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INFLUENCE OF FEEDING RATS WITH STARCH COMBINED WITH CASEIN AND CASEIN HYDROLYSATES ON CHANGES IN THE ABSORPTION OF GLUCOSE AND AMINO ACIDS INTO THE BLOOD

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ABSTRACT

The work studied in vitro the effect of feeding rats with starch together with casein and casein hydrolysates on changes in the absorption of glucose and amino acids into the blood. The study found that feeding rats with starch together with casein contributes to lower blood glucose levels compared to similar results of feeding rats only with starch and amino acids only with casein. At the same time, feeding rats with starch together with casein hydrolysate promotes a more pronounced decrease in glucose, which was less than the indicators of feeding only starch, and starch together with casein. Also, a pronounced decrease in amino acids, less than the indicators of feeding only casein, and starch together with casein. These changes may be associated with the formation of starch-casein, as well as starch-peptide complexes that prevent the hydrolysis of starch by salivary amylase and casein and casein hydrolysates by gastric juice.

Key words: starch, casein, interaction, digestion, absorption, feeding, rats, glucose, amino acids.

There is a growing interest in the role of carbohydrate intake in the development of metabolic syndrome and its subsequent health consequences associated with glycemia and type 2 diabetes mellitus [6]. Food intake that leads to a reduced rate of carbohydrate absorption results in a more controlled release of glucose into the bloodstream. Such foods have a lower glycemic index (GI) value. The GI system ranks carbohydrate-containing foods based on their postprandial blood glucose concentration [3]. Foods with a high GI are associated with rapid increases in blood glucose and insulin levels after consumption [2]. Hyperinsulinemia can reduce insulin efficiency by downregulating insulin receptors, which leads to insulin resistance [8]. This, along with impaired pancreatic β-cell function, is a major risk factor for the development of type 2 diabetes mellitus [4]. Studies have reported several benefits of low-GI diets in relation to metabolic syndrome, including improved blood glucose control and glucose tolerance [10], enhanced insulin sensitivity [9], and decreased insulin resistance [7].

It is well known that proteins in a specific food matrix can influence the rate at which carbohydrates are metabolized in vivo [7]. It has been suggested that the addition of milk protein fractions, α- or β-casein, to waxy corn starch may produce a postprandial glucose-lowering effect. Previous rheological, microscopic, and simulated gastrointestinal digestion studies have shown that starch gelatinized in the presence of casein fractions exhibits limited granule swelling and reduced maltose and glucose levels. These effects were not observed when starch was gelatinized with whey protein fractions (α-lactalbumin and β-lactoglobulin) [5].

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Objective of the Study:

To investigate the effect of feeding rats starch combined with casein and casein hydrolysates on the absorption of glucose and amino acids into the blood.

Materials and Methods:

The study was conducted *in vitro* on 72 rats to examine the effect of starch interaction with casein and casein hydrolysates on the rate of their absorption into the bloodstream. Biochemical analyses were performed to determine blood glucose and amino acid concentrations. Measurements were taken before feeding, and then 1 hour and 3 hours after feeding.

- In **Group 1**, the rats were fed starch;
- In **Group 2**, casein;
- In **Group 3**, a mixture of starch and casein in a 1:1 ratio;
- In **Group 4**, starch and casein pre-hydrolyzed by gastric juice in a 1:1 ratio.

Blood glucose levels were determined using biochemical methods with standard diagnostic kits (ZAO "Vector-Best," Russia). Amino acid concentrations in the blood were determined using the ninhydrin reaction method [1].

The obtained data were subjected to statistical analysis using Microsoft Excel 2007. Mean values (M), standard errors (m), and the Student–Fisher *t*-test were calculated to determine the reliability of differences between the mean values.

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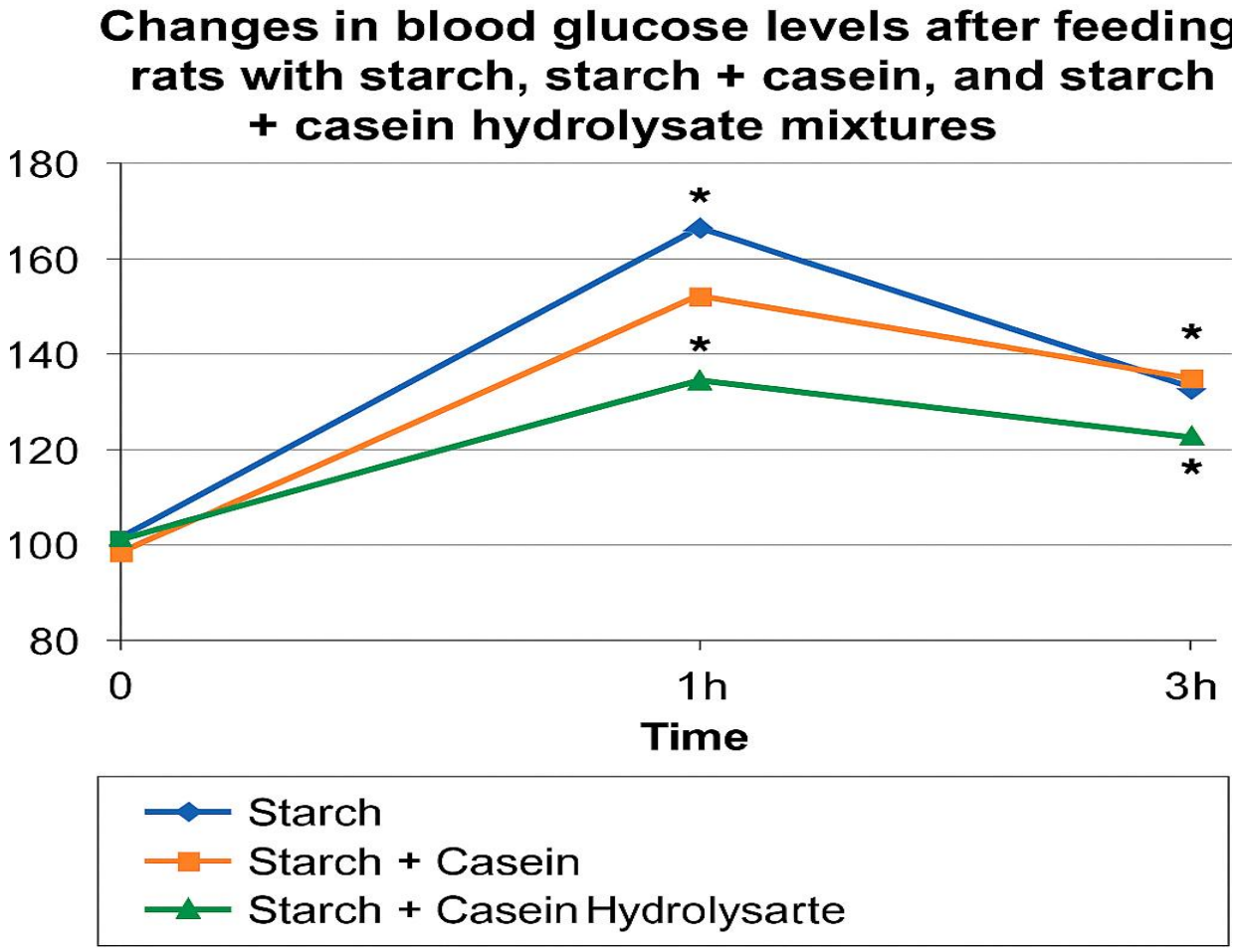


Figure 1. Changes in glucose absorption in rats before and 1 and 3 hours after feeding with starch, starch combined with casein, and starch combined with casein hydrolysate pre-digested with gastric juice.

- *Significantly different values compared with pre-feeding levels ($p < 0.05$).*

Results.

Based on the experimental data obtained from the rats, it was established that in animals fed with starch, the blood glucose concentration one hour after feeding was **172 ± 15.8%** relative to pre-feeding levels, which was significantly higher than the values recorded before feeding. However, three hours after feeding, the glucose concentration decreased to **125 ± 10.6%**, which was not significantly different from the baseline values.

In rats fed with a mixture of starch and casein (1:1), the blood glucose concentration one hour after feeding was **153 ± 13.7%**, which was also significantly higher than that of unfed rats. Three hours

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after feeding, this parameter reached **135 ± 11.9%**, remaining significantly higher than the pre-feeding levels.

At the same time, in the group of rats fed with a mixture of starch and casein hydrolysate, the blood glucose concentration one hour after feeding was **134 ± 11.3%**, which was significantly higher compared with unfed rats. However, in this group, glucose levels were slightly but not significantly lower than those in rats fed starch alone or starch combined with casein. After three hours of feeding with starch and pre-hydrolyzed casein, the glucose concentration was **129 ± 10.8%**, which was not significantly higher than the pre-feeding values and did not differ significantly from those of the starch-only group (*Figure 1*).

The conducted experiments on rats also revealed that in animals fed with casein, the blood amino acid concentration one hour after feeding reached **159 ± 13.6%** relative to pre-feeding levels, which was significantly higher than in unfed rats. After three hours of feeding, this indicator decreased to **128 ± 10.6%**, which still remained significantly higher than the values recorded before feeding.

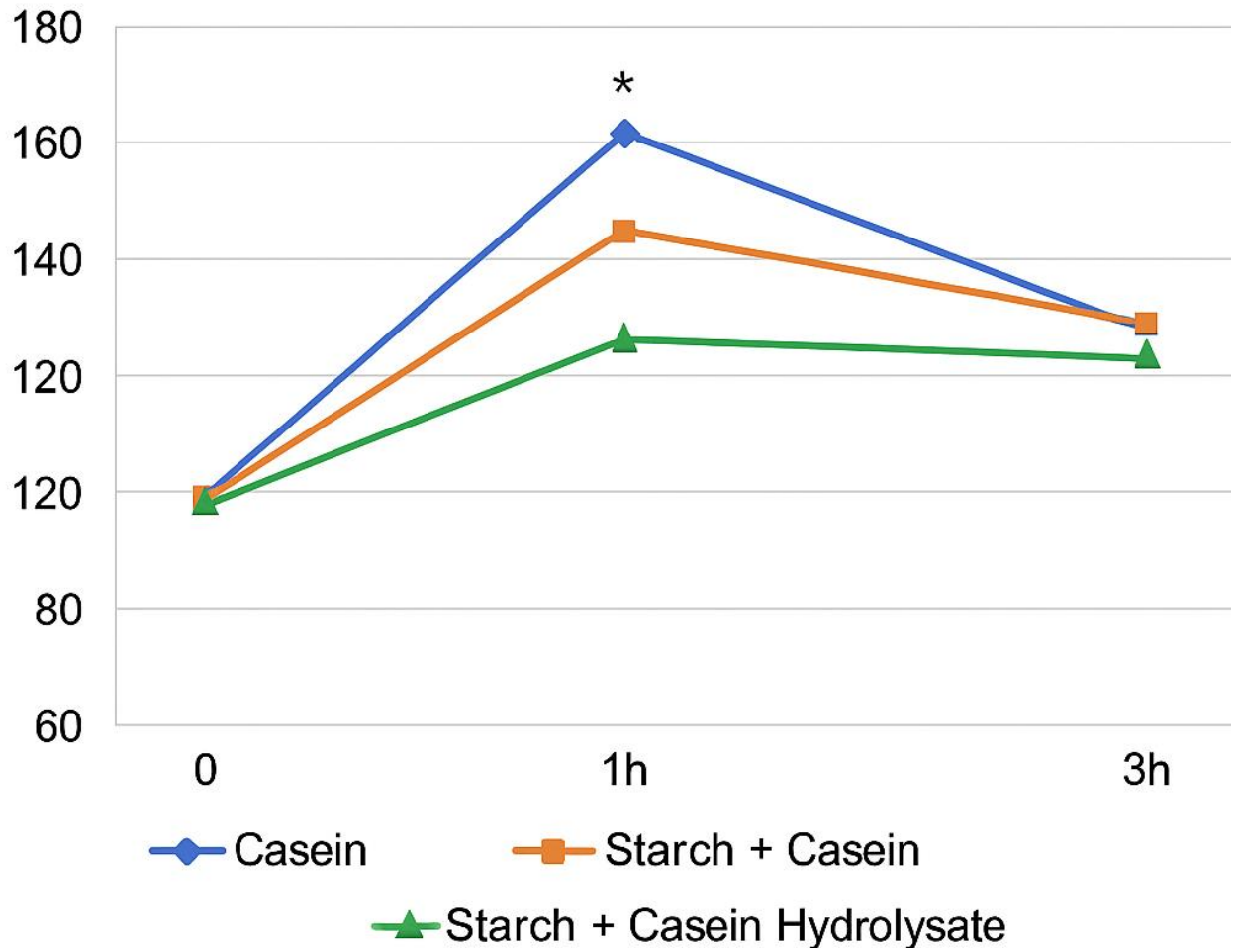


Figure 2. Changes in amino acid absorption in rats before and 1 and 3 hours after feeding with casein, starch combined with casein, and starch combined with casein hydrolysate pre-digested with gastric juice.

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- *Significantly different values compared with pre-feeding levels ($p < 0.05$).*

In rats fed with a mixture of starch and casein in a 1:1 ratio, the blood amino acid concentration one hour after feeding was **142 ± 12.5%** relative to pre-feeding values, which was significantly higher than in unfed rats. Three hours after feeding with starch and casein, the blood amino acid concentration was **129 ± 11.2%**, which was not significantly higher than before feeding. In rats fed a mixture of starch and pre-hydrolyzed casein, the blood amino acid concentration one hour after feeding was slightly higher but not significantly different from pre-feeding levels. After three hours, the amino acid concentration was **121 ± 10.6%**, which was not significantly higher than baseline values (*Figure 2*).

Discussion of Results.

The results of this study demonstrated that feeding rats with starch alone led to a significant increase in blood glucose levels one hour after feeding compared with pre-feeding values. After three hours, a substantial decrease in glucose levels was observed, returning to a value that was not significantly lower than at one hour and not significantly higher than before feeding. When rats were fed a mixture of starch and casein, glucose levels also rose significantly after one hour compared to baseline, although this increase was slightly but not significantly lower than that observed in rats fed starch alone. After three hours, glucose levels declined, remaining lower than the one-hour value but still significantly higher than pre-feeding levels.

Similarly, in rats fed a mixture of starch and casein hydrolysate, a significant increase in glucose concentration was observed at one hour, although the magnitude of this rise was smaller than in the starch and starch + casein groups. After three hours, glucose levels decreased further, being slightly lower than at one hour and not significantly different from baseline values.

The study also revealed that in rats fed casein alone, blood amino acid levels increased significantly one hour after feeding compared to pre-feeding values. After three hours, amino acid levels decreased, showing no significant difference from the one-hour value or the pre-feeding baseline. When rats were fed a mixture of starch and casein, amino acid concentrations also increased significantly after one hour compared to baseline, but this rise was slightly lower than that observed with casein alone. After three hours, amino acid levels declined, showing no significant difference from the one-hour values or the pre-feeding levels. Furthermore, feeding rats a mixture of starch and casein hydrolysate resulted in a modest, non-significant increase in amino acids after one hour; this increase was smaller than in the starch + casein group and markedly lower than in the casein-only group. After three hours, a further decline in amino acid concentration was observed, remaining slightly below the one-hour value and not significantly different from pre-feeding levels.

Thus, feeding rats with starch combined with casein resulted in lower blood glucose concentrations compared with feeding starch alone. Feeding with starch and casein hydrolysate produced an even greater decrease in glucose concentration than either starch alone or starch combined with casein. A similar pattern was observed in amino acid absorption: feeding with starch and casein resulted in lower blood amino acid concentrations than feeding with casein alone, while feeding with starch and casein hydrolysate caused an even more pronounced reduction. These changes are likely related to the

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formation of **starch–casein and starch–peptide complexes**, which may hinder the enzymatic hydrolysis of starch by salivary amylase and of casein and its hydrolysates by gastric enzymes.

Conclusions.

Feeding rats with starch combined with casein led to lower blood glucose levels compared with feeding with starch alone, and lower amino acid concentrations compared with feeding with casein alone. Feeding with starch combined with casein hydrolysate produced an even more pronounced reduction in both blood glucose and amino acid concentrations compared with the other feeding combinations. These results suggest that the observed effects are associated with the formation of starch–casein and starch–peptide complexes, which inhibit the enzymatic hydrolysis of starch by salivary amylase and of casein and casein hydrolysates by gastric juice.

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