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"Assessment of the Physiological Effects of Aspartame, Stevia, and Omega-3 on Body Weight and Organ Weights of Male Rabbits"

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Abstract:

Background: The widespread consumption of artificial and natural sweeteners has raised concerns regarding their potential physiological effects, particularly on reproductive and neural health. Omega-3 fatty acids are known for their anti-inflammatory and protective properties. **Objective:** This study investigated the effects of dietary supplementation with aspartame, stevia, and omega-3 fatty acids, alone or in combination, on body weight, testes weight, and brain weight in adult male rabbits. **Methods:** Thirty male rabbits were randomly divided into six experimental groups (n=5 each): Control, Aspartame, Stevia, Omega-3, Stevia + Omega-3, and Aspartame + Omega-3. Body weights and organ weights (testes and brain) were recorded after the experimental period. Data were analyzed using one-way ANOVA. **Results:** No significant differences ($P > 0.05$) were observed in final body weights among all groups. However, significant variations were detected in testes weight ($F = 3.35$, $P = 0.019$) and brain weight ($F = 4.18$, $P = 0.007$). The Omega-3 group exhibited the highest testes (5.430 ± 0.242 g) and brain (5.340 ± 0.361 g) weights. Aspartame-treated rabbits showed the lowest testes weight (3.50 ± 0.30 g), while the Aspartame + Omega-3 combination resulted in the lowest brain weight (3.400 ± 0.245 g). **Conclusion:** While sweeteners and omega-3 supplementation did not affect overall growth performance, they significantly influenced reproductive and neural organ weights. Omega-3 demonstrated protective effects, whereas aspartame showed potential adverse effects on testicular and brain tissues.

Keywords: Aspartame, Stevia, Omega-3, Male Rabbits, Body Weight, Organ Weight.

Introduction

Artificial sweeteners and nutritional supplements have gained considerable attention in recent years because of their widespread use in food industries and dietary management programs [1]. Aspartame is one of the most commonly used artificial sweeteners worldwide and is frequently added to beverages, desserts, and low-calorie food products as a sugar substitute. It is approximately 180–200 times sweeter than sucrose and is extensively consumed by individuals seeking weight control or diabetic-friendly diets [2-5]. Despite its popularity, concerns have been raised regarding the possible physiological and biochemical effects associated with long-term aspartame consumption, particularly on different body organs and metabolic functions. Stevia, a natural sweetener extracted from the leaves of *Stevia rebaudiana*, has emerged as an alternative to synthetic sweeteners. Stevia contains steviol glycosides that provide intense sweetness with minimal caloric value [6-10]. Unlike artificial sweeteners, Stevia is often considered a safer natural substitute because of its plant origin and its reported antioxidant and anti-inflammatory properties. Several studies have investigated the biological activities of Stevia and suggested that it may exert beneficial effects on glucose metabolism, oxidative stress, and organ function [11-15]. However, experimental findings regarding its physiological influence remain variable and require further investigation. Omega-3 fatty acids are essential polyunsaturated fatty acids known for their important biological functions in maintaining cellular integrity and physiological homeostasis. Omega-3 compounds, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are widely recognized for their beneficial effects on cardiovascular health, nervous system development, reproductive performance, and inflammatory regulation [16-20]. Dietary supplementation with Omega-3 has been associated with improvements in tissue function and organ protection in several experimental and clinical studies [21-30]. Consequently, Omega-3 supplementation is frequently investigated for its potential protective role against physiological alterations induced by dietary or environmental factors [31]. The testes and brain are among the most sensitive organs to nutritional and metabolic changes. Testicular tissue plays a critical role in male reproductive function through spermatogenesis and hormone production, whereas the brain is responsible for the regulation of neurological and physiological activities. Alterations in organ weight may reflect underlying physiological responses to dietary supplementation or exposure to certain compounds. Therefore, assessment of testes and brain weights represents an important experimental parameter for evaluating the biological effects of different treatments in animal studies. Rabbits are commonly used as experimental models in biomedical and nutritional research because of their physiological similarities to humans and their sensitivity to dietary interventions. The use of male rabbits in the current study provides an appropriate experimental approach for

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evaluating the effects of artificial sweeteners, natural sweeteners, and nutritional supplements on body and organ weight parameters [32-40]. Therefore, the present study was designed to investigate the comparative effects of Aspartame, Stevia, and Omega-3 supplementation on body weight, testes weight, and brain weight in male rabbits. In addition, the study aimed to evaluate whether combined administration of sweeteners with Omega-3 could influence organ weight parameters under controlled experimental conditions.

Materials and Methods

Thirty healthy adult male rabbits were used in the present experimental study. The animals were obtained from a local breeding source and housed under standard laboratory conditions. Rabbits were maintained in clean cages under controlled environmental conditions, including suitable temperature, humidity, and a 12-hour light/dark cycle. All animals were allowed free access to water and a standard laboratory diet throughout the experimental period. The rabbits were acclimatized for two weeks before the beginning of the experiment to minimize environmental stress and ensure physiological stability. The rabbits were randomly divided into six equal experimental groups, with five rabbits in each group, as follows: Control Group: Rabbits received a standard diet without any supplementation. Aspartame Group: Rabbits received Aspartame supplementation. Stevia Group: Rabbits received Stevia supplementation. Omega-3 Group: Rabbits received Omega-3 fatty acid supplementation. Stevia + Omega-3 Group: Rabbits received combined supplementation of Stevia and Omega-3. Aspartame + Omega-3 Group: Rabbits received combined supplementation of Aspartame and Omega-3. The experimental treatments were administered daily for the designated study period under controlled laboratory conditions. Aspartame and Stevia were prepared in appropriate concentrations and administered orally according to the experimental protocol. Omega-3 fatty acids were also administered orally using suitable doses prepared freshly during the study period. The combined treatment groups received both supplements simultaneously at the specified doses. All administered materials were prepared under hygienic laboratory conditions to ensure treatment consistency. Body weight measurements were recorded for all rabbits using a sensitive digital balance. Final body weight values were obtained at the end of the experimental period and expressed as grams (g). Mean values and standard errors were calculated for each experimental group. At the end of the experimental period, rabbits were sacrificed under appropriate laboratory procedures. The testes and brain were carefully dissected, cleaned from surrounding tissues, and weighed immediately using a digital analytical balance. Organ weights were recorded in grams (g) for each rabbit. **Statistical Analysis**: The obtained data were statistically analyzed using one-way analysis of variance (ANOVA) to evaluate the significance of differences among the experimental groups. Results were expressed as Mean ± Standard Error (Mean ± S.E.). Statistical significance was considered at a probability level of $P < 0.05$. Different superscript letters were used to indicate significant differences among group means. Statistical analyses and graphical presentations were performed using appropriate statistical software.

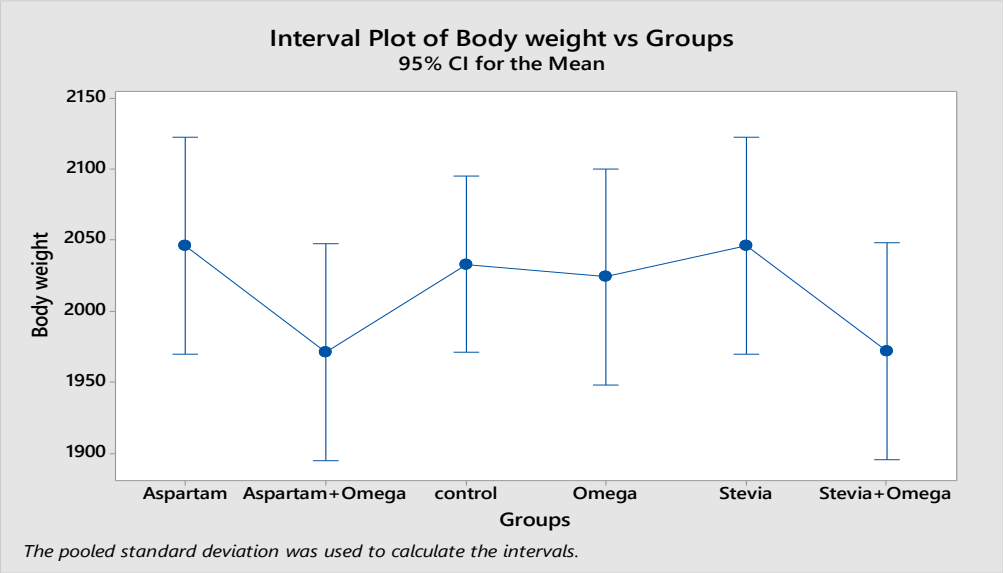
Results

The results of body weight measurements for the different experimental groups are summarized in Table 1 and illustrated in Figure 1. Statistical analysis via one-way ANOVA (Table 2) revealed that there were no significant differences ($P > 0.05$) in the final body weights among all experimental groups, including the control, sweeteners (Aspartame and Stevia), and Omega-3 supplemented groups. The control group maintained a mean body weight of 1946.80 ± 71.29 g. Rabbits treated with Aspartame and Stevia showed slightly lower mean weights (1913.00 ± 32.74 g and 1937.00 ± 45.81 g, respectively) compared to the control, although these variations did not reach statistical significance ($P = 0.518$). The Omega-3 supplemented group exhibited the highest numerical mean weight at 2036.00 ± 57.33 g. Furthermore, the combination groups (Stevia + Omega-3 and Aspartame + Omega-3) showed remarkably similar weight profiles, with means of 1972.00 ± 30.03 g and 1971.00 ± 32.35 g, respectively. The presence of the same superscript letter (a) across all groups in Table 1 confirms that the administration of natural or synthetic sweeteners, whether alone or in combination with Omega-3 fatty acids, did not induce a statistically significant alteration in the growth performance or total body mass of the rabbits under the current experimental conditions.

Table 1: Effect of Dietary Supplementation with Stevia, Aspartame, and Omega-3 Fatty Acids on Body Weight Dynamics in Adult Male Rabbits."

Experimental Group	Body Weight (gm) (Mean ± S.E.)
Control	1946.80 ± 71.29[a]
Aspartame	1913.00 ± 32.74[a]
Stevia	1937.00± 45.81[a]
Omega-3	2036.00 ± 57.33[a]
Stevia + Omega-3	1972.00 ± 30.03[a]
Aspartame + Omega-3	1971.00 ± 32.35[a]

"Values are expressed as Mean ± Standard Error (S.E.). Means within the same column carrying the same superscript letters are non-significantly different at ($P > 0.05$)."



"Figure 1: Mean Body Weight Variation in Rabbits Fed with Aspartame, Stevia, and Omega-3 Supplementation."

"Table 2: One-WAY analysis OF variance (anova) for the effects OF different treatments on rabbit body weight."

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Groups	5	125707	25141	0.85	0.518
Error	124	36675771	29643		
Total	129	3801477			

Table 3 presents the comparative effects of Aspartame, Stevia, and Omega-3 supplementation on testes and brain weights in male rabbits. The highest testes weight was recorded in the Omega-3-treated group (5.430 ± 0.242 g), while the lowest value was observed in the Aspartame-treated group (3.50 ± 0.30 g). The control group showed a testes weight of 4.73 ± 0.28 g. The Stevia, Stevia + Omega-3, and Aspartame + Omega-3 groups demonstrated intermediate values of 4.43 ± 0.507 g, 4.460 ± 0.223 g, and 4.30 ± 0.193 g, respectively. Statistical lettering indicated significant differences between some experimental groups. Regarding brain weight, the Omega-3 group exhibited the highest mean value (5.340 ± 0.361 g), followed by the control group (5.014 ± 0.399 g). The Aspartame-treated group showed a brain weight of 4.78 ± 0.550 g, whereas the Stevia group recorded 4.890 ± 0.297 g. Lower values were observed in the Stevia + Omega-3 group (4.400 ± 0.510 g) and the Aspartame + Omega-3 group (3.400 ± 0.245 g), which represented the lowest brain weight among all experimental groups. The interval plots shown in Figures 2 and 3 demonstrated clear variations in testes and brain weights among the treatment groups. Figure 2 indicated that the Omega-3-treated rabbits had the highest testes weight values, whereas the Aspartame-treated group showed the lowest values. Similarly, Figure 3 illustrated higher brain weight values in the Omega-3 and control groups compared with the other experimental groups. The statistical analysis presented in Table 4 revealed significant differences among the experimental groups in testes weight, as demonstrated by one-way ANOVA analysis ($F = 3.35$, $p = 0.019$). In addition, Table 5 showed significant differences in brain weight among the studied groups, with an F-value of 4.18 and a p-value of 0.007. These findings indicate measurable variations in organ weights between the different treatment groups.

Table 3. Comparative Effects of Aspartame, Stevia, and Omega-3 on Testes and Brain Weights of Male Rabbits.

Experimental Group	Testes Weight (g) (Mean ± S.E.)	Brain Weight (g) (Mean ± S.E.)
Control	4.73 ± 0.28ab	5.014±0.399a
Aspartame	3.50 ± 0.30b	4. 78±0.550ab
Stevia	4.43± 0.507ab	4.890± 0.297ab
Omega-3	5.430 ± 0.242a	5.340 ± 0.361a
Stevia + Omega-3	4.460 ± 0.223ab	4.400 ± 0.510ab
Aspartame + Omega-3	4.30 ± 0.193ab	3.400 ± 0.245b

"Values are expressed as Mean ± Standard Error (S.E.). Means within the same column carrying the same superscript letters are non-significantly different at ($P > 0.05$)."

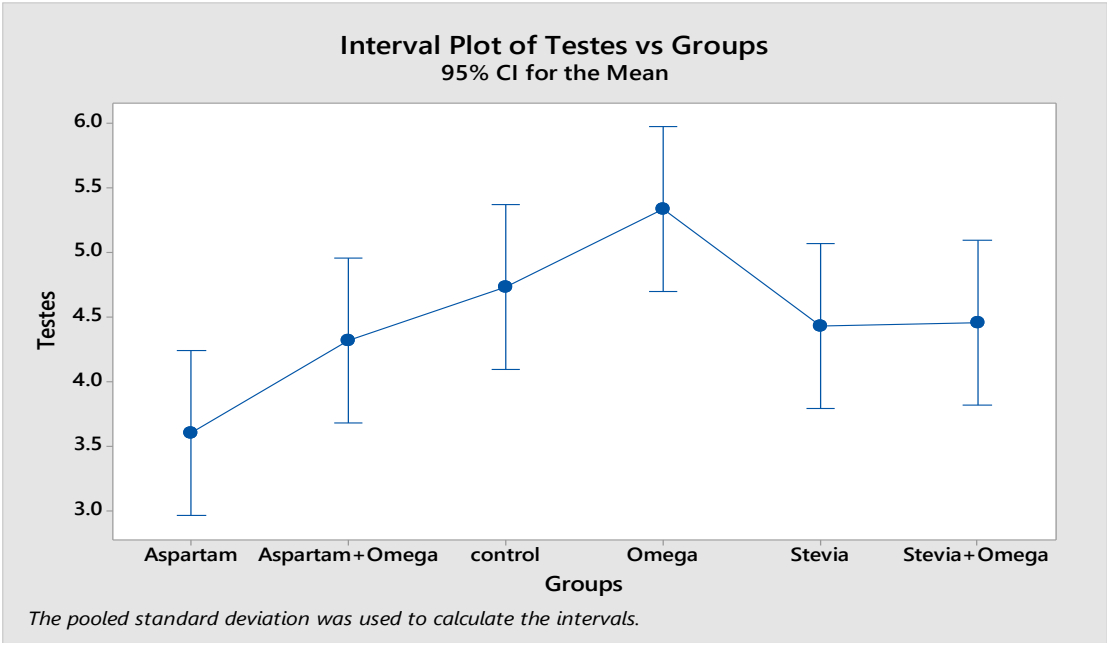


Figure 2. Effects of Aspartame, Stevia, and Omega-3 Supplementation on Testes Weight in Male Rabbits .

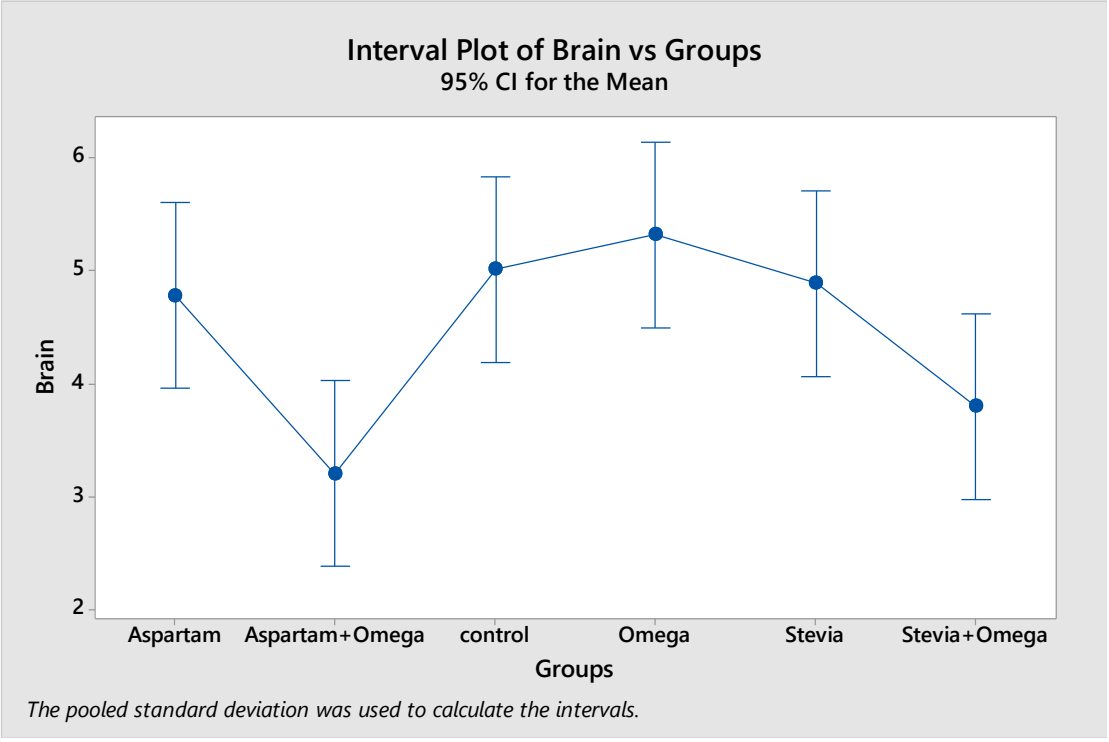


Figure 3. Effects of Aspartame, Stevia, and Omega-3 Supplementation on brain Weight in Male Rabbits

"Table 4: One-way analysis of variance (ANOVA) for the effects of different treatments on rabbit testes weight ."

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Groups	5	8.017	1.6035	3.35	0.019
Error	24	11.482	0.4784		
Total	29	19.499			

"Table 5: One-way analysis of variance (ANOVA) for the effects of different treatments on rabbit brain weight ."

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Groups	5	16.74	3.3484	4.18	0.007
Error	24	19.20	0.8001		
Total	29	35.95			

Discussion

The present study showed that supplementation with aspartame, stevia, and omega-3, alone or in combination, did not significantly affect final body weight in adult male rabbits, suggesting no major disruption of energy metabolism or growth at the given doses [41-44]. A non-significant increase in body weight in the omega-3 group may reflect its known anabolic and anti-inflammatory effects, while slight reductions in the artificial sweetener groups may be related to minor metabolic or gut-related changes. A significant effect was observed on testes weight (P = 0.019). Omega-3 produced the highest testes weight, likely due to its role in improving spermatogenesis, membrane integrity, and antioxidant protection in reproductive tissues. In contrast, aspartame showed the lowest testes weight, possibly due to oxidative stress and potential toxic metabolites affecting testicular cells [45-49]. The combination of omega-3 with aspartame showed partial improvement, suggesting a protective effect of omega-3 against sweetener-induced damage. Stevia showed values close to control, indicating a safer reproductive profile compared to aspartame [50]. Brain weight also showed significant differences (P = 0.007), with the highest values in the omega-3 group, consistent with the essential role of DHA in brain structure and function. Aspartame groups showed relatively lower brain weights, possibly linked to excitotoxic or oxidative mechanisms. Interestingly, combination groups did not show a clear improvement, suggesting possible interactions that may reduce the neuroprotective effect of omega-3 when combined with sweeteners [51-52]. Overall, the findings highlight clear differences between natural and artificial sweeteners, with omega-3 showing protective effects mainly in reproductive tissue, while brain responses appear more complex and potentially influenced by dietary interactions.

Conclusion:

This study provides novel evidence that dietary supplementation with natural (stevia) and synthetic (aspartame) sweeteners, alone or combined with omega-3 fatty acids, does not significantly affect overall body weight or growth performance in adult male rabbits. However, significant differences were observed in testes and brain weights, indicating organ-specific responses to these dietary interventions.

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