calves was calculated from birth until weaning.

Statistical Analysis: All data were subjected to statistical analysis according to Snedecor and Cochran [21]. Data were analyzed by one way ANOVA implying a completely randomized design after angular (Log) 1989. The difference between treatments were further compared by Duncan multiple range test using 3.03 version of Cost.

RESULTS

Data analysis (Table 1) revealed that blood glucose levels of treated group were higher (P<0.01) on days 30 and 60 post-partum as well as on day 45 (P<0.001) post-partum than those of the control group.

A non-significant increase in serum T3at days of treatment was noticed with an increase (P<0.01) at day 60 post-partum. Also, T4 increased at day of birth and at days 45 and 60 post-partum (P<0.01).

Table 3 reveals that, progesterone concentration in malate treated group increased (P<0.01) at the 3rd day after first ovulation and at the 3rd and 7th days after estrus and service as well as at day 21 after conception compared with the control group.

Reproductive Performance: Concerning the effect of malate supplementation on post-partum reproductive parameters (Table, 5) it was found that number of days to first ovulation, first detected estrus and to conception (days open) decreased in the treated group than in the control one. Regarding to conception rates and number of services per conception, data presented in the same table revealed that there was an improvement in conception rate as well as a marked decrease in the number of services per conception in malate treated group than that in the control one.

Calves Performance: Regarding calves performance, the present data (Table, 6) revealed that there was a non-significant difference in birth weight of calves from

Table 1: Serum glucose (mg/dl) concentration of control and treated groups (Means±SE)

Groups	Day of calving	Day 15 postpartum	Day 30 postpartum	Day 45 postpartum	Day 60 postpartum
• Control	64.28±3.60 ns	52.76±2.82 ns	53.42±1.53 b	56.14 ±2.01 b	60.86±2.02 b
• Treated	65.58±3.10 ns	53.18±2.39 ns	65.82±2.28 a	72.26 ±2.45 a	72.22±2.28 a

Means with different superscripts letters in the same column are significantly different at least at P<0.01.

Table 2: Serum triiodothironin (T3) and thyroxin (T4) concentrations of control and treated groups (Means±SE)

	T3 (ng /ml)		T4 (ug/dl)	
Day	Control	Treated	Control	Treated
of birth	0.99±0.07	1.23±0.04	9.84±0.65 b	12.04±0.63 a
15 pp	1.18±0.07	1.08±0.07	4.16±0.16	4.04±0.50
30 pp	1.50±0.26	1.18±0.08	14.84±1.11	12.90±0.35
45 pp	1.18±0.06	1.11±0.10	10.93±0.55 b	12.78±0.35 a
60 pp	0.96±0.04 b	1.30±0.15 a	11.02±0.29 b	12.51±0.42 a

Means with different superscripts letters in the same row are significantly different at least at P<0.05

Table 3: Serum progesterone (ng/ml) level of control and treated groups (Means±SE)

	For all buffaloes			For conceived buffaloes
	3rd day after first ovulation	3rd day after estrus and service	7th day after estrus and service	21 days after conception
• Control	0.59±0.01 b	0.85±0.02 b	1.09±0.02 b	2.47±0.16 b
• Treated	0.76±0.04 a	0.99±0.02 a	1.33±0.07 a	3.12 ±0.07 a

Means with different superscripts letters in the same column are significantly different at P<0.01.

Table 5: Effects of Malate supplementation on some reproductive parameters of control and treated groups

Groups	Days to first ovulation	Days to first detected estrus	Conception rates	Days to conception	Number of services/ conception
• Control	77.6±5.35 b	119.2±2.63 b	60%	157.2±9.6 b	2.8
• Treated	48.2±2.08 a	79.4±3.13 a	100%	96.2±5.11 a	1.8

Conception rate calculated at 100 days in milk Means with different superscripts letters in the same column are significantly different at P<0.5 and P< 0.01

Table 6: Birth weight, weaning weight and average daily gains of calves as affected by sex and treatment of their dams on malate during late pregnancy and suckling periods (Means±SE)

	Treatment		Sex	
	Control	Treated	Male	Female
Birth Weight (kg)	46.00±2.35 ns	47.80±1.93 ns	50.40±1.47 a	43.40±1.17 b
Weaning Weight(kg)	80.80±3.03 b	91.60±3.19 a	93.20±2.31 a	79.20±2.01 b
Average Day Gain (g/d)	579.87±22.76 b	729.10±27.66 a	713.33±37.89 a	596.53±31.08 b

Means with different superscripts letters in the same row are significantly different at least at P<0.05.

Table 7: Milk fat, protein, lactose, urea, total solids and SNF of control and treated groups (Means±SE)

Parameters	Control	Treated
• Fat (%)	6.88±0.35 ns	7.18±0.25 ns
• Protein (%)	3.66±0.18 b	4.48±0.22 a
• Lactose (%)	4.06±0.17 b	5.08±0.18 a
• Urea (mg %)	17.04±0.25 ns	16.74±0.22 ns
• Total Solid (%)	15.76±0.30 ns	16.44±0.33 ns
• Solid Not Fat (%)	9.06±0.19 b	9.78±0.22 a

Means with different alphabetical letters in the same row are significantly different at least at P<0. 05.

malate treated group and those from the control one. Meanwhile, the calves weaning weight and average daily gain were greater (P<0.05) and faster (P<0.01), respectively, in calves from malate treated dams than that of the control one.

Milk Composition: The results of milk samples analysis are shown in Table 7. It was found that, there was a non-significant increase in milk fat percent of malate treated group when compared with the control one. Milk lactose content increased (P<0.01) in the treated group than the control one. Also, results showed that the milk protein percent as well as solid not fat percent were higher (P<0.05) in milk from malate treated group than that from the control one. Data analysis showed a non-significant effect in the percentage of total solid due to malate supplementation.

DISCUSSION

The present study revealed that blood glucose levels of malate treated group were significantly higher at days 30, 45 and 60 post-partum than those of control group. These findings were in agreement with those of Wang *et al*. [14] and Waterman *et al*. [22]. Such significant increase in blood glucose level may be attributed to a significant increase in ruminal propionate production due to malate supplementation [23, 24]. Propionate is transported from rumen to the liver where as it is actively transferred to glucose by gluconeogenesis [25]. It has been estimated that between 20 and 50 % of glucose in ruminants is formed from propionate [26]. Meanwhile, there was a non-significant effect of malate supplementation on blood glucose level before day 30 post-partum. These results indicate that blood glucose level began to increase after the metabolic system adaptation to the sudden increase of glucose precursor (propionate), seen with malate supplementation [27].

Concerning serum T3 and T4 level, there was a non-significant increase in serum T_3 at days of treatment and showed only a significant increase at day 60 post-partum. Also, T_4 showed a significant increase at day of birth and at days 45 and 60 post-partum. These results consistent with those of Todini *et al*. [28] who recorded that the higher concentrate diet induced higher plasma T_3 and T_4.

A significant increase in progesterone level was observed in malate treated group at the 3rd day after first ovulation and at the 3rd and 7 thdays after estrus and service as well as at day 21 after conception compared with the control group. These findings were similar to those of Bushmich *et al.* [29] who found an increase in mean luteal progesterone level per heifer and per corpus luteum in heifers with increased molar proportions of ruminal propionate. This increment in progesterone production with increased ruminal propionate production in malate treated group may be attributed to increased serum insulin level [14]. As the presence of insulin receptors have been reported on bovine corpus luteum [30]. So the increase in progesterone production might be due to direct effect of insulin on corpus luteum or due to increasing the ovulation rate [31].

Concerning the effect of malate supplementation on post-partum reproductive parameters, it was found that number of days to first ovulation and to first detected estrus decreased in the treated group than in the control one. This result was in agreement with Waterman *et al*. [22] who found that increasing glycogenic potential stimulated resumption of estrus with shorter interval to first ovulation and first estrus. The resumption of normal episodic LH release is necessary for preovulatory follicular development has been proposed as a key event in the return to ovarian cyclicity in early post-partum dairy cows [32-34]. Malate supplementation had clear effect on LH release, which may be mediated through various metabolic signals; one of these signals is the increased ruminal propionate production by malate supplementation, as propionate infusion has been shown to enhance LH secretion in response to GnRH in heifers [27]. Also, Bushmich *et al*. [29] showed an enhanced ovarian sensitivity to gonadotropins in heifers with increased molar proportion of ruminal propionate. As propionate is the major glucose precursor in

ruminants [35] and increased glucose availability to the gonadotrophs might be mediating the enhanced capacity of the pituitary to response to GnRH by increasing the cells' available energy to perform its general metabolic function [27]. Another metabolic signal by which the effect of malate supplementation on LH release can be mediated is the elevation of serum insulin level associated with malate supplementation [14], which may play a role in the regulation and synthesis of LH [27]. Insulin receptors have been localized in the arcuate nucleus and medial basal hypothalamus (region of the brain containing gonadotropins-releasing hormone (GnRH) neurons) in rats [36]. Also, studies in diabetic rats and sheep indicated absolute requirement for insulin for normal LH pulsality and induction of LH surge [37, 38]. At ovarian level, insulin receptors are widely distributed throughout all ovarian compartments including granulose, thecal and stromal tissues [39]. Insulin increase ovarian responsiveness to LH and resulted in increased level of circulating estradiol and progesterone, with an enhancement of reproductive performance in post-partum dairy cows [40]. Regarding to conception rates, data presented in the same table revealed that there was an improvement in conception rate in malate treated group as compared with the control one. Such improvement in conception rate has been associated with a high circulating level of progesterone during luteal phase, as in the current study, before [41] and after [42] insemination. Progesterone required for embryo implantation and maintenance of pregnancy and it has been shown that low progesterone level associated with enhanced secretion of PGF2á which may induce luteolysis of the corpus luteum and termination of pregnancy [43]. On the same hand, early resumption of ovulatory cycles post-partum has been shown to enhance conception rate to first insemination [44, 45]. Also, the present results revealed that malate supplementation to post-partum buffalo-cows decrease the number of days to conception (days open) when compared with that of the control. Such effect could be explained as malate supplementation shortens the post-partum interval to first ovulation and to first detected estrus [22]. Also, the present results showed a significant decrease in number of services per conception in malate treated group than that in the control one. Such decrease reflects a higher rate of conception in malate treated group than that in the control one.

In the current study, the effect of malate supplementation on calves birth weight was non-significant, meanwhile, there were a significantly higher calves weaning weight as well as faster average daily gain in calves from malate treated dams than those of the control one. The increase in calf weaning weight and daily weight gain observed in calves from malate fed group may be attributed to greater milk production by their dams. As malate supplementation to dairy cows increases milk production and its persistence [10, 11, 13, 14] and considering that milk production is moderately to highly correlate with calf performance [46], it is likely that malate supplementation improve calves performance as well as milk production of their dam.

It was found that, the effect of malate supplementation on milk fat percent was non-significant. These results strongly agreed with those of Kung *et al*. [13] and Wang *et al*. [14] who found that malate supplementation to dairy cows tended to increase non-significantly milk-fat percent. There is a close relation between milk fat percent and ruminal VFAs proportion, about 76 % of the variation in milk fat percent being related to acetate: propionate ratio [47]. However, there is a general agreement that the depression of milk fat percent with low-roughage diet (high concentrate diet) is usually associated with high proportions of propionic acid in the rumen VFAs [48] and as the malate supplementation to dairy cows increases the ruminal propionic acid as well as the acetic acid [13, 12] that resulted in a slight increase in milk fat percent or prevents its decrease with high concentrate diet.

The reported increase in milk lactose content in the treated group was in agreement with Wang *et al*. [14] and Waterman *et al* [22] who attributed that to the increased amount of blood glucose level in malate supplemented group which converted to lactose in the mammary tissue.

In this study, the significant increase in milk protein percent in malate treated group may be attributed to the higher propionic acid production, which is the precursor of glucose and spares amino acids for milk protein synthesis [10]. Also, a significant increase in the percent of solid not fat was found due to malate supplementation in the milk of treated group than that of the control one. Meanwhile, there was a non-significant effect in the percentage of total solid due to malate supplementation which agreed with Kung *et al*. [13] who found that the total solid percent of milk from malate treated group tend to be higher. It was found that malate supplementation has a non-significant effect on milk urea content.

It could be concluded that, supplementation of multiparous Egyptian buffaloes with 85 g / head /day carboxylic acid salt (malate) in early lactation (from day of birth till day 60 post-partum) increased serum glucose level. Increased serum progesterone level, improved

the reproductive and calves performance. Malate has little effect on serum T3 activity while it has more effect on serum T4 activity. Also, malate supplementation significantly increased milk protein, lactose and SNF percentage, while the increase in milk fat percentage was non-significant.

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