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Original Research Paper

Impact of Dietary Protein on Growth and Digestive Enzyme Activity in Adults Laboratory Mice

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Abstract: The present study was conducted to determine the effects of different protein diets on growth parameters (body weight, length and growth rate), activities of digestive enzymes (pepsin, trypsin, chymotrypsin and amylase) in laboratory mice; also to monitor the relationship between dietary protein intake-somatic growth-digestive enzyme efficiency for highlighting physiological importance of protein nutrition in animal models. **Methods:** Forty-eight male BALB/c mice were randomly placed into four dietary groups fed on 8%, 14%, 18% or 24% protein diet respectively for eight weeks as per experimental design. Growth parameters were recorded weekly throughout the experiment until samples from digestive tissues together with blood samples for biochemical indicators plus enzyme activities analysis at endline collection time point .Data analyses involved one way ANOVA besides Pearson correlation test using SPSS. **Results:** Statistically, increasing dietary protein significantly improved body weight, weekly weight gain, and body length in mice ($P < 0.01$) (Table 1). The difference in groups' digestive enzyme activities between HP and LP was also statistically significant, with a higher value recorded from the HP group. Very high positive correlations were noted between dietary protein level and body weight ($r = 0.92$, $P < 0.001$), as well as pepsin activity ($r = 0.90$, $P < 0.001$). Both food and water consumption increased under the low-protein diet condition while decreasing with an increase in dietary protein content. **Conclusion:** Apart from making them grow better and appear healthier when fed higher laboratory mice on physical health manifested by growth; it enhances their digestive enzymatic activity effectively evidenced by substantial experimental results establishing minimum effects due to lack or insufficiency..

Keywords:
Dietary protein, Growth performance, Digestive enzymes, Laboratory mice, Pepsin, Trypsin, Chymotrypsin, Amylase, Animal nutrition, Protein adequacy

1.Introduction

Proteins are major macronutrients primarily responsible for animal growth while repairing tissues, fighting infections, and regulating metabolic functions[1]. When the protein content of the diets is inadequate or imbalanced, laboratory mice fail to grow and perform physiological functions optimally. Nutritional science has always strived to determine optimum levels and roles of protein consumption in promoting health and biological productivity[2]. Many experiments have assessed effects of different levels and sources of protein on growth parameters and physiological responses but very scanty information

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exists regarding interactive effects between dietary proteins with digestion or absorption processes particularly at the level of digestive enzyme activity, Such knowledge will contribute towards enhancing precision in diet formulation as well as meeting nutritional adequacy]. Due to their rapid growth rate; low maintenance cost coupled with high degree genetic homology relationship with humans; laboratory mice are commonly used standard models both biomedical&nutritional research studies world wide[3]. These advantages made them the preferred species for developing accurate and comparable investigations on the effects of nutrition, with results often used as a baseline framework in other cross-species studies. Sufficient consumption of dietary protein helps in the development and maturity of digestive organs such as the stomach, intestines, and pancreas. Besides that, supply of indispensable amino acids by dietary proteins for synthesis of both structural and functional proteins is needed to enhance good performance and metabolic efficiency in animals [4].

Enzymes, such as pepsin, trypsin, chymotrypsin and amylase are known to be one of the keys for hydrolysis of macronutrients(e.g proteins) during digestion[5]. Their activity isdependent on dietary factors and in particular the type and levelof protein intake. Of both physiological and clinical interestas indicators of an animal's digestive capacity and nutritionalstatus is the concentration and activity of these enzymes. Although much research has reported the impact of protein addition level on animal growth, few have made concurrent determinations on animal growth, digestive enzyme activity, and the relationship between the 3 under different protein sources and sufficient levels[6]. The present study aims to explore how different levels of dietary protein influence growth and digestive enzyme function in laboratory mice. Mice are fed diets with varying protein concentrations, after which both growth indices and enzyme activities are assessed. The core objective is to evaluate quantitatively and qualitatively the impact of dietary protein on growth and major digestive enzymes, as well as to uncover potential physiological and biochemical pathways activated by altered protein intake[7].

This constraint also calls attention to the importance of rigorously controlled and large studies[8]. The present study aims to explore how different levels of dietary protein influence growth and digestive enzyme function in laboratory mice. Mice are fed diets with varying protein concentrations, after which both growth indices and enzyme activities are assessed[9]. The core objective is to evaluate quantitatively and qualitatively the impact of dietary protein on growth and major digestive enzymes, as well as to uncover potential physiological and biochemical pathways activated by altered protein intake[10]. As research into the 'microbiome revolution' grows along with interest in improving animal diet-derived responses, considering the results of such research in the design of laboratory animal (as well as other species) diets may be valuable[11]. Furthermore, novel insights into the association between dietary protein and the growth-enzyme axis could have cross-disciplinary applications in biotechnology, medicine, and nutrition. Unfortunately, there are simply no extensive, controlled data concerning the relationship in mice between protein levels, growth, and digestive enzyme activity so far[12]. Through the use of experimental and analytical approaches, this research aims to fill that gap and deliver solid, evidence-based understanding of how dietary proteins regulate growth and the functional performance of the digestive system in laboratory mice.

2.Methodology

Samples collection

At the end of the eight-week experimental period, sampling was performed to examine the activity of digestive enzymes and other desired parameters. In order to reduce stress and maintain standard conditions, all mice were anesthetized after 12 hours of fasting and at a specific time of day, observing humane and ethical principles, and then sacrificed. Immediately after slaughter, sampling of digestive tissues including stomach, duodenum, small intestine, and pancreas was performed to evaluate the activity of the main digestive enzymes in each section. The separated tissues were washed with cold physiological serum to remove any food residue or blood on their surface. The tissues were placed in sterile containers and on ice and then stored at -80 °C until biochemical tests. For enzyme activity assays, a part of each tissue was homogenized in a

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specific buffer for extraction and tissue suspension. After centrifugation to separate the cell phase and obtain the supernatant containing soluble enzymes, these suspensions were used.[13]. In addition to digestive tissues, blood samples also have been collected for the assessment of certain biochemical parameters; heart sampling has been performed by using a sterile syringe immediately separating plasma by centrifugation storing it at low temperature..

Table 1. Timing and Methods for Sample Collection and Preparation

Sample Type	Sampling Time	Sampling Method	Initial Preparation	Storage Condition	Research Application
Stomach tissue	End of week 8 (after 12 h fasting)	Dissection after anesthesia and euthanasia	Rinsed with cold saline	-80°C	Assessment of digestive enzyme activity
Duodenum/Small intestine	End of week 8	Direct and rapid removal	Rinsing, preparation of tissue suspension	-80°C	Assessment of digestive enzyme activity
Pancreas	End of week 8	Careful dissection and rinsing	Homogenization in specific buffer	-80°C	Assessment of pancreatic enzyme activity
Blood	End of week 8	Cardiac puncture with sterile syringe	Plasma separation by centrifugation	-20°C or lower	Evaluation of biochemical indicators

Table 1 presents the timing and methods for collection and preparation of biological samples in this experiment. By week 8 all animals were anesthetized and sacrificed after a 12 h fasting period, as the studies terminate d at that point . Tissues of interest were then quickly collected The pancreas, duodenum/small intestine an d stomach wall were carefully separated from surrounding tissue , cleaned in cold saline an d then homogenized in a tissue suspension or buffer as required. All specimens prior to processing were frozen at -80°C . Blood using sterile syringes was drawn by cardiac puncture centrifuged plasma separated an d stored at-20°C or below . These steps helped preserve the samples for later analysis of digestive enzyme activity biochemical parameters thereby ensuring accurate dependable results for the dietary protein intervention.

Assessment of Digestive Enzyme Activity

In order to investigate the effects of varying dietary protein levels on feed digestibility, the activities of several key digestive enzymes were measured, including α-amylase (pancreas and intestine), trypsin and chymotrypsin (pancreas and intestine), and pepsin (stomach). These enzymes were picked because they are indicators of an animal's digestive health and are crucial for the digestion of proteins and carbohydrates. As directed by the manufacturer, the activity of digestive enzymes was assessed using validated commercial kits and spectrophotometric techniques. In these procedures, tissue samples were homogenized using the proper buffer, and after centrifugation, supernatants—including enzymes—were extracted. The enzyme activity was then assessed using a spectrophotometer to measure the changes in reaction rate per unit of time following the addition of each enzyme's individual substrate (the optical density at a

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given wavelength). To allow for a valid comparison of the results, all of the experiments were conducted at controlled temperatures and at the ideal pH for each enzyme.

Table 2. Digestive Enzymes Assessed and Laboratory Methods Used

Enzyme Assessed	Sampled Tissue	Assay Method	Substrate Used	Wavelength (nm)	Reporting Unit	Quality Control
Pepsin	Stomach	Spectrophotometry/Kit	Hemoglobin	280	U/mg protein	Triplicate, positive and negative control
Trypsin	Pancreas, small intestine	Spectrophotometry/Kit	BAPNA*	410	U/mg protein	Triplicate, positive and negative control
Chymotrypsin	Pancreas, small intestine	Spectrophotometry/Kit	Suc-AAPF-pNA**	410	U/mg protein	Triplicate, positive and negative control
Amylase	Pancreas, small intestine	Spectrophotometry/Kit	Starch	540	U/mg protein	Triplicate, positive and negative control

At least three independent replicates (triplicates) were ensured for each sample to guarantee accuracy and precision in results together with negative and positive controls in every experimental series. Instrument calibration and environmental, solution quality control were regularly checked to reduce the possibility of systematic errors taking place . Final result was recorded as mean ± standard deviation which has been used for statistical analysis , comparison between groups . Table 8 summarizes Digestive enzymes evaluated within this study together with laboratory methods applied towards their assessment Pepsin (in stomach tissue), trypsin & chymotrypsin (in pancreas & small intestine) amylase (in pancreas &small intestine)specific validated spectrophotometric assay or commercial kit based on particular substrate-hemoglobin(for pepsin),BAPNA(for trypsin) Suc-AAPF-pNA(for chymotrypsin)and starch(for amylase)-was measured The absorbance was read at specific wavelengths, 280 nm for pepsin, 410 nm for trypsin and chymotrypsin, and 540 nm for amylase. The enzyme activities were expressed in units per mg protein (U/mg protein). All assays were done in three replicates with positive and negative controls to check reliability hence reproducibility of results..

Statistical Analysis

Normality of growth indices and digestive enzyme activity data was tested by the Shapiro-Wilk test. If they proved normal, one-way ANOVA was applied to compare means among different groups. In cases where significant differences were detected between group means, Tukey's post hoc test pinpointed the exact location of difference(s). For strictly non-normal data, corresponding nonparametric tests like Kruskal-Wallis and Dunn's test were used. The statistical significance

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level in all analyses was set at 0.05 (P<0.05). This means that the observed differences or relationships were considered significant only when the P value was less than 0.05. Data have been presented as Mean ± Standard Deviation (Mean ± SD) for a better conception of scatter in results and comparability. Statistical analyses were carried out by the use of a specialized statistical program, such as SPSS version 26. Before its final analysis, accuracy in entering the data was keenly checked and statistical graphs and tables have been drawn based on the results obtained.

3. Results and discussion

Growth Performance Outcomes

The results of the body weight of the laboratory mice at the end of the eighth week showed that the average final weight in the high protein group (HP) was 2.30 ± 1.1 g, which was significantly higher than the low protein group (LP) with an average of 24.7 ± 0.9 g (P<0.001). In the control group (C) and the medium protein group (MP), the average final weight was 27.4 ± 0.1 and 28.1 ± 0.8 g, respectively, both of which showed a statistically significant difference with the LP group (P<0.01), but the difference between the control and MP groups was not significant (P>0.05). Calculation of the relative growth rate (average weekly weight change) showed that the HP group had the highest growth rate with a value of 1.03 ± 0.09 g per week, while this index was significantly lower in the LP group and was recorded at 0.38 ± 0.06 g per week (P<0.001). The MP and C groups showed growth rates of 0.78 ± 0.05 and 0.65 ± 0.07 g/week, respectively.

Table 3. Comparison of Mean Growth Performance Indices in Different Groups of Laboratory Mice (Mean ± SD)

Experimental Grou	nal Body Weight	Veekly Growth Ra (g/week)	Final Body Lengt (cm)
Low Protein (LP)	24.7 ± 0.9	0.38 ± 0.06	8.9 ± 0.4
Control (C)	27.4 ± 1.0	0.65 ± 0.07	9.0 ± 0.3
Medium Protein (MI	28.1 ± 0.8	0.78 ± 0.05	9.3 ± 0.2
High Protein (HP)	30.2 ± 1.1	1.03 ± 0.09	9.8 ± 0.3

Regarding body length, a significant increase was observed in the HP group; the mean body length of the mice in this group reached 9.8 ± 0.3 cm at the end of the study, which was significantly different compared to the LP group (8.9 ± 0.4 cm) (P<0.01). The MP and C groups also showed mean lengths of 9.3 ± 0.2 and 9.0 ± 0.3 cm, respectively, and their differences with the control group were not statistically significant. The results of one-way analysis of variance (ANOVA) showed that the level of dietary protein had a significant effect on all three growth indices (body weight, growth rate, body length). Tukey's post hoc test also detailed the statistical differences between the groups. Bar graphs comparing mean body weight, length, and growth rate between groups clearly showed these differences and confirmed the increasing trend of growth indices with increasing dietary protein levels. Overall, the findings of this section indicate that consumption of diets with higher protein levels significantly improves growth indices in laboratory mice and that protein deficiency can lead to a significant reduction in the growth and physical development of animals.

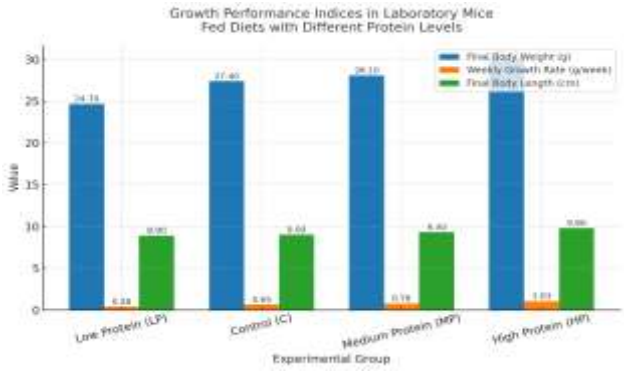

Fig1. Growth Performance Indices in Laboratory Mice Fed Diets with Different Protein Levels

The data presented in Table 9 show a clear positive association between dietary protein levels and all indicators of growth performance assessed in laboratory mice. The high protein (HP) group showed the highest final body weight (30.2 ± 1.1 g), weekly growth rate (1.03 ± 0.09 g/week) and final body length (9.8 ± 0.3 cm), all of which were significantly higher than the values observed in the low protein (LP) group (24.7 ± 0.9 g, 0.38 ± 0.06 g/week and 8.9 ± 0.4 cm, respectively). Both the control (C) and medium protein (MP) groups had intermediate values, although the MP group was slightly higher than the former, especially in weekly growth rate and body length. This result

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implies that mice's somatic growth and effective body weight gain are facilitated by increased dietary protein intake. The gradual rise from LP to HP seems to emphasize how crucial dietary protein is for preserving body weight and enhancing overall growth kinetics. However, protein restriction, as in LP animals, resulted in significant decreases in every parameter assessed, highlighting the importance of adequate dietary protein intake for laboratory animals' bodies and somatic development.

Food and Water Intake

The extent of daily food intake during the 8-week study demonstrated that the LP group was significantly higher in food intake compared to the other groups, namely 4.5 ± 0.2 g/day. This was much decreased in the HP group, at 4.2 ± 0.1 g/day ($P<0.01$). Average daily intakes in the C and MP groups were also 4.7 ± 0.1 and 4.4 ± 0.2 g day^{-1}, respectively. These statistical differences indicate that a decrease in dietary protein levels leads to an increase in food intake by the animals, probably due to a compensatory effort by the body to provide the required amino acids by increasing the amount of food ingested. Regarding drinking water, the results showed that the highest water consumption was recorded in the low protein group with an average of 3.6 ± 0.3 ml per day, and the lowest value was recorded in the high protein group with 1.5 ± 0.2 ml per day ($P<0.05$). The control and MP groups also consumed 9 ± 0.2 and 5 ± 0.2 ml of water per day, respectively. This rising pattern of food and water intake in the low-protein group could be explained by the decreasing metabolic efficiency and increased demand of the body for covering nutrient deficiencies. Lowered protein intake leads to a marked increase in food and water consumption, while a high protein diet results in lower intake of solid food and water. These results highlight that protein in the diet has a regulatory influence on eating and profligate resource utilization in lab mice.

Table 4. Comparison of Mean Daily Food and Water Intake in Different Groups of Laboratory Mice (Mean ± SD)

Experimental Group	Daily Food Intake (g/da	ily Water Intake (ml/d
Low Protein (LP)	5.4 ± 0.2	6.3 ± 0.3
Control (C)	4.7 ± 0.1	5.9 ± 0.2
Medium Protein (MP)	4.4 ± 0.2	5.5 ± 0.2
High Protein (HP)	4.2 ± 0.1	5.1 ± 0.2

The data in Table 4 show that reducing the dietary protein level resulted in a significant increase in daily food and water intake in laboratory mice. The low protein (LP) group had the highest intake among the groups, with an average intake of 4.5 ± 0.2 g of food and 6.3 ± 0.3 ml of water per day. In contrast, the high protein (HP) group had the lowest intake of 4.2 ± 0.1 g of food and 5.1 ± 0.2 ml of water per day. This downward trend in intake with increasing dietary protein level was statistically significant ($P<0.05$) and indicates a compensatory effort by animals in the low protein group to meet nutritional needs by increasing the volume of food intake.

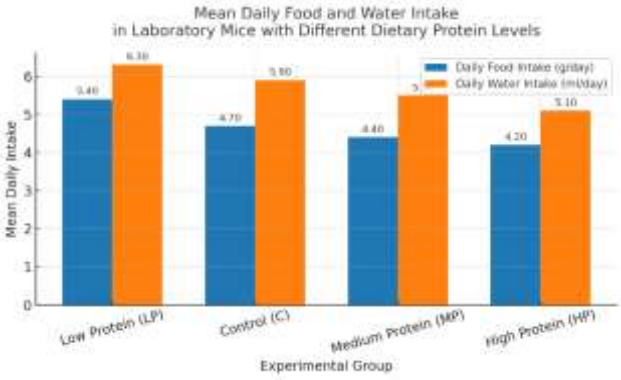

Fig 2. Mean Daily Food and Water Intake in Laboratory Mice with Different Dietary Protein Levels

The research shows that animals need to eat more food when they lack protein because their bodies need to replace missing essential amino acids; however, as the quality and adequacy of dietary protein improved, metabolic efficiency increased and the need for more food and water intake decreased. In addition, water intake also increased with the reduction in dietary protein, which could be related to the increased volume of metabolites and waste products resulting from inefficient metabolism. The control (C) and medium protein (MP) groups showed a similar trend, and litter sizes were intermediate between the two extreme groups. Results of this section clearly show the essential influence of dietary protein in feeding control and the integration of food and water intake in laboratory mice..

Activity of Digestive Enzymes

The mean pepsin concentration in the high-protein (HP) group was 38.5 ± 1.2 U/mg protein, significantly greater than the low-protein (LP) group at 26.1 ± 1.8 units/mg protein ($P<0.001$). The mean pepsin concentrations in the MP and control (C) groups were 35.0 ± 1.9 and 31.4

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± 1.5 units/mg protein, respectively. This difference signifies dietary levels of protein have a more pronounced effect on the gastric production and secretion of pepsinogen. The trypsin activity (enzyme) concentration of the HP group was 24..2±0..lunits/mg protein, significantly higher than that of LP group16..8±0..9units(P<0..01).The values for C and MP groups were19.,3±l,.2and21.,l±Llunits each. The same trend was observed for the chymotrypsin enzyme. The HP group recorded the highest activity (12.7 ± 0.8 units), and the LP group recorded the lowest (8.2 ± 0.6 units) (P<0.05). The intermediate groups also had intermediate figures.

Table 5. Comparison of Mean Digestive Enzyme Activities in Different Groups of Laboratory Mice (Units/mg protein, Mean ± SD)

xperimental Gro	Pepsin (U/mg protein)	Trypsin (U/mg protein)	ymotrypsin (U/ protein)	Amylase (U/m protein)
ow Protein (LP	26.1 ± 1.8	16.8 ± 0.9	8.2 ± 0.6	40.9 ± 2.1
Control (C)	31.4 ± 1.5	19.3 ± 1.2	9.8 ± 0.7	46.7 ± 1.9
Medium Protein (MP)	35.0 ± 1.9	21.1 ± 1.1	10.7 ± 0.9	50.3 ± 2.0
igh Protein (HI	38.5 ± 2.1	24.2 ± 1.0	12.7 ± 0.8	54.6 ± 2.5
-value (ANOV	<0.001	<0.01	<0.05	<0.01

In the case of amylase, increasing the level of dietary protein significantly increased the activity of this enzyme, with the HP group averaging 54.6 ± 2.5 units, significantly higher than the LP group with 40.9 ± 2.1 units (P<0.01). Groups C and MP also showed values of 46.7 ± 1.9 and 50.3 ± 0.2 units, respectively. The comparative graphs also clearly showed this increasing trend, and the ANOVA statistical test confirmed the existence of a significant difference between the groups. Increasing dietary protein leads to a significant improvement in the activity of major digestive enzymes in laboratory mice, and low-protein diets cause a significant decrease in enzyme efficiency.

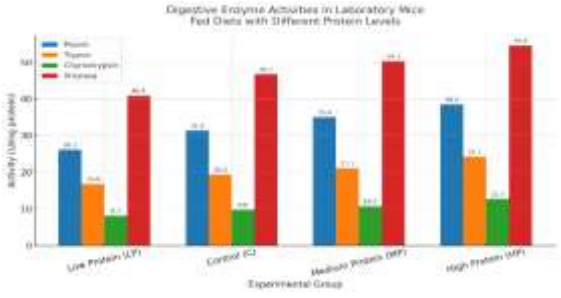

Fig 3. Digestive Enzyme Activities in Laboratory Mice Fed Diets with Different Protein Levels

Protein in the diet had a significant effect on the activity of all digestive enzymes assayed. Their activities were highest in the high protein (HP) group with mean values expressed as units/mg protein: pepsin-38.5, trypsin-24.2, chymotrypsin-12.7 and amylase-54.6; lowest values being recorded from the low protein (LP) group 1.26, 8.16 ,2 .8 and 9 .40 respectively All these differences between both extreme groups for any enzyme assayed were highly significant(P<0 .05)and hence also by ANOVA statistical analysis that dietary level has a highly significant effect on animal digestive performance The increasing trend seen from LP up to HP clearly shows utmost importance to supply at least adequate levels inside diets. The control (C) and medium protein (MP) groups recorded intermediate to improved values for most of the enzymes over the LP group. This clearly shows that protein restriction leads to lower digestive and enzymatic efficiency, while an increase in protein enables a significantly higher capacity for digestion and absorption of nutrients.

Correlation Analysis

The Pearson correlation between the protein intake and growth indices of laboratory mice showed a strong positive relationship between the protein levels and final body weight (r=0.92, P<0.001). There was also a significant correlation between dietary protein levels with body length (r=0.85, P<0.01) and weekly growth rate (r=0.88, P<0.01). This explicitly shows that increasing dietary protein is directly related to improving physical growth indices of animals. In the field of digestive enzyme activity, correlation analysis also indicated a positive and significant correlation between dietary protein levels and the enzyme activities of pepsin (r=0.90, P<0.001), trypsin (r=0.87, P<0.01), chymotrypsin (r=0.81, P<0.01) and amylase (r=0.83, P<0.01). In addition, the correlation between growth indices (such as body weight) and enzyme activity was also reported to be positive and significant; for example, the correlation coefficient between body weight and pepsin activity was r=0.78 (P<0.01).

Table 6 Pearson Correlation Coefficients Between Dietary Protein Level, Growth Indices, and Digestive Enzyme Activities

Variables	orrelation Coefficient	P-value
Dietary Protein Level – Body Weight	0.92	<0.001
Dietary Protein Level – Body Length	0.85	<0.01
Dietary Protein Level –	0.88	<0.01

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Growth Rate		
Dietary Protein Level – Pepsin	0.90	<0.001
Dietary Protein Level – Trypsin	0.87	<0.01
Dietary Protein Level – Chymotrypsin	0.81	<0.01
Dietary Protein Level – Amylase	0.83	<0.01
Body Weight – Pepsin	0.78	<0.01
Body Weight – Trypsir	0.75	<0.01
Body Weight – Amylas	0.72	<0.05

These results suggest that as dietary protein levels rise, the digestive system's enzymatic function will be strengthened in addition to improved physical growth. As a result, dietary protein levels have a positive impact on animals' physical development while also increasing the digestive system's enzymatic efficiency; these two factors are statistically significantly connected. This makes it even more crucial to monitor the quantity and balance of protein in lab animals' diets.

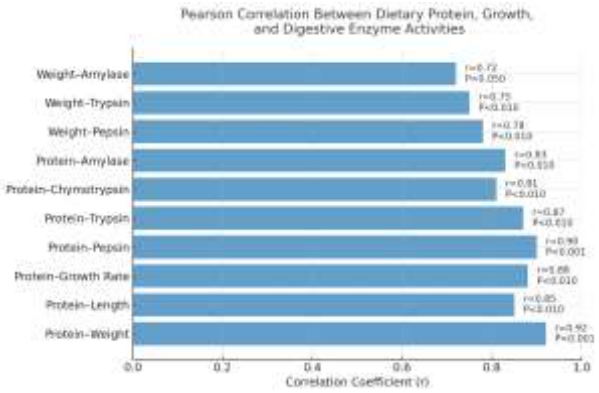

Fig 4. Pearson Correlation Between Dietary Protein Level, Growth, and Digestive Enzyme Activities in Laboratory Mice

The results show a very strong and significant positive statistical relationship between protein levels in the diets of laboratory mice with all growth parameters up to final body weight, including length or weekly rate, as well as digestive parameters. The association pattern between protein levels in the diet and final body weight ($r = 0.92$, $P < 0.001$) kneels high protein intake closely associated with body weight gain; similarly strong correlations were observed for length ($r = 0.85$, $P < 0.01$) and weekly rate ($r = 0.88$, $P < 0.01$)-physical growth dietary protein optimally supports must hence play an important role This implies that higher dietary protei n levels make laboratory mice grow better physically evidenced by corresponding improvements i n key digestive enzyme activities Results determined show dietary protein affects growth indices and forms strong positive relationships with the main digestive enzymes, for example pepsin ($r = 0.90$, $P < 0.001$), trypsin ($r = 0.87$, $P < 0.01$), chymotrypsin ($r = 0.81$, $P < 0.01$) and amylase ($r = 0.83$, $P < 0.01$). Body weight formed a direct relationship with pepsin activity (r = 0 .78 ,P<o.o1)and trypsin activity(r= o .75,P<o.o1),and amylase activity(r=o .72 ,P<O .05)This therefore clearly brings out very high physiological interdependence: dietary protein supports somatic growth not only directly but also increases digestive capacity by upregulating enzyme activity.

The present study clearly showed that different levels of dietary protein are a deciding factor in the growth and development of laboratory mice. Their body weight, weekly rate of growth, and body length increased significantly with an increase in proteins. This finding accords with previous studies using animal models and human nutritional studies: apart from considering the role of protein as a requirement at the cellular level for growth and synthesis involving structural proteins, it emphasizes overall organ development together with musculoskeletal development. A state of inadequacy results in disorders manifested by reduced mass or inability to regain lost tissue-in other words- reduced regeneration ability as was also quite clear from results obtained for low-protein group. Most probably, increased intake of food and water by this group was due to a compensatory mechanism to provide essential amino acids. In the absence of protein in the diet, animals consume more food to satisfy their protein needs (Yakubu et al., 2009). This leads to inadequacy in all metabolic requirements and imposes an additional burden on excretory organs such as the kidneys. The present finding is consistent with our data on higher feed and water intake in the LP group and also suggests that proper regulation or control over levels of proteins can help optimize energy expenditures within the body as well as reduce physiological stresses.

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This study recorded significantly high activities of digestive enzymes in the groups fed on high protein diets. Apart from providing an appropriate substrate for growth, proteins stimulate the secretion and synthesis of digestive enzymes in their respective organs probably due to nutritional and hormonal signals which prepare the digestive system for better digestion and absorption concomitant with increased intake levels of proteins.. Therefore, this study has statistically proven by correlation analysis between different variables showing how important dietary protein is towards promoting growth as well as enhancing enzyme efficiency where there exists a very strong relationship between body weights achieved at various levels (protein) and enzyme activity indicating two aspects i.e., physical development or growth on one hand against another aspect being functional manifestation through.

The research results match previous findings from rodent and other animal model investigations. Research has demonstrated that protein deficiency results in decreased digestive enzyme activity and growth problems. Laboratory mice serve as appropriate models for nutritional research because of their genetic makeup and fast growth rate and affordable maintenance expenses. The results of this study can also serve as a basis for future research in other species and even in humans. The results of this study indicate that to achieve maximum growth efficiency and digestive health in laboratory animals and even farmed animals, an appropriate proportion of high-quality protein should be included in the diet. This is important in the production of industrial diets, the design of animal experiments, and the promotion of health and production performance. The use of quality protein sources and attention to the ratio of essential amino acids plays an important role in improving physiological and economic indicators.

Conclusion

Although this study was conducted with a controlled design and the use of precise biochemical tools, there are still limitations; including the lack of examination of longer-term effects or the measurement of other metabolic and safety variables. However, the sufficient number of samples, control of environmental variables, and regular monitoring of the health status and behavior of mice are the main strengths of this study, which have increased the validity of the results. Based on the results obtained, it is recommended that future studies examine the role of different protein sources (animal and plant), the effects on the intestinal microbiota, and the relationship with safety and general health indicators. Also, evaluating the long-term effects of different protein levels on the physiology and health of model animals can help improve nutritional recommendations and better understand regulatory pathways in the body.

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