RESEARCH ARTICLE

F.U. Vet. J. Health Sci.
2026; 40 (1): 66 - 71
http://www.fusabil.org

Investigation of Protein Content and Digestibility of Various Protein Powders for Athletes *

Yusuf TÜRKOĞLU [1, a]
Adalet DIŞHAN [2, b]
Zafer GÖNÜLALAN [1, c]

[1] Erciyes University, Faculty of Veterinary Medicine, Department of Veterinary Public Health, Kayseri, TÜRKİYE

[2] Yozgat Bozok University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Yozgat, TÜRKİYE

[a] ORCID: 0009-0004-7002-2544
[b] ORCID: 0000-0001-8097-1648
[c] ORCID: 0000-0002-3935-6296

This study was conducted to determine the protein content, label accuracy, and in vitro digestibility of different protein powders commonly used as dietary supplements by athletes using the Dumas method. Eighteen different protein powders were provided to determine protein and digestibility levels: whey powder, bovine milk casein powder, soy powder, egg white powder, beef protein powder, and pea powder. The protein contents reported on the labels of the protein powder samples examined in the study and the protein levels determined within the scope of the study were 76-72.87%, 86-83.83%, 91-76.01%, 88-99.13%, 85-85.93%, and 84-77.32% for whey, casein, soy, meat, egg white, and pea powders, respectively. The protein powder most compatible with the label information was egg white powder, while the least compatible was soy powder. The protein powder with the highest digestibility rate was meat powder protein (66.21%), followed by casein powder (60.89%), standard bovine milk casein (56.75%), whey protein powder (54.50%), and pea protein powder (53.66%), followed by soy protein powder (43.76%). The protein powder with the lowest digestibility rate was egg protein (36.87%). This study emphasized the importance of informing consumers about the methods used to produce protein powders, as these methods affect digestibility and the rate of protein conversion into body protein. It emphasized that protein powders should be marketed with measurable and rational label information, such as digestibility and the rate of protein conversion into body protein.

Key Words: *Athlete nutrition, dumas method, in vitro digestibility, protein content*

Bazı Sporcu Protein Tozlarındaki Protein İçeriği ve Sindirilebilirliğinin Araştırılması

Bu çalışma, sporcular tarafından besin takviyesi olarak yaygın olarak kullanılan farklı protein tozlarının protein içeriklerini, etiket uyumluluğunu ve in vitro sindirilebilirlik oranlarını Dumas yöntemi ile belirlemek amacıyla yürütülmüştür. Protein ve sindirilebilirlik düzeylerini belirlemek için peynir altı suyu tozu, sığır sütü kazeini tozu, soya tozu, yumurta akı tozu, sığır eti proteini tozu ve bezelye tozu olmak üzere toplamda 18 adet farklı protein tozu temin edilmiştir. Çalışmada incelenen protein tozu örneklerinin etiketlerinde bildirilen protein içerikleri ve çalışma kapsamında belirlenen protein düzeyleri peynir altı suyu, kazein, soya, et, yumurta akı ve bezelye tozları için sırasıyla %76-72.87, %86-83.83, %91-76.01, %88-99.13, %85-85.93, %84-77.32 olarak belirlenmiştir. Sindirilebilirlik oranı en yüksek protein tozunun et tozu proteini (%66.21) olduğu, kazein tozunun ikinci sırada (%60.89) geldiği, kazein tozunu standart sığır sütü kazeini (%56.5), peynir altı suyu proteini tozu (%54.50) ve bezelye proteini tozunun (%53.66) izlediği, soya proteini tozunun (%43.76) ve en düşük sindirilebilirlik oranına sahip protein tozunun ise yumurta proteini (%36.87) olduğu belirlenmiştir. Bu çalışmada, protein tozlarının üretiminde kullanılan yöntemlerin sindirilebilirlik ve proteinin vücut proteinine dönüşüm düzeyi ile ilişkili olması nedeniyle tüketicinin bu konuda bilgilendirilmesinin önemi vurgulanmış olup protein tozlarının sindirilebilirlik ve proteinin vücut proteinine dönüşüm oranı gibi ölçülebilir ve akılcı etiket bilgileri ile pazarlanması gerektiği vurgulanmıştır.

Anahtar Kelimeler: *Sporcu beslenmesi, dumas metodu, in vitro sindirilebilirlik, protein içeriği*

Received : 13.01.2026
Accepted : 26.01.2026

Correspondence

Adalet DIŞHAN
Yozgat Bozok University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology
Yozgat – TÜRKİYE

adalet.dishan@bozok.edu.tr

Introduction

Nutrition is one of the primary factors affecting an athlete's performance. The Turkish Food Codex Sports Nutrition Regulation defines sports nutrition as "foods or food mixtures specifically formulated to help athletes meet their specific nutritional needs or perform at their best" (1). Protein supplements are an attractive strategy in athlete supplementation due to their ability to stimulate muscle protein synthesis (2). Protein powder supplements, in particular, are among the most commonly consumed dietary supplements by both professional and amateur athletes, as well as those exercising for non-sports purposes (3). Although high-protein sports nutrition supplements are available in powder form providing essential amino acids and protein, whey proteins, caseins, or meat hydrolysates are more common (4). Soy can also be added to commonly available protein sources (5). The quality of the extract can vary depending on the type and quality of the manufacturing process. Whey and casein proteins are considered complete protein sources for maximizing training adaptations, particularly due to their high leucine content, which plays a crucial role in muscle protein

* This research article was supported by Erciyes University Scientific Research Projects Unit with project number TYL-2019-9167.

synthesis (6). These types of proteins are also frequently used due to their ease of consumption and practicality. Therefore, widely available commercial protein sources were selected for this study, including egg white and pea protein sources.

Protein digestibility is typically defined as the rate at which ingested protein is hydrolyzed to amino acids, dipeptides, and tripeptides suitable for absorption (7). In vivo methods using animals or humans, while providing the most accurate results, are time-consuming and costly. Therefore, in vitro digestion models offer a valuable alternative to animal and human models, enabling rapid screening of food components (8). Static in vitro digestion involves in vitro oral, gastric, and/or small intestinal digestion experiments performed under constant physicochemical conditions in each simulated phase (9). These digestion models are widely used to study the structural changes, digestibility, and release of food components under simulated gastrointestinal conditions (8, 10).

In this study, simple, economical, and reproducible in vitro digestion models based on human physiology were developed to investigate the digestibility of protein powders under simulated gastrointestinal conditions. The amino acid bioavailability of a protein source is best conceptualized as the amount and diversity of amino acids digested and absorbed into the bloodstream after ingestion (11). However, data on the digestibility of commercially available powdered protein sources commonly used in sports nutrition, especially comparative analyses under standardized in vitro conditions, are lacking in the literature. The need to integrate information on protein consumption is critical for both practical and future research. The Dumas method involves incinerating a sample of defined mass at high temperature in the presence of oxygen; the gases produced are then reduced with copper and dried while trapping CO_2, and finally nitrogen is quantified using a universal detector. The Dumas method for quantitative nitrogen determination is an alternative to classical methods for analyzing the protein content of foods by measuring the molecular weight of an unknown gas (12).

This study was conducted to determine the protein content, label compliance, and digestibility rates of various standard protein powders commercially available to athletes as dietary supplements using the Dumas method in a simulated in vitro environment.

Materials and Methods

Research and Publication Ethics: Ethical approval was not required, as no live animals were used in this study.

Study Design and Sample Collection: Eighteen samples (n=18) were used to determine protein and digestibility levels by purchasing commercially available athlete protein powders, including three each of whey, bovine milk casein, soy, egg white, beef protein, and pea. The origin and brand categorization of the products are given in Table 1. Bovine milk casein sodium salt (Sigma C3400) was used as a standard.

Table1. Information on protein powders

Types of Products	Origin	Brand Category
Whey Powder (n:3)	Türkiye	Brand A
	Türkiye	Brand B
	Canada	Brand C
Casein Powder (n:3)	USA	Brand D
	USA	Brand E
	Türkiye	Brand F
Soy Powder (n:3)	China	Brand G
	USA	Brand H
	Türkiye	Brand I
Meat Powder (n:3)	USA	Brand D
	USA	Brand J
	Türkiye	Brand K
Egg White Powder (n:3)	Türkiye	Brand L
	Türkiye	Brand M
	Germany	Brand N
Pea Powder (n:3)	Türkiye	Brand L
	UK	Brand O
	China	Brand P

Gastric Digestion Simulation: The protein powder samples were weighed to yield 5.0 g each. HCl (0.2 N, 34.0 mL) was added to each flask containing the sample. The mixture was shaken for 5 minutes, then its pH was adjusted to 1.9 with 1.0 N NaOH, and the mixture was placed in a water bath at 37°C. At the same time, 2.08 g of pepsin was added to a flask and dissolved in 80 mL of distilled water. The fresh pepsin solution prepared for each flask was added to the mixture, whose pH was adjusted to 1.9, and the mixture was kept for 30 minutes with occasional stirring. At the end of the period, 0.2 N Na_2HPO_4 $2H_2O$ buffer was added to the mixture, and the pH was adjusted to 7.5 to terminate pepsin enzyme activity.

Small Intestine Digestion Simulation: In this stage of the study, 16.00 g of pancreatin was put into a conical flask and dissolved in 80 mL of distilled water, and 1.0 mL of fresh pancreatin solution was added to each conical flask (0.200 g of pancreatin for each conical flask) after the stomach digestion stage was completed. It was kept for 150 minutes, stirring occasionally.

Dumas Method: After the digestion time with both enzymes, the mixture was removed from the water bath. Two mL of the mixture was taken, 1.25 mL of saturated TCA was added to stop the digestion and precipitate the undigested proteins, and the mixture was made homogeneous by shaking. From this mixture, after the digestion was complete and the mixture was thoroughly shaken to make it homogeneous, 0.2 mL was taken serially with a micropipette, and the nitrogen content was measured using the Dumas method. The values were used to determine the amount of nitrogen before centrifugation.

To determine the nitrogen amount, analysis was performed on the supernatant obtained by centrifuging the mixture after stopping small intestine digestion for 20 minutes at 12500 rpm. The nitrogen amount determination from the mix before centrifugation was used to determine the total protein amount of the sample, and the nitrogen amount determination from the supernatant after centrifugation was used to determine the total protein amount digested proportionally (13-15). The nitrogen factor used in calculating protein content was 6.38 for whey, 6.36 for casein, 5.70 for soy, and 6.25 for egg white, meat, and pea powders. The protein content was obtained by multiplying the nitrogen content by the nitrogen factor. The digestion rate was calculated with the following formula:

% Digestibility Rate = (Supernatant protein amount / Pre-centrifugation mixture protein amount) x 100

Statistical Analysis: Three measurements were taken from each protein powder sample, resulting in a total of nine replications for each protein. Nine replications were obtained for the standard. The normality distribution of the determined observation values was measured using the Shapiro-Wilk normality test. Homogeneity of variance was checked using the Bartlett test. Statistical significance between digestibility, Nitrogen Amount Before Centrifugation (mg), and Nitrogen Amount After Centrifugation (mg) values among protein powders was determined using analysis of variance and the Tukey multiple comparison test in case of significance. All statistical analyses were performed using the R statistical software (www.r-project.org/). The significance level was determined as $p<0.05$.

Results

The percentage protein rates stated on the sample labels and the percentage protein contents determined by the analyses are given in Table 2. The protein standard (bovine casein) label information lists the protein content as 87-94% (16). According to the Dumas method, the protein content is 87.3%, consistent with the label information. In line with the study's objective, this eliminates any concerns regarding the reliability of the method.

Table 2. Protein contents of samples

Powder Types	Protein %	Standard Deviation	Protein % Indicated on the Label	Validity of Label Information %
Whey Powder	72.87	0.48	76	95.89
Casein Powder	83.83	0.67	86	97.47
Soy Powder	76.01	0.94	91	83.52
Meat Powder	99.13	0.74	88	112.65
Egg White Powder	85.93	1.04	85	101.09
Pea Powder	77.01	2.50	84	91.68

Table 3. Digestion rate of protein powders

Powder Types	N	Nitrogen Amount Before Centrifugation (mg)	SD	Nitrogen Amount After Centrifugation (mg)	SD	Digestion Rate %	SD	Min	Max
Whey Powder (3)	9	0.918[f]	0.006	0.500[d]	0.018	54.50[c]	1.77	52.64	56.16
Casein Powder (3)	9	1.121[c]	0.003	0.683[b]	0.023	60.89[b]	1.94	56.40	63.08
Soy Powder (3)	9	1.160[d]	0.006	0.508[d]	0.011	43.76[d]	1.01	42.60	44.43
Meat Powder (3)	9	1.425[a]	0.004	0.944[a]	0.011	66.21[a]	0.70	65.51	66.92
Egg White Powder (3)	9	1.139[e]	0.001	0.420[e]	0.037	36.87[e]	3.32	33.66	36.64
Pea Powder (3)	9	1.231[b]	0.009	0.660[c]	0.006	53.66[c]	0.91	53.01	54.70
Bovine Milk Casein (Standard,1)	9	1.227[b]	0.015	0.696[b]	0.032	56.75[c]	2.85	52.85	61.63

a, b, c, d, e, f; means shown with different letters in the same column are different from other groups ($p<0.05$)

SD: Standard deviation

N: Observation size

Table 4. One-way ANOVA data according to the R statistical program

	Test	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Digestion Rate	Protein$Digestion	6	5390	898.4	185.8	<0.0001
	Residual	56	271	4.8		
Nitrogen Amount Before Centrifugation	Protein$PreN	6	1.2490	0.20816	1489	<0.0001
	Residual	56	0.0078	0.00014		
Nitrogen Amount After Centrifugation	Protein$PostN	6	1.6422	0.2737	459.3	<0.0001

Df: Degrees of Freedom

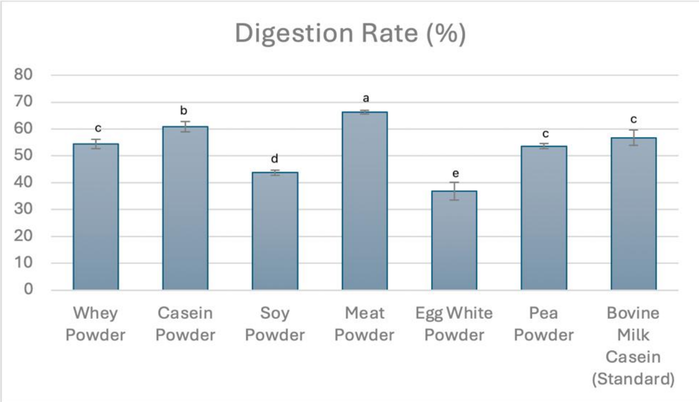

Figure 1. Digestion rates of protein powder bars

Discussion

In the study, using the Dumas method, it was found that meat powder and egg white powder had higher average protein content than the protein powders of different origins and brands available for commercial sale. In contrast, whey, casein, soy, and pea powders had average protein contents lower than those listed on the labels. Schönfeldt et al. (17) stated that the protein levels of food supplements produced from whey using the Dumas method are compatible with the protein amounts stated on the product label. The protein contents of the meat powders analyzed in our study differ from the values stated on their labels. It is critical not to misstate the protein content of protein powders sold as sports food supplements on the label, as this will mislead consumers. It is essential to inform consumers about the methods used in the production of protein powders, as these affect digestibility and the conversion rate of protein into body protein. Protein powders should be marketed with measurable, rational label information, such as the rate of conversion of the protein into body protein.

The digestion rates for each sample are shown in Table 3. Table 4 also shows statistical data. According to the findings and statistical evaluations, the highest digestibility rate among the protein powder groups was meat powder protein (66.21%). Casein powder digestibility ranked next (60.89%), followed by standard bovine milk casein (56.75%), whey protein powder (54.50%), pea protein powder (53.66%), and soy protein powder (43.76%). In comparison, the protein powder with the lowest digestibility rate was egg white protein (36.87%). The difference between the groups was found to be statistically significant for the samples analyzed ($p<0.05$). In the Turkish Food Codex Sports Food Communiqué, it was emphasized that protein with a minimum net protein utilization of 70% (from egg, milk, and meat) offered as sports foods should be more than 70% in dry matter. In our study, all of the powders complied with the Turkish Food Codex Sports Nutrition Communiqué (2).

This study determined the protein content and in vitro digestion rates of diverse types of protein powders commonly used as dietary supplements by athletes using the Dumas method. It was noteworthy that egg white protein powder digestibility in a simulated in vitro environment, determined by the Dumas method, was relatively low. The amino acid composition of egg proteins has long led to their acceptance as a reference protein for humans (18). Egg white powders are subjected to thermal processing, dry heating, or spray drying. Although heat-treated egg protein is known to be more digestible than raw egg protein, ovomucoid does not react with human trypsin and is also relatively heat stable (19, 20). Ovomucoid is quantitatively the most critical trypsin inhibitor. The underrepresentation of ovomucoid-derived peptides may be explained by the known resistance of ovomucoid to digestion even after heat treatment (18). Therefore, the low digestibility level of egg white powder protein may be due to the high heat resistance of ovomucoid. The high digestibility of meat powder proteins may be due to the high particle size and high bioaccessibility of the spray-dried powder, as calculated by digestibility factors (21).

Almeida et al. (22) investigated the in vitro digestibility of commercially available whey proteins from the United States and Brazil. In vitro protein digestibility experiments revealed significantly higher digestibility for whey and standard caseinate powder than the current study's findings. Soy powder digestibility, however, is more comparable to that of other protein sources. The lower digestibility rates of the standard caseinate and soy powder used in our study may be due to the three-hour digestion time we limited compared to other studies and the method used. The low digestibility of soy protein compared to other protein sources is consistent with the findings of Pires et al. (23). Pires et al. (23) explained the low digestibility of plant proteins by the formation of protein complexes with anti-nutritional factors in their structures, thereby reducing their digestibility. Mokrane et al. (24) subjected the seeds of seven Algerian sorghum plants to in vitro protein digestion tests, using the Dumas method for protein determination, and reported that the low digestibility, below 50%, was due to the resistance of kafirin protein, the main proteins in sorghum, to peptidase enzyme due to its rich disulfide cross-links. Sindayikengera and Xia (25) found significantly higher digestibility levels for whey protein concentrate and sodium caseinate, which contain protein, compared to the current study. This may be due to the Kjeldahl method being used for protein analysis after in vitro digestion with a commercial complex prepared from Bacillus enzymes.

Amino acid scores are an important parameter in determining the digestibility of protein powders. It is also worth noting that the most widely accepted methods for assessing protein quality are the protein digestibility-corrected amino acid score (PDCAAS) and the digestible indispensable amino acid score (DIAAS) (26). The low or high protein digestibility of foods significantly affects the PDCAAS and DIAAS value (7, 27). Therefore, this study is promising to fill this gap in the literature, as future studies plan to determine the amino acid score in addition to the digestibility of protein powders.

Consequently, this study compared the protein content and digestibility rates of commercially available animal and plant protein sources using the Dumas method in a simulated environment. This study also addressed label compliance of commercial protein powder sources. Accurate food labeling allows consumers to know exactly what ingredients a product contains and helps them make more informed health and nutrition choices. Regulations stipulate that compliance is mandatory, but they may not always provide a process for resolving non-compliance issues. This study highlights discrepancies in label information for commercially available protein powders and will guide consumer preferences. Furthermore, it will serve as a guide for future studies on determining amino acid scores in protein powders or assessing digestibility beyond digestibility in peptidomic studies using mass spectrometry.

Acknowledgment

We would like to thank the Proofreading & Editing Office of the Dean for Research at Erciyes University for copyediting and proofreading service for this manuscript.

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