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Fishery Technology 54 (2017) : 197 - 201

Thiamine and Pyridoxine Loaded Vanillic Acid Grafted Chitosan Modulates Lactate and Malate Dehydrogenase in Albino Rats

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Abstract

Feeding trials was conducted to study the response of metabolic enzymes activity such as lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) through dietary supplementation of thiamine and pyridoxine loaded vanillic acid grafted chitosan microparticles (TPVGC) in male and female Wistar strain albino rats for a period of 45 days. Forty-eight animals both male and female were randomly distributed into eight experimental groups and were fed with basal feed along with different levels of TPVGC. At the end of the experiment, the LDH activity in both male and female Wistar strain albino rats were found to be significantly higher ($p<0.05$) in the control groups and found to decrease with the increase in the inclusion level of dietary TPVGC in the diet. MDH activity was significantly ($p<0.05$) reduced only in case of female rats. The results obtained in present study shows that dietary supplementation of TPVGC at a level of 0.8% could uphold better metabolic homeostasis of lactate dehydrogenase and malate dehydrogenase.

Keywords: Lactate dehydrogenase, malate dehydrogenase, dietary supplementation, albino rats, chitosan derivative

Introduction

Thiamine and pyridoxine are water soluble vitamins which play a vital role in many physiological and metabolic functions in the body. Thiamine is a pivotal engine in metabolic activity and acts as coenzyme in the metabolism of carbohydrates, in addition it is also involved in metabolism of proteins and fats. Pyridoxine is the key element in protein metabolism (IMFNB, 1998). The vitamin B complex mediates the conversion of food into fuel which in turn can be used to produce energy in the body. Thiamine and pyridoxine arbitrate in biosynthesis of neurotransmitters such as acetylcholine, gamma amino butyric acid (GABA), serotonin (Tanphaichitr, 1998).

Lactate dehydrogenase is the decisive enzyme of the glycolysis pathway. LDH converts lactate to pyruvate in the presence of coenzyme NADH which is converted to NAD^+. Thus, lactate dehydrogenage helps in maintaining the glycolysis cycle by supplying NAD^+. In the presence of enough oxygen, pyruvate enters the Kreb's cycle, but when there is an oxygen shortage in the tissue system, pyruvate is converted to lactate (Everse & Kaplan, 1973; Murray et al., 2000). This particular reaction is reversible and the reaction favors the reduction of pyruvate to lactate (Kaneko et al., 2008; Panteghini et al., 2008). It is well documented that changes occurring in the tissues of ageing rats / animal will alter the activity of several enzymes (Singh & Kanungo, 1968). Elevated level of serum LDH has been well recorded during the renal infarction (Winzelberg et al., 1979). On the other hand, Drent et al. (1996) has reported that LDH serve no function in body cavity effusions but it acts as an indicator of disturbed cellular integrity induced by pathological conditions. Any damage in the tissue level can lead to significant elevation of LDH activity; therefore, this assay can be used to identify cell injury or cell death (Panteghini et al., 2008). Exposing tilapia to endosulfan resulted in significant increase in kidney LDH activity and found to be influenced by the concentration and period of exposure (Rani & Ambili, 2006).

Received 17 September 2016; Revised 01 July 2017; Accepted 25 August 2017

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Malate dehydrogenase (MDH, EC1.1.1.37) is the ultimate enzyme in the mitochondrial tricarboxylic acid (TCA) cycle. It is a homodimeric (α) sulfhydryl enzyme which oxidizes malate to oxaloacetate, using NAD^+ as the electron acceptor. Malate dehydrogenase activity is expected to increase during gluconeogenesis. The oxaloacetate formed from the deamination of aspartate and the pyruvate formed from deamination of various amino acid *viz*., alanine, serine, tryptophan are converted to malate or phosphoenol pyruvate (PEP) in the mitochondria. The malate and PEP are transferred to cytosol where they are converted to glucose. The conversion of oxaloacetate to malate in the mitochondria and the reverse in cytoplasm is catalyzed by MDH (Das et al., 2006). Recent reports in the field of medical science have also show that the patients who died with Alzheimer's disease were found in *Labeo rohita* fingerlings due to to have elevated MDH activity in brains (Velde & Stam, 1976; Bubber et al., 2005). High MDH activity was also found in endosulfan-induced stress indicates increased activity of TCA cycle due to higher energy demand (Akhtar et al., 2010).

In our earlier studies, vanillic acid grafted chitosan derivatives were developed and evaluated for their antioxidant and antimicrobial properties (Chatterjee et al., 2016). In the present study, thiamine and pyridoxine loaded vanillic acid grafted chitosan micro-particles were evaluated as dietary supplement in male and female albino rats with reference to LDH and MDH activity. The different organs/ body parts like muscle, kidney and liver tissues were targeted for LDH and MDH activities.

Materials and Methods

Thiamine and pyridoxine loaded vanillic acid grafted chitosan was synthesized per our earlier report (Tejpal et al., 2017). In brief, 20 g of chitosan was dissolved in 2 L of 2% acetic acid solution (v/v). An aliquot of 20 mL of 1 M H_2O_2 containing 1.08 g of ascorbic acid was added drop wise to the chitosan solution, followed by addition of 20 g vanillic acid dissolved in 100 mL ethanol. The reaction was maintained under nitrogen environment for 24 h at 25°C with constant stirring. The reaction mixture was dialyzed against distilled water for 72 h to remove unreacted vanillic acid. The dialysate (3 L) was then mixed with thiamine and pyridoxine (1 g each) in deionized water followed by homogenization at 16 000 rpm for 15 min. The homogenate was spray dried (inlet temperature 140°C, outlet temperature 77°C, feed pump flow rate 10 mL min^{-1}) to obtain microencapsulated thiamine and pyridoxine.

Male and female Wistar strain albino rats, aged 4 weeks old, weighing 30-38 g were selected for the study. The animals were housed individually in polypropylene cages under hygienic and standard environmental conditions (28±2°C; humidity, 60–70%; 12 h light/dark cycle). During the acclimatization period animals were fed with a standard diet and water *ad libitum*.

Seven days after acclimatization, forty-eight animals were randomly distributed and each group was fed with a diet supplemented with either 0, 0.4, 0.8 and 1.6% of thiamine and pyridoxine loaded vanillic acid grafted chitosan microparticles. Totally, eight experimental groups *viz.,* Control Male (Basal feed + 0% TPVGC); T_1M (Basal feed + 0.4% TPVGC); T_2M (Basal feed + 0.8% TPVGC); T_3M (Basal feed + 1.6% TPVGC); Control Female (Basal feed + 0% TPVGC); T_1F (Basal feed + 0.4% TPVGC); T_2F (Basal feed + 0.8% TPVGC); T_3F (Basal feed + 1.6% TPVGC) were arranged in triplicates following a CRBD. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by the Institutional Animal Ethics Committee of the Central Institute of Fisheries Technology, Cochin.

The standard diet was supplied by Krish Feeds (Scientist's Choice Laboratory Animal Feeds, Bangalore, India). The diet contained carbohydrate (Nitrogen free) 56.2%, crude protein 22%, ash 7.5%, crude fat 4.2%, crude fibre 3%, glucose 2.5%, vitamin 1.8%, sand silica 1.4%, calcium 0.8%, phosphorus 0.6%, and provided metabolizable energy of 3600 kcal. Experimental diet was prepared by incorporation of TPVGC to the standard diet at different concentrations (0, 0.4, 0.8 and 1.6%).

At the end of the feeding trial, experimental animals were sacrificed using chloroform anaesthesia. For enzyme assay, separate homogenates were prepared for each tissue (muscle, kidney and liver). The tissue was homogenized with chilled 0.25 M sucrose solution using a mechanical tissue homogenizer. The homogenized samples were centrifuged at 5000 g for 10 min at 4°C and supernatants were collected and stored at −20°C for further analysis. The supernatants were used as the source of enzymes.

LDH and MDH activities were measured by the change in optical density (OD) at 340 nm for 5 min using the method of Wrobleuiski & Ladue (1955) and Ochoa (1955), respectively. For LDH and MDH assay, Sodium pyruvate and Oxalo-acetate were used as substrates, respectively.

The experiments were conducted in triplicate. The homogeneity of variance was analyzed using one-way ANOVA. The significant difference was tested using Duncan's multiple range comparison Test. All the analysis was performed using statistical software SPSSTM version 16.

Results and Discussion

Lactate dehydrogenase activity was assayed in different tissues such as muscle, liver and kidney of male and female albino rats. The results are depicted in Table 1. The highest LDH activity was recorded in muscle tissue of male in control groups and further it was found to follow a decreasing trend with increase in TPVGC concentration. When we compared muscle tissue of female with liver and kidney in control group, the LDH activity was higher in muscle tissue and the activity was found to increase in the muscle with increase in TPVG concentration up to 0.8%, beyond which a steep decrease in the activity was observed at 1.6% of TPVGC. It is well established during the illness or cell injuries or under stress, the LDH level increases (Moore & Yontz, 1969). Usually, low LDH level is not harmful. In living organisms, five different isoforms of LDH are expressed at different tissues. In liver and muscle, LDH-5 and in kidney LDH-4 is expressed. In general, expression of all the isoforms of LDH, in the serum sample due to leakage of enzymes from the organs, indicates the multiple organ failure. In the present study, the LDH activity in liver was found to be reduced with increase in the level of TVCGP in both male and female compared to control group. However, slightly higher LDH activity was recorded in liver of male compared to female animals irrespective of the treatments. A similar effect in the expression of LDH in kidney was observed. Brinkworht & Masters (1978) have found low level of LDH activity in the mouse fed with thymine enriched diet and higher level of LDH activity in thiamine deficient diet similar to results in the present study. In general, vitamins play an important role in maintenance of metabolic homeostasis at tissue level. Feed supplements such as levan, tryptophan and pyridoxine in fish was also found to lower LDH activity (Tejpal et al., 2009; Akhtar et al., 2010; Gupta et al., 2014). From the results, it can be assumed that the control group expressed more LDH due to high energy requirement for maintaining normal metabolic process and inclusion of graded levels of dietary TPVGC in the treatment groups helped to maintain the metabolic homeostasis. Study clearly showed that vitamins in the encapsulated form along with vanillic acid grafted chitosan as a wall material and in the diet as food supplement could be beneficial to health.

Malate dehydrogenase activity was assayed in different tissues such as muscle, liver and kidney of male and female albino rats (Table 2). Higher

Table 1. Impact of dietary supplementation of thiamine and pyridoxine loaded vanillic acid grafted chitosan microparticles on lactate dehydrogenase activity in muscle, liver and kidney tissue of male and female albino rats

	Tissue	Treatments			
		Control (0% TPVGC)	T_1 (0.4% TPVGC)	T_2 (0.8% TPVGC)	T_3 (1.6% TPVGC)
LDH Male	Muscle	$1.34^{a} \pm 0.02$	$1.20^{a} \pm 0.10$	$1.25^{a} \pm 0.06$	$1.17^{a} \pm 0.03$
	Liver	$0.25^{b} \pm 0.01$	$0.21^{b} \pm 0.01$	$0.18^{a} \pm 0.03$	$0.17^{a} \pm 0.01$
	Kidney	$0.23^{b} \pm 0.01$	$0.21^{b} \pm 0.01$	$0.11^{a} \pm 0.01$	$0.12^{a} \pm 0.01$
LDH Female	Muscle	$1.26^{b} \pm 0.14$	$1.34^{b} \pm 0.03$	$1.36^{b} \pm 0.06$	$0.87^{a} \pm 0.16$
	Liver	$0.22^{b} \pm 0.01$	$0.14^{a} \pm 0.02$	$0.15^{a} \pm 0.02$	$0.14^{a} \pm 0.01$
	Kidney	$0.28^{b} \pm 0.03$	$0.19^{ab} \pm 0.02$	$0.11^{a} \pm 0.04$	$0.13^{a} \pm 0.01$

Different superscript (a & b) in the row indicate significant difference ($p<0.05$) among the control and treatment groups (control, T1, T2 and T3) (Duncan's multiple range test, $\alpha = 0.05$). Values are expressed as mean± SE (n= 6).
Units: nano-moles of pyruvate utilized/mg protein/min (LDH)

Table 2. Impact of dietary supplementation of thiamine and pyridoxine loaded vanillic acid grafted chitosan micro particles on malate dehydrogenase activity in muscle, liver and kidney tissue of male and female albino rats.

	Tissue	Treatments			
		Control (0% TPVGC)	T_1 (0.4% TPVGC)	T_2 (0.8% TPVGC)	T_3 (1.6% TPVGC)
MDH Male	Muscle	$9.11^{b} \pm 0.95$	$8.94^{b} \pm 0.31$	$6.61^{a} \pm 0.49$	$6.01^{a} \pm 0.25$
	Liver	$6.63^{a} \pm 0.78$	$6.33^{a} \pm 0.69$	$5.64^{a} \pm 0.22$	$5.01^{a} \pm 0.53$
	Kidney	$4.28^{a} \pm 0.48$	$4.13^{a} \pm 0.84$	$3.32^{a} \pm 0.35$	$2.63^{a} \pm 0.60$
MDH Female	Muscle	$9.85^{b} \pm 0.45$	$9.90^{b} \pm 0.47$	$8.89^{ab} \pm 0.17$	$6.94^{a} \pm 1.07$
	Liver	$7.82^{b} \pm 0.40$	$7.26^{ab} \pm 0.08$	$6.39^{a} \pm 0.62$	$5.97^{a} \pm 0.22$
	Kidney	$3.60^{b} \pm 0.42$	$3.96^{b} \pm 0.33$	$3.66^{b} \pm 0.17$	$2.62^{a} \pm 0.10$

Different superscript (a & b) in the row indicate significant difference ($p<0.05$) among the control and treatment groups (control, T1, T2 and T3) (Duncan's multiple range test, $\alpha = 0.05$). Values are expressed as mean± SE (n= 6).
Units: nano-moles of Oxaloacetate utilized/mg protein/min (MDH)

activity of MDH indicates greater activity of tricarboxylic acid (TCA) cycle due to increased energy demands from a particular animal. The higher MDH activity in tissue and serum indicates the cellular injury and diseased conditions (Kawai & Hosaki, 1990). In the present study, muscle tissue showed higher MDH activity compared to liver and kidney. The MDH activity in muscle and liver tissue of both male and female decreased with the increase in the level of TPVGC in diet. A similar decreasing trend was observed in the kidney tissue of male animals. However, a different trend in MDH activity was recorded in kidney tissue of female animals. In recent years, MDH assay is used as a biomarker in acute hepatic disease (Schomaker et al., 2013). Similar to LDH, during the diseased conditions or cell injuries or under stress, the MDH level increases (Das et al., 2006; Tejpal et al., 2009; Gupta et al., 2014). The dietary supplementation of thiamine and pyridoxine encapsulated using vanillic acid grafted chitosan in the diet could efficiently maintain the homeostasis of metabolic enzymes.

From the present study, it can be concluded that the dietary supplementation of thiamine and pyridoxine encapsulated using vanillic acid grafted chitosan could modulate the expression of energy metabolic enzymes, lactate dehydrogenase and malate dehydrogenase in turn maintain metabolic homeostasis of albino rats in dose dependent manner. Thus, the thiamine and pyridoxine loaded vanillic acid grafted chitosan can be a potential functional food supplement to improve the health status.

Acknowledgement

The authors gratefully acknowledge the Director, ICAR-Central Institute of Fisheries Technology, Cochin for providing the necessary facilities. The authors also wish to express their gratitude to technical staff of Biochemistry and Nutrition Division, CIFT for the support rendered during the research.

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