

**CONCENTRATION OF PROTEIN FOUND IN PROTEIN SUPPLEMENTAL
POWDERS VS. LABEL CLAIMS**

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Abstract

Protein is vital to the human body and especially important for athletes who want to build lean muscle. A way of meeting adequate protein needs is protein powders. The purpose of this research is to evaluate three popular protein powders to see if their actual protein concentration matches the label's content. The hypothesis states there is a difference between the amount of protein recorded on the supplement label and the amount of actual protein as determined by experimental methods. Three companies were chosen to be tested: Dymatize Nutrition, Champion Nutrition, and Ultimate Nutrition. The companies' whey supplement was tested but three different lot numbers for each product were used. By using the Bradford method, concentrations were found for the three powders tested. Protein standards were used to generate a standard curve. The protein powders were diluted to an expected concentration of .75mg/ml. 20 μ l of the diluted powder were combined with 1ml of protein dye into a curvet. Three test samples of each lot number were read in a spectrophotometer and three absorbance readings were taken for each curvet. The absorbance readings were averaged for each lot number and their concentrations were found using the curve generated by the protein standards.

Dymatize Nutrition had the lowest variance between the concentration and its label while Universal Nutrition had a slightly higher variance. Champion Nutrition had the most variance from its label. A one-way ANOVA test ($\alpha=.05$) was conducted for each companies' products against the control rejecting the null hypothesis. There was a statistically difference between the protein powders and the controls. The data supports the hypothesis that there were differences between the label claim and the protein concentration determined by experimentation.

Introduction

Why is protein so vital to human beings? Protein is important to the human body because it helps the body perform many functions including: fighting illness and disease, building and repairing body tissues, producing enzymes, carrying nutrients throughout the body, hormone production, creating new cells, maintaining fluid balance, transporting oxygen throughout the body, source of energy. (Applegate 2002) Under the Dietary Supplement Health and Education Act of 1994, the dietary supplement manufacturer is responsible for ensuring that a dietary supplement is safe before it is marketed. “The government does not regulate dietary supplements by the same strict laws that it uses to regulate food. That means, for the most part, that what supplement makers claim on the bottle or on their websites does not necessarily have to be true.” (Applegate 2002). In the *American Health Journal*, there has been experiments showing dangerously high amounts of Vitamin B and C found in a multi-vitamin thus leading to this experiment being conducted (Webb, Densie 1995). A 2010 study conducted by a private laboratory found heavy metals in supplements, raising concern for consumers. “They found nearly 1,000 reports within a 10 month period of specific health problems linked to supplements, including nine deaths, 64 life-threatening illnesses and 234 hospitalizations.” A common supplement used by many is protein powders which may pose an interest in the integrity of the companies.

“Protein powders are made from four basic sources: whey, casein, egg, and soy. These concentrated sources of protein are processed into powder form.” (What is Protein, 2010). Whey protein has great benefits to your health, whether you are a dieter, a bodybuilder, or an average person. Having enough protein in your diet, helps regulate hormones and cellular functions, help boost your immune system, and along with adequate exercise can support muscle growth (Nollet and Toldrá 2009). Supplementing protein has great appeal to athletes because the powders provides high amounts of proteins without having to eat the equivalent amount found in food (Paul, Ron 2005). When muscles are exercised, they become fatigued and develop micro-tears. Protein rebuilds these broken down muscle fibers, and adapts to the stress placed on it, making the fibers bigger and stronger than before. This process allows for athletes to train their bodies to get faster, more explosive, and stronger (Bjerklie 2004) The government has wanted to regulate the sales of supplements (Blendon, 2001). This power over the supplement market means permission has to be granted to buy certain supplements. Ron Paul, a laboratory research for *Alternative Therapies*, would argue that this would violate America’s health freedom. The

FDA's loose regulations on the market, allow for bodybuilders and athletes to gain any competitive edge possible which is important at the professional level (Kurtzweil, Puala 1998). Each form of protein has a different biological value. The biological value is how closely matched the amino acids are in relation to the body's requirements, measuring the percentage of protein that is used for muscle and connective tissue growth and repair (Anderson 2010). Whey protein has a 104 rating which makes whey a reliable source of protein to repair your body. There are advantages to each whey and casein: whey is rapidly absorbed into the blood stream and is metabolized. This experiment will use the Bradford Assay Method which incorporates Beer's Law: When a solute absorbs light of a particular wavelength, the absorbent is directly proportional to the concentration of substance in solution. The measurement most commonly used in protein assays is the absorbent of light (Rice University 2010). The purpose of this research is to evaluate three popular protein powders to see if their actual protein concentration matches the label's content. The powders chosen to be tested are from supplement companies who have a good reputation on the supplement market today. Either the protein powders will contain the amount given or will not meet the label's content.

Materials:

- Electronic Gram Scale
- Bio-Rad Bradford Assay Kit, Coomassie Blue G-250 (1L)
- Bio-Rad bovine-globulin standards (.0mg/ml - 2 mg/ml)
- 1.5ml disposable polystyrene cuvettes
- spectrophotometer
- 500 ml beakers
- Bio-Rad micropipettes
- Dymatize Elite Whey Protein Isolate
- Ultimate Nutrition Muscle Juice 2600
- Champion Nutrition Weight Gainer 900

Hypothesis: There is a difference between the amount of protein recorded on the supplement label and the amount of actual protein as determined by experimental.

Null Hypothesis: There is no difference between the amount of protein recorded on the supplement label and the amount of actual protein as determined by experimental methods.

IV: protein powder supplement								
Dymatize Elite Whey Protein Isolate			Ultimate Nutrition Muscle Juice 2600			Champion Nutrition Weight Gainer 900		
3005816913	3001673489	3002136549	07-025-492	07-015-619	07-001-493	2002251-9040	2002261-9140	2002272-9004

Dependent Variable: Grams of Protein in supplement determined by spectrophotometric absorbance

Constants: amount of protein measured, amount of coomassie blue G-250, type of spectrophotometer, micropipettes

Methods

The protein standards were prepared for spectral analysis. 20µl of the bovine-globulin standards were added into a curvet. 1ml of coomassie blue G-250 was added into the curvett. The curvett was inverted to mix the dye and the diluted standard. The curvett was placed into the spectrophotometer, the wavelength was set at 595 nanometers (yellow-orange). 595nm is the wavelength for maximum absorption which correlates to Beer’s Law: when a solute absorbs light of a particular wavelength, the absorbent is directly proportional to the concentration of substance in solution. Three absorbance readings were taken for each known standard then averaged. The protein powders were diluted to .75mg/ml by information given from the labels. 20µl of the diluted powders were added to the curvett along with 1ml of coomassie blue G-250. The curvett was inverted to mix the powder and dye. The curvett was placed in the spectrophotometer. Three curvetts were prepared for each lot number. For each curvett three absorbance readings were taken. The absorbance readings for each lot number were averaged. The Standard curve generates a y-value from which the absorbance readings of the powders are used in the equation to calculate the concentration. By taking the expected concentration for all

the powders, .75mg/ml, subtracting the actual concentration then dividing by expected concentration, the percent difference between the label and actual concentration is calculated.

Results

Figure 1: Bovine- Globulin Standards vs Absorbance

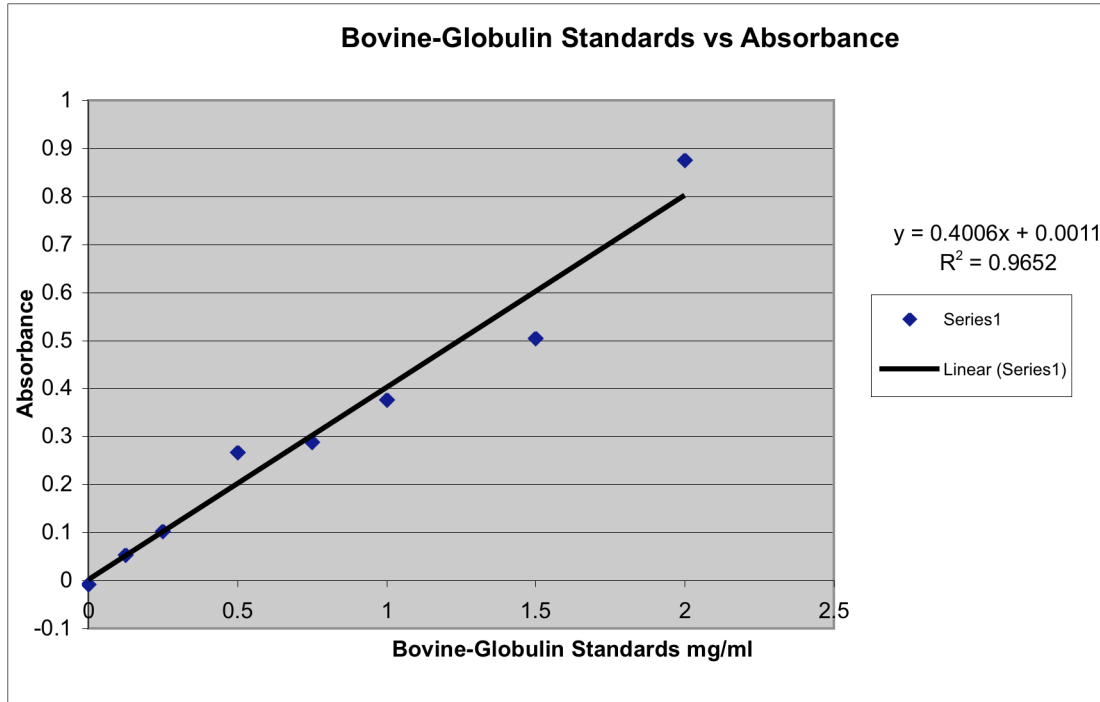


Figure 1 displays the bovine-globulin standards versus their absorbance readings. The known standards generate a y-value so that the protein powder's concentration can be calculated.

Table 1: Protein Concentration (g)

Protein Concentration (g)	Protein Powder Supplement								
	Champion			Ultimate			Dymatize		
	1	2	3	1	2	3	1	2	3
Trial 1	7.27	10.05	6.74	31.81	30.36	34.84	15.41	18.77	15.17
Trial 2	7.04	10.45	7.87	34.77	33.33	32.44	15.38	15.24	15.29
Trial 3	9.11	7.55	6.25	30.31	36.29	35.30	15.07	12.52	15.37
Mean	7.80	9.35	6.95	32.30	33.33	34.19	15.29	15.51	15.28
Range	2.07	2.90	1.62	4.46	2.86	2.86	0.35	6.25	0.19
St.Dev	1.13	1.57	0.83	2.27	2.97	1.54	0.19	3.14	0.10

Table 1 displays every three concentrations for each lot number for each company. It displays the mean, range of concentrations in each lot number, and the standard deviation in each lot number. This table reveals the companies were consistent in concentration within their product.

Table 2: % Difference Between Actual and Label Claim

% Difference Between Actual and Label Claim	Protein Powder Supplement								
	Champion			Ultimate			Dymatize		
	1	2	3	1	2	3	1	2	3
Trial 1	(72.00)	(71.28)	(80.74)	(42.16)	(44.81)	(36.65)	(35.77)	(21.78)	(36.77)
Trial 2	(79.89)	(70.14)	(77.52)	(36.78)	(39.40)	(41.02)	(35.90)	(36.50)	(36.27)
Trial 3	(73.98)	(78.42)	(82.15)	(44.89)	(34.01)	(35.82)	(37.23)	(47.84)	(35.96)
Mean	75.29	73.28	80.14	41.28	39.41	37.83	36.30	35.37	36.34
Range	7.89	(8.28)	4.63	(8.11)	(10.80)	5.20	1.45	26.06	0.81
St.Dev	4.10	4.49	2.38	4.13	5.40	2.80	0.81	13.07	0.41

Table 2 displays the percent difference between the actual concentration and label claim. It displays the mean, range, and standard deviation for each lot number for each company. Champion Nutrition had the greatest percent difference between its label and actual concentration. Dymatize Nutrition had the lowest percent difference between its label and actual concentration.

Figure 2: Label Claim vs: Actual Concentration

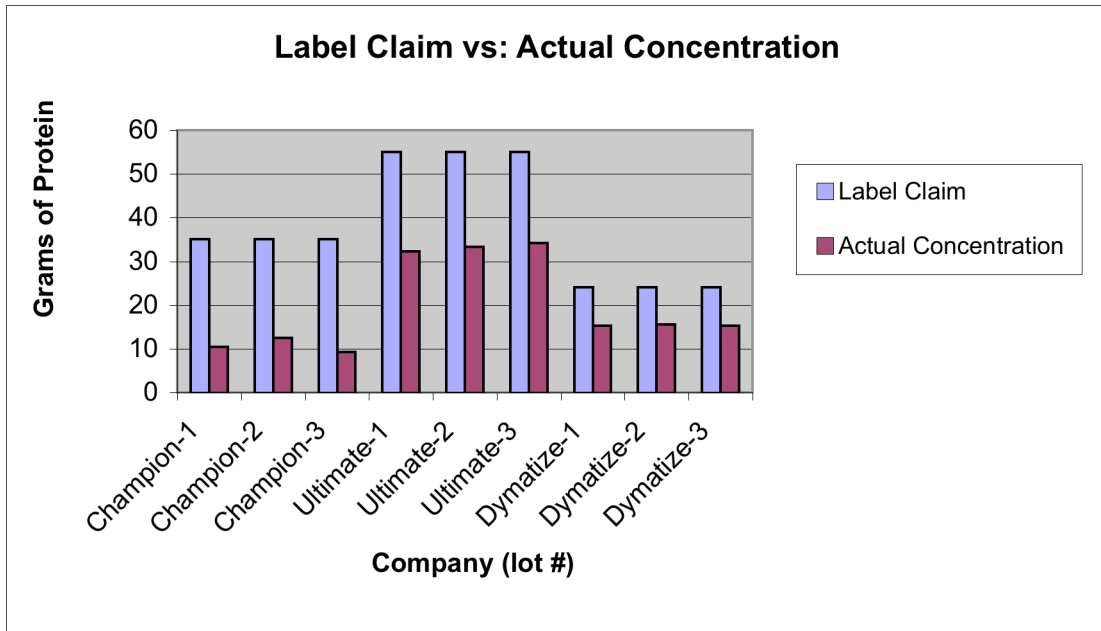


Figure 2 displays each company's lot numbers actual concentration against the company's label claim. The graph provides a visual aid on how much protein is missing from the powders. This figure shows that Dymatize has the highest actual concentration compared to its label among its competitors and has the lowest standard deviation between its concentration and label. Champion had the greatest difference between its actual concentration and its label which correlates to a high standard deviation. There was no statistically significant difference between the actual concentrations of different lot numbers of a given product. This shows that the companies are consistent between lot numbers as to the concentration of the protein.

Analysis

Through data analysis the protein powders now have more accurate concentration readings. The absorbance readings from the protein standards generated a curve from which the protein powders could be plotted (Figure 1). Champion Nutrition Weight Gainer 900 had a 0.22mg/ml concentration; the average standard deviation of each lot number for Champion is 0.034 (Table 1). Ultimate Nutrition Muscle Juice 2600 had a 0.453333mg/ml concentration; the average standard deviation of each lot number for Ultimate was 0.013013 (Table 1). Dymatize Elite Whey Protein Isolate had a 0.48mg/ml concentration; the average standard deviation of each lot number for Dymatize was 0.004 (Table 1). Using the percent difference of Champion, -69.38333%, the actual concentration of the protein powder was 10.717g of protein compared to its label claim of 35 grams (Table 2). Using the percent difference of Ultimate, -39.50667%, the actual concentration of the protein powder was 33.269g of protein compared to its label claim of 55 grams (Table 2). Using the percent difference of Dymatize, -36.00333%, the actual concentration of the protein powder was 15.36g of protein compared to its label claim of 24 grams (Table 2). ANOVA testing was conducted for every lot number against the expected concentration. The P-Value for all the numbers was significant.

Conclusion

The purpose of this study was to determine if there was a difference between a company's protein level claim in their supplements and the actual concentration. It was hypothesized that there is a difference between the amount of protein recorded on the supplement label and the amount of actual protein as determined by experimental. Champion Nutrition Weight Gainer 900 contained just 30.62% of protein in comparison to its claim. Ultimate Nutrition contained only 60.49% of the protein stated on the label of Ultimate Nutrition Muscle Juice 2600. Dymatize contained 63.99% of the actual protein stated by Dymatize Elite Whey. The data supported the experimental hypothesis that there is a significant difference between the label claim and actual concentration. The experiment did reveal that supplement companies could claim anything they want to make their product more marketable just as Liz Applegate warned in her book, "The government does not regulate dietary supplements... supplement makers claim on the bottle or on their websites does not necessarily have to be true." The supplement market is saturated with outrageous claim such as this one found on a supplement company's website: "10 to 20 lb. gains

of pure muscle in just six to twelve weeks with Hyper Gain. Increases in muscle strength and replaces energy components within the muscle cell 323% faster. DRASTIC increases in lean body mass... in record time.” The human body is not capable of performing such dramatic changes. Actually there was no valid research to support their claims. The supplement market is a 25 billion dollar industry, but does this mean the FDA should not impose? While this experiment was conducted in triplicate, the results would need to be verified by repeating the entire experiment. This experiment could be extended to include more brands or more lot numbers. Further research should test for contaminants in supplements such as heavy metals secondary to prior studies suggesting high levels of contamination. It is important to know what is in the supplements people use every day. As a result of the lack of protein found in the supplements and the possible negative impact of heavy metals, do the benefits outweigh the consequences?

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