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Efficacy of multiple blend nutraceutical bar supplementation on weight loaded swimming performances in animal model

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Abstract
Nutraceutical is the hybrid of “nutrition” and “pharmaceutical”. Nutraceuticals; in broad, are food or part of food playing a significant role in modifying and maintaining normal physiological function that maintains healthy human beings. They are also known as medical foods, nutritional supplements and a foodstuff (as a fortified food or dietary supplement) that provides health benefits in addition to its basic nutritional. Nutraceuticals are active substances extracted from vegetal (as phytocomplexes) or animal origin food concentrated and administered in a suitable pharmaceutical form. These are multi-component, micro-nutrient based, containing dietary ingredients, for example, vitamins, minerals, amino acids, or other botanical or dietary substances, which are not specifically addressed to prevent or cure an health issue. Dietary protein plays a critical role in countless physiological processes in the body. It is also required to maximize the adaptive response of skeletal muscle to more prolonged resistance-type exercise training. It has been well established that ingestion of dietary protein before resistance-type exercise increases post exercise muscle protein synthesis rates and inhibits muscle protein breakdown, thereby allowing net muscle protein accretion during the acute post exercise recovery period. Energy balance is an important factor in sustaining training load and maintaining high performance during strenuous exercise. It has been reported that the intake of high-energy foods increases muscle glycogen content, which can prolong exercise time and delay fatigue. Recently, several studies have indicated that high protein high energy supplementation high protein supplementation provide the following benefits: enhancing muscle glycogen and sports performance, extending endurance times, resistance to fatigue, decreasing oxidative stress after strenuous exercise, and detoxifying the body. Therefore, the aim of this study was to examine the efficacy of multiple blend nutraceutical bar supplementation on endurance performance in a weight loaded swimming test in animal model.

Keywords: nutraceutical bar, supplementation, high protein high energy bar, swimming performance, glycogen content

Introduction

Experimental design/Methodology

i) Development of high protein high energy bar

For the supplementation, a high protein high energy bar were developed using dehydrated beetroot cubes, dates, oats, ground nut, white sesame seed, flax seed, pumpkin seed, muskmelon seed, jaggery, chocolate at different level of incorporation. The raw ingredient selected were according to the guidelines approved by Athlete’s Guide to sports supplements (AGSS, 2013) and Dietary Supplement Health and Education Act (DSHEA, 1994) which should be rich in protein mainly branched chain amino acid (valine, isoleucine, leucine), energy, crude fiber, fatty acid, minerals, inorganic nitrite, antioxidant, phytochemicals.

ii) *In-vivo* efficacy of the developed product

The animals used for this experiment were male adult healthy Wistar rats (between 150 to 250 gram) procured from Chakra borty Enterprises, Kolkatta. All the procured rats were raised in Department of Pharmacology and Toxicology, College of Veterinary Sciences, AAU Khanapara. The test diet used (high protein high energy bar) were developed in the Department of Food Science And Nutrition, College Of Community Science, Assam Agricultural University, Jorhat.

Rats were housed in polypropylene cages in small groups of 6 rats per cage. The rats kept in a controlled condition, 12 hours light and dark cycle for acclimatization. Deionized distilled water was offered *ad libitum*. The animals were grouped into five groups, namely Control group, Group I, Group II, Group III and Group IV respectively. Each group consists of 6 animals in each group, notably, with no statistical differences. Control Group was fed with rat ration from M/S Hindustan lever rat ration. Standard Group was fed with rat ration.

The experiment was carried out following the guidelines of the Institutional Animal Ethics Committee of an approval No. 770/GO/Re/S/03/CPCSEA/FVSC/AAU/IAEC/18-19/703.

In total, 30 male rats (4 week old) were divided into five groups (n = 6 per group): Control Group fed with 100% rat ration, Group I fed with 100% test diet (High protein high energy bar), Group II fed with 75% test diet (High protein high energy bar)+ 25% rat ration, Group III fed with 50% test diet(High protein high energy bar)+50% rat ration, Group IV fed with 25% test diet (High protein high energy bar)+75% rat ration. Experimental rats in each group underwent strength capacity test for 4 weeks. For measuring the strength capacity of experimental rats rotarod test was performed to evaluate the motor coordination and balance of experimental rats in terms of time and distance covered and analyzed by latency to fall time and speed at fall parameters. Experimental rats were placed in testing room for at least 1hr before testing to minimize effects of stress on behavior during testing. They were trained to walk forward on rotating rod. Experimental rats were taken out individually and placed in separate lanes on rod rotating at 10rpm and left for walking forward to keep balance. Apparatus was set to accelerate from 10rpm, 15rpm and 20rpm. The use of a rat model in this study was approved and conducted under the guidelines of the Institutional Animal Ethics Committee.

Results and Discussion

The maximum swimming time in Group I fed with 100% test diet increased from initial timing of 2.46±0.07min to 6.95±0.25min at the end of the supplementation period. In Group II, Group III and Group IV fed with 75%, 50% and 25% of test diet, the swimming time increased from an initial timing of 2.45±0.05min to 6.01±0.12min, 2.43±0.05 min to 5.76±0.19 min and 2.39±0.11 min to 4.88±0.06 min respectively at the end of supplementation period. In Control group fed with 100% rat ration, the swimming time increased from 2.20±0.20 min to 2.74±0.07 min. at the end of the supplementation period.

The mean increase in swimming time from initial day to the end of supplementation period by experimental groups namely Group I, Group II, Group III and Group IV was 4.49 min, 3.56 min, 3.33 min and 2.49 min respectively. The mean increase in swimming time taken by the experimental rats were significantly higher when compared to the control group (p<0.05) which had a mean increase of 0.43 min at the end of the 28 days supplementation period. Supplementation of high protein high energy test diet on exercise durability showed that there was significant improvement and significantly longer (p<0.05) swimming time in all the experimental groups fed on different test diets. A significant reduction (p<0.05) in exhaustion time of the experimental group namely Group I, Group II, Group III and Group IV fed with test diets when compared to control group fed with 100% rat ration.

The exhaustion time in Group I fed with 100% test diet reduced from initial timing of 5.32±0.06 min to 1.75±0.09 min at the end of the supplementation period. In Group II, Group III and Group IV fed with 75%, 50% and 25% of test diet, the exhaustion time decreased from initial timing of 2.39±0.03 min to 1.50±0.15 min, 2.28±0.57 min to 1.73±0.08 and 2.16±0.04 min to 1.71±0.08 min at the end of 28 days supplementation period. In Control group fed with 100% rat ration, the exhaustion time decreased from initial timing of 2.12±0.10 min to1.70±0.01 min at the end of the supplementation period.

The mean decrease in the exhaustion time (Table 11) by experimental groups namely Group I, Group II, Group III and Group IV were 3.57 min, 0.89 min, 0.55 min, 0.45 min respectively. The mean decrease in the time taken by the experimental rats were significantly higher when compared to the control group (p<0.05) which had a mean exhaustion time of 0.42 at the end of the supplementation of 28 days supplementation. Shortness of the length of exhaustion time, indicates the degree of fatigue. The results indicated that supplementation of multiple blend nutraceutical bar enhances the endurance capacity by delaying the onset of physical fatigue in rats. The mean increase in swimming time, decrease in exhaustion time may be due to the composition of the multiple blend nutraceutical bar of food ingredients rich in branched chain amino, carbohydrate, iron, magnesium, phosphorous and antioxidant that are responsible for enhancing strength and endurance capacity, muscle tissue repair, fuel usage, boost up immune system, improve body composition and decreases muscle fatigue.

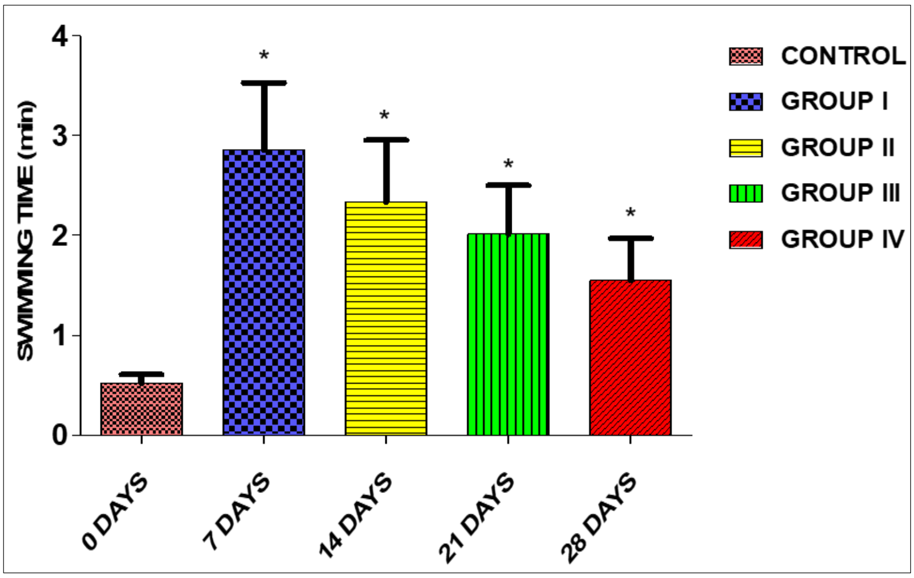

Fig 1: Mean increase of swimming time taken to perform a weight loaded swimming test by experimental rats at the end of supplementation

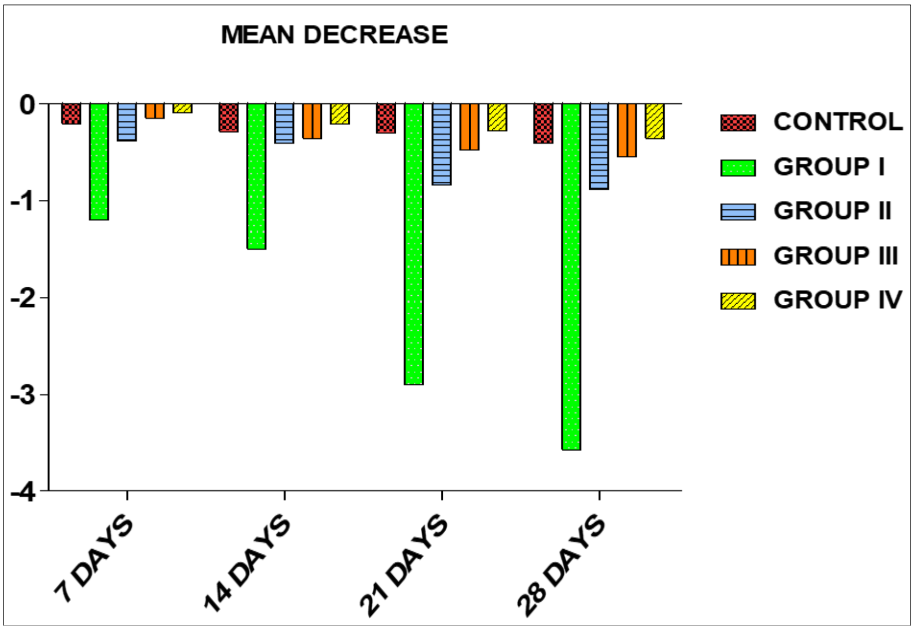

Fig 2: Mean /decrease of swimming time taken to perform a weight loaded swimming test by experimental rats at the end of supplementation

Morphological data from each experiment group are summarized in Table 1. There were significant changes in the body, liver weights and body weights among Control, Group I, Group II, Group III and Group IV and thus the short term supplementation of high protein high energy would affect the body growth.

Table 1: General characteristics of the experimental groups

Characteristics	Control	Group I	Group II	Group III	Group IV
Initial BW (g)	139.49±1.21	134.11±2.68	132.43±2.89	133.71±3.00	133.95±2.88
Final BW (g)	163.44±1.31	157.96±2.52	171.81±2.08	168.71±3.46	169.95±2.05
Liver glycogen content(mmole/glucose unit)	30.14±3.88	42.59±6.03	41.06±4.04	40.50±4.84	33.14±3.94
Muscle glycogen content (mmole/glucose unit)	36.70±4.65	43.00±5.42	41.12±5.10	40.43±5.05	39.22±3.31

Values are expressed in mean ± SD (Standard Deviation)
Means within rows separated by Duncan's multiple range test P=0.05

In this present study, the increase in swimming time may be attributed to the presence of branched chain amino acid, fructose, minerals, antioxidants in the developed multiple blend nutraceutical bar that has a high impact on increased oxidative capacity of muscle and decreases the physical fatigue. The increase in swimming time of experimental rats supplemented with high energy high protein bar may be due to the psycho stimulating and ergogenic effects of bioactive compounds namely carotenoids, betacyanines, folates, biologically accessible anti-oxidants, polyphenols and flavonoids (Gokhale and Lele, 2011) [4] present in test diet that improve the glycolytic flux and increased effort at high endurance test.
The gain in body weight of experimental rats supplemented with test diet (high energy high protein bar) may be due to combination of different food ingredients that include protein rich food ingredients like pumpkin seed, muskmelon seeds and fructose containing dates and flavonal epicatechin present in dark chocolate which increases mitochondrial biogenesis during exercise to respond the higher energy demand and body weight that has an impact on protein synthesis and affect muscle hyperotrophy by enhancing muscle tissue repair. Studies have also reported that high protein and high energy diet consisting of pumpkin seeds, dates, oats rich in fatty acids such as palmitic acid, steric acid, oleic acid, linoleic acid, vitamin E and vitamin A, minerals, fructose and complex starches like soluble fiber significantly increases the body weight of experimental rats in training.(Daenzer *et al.*, 2002) [2].

Muscle glycogen content and liver glycogen content of experimental rats were significantly higher ($p<0.05$) than that of control group. This indicate that high protein high energy supplementation may boost muscle glycogen. The experimental group had a longer running time to exhaustion compared to control group the running time till exhaustion was significantly related to muscle glycogen and liver glycogen
The results were similar to the study by Murakami *et al.* (2019) [7] where supplementation of high protein high energy diet rich in fructose (20%) resulted in significant increase in muscle glycogen content and liver glycogen content of 37 m moles of glucose units x kg^{-1} and 42 m moles of glucose units x kg^{-1} for 4 weeks as compared to control diet (22.3 m moles of glucose units x kg^{-1}). These results indicates that both short-term intake of the high protein high energy diet and exercise training synergistically increased glycogen in both muscle and liver tissue.
Anthony *et al.* 2007 [1] reported that short term intake of the high protein diet consisting of branched chain amino acid powder (30%), casein (20%) and oats (6%) had significantly higher level of muscle glycogen content (41±7.11 m moles of glucose units x kg^{-1}) and liver glycogen content (40±4.41 m moles of glucose units x kg^{-1}) as compared to control group (35.34±3.56) at 28 days of supplementation.
Kanda *et al.* (2010) [5] studied that short-term feeding of high protein high energy consisting of whey protein, branched chain amino acid, casein hydrolysates increased skeletal muscle glycogen levels and improves exercise performance in

rats and the results indicated that short-term feeding of high protein high energy is more effective for increasing glycogen content in skeletal muscle, and improving exercise performance due to inhibition of glycogen phosphorylase by fructose 1- phosphate produced from energy and protein dense diet is responsible for enhancing net glycogen store in liver (Galbo *et al*., 2012) [3].

In 2018 Lamb *et al*. [6] observed that supplementation of high protein diet consisting of casein and cornstarch resulted in significantly higher level of muscle glycogen concentration in experimental rats than control group after a weight loaded endurance exercise.

Conclusion

It can be concluded that multiple blend nutraceutical bar which is rich in protein, essential fatty acids, minerals such as iron, zinc, magnesium and phosphorus and soluble fibres played an important role in physiological protection and performance elevation with strength and endurance exercise of experimental rats. Present study provided substantial evidence that supplementation of multiple blend nutraceutical bar resulted in increase in liver and muscle glycogen storage of experimental rats which contributed to extending the running time and swimming time. Multiple blend nutraceutical bar increased the activity of antioxidant enzymes and anti-fatigue activity by increasing haemoglobin level, liver and muscle glycogen depletion thereby elevating exercise performance. The present study provided a science-based evidence to support that multiple blend nutraceutical bar could be a promising anti- fatigue agent and an ergogenic aid.

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