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Impact of Supplementation with Carboxylic Acid Salts on Growth Rate and Some Blood Biochemical Values in Male Lambs

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Abstract: The objective of this study was to evaluate the effects of carboxylic acid supplementation on growth rate and some relevant biochemical values in growing lambs. Ten Egyptian male lambs (12-18 keg live body weight) were divided into two equal groups. Group 1 lambs, did not receive any supplement (The control group). Group 2 lambs were supplemented with 4g /head /day of carboxylic acid salt = malate. Lambs were fed a balanced ration *ad libidum*. Body weights were monthly recorded and blood samples were taken each two weeks. Results indicated that the body weight gain increased ($P<0.05$) in supplemented than the control lambs. Concentrations of serum glucose, cholesterol and triglycerides were higher in supplemented than control lambs. Serum total protein and globulins obviously increased in the supplemented lambs, Vitamin C and glutathione-S-transferase serum concentrations increased ($P<0.05$) during most times of the experiment. Plasma malondialdehyde decreased ($P<0.05$) in supplemented lambs. The levels of serum copper and zinc increased ($P<0.05$) in the most times of the study, especially serum zinc values after withdrawal. B- hydroxybutyric acid concentration increased ($P<0.05$), especially after first two weeks of the trial. Serum testosterone level increased ($P<0.05$) in supplemented than control lambs. It was concluded that malate can be used safely as feed additive in growing male lambs as it improved weight gain and increased values of glucose,cholesterol, triglycerides, total protein, globulins and antioxidants (vitamin C, glutathione S-transferase, copper and zinc) and testosterone with decreased value of lipid peroxide (malondialdehyde).

Key words: Carboxylic acid salt · Body weight · Blood parameters · Sheep · Antioxidant · Testosterone

INTRODUCTION

Nowadays, feed additives are frequently used for effective improving nutrient utilization and consequently lead to high feed conversion and better performance [1]. Organic acid (Malate) is a four carbon dicarboxylic acid that is commonly found in biological tissues [2]. A recent research has shown that organic acids stimulate growth of prominent bacterium *Selnomonas ruminantium*, favorably alter the mixed ruminal microorganism fermentation and improve performance of feed lot steers [3]. Excessive starch supply stimulates the production of lactic acid which can lead to the appearance of acidosis and consequent reduction in production with negative effect on healthy condition [4]. Carboxylic acid in the medium activate transformation of lactic acid into propionic acid through *Selnomonas ruminantium* bacterium by using the succinate-propionate pathway [2], it therefore prevent pH reduction in the rumen [3]. Propionic acid is the essential substrum for glucose synthesis [5], by this way carboxylic acid salts increase the available energy for growth [6]. Moreover,there is a recorded obvious increase in serum glucose values of malate supplemented buffaloes [7] and cows [8]. Wang *et al*., [8] reported that the concentration of β-hydroxybutyric acid was lower in cows fed malate. Wu *et al*., [9] recorded that dietry L-malate reduces the accumulation of the reactive oxygen species (ROS) and significantly decrease the level of lipid peroxidation. Carboxylic acids stimulate a number of antioxidative enzymes as glutathione-S-transferase (GST) and other antioxidative agents as vitamin C and zinc to decrease oxidative stress [10]. Malate increases energy value of the ration to perfuse its use in critical periods as the first days of fattening [11]. Therefore the present study was designed to evaluate the effects of L-malate

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supplementation on weight gain and some relevant biochemical values including some antioxidant parameters, malondialdehyde, nitric oxide lysozyme values and testosterone level in growing male lambs.

MATERIALS AND METHODS

This study was conducted during the period from December, 2007 to June, 2008 in the experimental sheep farm of the Animal Reproduction Research Institute (ARRI), El Haram-Giza Egypt.

Animals and Experimental Design: The experiment was carried out on ten apparently healthy weaning male lambs (2-3 months' old and 12-18 Kg live body weight). All animals were housed in an open yard system under natural light and temperature and were fed with balanced ration according to ARRI management. Lambs were randomly allotted into two equal groups (5 lambs /group). Group: I (the control group) was fed on basal diet and received no treatment while Group II (The treated group) was fed on basal diet plus the treatment of 4g /head /day malate for a period of 4 months.

*Carboxylic acid salt = Malate (Rumalato) was obtained from Norel and Nature,
Norel-Miser Suez Gulf P.O.B. 157 Suez-Egypt.

Measurements and Collection of Samples: Body weights were recorded before the experiment and on each subsequent month throughout the trials. Blood samples were collected each two weeks from both control and treated lambs via jugular vein puncture and received into two vacutainer tubes. A tube was plain for serum separation (biochemical and hormonal analysis), the other was heparinized vacum tube for plasma separation (Antioxidant, lysozyme and nitric oxide measurements). Both serum and plasma were stored frozen at -20°C until biochemical analyses.

Biochemical Analyses: Serum and plasma were analyzed by using diagnostic reagent kits.

Glucose [12], triglycerides [13], total cholesterol [14],total protein [15], albumin [16], vitamin C [17], glutathione-S-transferase [18], Malondialdehyde [19], β-hydroxybutyric acid [20] values were assayed. Biochemical analysis were done by spectrophotometer (Dr. Lang 400 Spain). Copper and zinc were measured by atomic absorption system (Model X 3300 Peikin Elmer U.S.A.). Nitric oxide level were measured according to Rajaraman *et al.*, [21] using ELISA reader, while lysozyme activity was estimated according to Peters and Vantrapen [22].

Hormonal Analysis: Serum was assayed for testosterone level by direct radioimmunoassay (RIA) using coat-count-kits (Diagnostic system laboratory kit DSL) and counted by Gamma counter (Multi crystal-Berthold LP 2103, Germany) according to the method described by Marcus [23]. The sensitivity of the assay was 0.04 ng/ml.

Statistical Analysis: All data were subjected to statistical analysis according to Snedecor and Cochran [24]. Using 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

The present study revealed an increase ($P< 0.05$) in body weight of malate supplemented than control lambs during the 4th month as well as after withdrawal (Table 1) as compared with the control group. Table 2 reveals that the supplemented lambs have increased blood glucose values during all the trial, especially during the 2nd and 6th weeks ($P<0.05$). Serum triglycerides values were higher in treated lambs allover the experiment, especially during the 6th and 14th weeks ($P<0.05$) after supplementation. Serum cholesterol levels increased ($P<0.05$) during 14th weeks in supplemented lambs and after withdrawal. In Table 3, total protein and globulin levels increased throughout the trial in treated group, especially during the 6th and 10th weeks ($P<0.05$). Serum albumin values increased ($P<0.05$) during the 14th week only as compared with the control lambs. Data in Table 4 revealed an increase in both vitamin C and glutathione-S-transferase, especially during the 2nd, 6th and 14th weeks ($P<0.05$) for vitamin C and the 10th, 14th and after withdrawal ($P<0.05$) for glutathione-S-transferase. There was a decrease in serum malondialdehyde level in supplemented group in allover the trial, especially during 6th week ($P<0.05$) than the control group. Serum copper values increased during 10th and 14th weeks ($P<0.05$), while zinc values increased during 6th, 10th weeks and after withdrawal ($P<0.05$) in supplemented than the control lambs.

Table 1: Impact of supplementation with carboxylic acid salts on body weights of growing male lambs (Means ± SE)

Items / Periods	Body weights (keg) Control	Supplemented
Before treatment	16.4±1.36	15.4±0.18
1st month	22.2±1.85	22.4±1.99
2nd month	27.0±0.68	30.0±1.52
3rd month	31.8±1.24	35.4±1.49
4th month	37.0±1.22[b]	41.8±1.3[a]
One month after withdrawal	41.8±1.06[b]	47.0±1.04[a]

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

Table 2: Impact of supplementation with carboxylic acid salts on glucose, triglycerides and total cholesterol of growing male lambs (Means ± SE)

Items	Glucose (mg/dl)		Triglycerides (mg/dl)		Total cholesterol (mg/dl)	
Periods	Control	Supplemented	Control	Supplemented	Control	Supplemented
2nd week	51.2±2.08[b]	60.05±2.43[a]	48.4± 1.49	48.22±2.05	41.44± 3.38	47.72± 1.11
6th week	93.08±3.43 [b]	107.00±5.17 [a]	43.82± 0.5[b]	49.54 ± 2.21[a]	50.42± 4.68	41.12 ± 2.83
10th week	87.3 ± 2.39	95.64± 2.74	45.9±2.3	49.54± 2.21	55.05 ± 3.13	60.75 ± 2.67
14th week	85.64± 2.07	96.11±2.82	44.46 ± 0.83[b]	47.37± 0.46[a]	52.2± 0.96[b]	60.45 ± 4.25[a]
One month after withdrawal	82.66±5.46	98.78± 7.44	47.35± 1.25	49.64± 2.06	51.16± 1.62[b]	63.49±3.31[a]

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

Table 3: Impact of supplementation with carboxylic acid salts on total protein,albumin and globulin of growing male lambs (Means ± SE)

Items	Total protein (gm/dl)		Albumin (gm/dl)		Globulin (gm/dl)	
periods	Control	Supplemented	Control	Supplemented	Control	Supplemented
2nd week	6.59±o.4	7.23±0.39	3.84±0.38	3.45±0.53	2.77±0.34	3.77±0.59
6th week	7.19±0.26[b]	10.7±o.59 [a]	4.22±1.28	5.15±1.31	2.62±0.9[b]	5.83±0.78[a]
10th week	8.72±0.76[b]	12.93±0.7[a]	4.95±0.6	4.75±0.91	3.77±0.59[b]	8.17±1.06[a]
14th week	9.79±1.04	12.2±0.71	4.63±0.55[b]	6.28[a]±0.63[a]	5.15±0.88	5.92±0.92
One month after withdrawal	7.13±0.8	9.17±0.52	4.35±1.12	4.75±0.69	2.77±0.28	4.52±0.28

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

Table 4: Impact of supplementation with carboxylic acid salts on vitamin C, glutathione-S-transferase and malondialdehyde of growing male lambs (Means ± SE)

Items	Vitamin C (mg/l)		Glutathione-S-transferase (µ/l)		Malondialdehyde (nmol/ml)	
periods	Control	Supplemented	Control	Supplemented	Control	Supplemented
2nd week	29.42±1.5[b]	32.57±0.76[a]	82.04±4.5	88.57± 4.08	15.5± 0.98	12.00±2.85
6th week	31.2±2.04[b]	38.91±1.51[a]	82.03±4.96	85.07± 3.31	17.37±0.3[b]	12.16±0.65[a]
10th week	32.96±2.04	37.52±1.16	64.73 ±5.7[b]	127.95±2.86[a]	14.36±0.6	13.65±0.85
14th week	34.6±2.44[b]	45.34±0.91[a]	80.01±5.04[b]	93.61±2.5[a]	20.00±2.02	22.31±2.66
One month after withdrawal	29.52±1.35	35.48±1.92	84.64±3.67[b]	166.29±8.52[a]	20.05±2.03	17.01±0.55

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

Table 5: Impact of supplementation with carboxylic acid salts on β-hydroxybutyric acid, copper and zinc of growing male lambs (Means ± SE)

Items	β-hydroxybutyric acid (mg/dl)		Copper (µg/dl)		Zinc (µg/dl)	
periods	Control	Supplemented	Control	Supplemented	Control	Supplemented
2nd week	7.28±0.62[b]	12.93±0.78 [a**]	2.52±2.47	2.68±1. 98	4.24±0.29	5.13±0.32
6th week	9.78±1.33	10.71±1.33	1.98±0.3[b]	1.96±1.77[a]	3.91±0.3[b]	7.01±1.05[a]
10th week	11.12±0.7	10.64±0.32	0.91±0.42[b]	1.62±1.04[a]	4.26±0.37[b]	6.86±0.94[a]
14th week	15.87±1.32	14.08±1.33	0.97±0.64[b]	1.19±0.004[a]	4.95±0.2	5.60±0.35
One month after withdrawal	9.16±0.73	12.9±1.41	1.45±1.44	1.63±0.01	6.12±0.3[b]	8.36±0.43[a]

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

Table 6: Impact of supplementation with carboxylic acid salts on lysozyme and nitric oxide of growing male lambs (Means±SE)

Items	Lysozyme (mmol/ml)		Nitric oxide (µm/ml)	
periods	Control	Supplemented	Control	Supplemented
2nd week	63.24±8.27	80.89±5.41	17.71±3.5	21.34± 3.01
6th week	111.79±14.0	111.78±12.11	17.06±1.68	17.65± 0.01
10thweek	169.15±5.4[b]	186.8±5.41[a]	19.23±1.39	19.18±1.36
14th week	200.04±6.99	186.8±15.04	28.71±2.27	28.09±1.67
One month after withdrawal	191.21±13.2	195.62±4.42	20.65±3.18	18.25±0.95

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

Table 7: Impact of supplementation with carboxylic acid salts on testosterone of growing male lambs (Means±SE)

Item	Testosterone (ng/ml)	
periods	Control	Supplemented
14th weeks of treatment	0.9±1.2[b]	2.06±0.14[a]

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

Results in Table 5 cleared that β-hydroxybutyric acid concentration increased during 2nd week (P<0.05) post supplementation. Plasma lysozyme values (6) increased during the 10th week (P<0.05) in malate fed than non-supplemented lambs, while there was no marked changes in plasma nitric oxide values between experiment and control lambs.

Serum testosterone level was higher (P<0.05) in supplemented growing lambs during the 14th week as compared with the control lambs (Table 7).

DISCUSSION

In the present study, malate supplementation tended to increase body weight at a greater rate relative to control diet. This response is similar to the study of Wang *et al.*, [8] who recorded that the malate supplementation increase body weight gain in dairy cows. This increase might be due to increase protein concentration in all over the trial due to nitrogen retention as reported by Khampa and Wanapat [25], whereas they found that improve average day gain (ADG) and feed efficiency in supplemented sheep and steers by malate. Serum glucose level was significantly increased in malate supplemented lambs as compared with the control group. This confirmed the finding of El- Nour *et al.*, [7], Wang *et al.*, [8] and Waterman *et al.*, [26]. This increase might be attributed to increased in propionic acid production due to carboxylic acid supplement as reported by Khampa and Wanapat [27] and Foley *et al.*, [28]. It has estimated that 20 and 50% of glucose in ruminant is formed from propionate [5], which is transported from rumen to the liver where it is actively transferred to glucose by gluconeogenesis [29]. The effect of malate supplementation in lambs caused a significant increase on triglycerides levels, this might be due to increase volatile fatty acids (VFA) [acetic, propionic and butyric acids]. These acids are absorbed through ruminal wall and then used as a source of energy and precursors of triglycerides [8, 30]. Acetic acid is predominant VFA in fiber diets; it is used to synthesize fatty acids and is the main precursors in the lipogenesis of adipose tissue [30]. Serum cholesterol level showed a significant increase in its level in malate supplemented lambs. This increase might be due to increase in the concentration of acetate production due to malate supplementation via *Selenomonas ruminantium* which use lactate as substrate to produce acetate [3], cholesterol synthesis initially involves the conversion of acetate to mevalonic acid [30]. The concentration of total protein was markedly increased in all over the trial, this result was in accordance with that obtained by Khampa *et al.*, [31] and Sanson and Stallcup [32] who reported that the supplementation of organic acid in ruminant diets has been shown to increased nitrogen retention in sheep and steers. This increase the microbial protein synthesis, feed digestibility and voluntary feed intake which lead to increase NH3-N levels and urea nitrogen (BUN) [33, 34]. The same results were obtained by Khampa *et al.*, [35] who mentioned that the nitrogen absorbed, excreted in feaces and in urine were all lower in malate fed animals. Also they reported that *Selnomonas ruminantium* bacteria can use lactic acid to produce propionic acid which could maintain pH in rumen, when ruminal pH increased can stimulate of chewing roughage increasing nitrogen return into the rumen via salivation and increase ammonia nitrogen, the total protein and globulin levels represent two of the important immunological parameters. In our data, there was a significant increase in plasma vitamin C, glutathione-S-transferase during the most periods of the trail in carboxylic acid supplemented lambs relative to control. These results agreed with that obtained by Wu *et al*., [9] who recorded that L-malate plays a central role in fostering the transport of cytosolic reduce nicotinamid adenine dinucleotide (NADH) into mitochondria. It is also important as a trigger for oxidation of acetyle-CoA and β-oxidation of fatty acids by virtue of its ability to form ATP [36, 37]. Wu *et al*., [38] reported that supplementation of L-malate could improve physical stamina and enhances the activity of the malate-aspartate shuttle and ATP metabolism. Wu *et al*., [9] cited that L-malate was found to enhance the oxidation defense system with an increase the activity of antioxidant glutation-S-transferase, L-malate could reduce the generation of ROS by increasing efficiency of electron transport and thus enhancing the activity of energy metabolism. The decrease of malondialdehyde in malate supplemented lambs was similar with results obtained by Wu *et al*. [9] who recorded that dietry L-malate reduced the accumulation of ROS and significantly the lipid peroxidation level. Lipid peroxidation is used as an index for measuring the damage that occurs in cell membranes as a result of free radical insult [39]. The present results showed significant increase in serum copper and zinc values of L-malate supplemented lambs. Boland [40] reported that copper and zinc are essential trace elements required for normal growth and developments of the animals. The biological role of copper is excreted through a number of copper containing proteins including ceruplasmin and superoxide dismutase.

Zinc is a co-factor for many proteins and enzymes. It activates several enzyme systems and is a component of many metalo-enzyme which plays a vital role in hormones secretion especially that related to growth, reproduction, immunocompetence and stress. Zinc is essential to the integrity of the immune system [40]. In these results there was a significant increase in blood β-hydroxybutyric acid in malate supplemented lambs compared with control at the first two weeks of the feeding and showed non significant effect in the other periods. This increase firstly might be due to increase VFA (acetic, propionic and butyric acid). Whereas, they increased by malate supplementation once butyric acid is absorbed through rumen epithelium, it is largely converted into ketones (β-hydroxybutyric acid) which account for more than 80% of all ketone bodies and used to synthesis fatty acid in adipose tissue [3]. This finding is confirmed with the results of Wang *et al*., [8] who suggested that there is an improve in energy balance and body weight with increasing malate supplementation causing lowering concentration of ketones in plasma of fed lambs. Cattle have at least 10 lysozyme genes, of which at least four are expressed in the rumen. Bovine lysozyme has an important role in gastric digestion by facilitate digestion of bacteria coming from a fermentative foregut [41]. Lysozymes are most active at low pH, in contrast to other lysozymes which function best at a more basic pH and have wider pH optima [42]. In these results there were fluctuations in concentration of lysozyme and this might be due to the effect of malate on the fermentation process which led to increase ruminal pH that accompanied by the recuirtment of lysozyme as a lytic digestive enzyme [43]. Moreover in the present study there was no changes in nitric oxide levels between supplemented lambs and control one throughout the trial, this might be the regulatory effect of vitamin C on it [44]. The concentration of blood testosterone hormone was increased significantly in malate supplemented lambs than control one. This increase may be due to improve energy balance of treated lambs via increase glucose level [7, 8]. Energy lead to increased LH response to GnRH and LH stimulates steroidogenesis and testosterone production by binding receptors on the plasma membrane of the leydig cells and activating adenylcyclase, thus increasing c.AMP (cyclic adenosine monophosphate). This action enhances the rate of cholesterol transport by steroidogenic acute regulating protein [30, 45].

It could be concluded that supplementation with carboxylic acid (Malate) could improved weight gain, energy availability as showed by higher blood glucose. Malate increased protein and globulins levels which is used as a parameter for detection of immune response. Malate protects rams in their reproductive period from different affects of oxidative stress. It improved antioxidant (vitamin C, glutathione-S-transferase, copper and Zinc) and lower free radical as lipid peroxidation (malondialdehyde). Malate has a little effect on lysozyme and nitric oxide. In addition, it has beneficial effect on testosterone which showed significant increase in fed lambs. So, it is recommended to use malate as a natural feed additive in the concentrate diet. Carboxylic acid has a positive effect on growth, reproduction and healthy condition of growing male lambs.

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