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RESEARCH ARTICLE

Evaluation of aminace, a proteolytic enzyme combined with whey protein supplement in comparison with whey protein alone for improvement of protein status in albino rats

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ABSTRACT

Background: Whey protein is a commonly used protein supplement among patients with malnutrition, post gastrointestinal surgeries, and chronic diseases. After consumption, this protein is broken down into smaller peptides by endogenous peptidases and absorption occurs within 90 min or else the larger undigested peptides are excreted, thus poor protein absorption. Furthermore, the larger peptides composed of more than seven amino acids may trigger an immune response, causing discomfort and even inflammation in the gut. To overcome such drawbacks, exogenous digestive peptidases like aminace, which is a proteolytic enzyme on addition with whey protein helps in effective degradation and absorption of proteins. **Aims and Objectives:** The aim of the study was to evaluate the efficacy of aminace, a proteolytic enzyme combined with whey protein compared with whey protein alone in the improvement of protein status in albino rats. **Materials and Methods:** A total of 18 male albino rats weighing about 150–300 g were divided into three groups of six rats each. Group 1 (standard) - whey protein (200 mg/kg) alone, Group 2 (test) - whey protein (200 mg/kg) with proteolytic enzymes (aminace), and Group 3 (test control) - proteolytic enzyme (aminace) alone were administered orally daily for 2 weeks. The efficacy parameters analyzed were body weight and the total serum protein levels. **Results:** One-way ANOVA was used to compare the mean of all the groups followed by *post hoc* Tukey's test. There was a statistically significant improvement in weight gain in Group 2 animals compared to other groups ($P < 0.001$). Though there was rise in the total serum protein levels among the Groups 2 animals, it was not statistically significant. **Conclusion:** Addition of aminace, a proteolytic enzyme, helped in better utilization of protein and hence could be taken along with protein supplements for better absorption.

KEY WORDS: Whey protein; Aminace; Protein status

INTRODUCTION

Protein supplementation is a common practice for improvement in the body protein status. It serves as an important adjunctive concern in the treatment of malnutrition associated with various infections and after gastrointestinal surgeries for increasing the lean body weight and immune response.[1] Apart from these, it is also used to promote muscle building in people with an active lifestyle and exercises.[2]

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Whey protein, a milk protein is commonly used protein supplement containing all the nine essential amino acids. It is more easily digested by the body than any other protein. Basically, milk consists of two types of protein whey protein and casein. Whey protein is a cysteine-rich protein source,

which is a byproduct obtained during the cheese making process. It is a mixture of beta-lactoglobulin (~65%), alpha-lactalbumin (~25%), and serum albumin (~8%), constituting ~20% of the total bovine milk protein.[3] Whey protein is one of the richest sources of all nine essential amino acids which the body is unable to produce on its own.[2] Whey protein is also a rich source of branched chain amino acids, which stimulates muscle protein synthesis in a dose-dependent manner. This property is attributed to the faster digestion and absorption kinetics of whey protein, which results in increase in plasma concentration of amino acids, which is further employed in skeletal muscle synthesis. Whey protein appears to increase antioxidant glutathione levels in the body as well to fight free radicals.[4]

For effective absorption, whey protein must be broken down into tripeptides and dipeptides by endogenous peptidase within approximately 90 min of consumption; otherwise, the protein goes undigested and excreted from the body.[5] Furthermore, during the digestion process, the larger peptides composed of more than seven amino acids may trigger an immune response, causing discomfort and even inflammation in the gut. These can be due to inhibition of endogenous digestive enzymes from over processing and a rapid small intestine transit time. In addition, if this whey protein is not broken down into the smallest composition, it creates large peptides that can cause discomfort such as bloating, nausea, and cramping. The combined effect of these factors may contribute to incomplete digestion and excretion, thereby limiting the absorption rate of protein before it reaches the caecum. These are the major drawbacks with protein supplements.[6]

Aminace is a unique digestive protease obtained from bacteria that help in quick and effective degradation of proteins to small peptides. Being exogenously administered proteolytic enzyme, it augments the protein breakdown process during over processing and thus improves the efficacy of substrate (whey protein) in improving the absorption rate and prevents unnecessary wasting of protein due to deficient digestive process.[7] Hence, the present study was undertaken to know the efficacy of aminace, a proteolytic enzyme in improving the protein status when given along with whey protein supplement.

MATERIALS AND METHODS

The study was conducted after the study protocol was approved by the Institutional Animal Ethics Committee, JJMMC/IAEC/14-2017. A total of 18 male albino rats weighing about 150–300 g were obtained from the animal house of JJM Medical College, Davangere. The animals were fed on standard pellet diet and water and were maintained under standard conditions of temperature, humidity, and light (12 h light/12 h dark cycle). The study inclusion criterion was healthy male albino rats weighing about 150–300 g and the exclusion criteria were rats >300 g or <150 g and rats used for other experiments in the preceding 6 months. The albino rats were divided into three groups of six animals each. Group 1 (standard) rats were fed on whey protein (200 mg/kg)[4] alone, Group 2 (test) rats were fed on whey protein (200 mg/kg) + proteolytic enzymes (aminace) - 10 mg/g of protein, and Group 3 (test control) rats were fed on proteolytic enzyme (aminace) alone - 10 mg/g of protein[7] for 2 weeks. Distilled water was used as a control. The whey protein used in the study was manufactured by Davisco, USA. The test drug aminace was manufactured by Goodman Pharmaceuticals, Puducherry, India, and marketed by Mylin Biotech Ltd., Bengaluru.

All the test components were dissolved in distilled water and administered per orally once daily in the morning for 14 days. The assessment was done on the 1st day (baseline) and 14th day (after 2 weeks) at the end of the study, after 60 min of oral administration of the test components. The parameters assessed were body weight and total serum protein levels. Body weight was measured using the electronic weighing scale and total protein levels using an autoanalyzer. Blood sample was collected from the retro-orbital plexus of each animal, under light anesthesia, according to the method of Cocchetto and Bjornsson on the 1st and 14th days.[8] About 1 ml of blood was collected from each rat and was made to settle for separation of serum, and then, total serum protein level was determined.[9]

Statistical Analysis

All the data were evaluated statistically with SPSS software. The body weight and total proteins levels were represented as mean ± standard deviation and compared with one-way ANOVA among groups. For intergroup comparison, *post hoc* Tukey's analysis of standard, test, and test control groups was performed. $P \leq 0.05$ was considered statistically significant.

RESULTS

The mean body weight and total serum protein were compared between the groups using one-way ANOVA and represented in Tables 1 and 2, respectively. The mean weight improvement in Group 1 animals fed on whey protein alone was from 197 ± 22.19 to 210 ± 5.06, in Group 2 animals which was fed on whey protein + aminace was from 259 ± 18.86 to 278.83 ± 22.66, and in Group 3 animals fed on aminace alone was from 210 ± 12.32 to 218.67 ± 9.6. The overall weight gain by Group 2 animals was statistically significant with $P < 0.001$ when compared to whey protein alone and aminace alone group as represented in Table 3.

The mean total serum protein levels of Group 1 receiving whey protein alone were 6.817 ± 0.397 on the 1st day and 7.233 ± 0.372 on the 14th day. In Group 2 receiving whey protein +

Table 1: Effect of whey protein and aminace on weight

Groups	Baseline (body weight in g)	After 2 weeks (body weight in g)
	Mean±SD	Mean±SD
Group 1	197.00±22.190	210.00±5.060
Group 2	259.67±18.864	278.83±22.666
Group 3	210.00±12.329	218.67±9.606

Table 2: Effect of whey protein and aminace on total protein levels

Groups	Baseline (total proteins g/dl)	After 2 weeks (total proteins g/dl)
	Mean±SD	Mean±SD
Group 1	6.817±0.3971	7.233±0.3724
Group 2	6.733±0.3445	7.200±0.3795
Group 3	7.033±0.3077	7.200±0.1789

Table 3: Comparison of body weight and the total protein levels between the groups

	Post hoc Tukey's test	
Groups compared	Body weight after 2 weeks	Total protein after 2 weeks
Group 1 versus Group 2	$P<0.001$	$P=0.983$
Group 1 versus Group 3	$P=0.567$	$P=0.983$
Group 2 versus Group 3	$P<0.001$	$P=1.000$

aminace, the serum protein levels were 6.733 ± 0.34 on the 1st day and 7.2 ± 0.379 on the 14th day, and in Group 3 receiving aminace alone, the serum protein levels were 7.033 ± 0.3077 on the 1st day and 7.2 ± 0.178 on the 14th day. Although there was a better improvement in protein levels in Group 2 animals compared to other groups, the results were not statistically significant. The results are represented in Table 3.

DISCUSSION

In the present study, we studied for the improvement in the protein status using two parameters, increase in body weight which is due to increase in lean body weight of the animals and increase in the total serum protein levels. There was a significant increase in the body weight of the animals when the proteolytic enzyme was added to whey protein, compared to the animals which were fed on whey protein alone. This justifies that addition of aminace has better utilization of whey protein.

According to few studies saturation of endogenous proteolytic enzymes is the rate-limiting step in the process of protein digestion. This can be explained as over processing, due to increased protein intake that could slow down the digestive enzyme; as a result, the complex peptides in the whey protein are not broken down to smaller peptides. Finally, this results in decrease in the absorption rate which limits the amount of absorption from the caecum.[6] The results of the present study are in accordance with a similar study done by Oben *et al.* suggested that addition of digestive aminogen, a patented blend of digestive proteases from *Aspergillus niger* and *Aspergillus oryzae*, increased the absorption rate of whey protein. Furthermore, *in vivo* studies produced a positive nitrogen balance and decrease C-reactive proteins levels which could be associated with a decrease in immune activities.[10,11] Another study done by Kobayashi *et al.* studied the weight of the extensor digitorum longus muscle, which was significantly suppressed in animals fed with protein free diet. However, this suppression was significantly reduced in animals which were fed on a whey protein diet, justifying that whey protein helped in increasing the lean body weight of the animals though no proteolytic enzymes was used in this study.[4] Apart from the benefit of enhanced protein absorption, few unpublished *in vitro* data have shown a similar protease enzyme, aminogen hydrolyzes whey, soy, and casein protein to produce peptides that inhibit angiotensin converting enzymes which may cause decrease in the diastolic blood pressure.[10] Another study done by Nagaoka *et al.* suggested that whey protein has favorable effects on lowering serum cholesterol by decreasing low-density lipoprotein (LDL) and very LDL cholesterol levels compared to soy and casein protein.[12]

Estimation of blood amino acid levels would have been an ideal parameter to measure the absorption of proteins; this was not carried out due to non-availability of local laboratory facilities and financial constrain, hence a limitation of this study. Although there was a better improvement in total serum protein levels in Group 2 compared to other groups, the results were not statistically significant. The reason for this could not be ascertained.

CONCLUSION

To conclude, it is well known through published literature that whey protein supplement helped in the improvement of protein status. Therefore, for better utilization of such compound addition of proteolytic enzymes like aminace is necessary for maximum digestion and absorption to improve body weight and amino acids status. Apart from improving the protein status, this exogenous peptidase has an additional beneficial profile on health. In summary, this preliminary study has shown the benefits of adding the protease enzyme aminace to whey protein supplement. Further studies on the combination of aminace with whey protein with estimation of amino acids profile are warranted.

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