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Antifatigue Effect of High Protein High Energy Supplementation Improves Motor Performance and Glycogen Content in Wistar Rats Subjected to Strength Capacity Test

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ABSTRACT: This study was designed to investigate the impact of high protein high energy formulation on exercise performance in relation to strength capacity tests. To assess the in-vivo efficacy of the developed product in terms of strength capacity, body weight, muscle, and liver glycogen level, the Wister rats were given the developed food formulations for 28 days. Four formulations namely TS_1, TS_2, TS_3, and TS_4 were developed and TS_4 exhibited the highest scores for all the sensory attributes, nutritive value, and antioxidant activity and was selected for in-vivo studies. The experimental rats were divided into five groups consisting of six rats in each group namely Control (100% standard rat ration), Group I (100% test diet), Group II (75% test diet + 25% standard rat ration), Group III (50% test diet + 50 % standard rat ration), Group IV (25% test diet + 75% standard rat ration). The highest significant ($p<0.05$) increase in running time, distance covered, muscle glycogen, and liver glycogen level was found in Group I fed with 100% test diet. The present study provided science-based evidence to support that high protein high energy formulation could be a promising anti-fatigue agent and an ergogenic aid.

Keywords: Strength capacity, nutrition, ergogenic aids, high protein high energy supplementation, motor performance, sports nutrition.

INTRODUCTION

Fatigue is a complex physiological process characterized by challenges in commencing or maintaining deliberate activity. Physical activity enhances the production of reactive oxygen species, which aid in the expansion of blood vessels; hence, it promotes an increase in blood circulation and the delivery of oxygen and glucose (Tornero-Aguilera *et al.*, 2022). Muscular tiredness during exercise is commonly ascribed to extended muscle activity that impacts cellular function (Constantin-Teodosiu and Constantin 2021). There is a widely held belief that weariness is a contributing factor to a decline in performance. Physical weariness is believed to result from multiple mechanisms. Hemoglobin, radical, clogging, and exhaustion theories are a few of these theories. According to physical fatigue theory, various energy sources are drained during activity, including glucose and liver glycogen. In exercise physiology and therapeutic settings, endurance and strength tests are crucial endpoints for determining physical significance. The efficacy of antifatigue can be assessed by the weight-loaded swimming test. Consuming an adequate amount of protein can enhance physical performance and have a good impact on the body's recovery mechanisms. Engaging in prolonged endurance sports while consuming a diet high in protein and energy can lead to a reduction in upper respiratory tract infections and an improvement in immune system function has been reported. Natural or conventional protein supplements have gained popularity among athletes looking to lower oxidative stress, speed up recovery, and boost performance (Mardiana *et al.*, 2023). It may also help increase the activity of genes that produce proteins needed to enhance bioenergetic pathways. Dietary protein serves a key function in various physiological processes in the body. Additionally, it is necessary to optimize skeletal muscle's adaptive response to longer-term resistance-training regimens. It is generally established that resistance-type exercisers who ingest dietary protein have decreased post-exercise muscle protein synthesis and breakdown. After exercise, muscle protein accumulates throughout the

acute recovery period (Narkhede *et al.,* 2015). The body needs more proteins available to repair damaged tissue from repetitive muscular contractions and to improve contractile capacity (hypertrophy), particularly during resistance training (da Rosa Lima *et al.,* 2018). The human body can develop muscle tissue more quickly and effectively with the help of proteins. Protein-rich supplementation before and/or after exercise can dramatically boost the synthesis of muscle protein (Zare *et al*., 2023). In sports nutrition, the ergogenic effect of protein supplementation is well established (Craddock *et al*., 2021). However, a thorough assessment of the impact of plant protein supplements on exercise and sports-related outcomes in the active and athletic population is lacking. Thus, the purpose of this study was to investigate the antifatigue effects of high-protein, high-energy supplementation on muscle weight and motor function in Wister rats that were put through a strength capacity test.

MATERIAL AND METHODS

A. Animal model

In this experiment, male Wistar rats (4 weeks old, post-weaning) were procured from Chakraborty Enterprises, Kolkata, weighing between 150 and 250 grams. Rats were raised at the College of Veterinary Sciences, AAU Khanapara, Department of Pharmacology and Toxicology. 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. High protein high energy formulation was developed using dehydrated beetroot cubes, dates, oats, ground nuts, white sesame seeds, flaxseed, pumpkin seeds, muskmelon seeds, jaggery, and chocolate at different levels of incorporation. The raw ingredients selected were according to the guidelines approved by Athlete's Guide to Sports Supplements (AGSS, 2013) and the Dietary Supplement Health and Education Act (DSHEA, 1994) which should be rich in protein mainly branched-chain amino acids (valine, isoleucine, leucine), energy, crude fiber, fatty acid, minerals, inorganic nitrite, antioxidant, phytochemicals.

B. Experimental Methods Adopted

A small group of six rats was housed in a polypropylene cage. A controlled environment was maintained in a temperature of (22 ± 1℃), and humidity (55 ± 5%) for acclimatization, with 12 hours of light and 12 hours of darkness. Deionized distilled water was offered ad libitum. A total of five groups were formed, namely the Control group, Group I, Group II, Group III, and Group IV. Each group contains 6 animals, with no statistical differences between them. The control group received rat ration from M/S Hindustan lever rat rations. Standard Group was fed with rat ration. Animal maintenance and experimental procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals (National Research Council of the National A.O.A.C., 2000) and were evaluated and carried out following the guidelines of the Institutional Animal Ethics Committee of approval No. 770/GO/Re/S/03/CPCSEA/FVSC/AAU/IAEC/18-19/703 dated 28.12.2018. 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. The food in the animal cages was changed every day.

(i) Determination of body weight gain, strength capacity test. Experimental rats in each group underwent a strength capacity test for 28 days. 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. The rotarod test was performed based on the methods described by Citraro *et al*. (2016). Experimental rats were placed in a testing room for at least 1hr before testing to minimize the effects of stress on behavior during testing. They were trained to walk forward on rotating rods. 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. Under the guidelines of the Institutional Animal Ethics Committee, rat models were used in this study (Fig. 1).

(ii) Blood Sample Collection. Sahli's acid haematin method was done to study the impact of supplementation of a test diet (high protein high energy bar) on haemoglobin level of experimental animals Principle: The haemoglobin is converted to acid haematin after adding dilute hydrochloric acid which is brown in colour and then matched with colour standards. In this study, haemoglobin concentration was estimated using the Sahli acid haematin method as described by Cheesebrough (2000). In a dilution tube containing 0.1 mol/l hydrochloric acid (HCL), 0.02 ml of blood was mixed to convert haemoglobin into acid haematin. 0.1 mol/l of HCl was added drop-by-drop, with mixing, after 10 minutes of reaction time. In bright diffuse day light with a sheet of white paper as a background, a permanent-colored glass comparison standard was positioned alongside the dilution tube in bright diffuse daylight and the solution (haemolysed blood) matched the color of the permanent-colored glass comparison standard. A graduation at the bottom of the meniscus on the dilution tube was used to determine hemoglobin concentration. By using the standard table of comparison provided, the relative hemoglobin value was converted to the absolute hemoglobin value. To reduce the level of imprecision and inaccuracy associated with this method of estimating hemoglobin, each sample was tested in triplicate.

(iii) Muscle and liver glycogen level estimation. Glycogen is a higher-branched polysaccharide containing glucose units and is exclusively found as a storage carbohydrate in the liver or muscles of animals. The glycogen is extracted from the known weight of fresh tissue and the amount is estimated by first hydrolyzing it to glucose and then estimating the glucose by any suitable method. The concentration of glucose obtained is multiplied by a factor of 0.93 to convert it to glycogen. Hydrolysis of glycogen- Reagents 1. Potassium hydroxide 30% solution, 2. Ethanol 95% and 60%, 3. Sulphuric acid 2N. Protocol- The liver tissue must be collected from the animal immediately after sacrifice and a small piece (approx. 1g) is taken, excess blood removed by blotting between folds of filter paper. It is put into weighed accurately to obtain the extract weight of the liver tissue. The tissue is digested in a boiling water bath for 90 min. The contents are then cooled in ice-cold water and 5 ml of 95% ethanol is then added and mixed. The mixture is heated in the water bath just to boil to avoid spurting. The tube is allowed to stand overnight in a refrigerator. The next day it is centrifuged at 3000 rpm for 10 min. and the supernatant is carefully drained out. The precipitate is dissolved in 2ml of warm water and the glycogen is reprecipitated with 4ml of 95% ethanol. The precipitate is centrifuged as above and washed 3 times with 4ml portions of 60% ethanol. The precipitate is then hydrolyzed with 2ml of 2N sulfuric acid in a boiling water bath 3-4 h. The solution is neutralized with NaOH using phenol red as an indicator, diluted to 200ml with water and filtered using Whatman filter paper. Glycogen analysis- Tissue glycogen was isolated and purified by precipitation with ethanol from a digest formed by the addition of 5·3 M-KOH, and then quantified by the phenol–sulphuric acid method (Lo *et al.,* 1970).

C. Statistical analysis

The statistical analysis was done by using SPSS for MS Windows version 7.5 and Microsoft Excel- 2010. The mean standard deviation (SD) was calculated for all the quantitative parameters. A paired 't' test was applied to compare the impact of a specific treatment on the sample individual (selected sample). Analysis of variance (ANOVA) in terms of ratio of between group variability to within group variability (F-test) was used in order to test the equality of different treatments on the time of interval. Critical differences were calculated to find out the treatment differences. The level of significance selected were: ($P \leq 0.05$) Significant *; ($P \leq 0.01$) Significant ** Formula used was from Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

A. Impact of supplementation of test diet (high protein high energy bar) on body weight of experimental rats

The impact of high protein high energy bar on body weight of the experimental rats after supplementation of test diet (high energy high protein bar) showed a significant increase ($p<0.05$) among the experimental groups namely Group I, Group II, Group III, Group IV fed on 100%, 75%, 50% and 25% test diet when compared to the control group fed on 100% standard rat ration. The highest significant ($p<0.05$) increase in body weight was found in Group I fed on 100% test diet from an initial weight of 134.11±2.68 gm to 180.11±2.52 gm at the end of the supplementation period (Fig. 2). 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 distance covered by experimental rats and analyzed by latency to fall time and speed at fall parameters. The mean running time (sec) of the experimental rats at different speeds (10, 15, and 20 rpm) after supplementation of high protein high energy test diet is presented in Fig. 4. It was observed from the present study that at 10 rpm, Group I (fed on a 100% test diet) was able to perform the rotarod test from an initial timing of 375.21±2.42 sec to 715.37±3.74 sec at the end of the supplementation period. The running time of Group II (fed on 75% test diet), Group III (fed on 50% test diet), and Group IV (fed on 25% test diet) increased from initial timing of 365.43±5.41 sec to 665.69±2.68 sec, 360.42±3.56 sec to 655.60±3.75 sec and 355.34±4.16 sec to 635.44±4.75 sec respectively indicating better performance. The mean time taken by control group fed with 100% rat ration at 10 rpm increased from an initial value of 350.34±4.34 sec to 555.5±3.23 sec. at the end of the supplementation period respectively.

When the speed was increased to 15 rpm, the running performance was significantly highest ($p<0.05$) in all stages of intervention for all the experimental groups treated on test diets. At 15 rpm the running time of Group I (fed on 100% test diet), Group II (fed on 75% test diet), Group III (fed on 50% test diet) and Group IV (fed on 25% test diet) significantly increased ($p<0.05$) from initial running time of 350.41±3.54 sec to 655.51±5.64 sec, 340.43± 3.51 sec to 590.54±5.78 sec, 335.42± 4.76 sec to 575.54±4.45 sec and 330.43±3.33 sec to 560.53±3.65 sec respectively. The mean time taken by the control group fed with 100% rat ration at 15rpm is presented in Fig. 3. At 20 rpm the running time of Group I (100% test diet), Group II (75% test diet), Group III (50% test diet) and Group IV (25% test diet) significantly increased from the initial running time of 301.14±2.23 sec to 602.72±3.34 sec, 300.16± 4.41 sec to 545.32 ±5.78 sec, 285.23± 5.34 sec to 515.74±5.12 sec and 280.21±3.41sec to 500.82±2.43 sec respectively. The mean time taken by the control group fed with 100% rat ration at 20rpm increased from an initial value of 295.12±2.34 sec to 490.50±5.15 sec at the end of the supplementation period respectively (Fig. 3).

The running time of Group I was significantly higher at 10 rpm, 15 rpm, and 20 rpm ($p<0.05$) compared to other experimental groups. The mean increase in running time of experimental rats supplemented with a test diet (high protein high energy bar) using rotarod test at 10 rpm, 15 rpm, and 20 rpm was observed. An increase in running at different rpm was observed in all

the experimental groups fed on the test diet compared to the control group. The running time of Group I was significantly highest at 10 rpm, 15 rpm, and 20 rpm ($p<0.05$) compared to other experimental groups (Fig. 4). Increase in running at different rpm was observed in all the experimental groups fed on test diet compared to the control group. The mean increment in running time in Group I at 10 rpm, 15 rpm, and 20 rpm was 195.36 sec to 340.16 sec, 170.42 sec to 305.11sec and 155.23sec to 301.56sec respectively at the end of the supplementation period. Though improvement in running time was observed in control groups but time taken was less compared to the experimental groups. At 20 rpm, a significant ($p<0.05$) increase was observed in running time for all the experimental groups at different stages of intervention (Table 1).

It was observed from the present study that consumption of high protein high energy bar resulted in high-intensity running performance in rotarod test measured as distance covered at the highest speed of 20 rpm. The combination of protein, energy, and fiber-rich food improved the nutritional quality of the product and the presence of soluble fiber decreases the susceptibility to infection during strength training and increases performance. The soluble fiber present in oats increases muscle and hepatic glycogen concentration (Donatto *et al.,* 2010). This study substantiates that high protein high energy bars developed from different food ingredients showed improve exercise performance in experimental rats by increasing the strength capacity in terms of running time and distance covered using the rotarod test may be due to the presence of antioxidants in the test diet.

In this present study, the increase in running time may be attributed to the presence of branched-chain amino acid, fructose, minerals, and 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 running time of experimental rats supplemented with high energy high protein bar may be due to the psycho-stimulating and ergogenic effects of bioactive compounds present in the test diet that improve the glycolytic flux and increase effort at high strength test (Jung *et al.,* 2004).

During prolonged moderate-to-high-intensity exercise muscle glycogen is the primary fuel source. Muscle glycogen content is associated with running time to exhaustion in animal studies. Fatigue or a decline in sports performance is attributable to reduced muscle glycogen content (Table 2). Therefore, increased muscle glycogen extends the time to exhaustion and delays fatigue. Increasing muscle glycogen through diet and before exercise *i.e.,* one method of enhancing endurance capacity. Evidence suggests that consuming carbohydrate and protein together work synergistically together for enhancing glycogen synthesis and subsequent performance improvements. There was a significant increase ($p<0.05$) in the muscle glycogen content among the experimental groups namely Group I, Group II, Group III, and Group IV after strength training at the end of the supplementation period compared to the control group fed with 100% rat ration (Fig. 5).

Muscle glycogen content and liver glycogen content of experimental rats were significantly higher ($p<0.05$) than that of control group. This indicates 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 mean hemoglobin level of experimental rats in Group I (fed on 100% test diet) was from 14.92±3.16mg/dl to 20.69±3.41mg/dl The mean increase in hemoglobin level of experimental groups namely Group I, Group II, Group III, and Group IV was 5.77 mg/dl, 3.36 mg/dl, 1.59 mg/dl, 1.45 mg/dl had significantly ($p<0.05$) higher value when compared to the control group which had a mean increase of 1.02 mg/dl at the end of the supplementation of 28 days of period. Kalicki *et al*. (2019) reported that protein supplements significantly increased body and muscle mass and increased hemoglobin concentration (18.45 mg/dl) due to increased percentage of white blood hemoglobin subpopulation (Lewicki *et al.,* 2014) (Fig. 6).

B. Correlation of strength capacity with body parameters of experimental rats

The correlation analysis of the strength capacity with body parameters of experimental rats is presented in Table 3. It was evident from Table 3 that there was a significant ($p<0.05$) moderately positive correlation between strength capacity and body parameters namely body weight ($r = 0.395$), muscle glycogen ($r = 0.202$), liver glycogen ($r = 0.215$), hemoglobin level ($r =0.185$) and liver weight ($r =0.127$) of experimental rats which indicated that increase in body parameters increases the strength capacity of experimental rats. Increased muscle glycogen and liver glycogen content led to increased strength capacity and improved performance level and decreased exhaustion time (Table 3).

C. Linear regression of different body parameters on strength capacity of experimental rats

Linear regression analysis of body parameters including body weight, muscle weight, liver glycogen, hemoglobin level, and liver weight on strength capacity of experimental rats revealed that 41.65 percent of the total variability on the strength capacity was determined by liver glycogen content, 34.43 percent of total variability on the strength capacity was determined by liver weight, 25.54 percent of total variability on the strength capacity was determined by muscle glycogen content, 23.41 per cent of total variability on the strength capacity was determined by body weight, 22.54 per cent of total variability on the strength capacity was determined by hemoglobin level (Table 4).

Table 1: Impact of supplementation of test diet (high protein high energy bar) on running capacity of experimental rats using rotarod test at 10rpm, 15rpm and 20 rpm.

Treatment	Mean running time (sec) taken to perform rotarod test at 10rpm, 15rpm and 20 rpm														
	0 Days			7 Days			14 Days			21 Days			28 Days		
	10rpm	15rpm	20 rpm	10rpm	15rpm	20 rpm	10rpm	15rpm	20rpm	10rpm	15rpm	20rpm	10rpm	15rpm	20rpm
Control	350.34± 5.36	325.65± 3.53	295.12±3 .73	450.45± 4.08	406.01± 3.89	355.47± 4.05	500.59± 4.36	425.80± 3.39	375.63± 4.05	535.37± 4.88	505.65± 3.63	482.45± 3.30	555.5±3 .78	535.46± 3.15	490.50± 3.87
Gr I	375.21± 3.54	350.41± 4.63	301.14±5 .20	570.57± 3.87	520.83± 4.19	451.49± 4.45	625.37± 3.38	580.58± 5.04	511.24± 4.06	670.39± 3.31	605.51± 4.96	547.45± 5.22	715.37± 4.36	655.51± 3.77	602.72± 5.14
Gr II	365.43± 4.06	340.43± 4.55	300.16±3 .61	555.65± 4.01	505.57± 4.14	450.51± 4.94	575.60± 4.41	520.69± 3.69	440.42± 3.45	615.49± 4.44	580.58± 2.77	530.41± 4.57	665.69± 3.72	590.54± 3.73	545.32± 4.13
Gr III	360.42± 3.26	335.42± 3.24	285.23±5 .51	545.42± 5.33	495.64± 3.64	430.55± 4.99	561.53± 4.80	495.77± 4.71	405.38± 5.78	602.02± 4.43	566.03± 4.47	505.98± 4.49	655.60± 5.34	575.54± 3.15	515.74± 4.88
Gr IV	355.34± 4.93	330.43± 4.32	280.21±4 .67	530.57± 3.98	485.54± 4.11	421.13± 3.70	545.45± 3.51	480.84± 3.39	390.62± 4.05	595.51± 8.77	550.58± 7.04	480.31± 5.37	635.44± 5.47	560.53± 4.70	500.82± 5.88

Factor	F value
For factor treatment	1040.22**
For factor time	1213.00*
For factor speed	3527.00**
For factor speed × time	47.52*
For factor speed × treatment	18.35*
For factor days × treatment	58.88*
For factor speed × time × treatment	6.39*

Table 2: Impact of supplementation of high protein high energy bar on muscle and liver glycogen content of experimental rats.

Experimental group	Muscle glycogen and liver glycogen content of experimental animals after exercise performance (m mole/glucose unit)			
	Mean Initial muscle glycogen	Mean muscle glycogen	Mean Initial liver glycogen	Mean liver glycogen
	Before feeding	After 28 days of feeding	Before feeding	After 28 days of feeding
Control	37.56	36.70±4.65	30.53	30.14±3.88
Gr I		43.00±5.42		42.59±6.03
Gr II		41.12±5.10		41.06±4.04
Gr III		40.43±5.05		40.50±4.84
Gr IV		39.22±3.31		33.14±3.94

Table 3: Correlation of strength capacity with body parameters of experimental rats.

Sr. No.	Body Parameters	Correlation co-efficient with strength capacity ($p<0.05$)
1.	Body weight	0.395 *
2.	Muscle glycogen	0.202*
3.	Liver glycogen	0.215 *
4.	Haemoglobin level	0.185*
5.	Liver weight	0.127 *

Table 4: Linear regression of different body parameters on strength capacity of experimental rats.

Body parameters	Intercept	Regression coefficient	't' value	r^2 (%) coefficient of determination
Body weight	6.76	0.243	0.78*	23.41
Muscle glycogen		0.341	2.98*	25.54
Liver glycogen		0.412	3.21**	41.65
Haemoglobin level		0.125	0.71*	22.54
Liver weight		0.116	0.66*	34.43

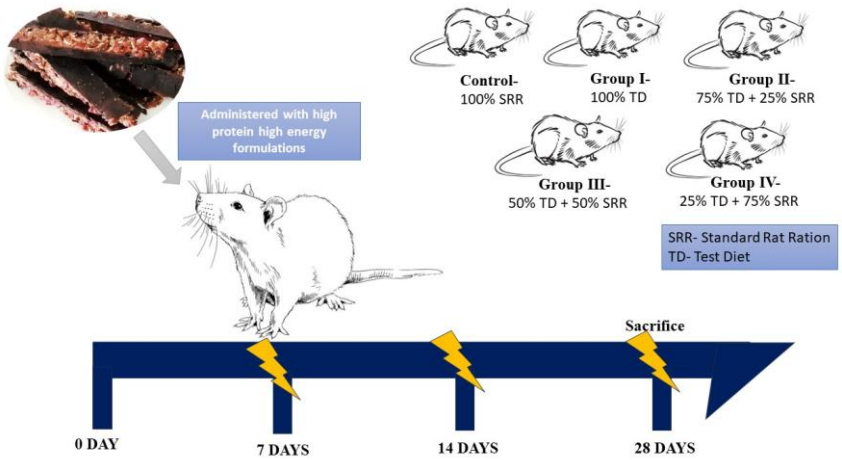

Fig. 1. Experimental timeline. The simplified graphic demonstrates that during the course of the 28 experimental days, each of the five mouse groups received daily doses of SRR and TD (the high protein, high energy supplement). To measure the strength capacity test in terms of running time and distance covered, the rotarod test was conducted seven days apart. On day 28, all of the mice were killed in order to estimate the weight and glycogen levels in the liver and muscles.

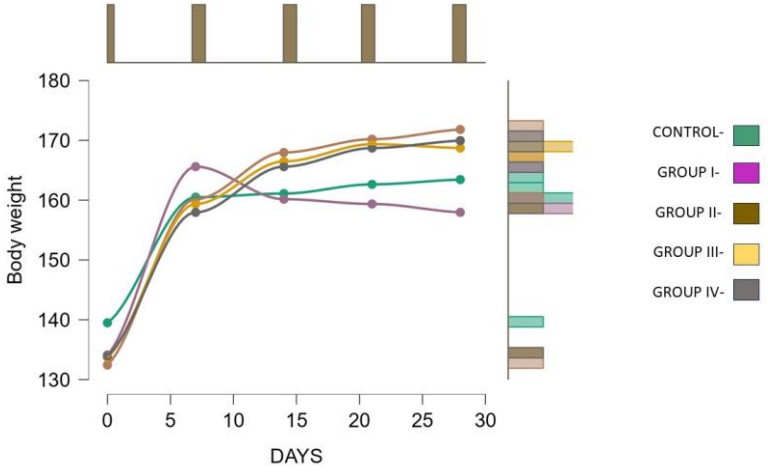

Fig. 2. Impact of supplementation of test diet (high protein high energy bar) on body weight of experimental rats. The bodyweight was measured every day. n = 6, $P < 0.05$, Data were analyzed using ANOVA, followed by Dunnett's multiple comparisons test, presented as mean ± standard error (S.E.M.).

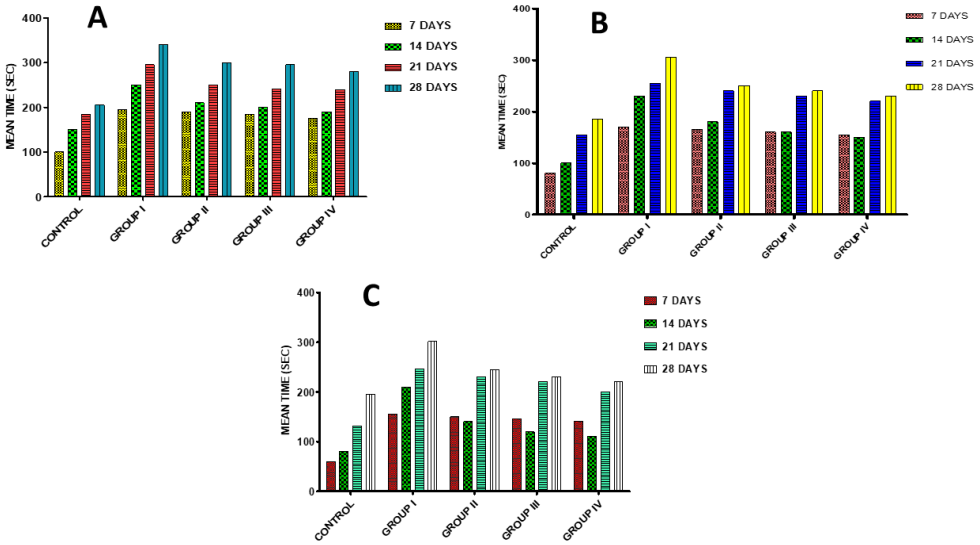

Fig. 3. Mean increase on strength capacity of experimental rats supplemented with high protein high energy bar (test diet) using rotarod test at A) 10 rpm, B) 15 rpm C)20 rpm, Data were presented as mean ± standard error (S.E.M.).

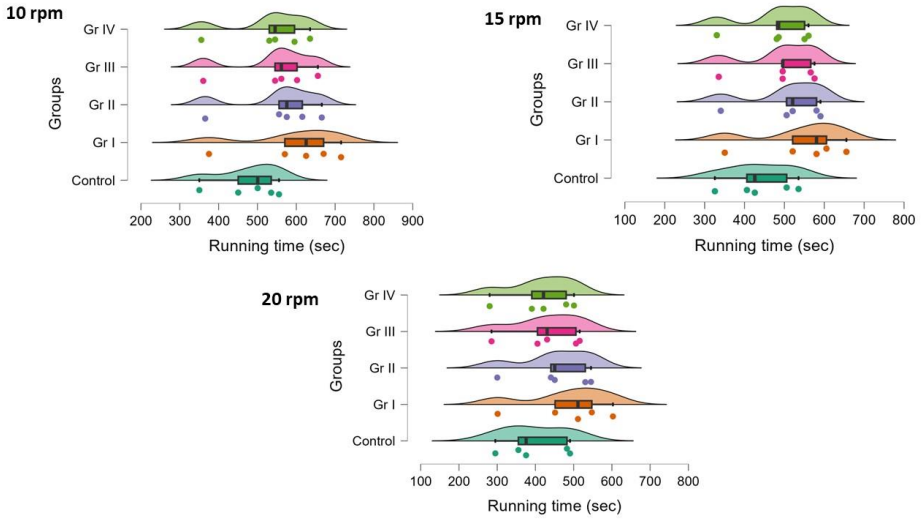

Fig. 4. Running time taken by experimental rats supplemented with high protein high energy bar (test diet) using rotarod test at 10 rpm, 15 rpm and 20 rpm The running time was measured every day. n = 6, P < 0.05, Data were analyzed using ANOVA, followed by Dunnett's multiple comparisons test, presented as mean ± standard error (S.E.M.).

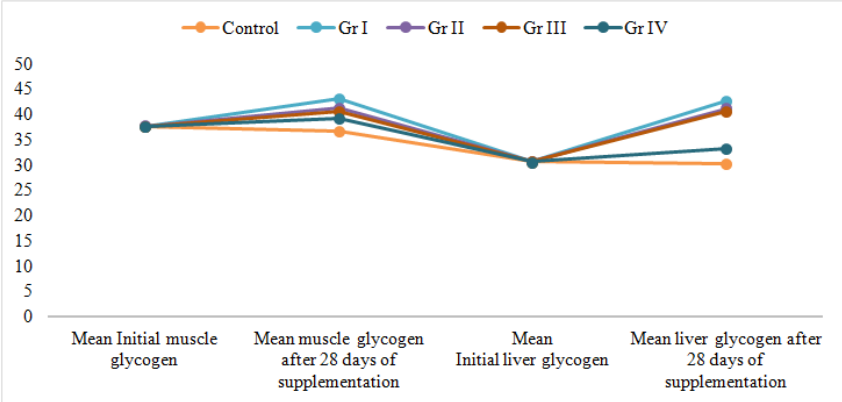

Fig. 5. Impact of supplementation of high protein high energy (test diet) on liver glycogen level of experimental rats, n = 6, P < 0.05, Data were presented as mean ± standard error (S.E.M.).

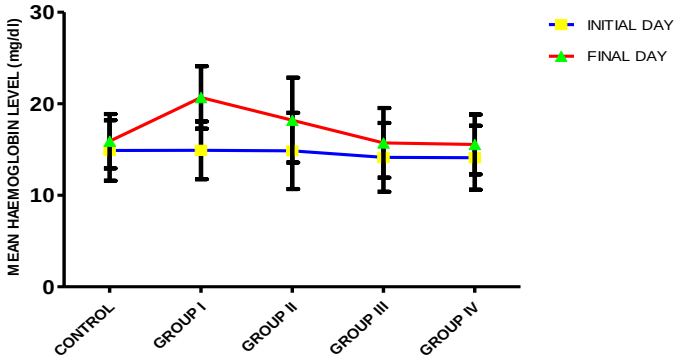

Fig. 6. Impact of supplementation of high protein high energy bar (test diet) on haemoglobin level of experimental rats. n = 6, P < 0.05, presented as mean ± standard error (S.E.M.).

CONCLUSIONS

It can be concluded that a high protein high energy 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. High protein high energy bar increased the activity of antioxidant enzymes and anti-fatigue activity by increasing hemoglobin level, liver and muscle glycogen depletion thereby elevating exercise performance. The present study provided science-based evidence to support that high protein high energy bar could be a promising anti- fatigue agent and an

ergogenic aid. 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 hemoglobin level, liver and muscle glycogen depletion thereby elevating exercise performance. The present study provided science-based evidence to support that multiple blend nutraceutical bars could be a promising anti-fatigue agent and an ergogenic aid.

FUTURE SCOPE

The outcome of the present study can be recommended to Ministry of food Processing Industries and Sports Authority of India to include the high protein high energy bar for popularization, consumption and improvement in sports performance by enhancing endurance and strength capacity of sports person. The nutrient rich high protein high energy bar can be included in different National sports Nutrition supplementation programme under Government of India to meet the nutritional requirement of the beneficiaries and to increase the nutritive value of the dietary intake of the sports persons. It can be recommended for Popularization in domestic sports market and entrepreneurship development.

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Conflict of Interest. None.

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