# The response of rat submandibular salivary gland to plant protein diet; Biological and histochemical study

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**Abstract**

**Objective:** This investigation aimed at studying the effect of plant protein diets on the salivary glands. It compares 3 protein's types on the submandibular gland structure and function. The submandibular gland was chosen because it contains all the elements of the salivary glands. The different protein types were chosen to answer if the plant protein or legume plant protein can be replaced for the animal proteins.

**Methods:** Thirty young albino rats were divided into 3 dietary groups: group I was fed 10% skim milk and acts as a control, group II was fed corn that is a cereal plant protein, while group III was fed 10% soybean, which is a legume plant protein. The animals were sacrificed after 2 months from the beginning of the experiment and the submandibular salivary glands were prepared for the biochemical analysis and histological, as well as, histochemical studies.

**Results:** The biochemical analysis revealed that total protein in soybean fed group was nearly the same as that of the control, while being significantly decreased in the corn fed group. The histological appearance of the submandibular salivary gland of both study groups showed shrinkage in the acini and ducts. The histochemical results showed an intense precipitation for total protein in the soybean group, while the corn fed group showed a mild precipitation. The reaction for DNA and RNA was intense in the control group as compared with both study groups.

**Conclusion:** From this study, it was apparent that the plant protein had adverse effects on the submandibular salivary gland and impairs its function. Thus, plant protein including the soybean cannot replace animal protein which is ideal for the normal growth and function of the gland.

**Key words:** Rat, submanibular salivary glands, animal protein, cereal plant protein and legume plant protein.

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## Introduction

In large areas of the world, there is not enough protein to meet people's need. Two-thirds of the world's people subsist almost entirely on plant proteins. [1]

Salivary glands play an important role in oral health. Their behavior is very sensitive to the general condition of the body and to a variety of systemic diseases. [2] Nevertheless, their functions are dependent upon food intake. [3] Deficiency in the quantity of protein reduced the size and weight of the submandibular gland. [4-7] Atrophic changes including shrinkage of the acinar cells, [4] loss of cytoplasmic material, [8] and degenerative changes of the subcellular organelles [9] in salivary glands were reported. The rate and volume of the salivary gland secretion were decreased in protein deficient animals, [7, 9, 10] and in malnourished human subjects. [11-13] The biochemical analysis of the saliva taken from protein malnourished animals and human showed changes in their composition. The total protein content was reduced in protein malnourished rats, and human subjects. There were reduced activities of the specific proteins, such as secretors IgA, [13, 14] IgG, [14] amylase, [10, 15] lysozyme, [11] arginase, [12] ferritin, [12] and bacteria-agglutinating glycoprotein. [16, 17]

However, protein deficiency should not be considered only in terms of quantity, but also in terms of quality. This is particularly true in case with most plant proteins. Therefore, the present investigation was done to study the histological, histochemical and biochemical effects of two different types of plant protein on rat submandibular salivary gland.

## Materials and Methods

Thirty young albino rats aging 21 days and weighing 40.7-43.2 grams were divided into three dietary groups.

Group I: was fed a diet containing 10% skim milk and acts as a control group.

Group II: was fed a diet containing 10% corn that is cereal plant protein.

Group III: was fed a diet containing 10% soybean that is legume plant protein.

All the diets were essentially the same in respect to mineral, vitamin and fat content, but differed in protein quality. Starch was added to the diet to compensate for the amount of protein content and to complete the weight of each diet to 1 Kg. The animals were housed singly and offered adlibitum amounts of the diet. After two months from the beginning of the experiment, the rats were sacrificed and the submandibular salivary glands of each rat were dissected out. The gland of one side was immediately frozen on dry ice and prepared for the biochemical assessment of total protein content according to the method of Bradford [18]. The gland of the other side was fixed in 10% formalin, then, processed for paraffin embedding.

### Histological and Histochemical analysis:

Sections of 4 µm thick were stained with haematoxylin and eosin and used for histological study, with mercuric bromphenol blue [20] for total protein and with methyl green-pyronin [19] for demonstration of DNA and RNA.

### Biochemical estimation of total tissue proteins:

Tissues of submandibular salivary glands were homogenized in ice cold lysis buffer, cell lysates were separated and used for estimation of total protein content according to Bradford method using Bradford reagents (Bio-Rad Laboratories, Inc.2000 Alfred Nobel Dr.Hercules, CA 94547, USA) according to instruction manual. [18]

### Statistical analysis:

Data of total proteins are presented as mean ± SD. Mean values of various groups are compared using student t-test. Values of $p<0.05$ are considered significant.

## Results

### *I. Biochemical findings:*

As shown in Table (1), total protein content of the submandibular salivary gland tissue of the corn fed group decreased significantly when compared with that of the control group. On the other hand, the soybean fed group showed a slight decrease than the control group, however, of no statistically significant difference.

**Table (1): Biochemical assessment of total protein content in rat submandibular salivary gland tissue.**

| Groups | Protein Content mg/mg tissue |
|---|---|
| I (control) | 179.1 ± 2.96 |
| II (corn fed) | 148 ± 6,13* |
| III (soybean) | 177 ± 2,49 |

**Note: values are mean ± SD.**
*** Significant at p<0.005.**

***II. Histological findings:***

Typical acini and ducts architecture of the submandibular salivary gland was observed in the control group (Fig.1). Meanwhile, in corn fed group, the acini and ducts were reduced in size, however, their architecture seemed to be normal (Fig.2). The acini and ducts of soybean fed group were smaller in size than the control group, but slightly larger than the corn fed group. Spaces indicative of edema were evident in between the acini and ducts leading to their squeezing (Fig.3).

***III. Histochemical findings:***

1. Total protein: In the control group, the protein content was found to be of moderate intensity. Protein was precipitated around all acini and within some of them (Fig.4). In corn fed group, there was a weak precipitation of protein within the acini, while being of a moderate intensity in the ducts (Fig.5). In soybean fed group, the precipitation of protein within the acini was found to be of great intensity, masking the nuclei of some acini (Fig.6).
2. DNA and RNA: In the control group, an intense reaction for both DNA and RNA was observed. The enzymatic activity showed an increase in DNA in the nuclei of the acinar cells. Meanwhile, DNA and RNA were found to be of a moderate intensity in the nuclei and cytoplasm of the ductal cells (Fig.7). In corn fed group, a mild reaction for both DNA and RNA was evident (Fig.8). Finally, in soybean fed group, a moderate reaction for both DNA and RNA was present (Fig.9).

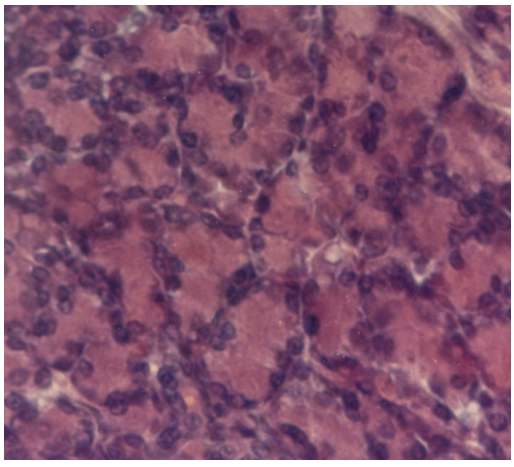

**Fig. (1). Submandibular salivary gland of a control rat showing typical duct and acinar architecture (H & E stain X 400).**

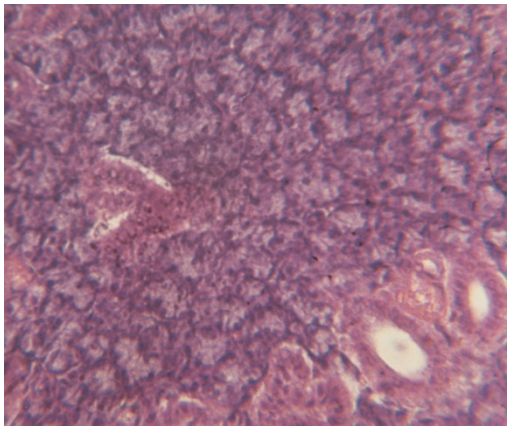

**Fig. (2). Submandibular salivary gland of a corn fed rat showing a smaller size of the acini and ducts. (H & E stain X 400).**

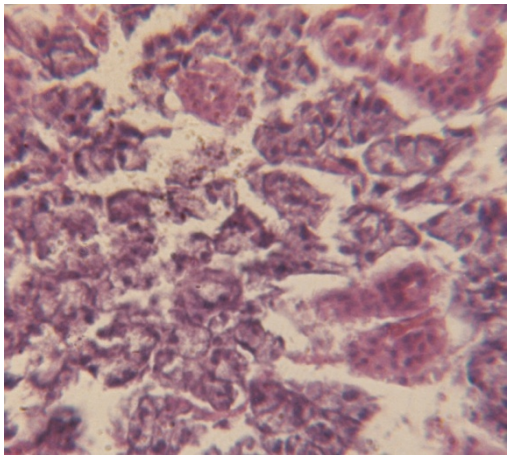

**Fig. (3). Submandibular salivary gland of a soybean fed rat showing small size of both acini and ducts with many spaces in between indicative of edema (H & E stain X 400).**

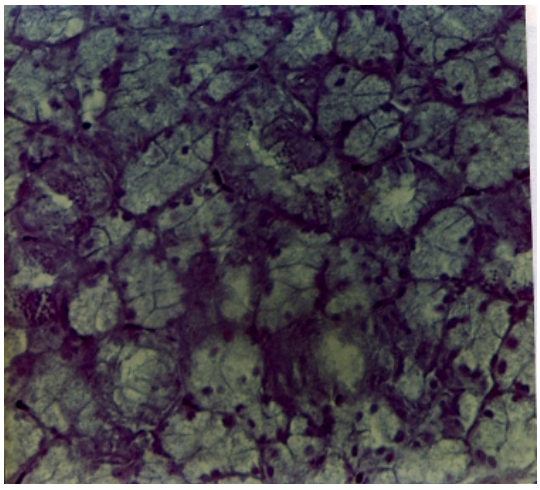

**Fig. (4). Submandibular salivary gland of a control rat demonstrating moderate reaction for total protein (Mercuric bromophenol blue X 400).**

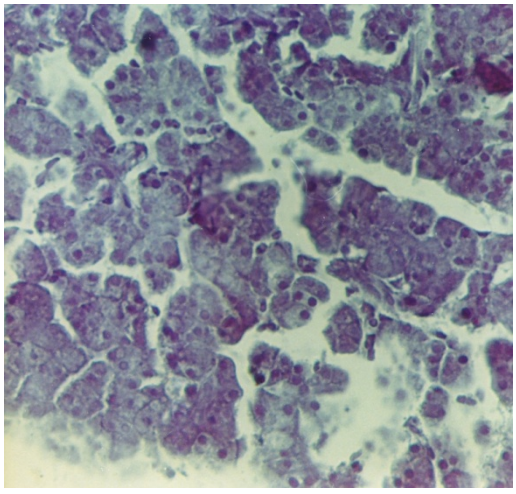

**Fig. (6). Submandibular salivary gland of a soybean fed rat showing intense reaction for total protein (Mercuric bromophenol blue X 400).**

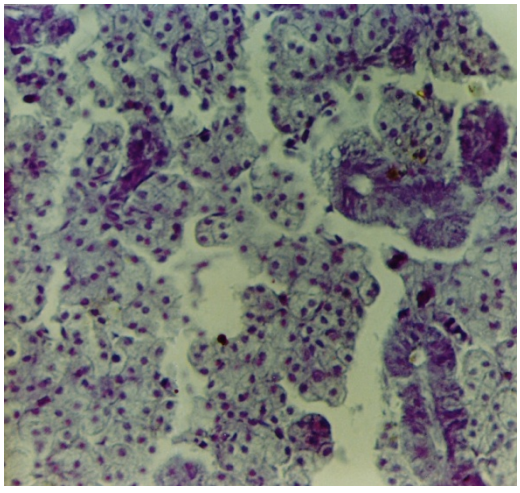

**Fig. (5). Submandibular salivary gland of corn fed rat, illustrating mild reaction for total protein in the acini while moderate reaction in the ducts (Mercuric bromophenol blue X 400).**

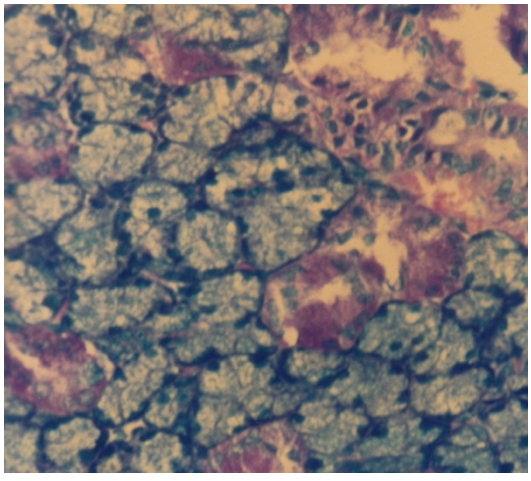

**Fig. (7). Submandibular salivary gland of a control rat showing intense reaction for both DNA and RNA (Methyl-green pyronin X 400)**

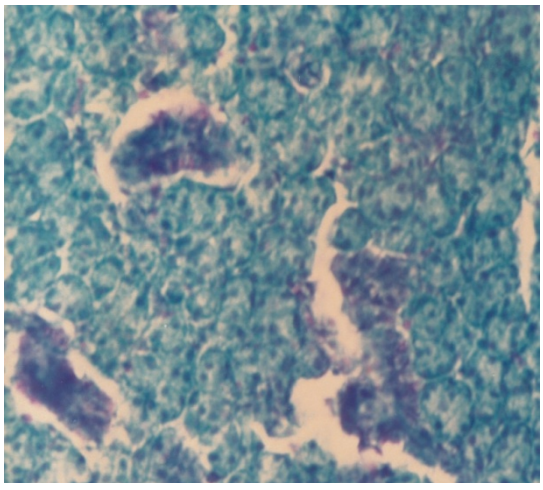

**Fig. (8). Submandibular salivary gland of corn fed rat showing weak reaction for both DNA and RNA (Methyl-green pyronin X 400).**

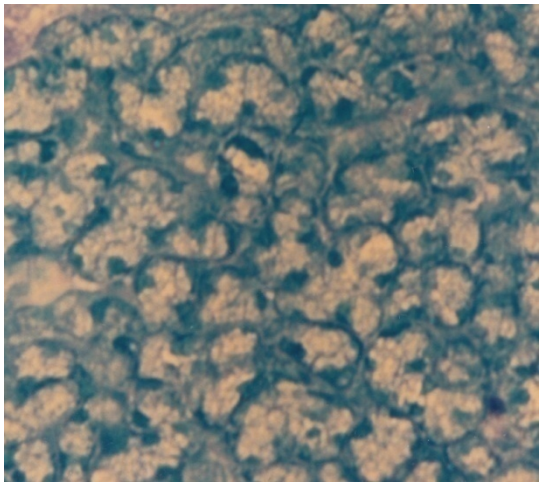

**Fig. (9). Submandibular salivary gland of a soybean fed rat showing moderate reaction for both DNA and RNA (Methyl-green pyronin X 400).**

## Discussion

The data from the quantification of total protein content in the tissues expressed on a unit concentration basis showed that the total protein content in the submandibular salivary glands of soybean fed group was nearly the same as the control group, while the corn fed group showed a significant decrease in total protein content. Thus, this would indicate a good biochemical activity and growth in the glandular tissue of the soybean fed group and poor biochemical activity and growth in the corn fed group. Different reports showed similar results. [11, 13]

The histological results of the corn group were found to be parallel with its biochemical analysis, as the acini and ducts showed marked reduction in size. On the other hand, the histological changes in the soybean fed group contradicted the biochemical results, where the glandular tissue showed a marked reduction in the acini and ducts with the presence of edematous areas the units. The existence of edema could be contributed to the decrease in albumin concentration. [21] Similar observations were noticed on rats kept on deficient quantity of animal protein diet. [4] Therefore, it seems visible that protein deficiency either in quantity or quality could lead to the same histological alterations.

The histochemical changes observed during plant protein feeding of rats are expressed here on the basis of total protein, RNA and DNA reaction that is the function of submandibular salivary gland tissue is evaluated by its ability to synthesize protein, DNA and RNA. The histochemical results revealed that the reaction of total protein in corn fed rats was weak around the acini and moderate within the ducts. This supports the biochemical findings. However, in soybean fed rats, the precipitation of protein was of greater intensity than that of the control rats. Since, the biochemical evidence revealed that in soybean group the concentration of total protein was nearly the same as in the control and while, the histological evidence showed that there was a reduction in the size of the acini, therefore, it is not surprising that the concentration of protein in the smaller acini and ducts should be more intense when compared with the control. In this respect, it is valid to assume that the synthetic activity of protein in soybean group was better than in corn fed group. The primacy of protein as the most limiting nutrient perhaps is most importantly reflected in the DNA content of the gland. It was proved that the DNA content of a tissue is an accurate reflection of the degree of cellularity of that tissue. [22] Other investigators [23] have shown that undernutrition during the critical period of cell hyperplasia (DNA replication) results in permanent stunting of growth. The results of this study accepted the latter assumption, and indicated that the

control group had more intense reactions of these compounds together with an increased acinar size, while the study groups showed moderate or weak reaction for DNA and RNA with smaller acinar cells.

The histological alteration, as well as the histochemical reactions in the study groups could be attributed to the various types of amino acid contents in corn and soybean. It is ascertained that the limiting amino acids of corn are lysine and tryptophan. [24] Meanwhile, there are two suggestions regarding the amino acid content of soybean. First, soybean is said to be a complete protein equal in quality to most animal proteins. [25, 26] Second, soybean is poor in methionine. [24] Our findings support the second assumption because, although the soybean fed rats showed similar biochemical behavior in the submandibular salivary gland like the controls, yet it showed histological alterations and histochmeical reactions that deviated from normal. Nevertheless, soybean fed group showed better biochemical and histochemical reactions than that of the corn fed group.

Finally, it should be emphasized that soybean plant protein cannot replace the animal protein which is the ideal protein essential for normal salivary gland development, growth and function.

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