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IMPACT OF DIETARY CITRATE SUPPLEMENTATION ON MUSCLE METABOLITES OF *RATTUS SP.:* A MODEL FOR LIVE ANIMAL INTERVENTIONS OF MEAT QUALITY

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I. INTRODUCTION

Citrate, and its role in glycolysis, changes in living muscle and postmortem muscle [1]. During the conversion of muscle to meat, glycolytic substrates undergo several reactions. The enzyme 6-PFK of glycolysis is inhibited by ATP, especially in the presence of citrate, which directs glucose-6-phosphate (G6P) to glycogen production and depresses glycolysis. Citrate supplementation may promote lipogenesis by being readily available in the diet, and not requiring transport out of the mitochondria, further depressing glucose utilization. Supplementing exogenous citrate could impact cellular citrate concentrations, possibly promoting various cellular pathways of importance to meat quality. Objectives were to determine if dietary citrate supplementation 1) influences cellular glycolytic metabolites in rat liver, *M. soleus*, *M. gastrocnemius*, and *longissimus dorsi*; and 2) impacts adipose tissue accumulation and body weight.

II. MATERIALS AND METHODS

Genetically similar *Rattus sp.* males (n = 24) were divided into three treatment groups (n = 8 rats/group): 1) CONTROL – sugar water supplementation; 2) LOW- 1.5% citrate inclusion in water; and 3) HIGH- 3% citrate inclusion in water. Citric acid was added to water (LOW: 0.015 g/ml; HIGH: 0.03 g/ml), stirred, and pH was adjusted utilizing sodium hydroxide to achieve a pH of 7.35-7.40. To entice consumption, 340 grams of sugar were dissolved in water solutions to achieve 17% concentration. Each group underwent treatment for a 2-week period, and body weight was collected daily. After treatment, rats were anesthetized utilizing sodium pentobarbital (60 mg/kg) and harvested through the guillotine method. Immediately following exsanguination, the epididymal fat pad was extracted immediately to quantify the effect of citrate supplementation on fatty acid synthesis. Liver, *M. soleus*, *M. gastrocnemius*, and the *longissimus dorsi* were dissected and immersed in liquid nitrogen. Frozen tissue samples were weighed, dissolved in 5 mL of 50% perchloric acid, neutralized with 200 μL of potassium bicarbonate, and then centrifuged at 1500 rpm for 30 min. Supernatant fractions were extracted into centrifuge tubes for subsequent metabolite analysis. Lactate and citrate levels were analyzed with a Beckman DU-7400 Spectrophotometer (Palo Alto, CA) set at 339 nm, following a modified version of Bergmeyer [2]. Data were analyzed with JMP Pro (v. 16.0.0; SAS Institute, Cary, NC). The Fit Model Standard Least Squares function was used for ANOVA comparisons, and mean comparisons were conducted using Tukey's test at an alpha of P <0.05.

III. RESULTS AND DISCUSSION

For final epididymal fat pad weight, a measure of lipogenesis, and final body weight, there was a treatment effect (P <0.0001). The HIGH group had the lowest fat pad weight (20.5 ± 4.3 grams), and the LOW and CONTROL groups did not differ (P = 0.64) (Table 1). For final weight, there was a treatment effect (P < 0.0001), where the CONTROL and LOW groups did not differ at the final weight post 2-week treatment period (P = 0.52); however, the HIGH group had the lowest final weight (Table 1). This difference in final weight may be attributed to the high sodium content from the sodium hydroxide that was added to the water to treatment water to bring final water pH to a level appropriate for drinking water. Additionally, the amount of sugar added to the water was held constant, regardless of the amount of sodium hydroxide added, which may have altered water palatability between groups. The availability of citrate is dictated by the amount of citrate transported out of the mitochondria by the citrate carrier (CIC) [3], dietary citrate supplementation and an increase in muscular citrate levels may limit this transport, possibly increasing lipogenesis and further depressing glucose utilization. Increase in lipogenesis was not noted in the current study, but the concentration of sodium hydroxide and palatability of the water solution may hinder that function.

For muscle lactate and citrate concentrations, there was a treatment effect, P = 0.0007 and P = 0.024, respectively. The HIGH group had the highest levels of lactate concentration, and the CONTROL and LOW groups did not differ (Table 1). The HIGH group also had the highest level of citrate in tissues, whereas the CONTROL and LOW groups did not differ in citrate concentration (Table 1). The differences in citrate and lactate concentrations were unexpected,

as increased citrate concentration is thought to depress glycolysis and lactate accumulation. Citrate supplementation possibly influences the rate of lactic acid accumulation in postmortem metabolism, which may impact the rate of pH decline and ultimate pH in postmortem muscle, impacting meat quality traits differently than originally anticipated. Mitochondrial activity postmortem is dependent on available substrates [4]. Increasing substrates pre-harvest would increase the time before substrates are depleted, therefore prolonging mitochondrial function. In postmortem muscle, citrate levels decrease [1]. If beginning levels of citrate are altered through supplementation, this may change postmortem mitochondrial metabolism and glycolysis and warrants further investigation.

Muscle lactate concentration also differed by tissue type (P = 0.0002), with *M. soleus* having the highest level of muscle lactate (0.46 ± 0.043 micromole/gram); whereas the liver, M. gastrocnemius, and the longissimus dorsi did not differ in lactate concentration (P > 0.05). This may be due to the composition of the *M. soleus,* which is primarily composed of type I muscle fibers that are characterized by a highly oxidative metabolism. The reliance of these fibers on the TCA cycle may contribute to the accumulation of lactate rather than conversion to pyruvate for energy production. Saitoh et al. [5] evaluated the effect of supplemental citrate and glucose together on the rate of glycogen replenishment post prolonged exercise. This combination significantly increased glycogen in both the liver and the *M. soleus* muscle. This suggests that feeding exogenous citrate would be able to influence glycolysis or other metabolic processes, and impact muscles differently [5].

Table 1 – Effects of dietary citrate inclusion on evaluated traits.

		Treatment						
Trait	n[2]	CONTROL		LOW GROUP		HIGH GROUP		P-value
Final Body Weight, g	24	323.25[a]	±9.11	309.00[a]	±9.11	219.63[b]	±9.11	<0.0001
Fat Pad Weight, g	24	51.69[a]	±4.27	46.24[a]	±4.27	20.54[b]	±4.27	<0.0001
Citrate Concentration, μmoles/ gram[1]	95	0.072[a]	±0.054	0.104[ab]	±0.055	0.272[b]	±0.054	0.0239
Lactate Concentration, μmoles/ gram[1]	95	0.255[a]	±0.036	0.253[a]	±0.037	0.429[b]	±0.036	0.0007

[a-c] Least squares means in the same row without common superscript letters differ (P<.05).
[1]Metabolite concentration is recorded as micromole of metabolite per gram of tissue sampled.
[2]Body weight and epididymal fat pad weight was recorded as one per animal (n=24). Metabolites were measured as tissues extracted (4 tissues) multiplied by the number of animals (n=24). One sample was lost in transit resulting in one less value.

IV. CONCLUSION

Dietary citrate supplementation in *Rattus sp.* males had a significant effect on muscle concentrations of lactate and citrate. These preliminary findings suggest dietary citrate supplementation in live animal models may present an avenue to alter meat quality traits. Cellular metabolites fuel metabolic pathways for oxygen consumption and mitochondrial function in postmortem muscle, potentially impacting meat color through ferric heme reduction. Citrate supplementation may impact postmortem pH decline, which could influence meat quality traits such as color stability and protein functionality.

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