THE EFFECT OF PRAWN PROTEIN ON 
THE BONE OF MATURE RATS

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The present study was undertaken in order to obtain data relative to the 
influence of the nutritional variations studied on the bones of normal limbs 
of mature rats. There is a wealth of knowledge pertaining to the effects 
of dietary deficiencies of minerals on the skeleton of young animals; and 
Sherman and co-workers (Sherman and Booher, 1939; Sherman and 
Sherman, 1938; Van Duyne et al., 1941; Sherman et al., 1941) have investigated 
extensively the beneficial effects of diets rich in calcium, on growth 
and calcium storage in young animals. While there is considerable informa-
tion (Conner and Sherman, 1936; Osborne and Mendel, 1926; Quinn et al., 
1929) dealing with several phases of the influence of protein intake on skeletal 
growth of young rats, there appears to be little published knowledge with 
regard to the effect produced by protein deficient diets on the bones of 
mature animals. And also the finding (Armstrong, 1948) that severe restriction 
of protein content of the diet of mature rats produced marked effect on 
the humeri, suggested the present problem of the influence of different levels 
of prawn protein intake on the bones of such animals.

METHOD

Diets containing respectively 5%, 10%, 12.5%, 15%, 17.5% and 20% 
of prawn protein given in Table I were prepared according to the method 
described by Armstrong (1948). Additional sucrose was added on an equal 
weight basis, as a replacement for protein in the 3 diets of lower protein 
content.

From a colony that was fed with a stock diet (Table II) well balanced 
as regards fat, carbohydrate, protein, minerals and vitamins, 60 albino mature 
rats were selected with weights between 240-50 gm. They were fed again 
on stock diet and carefully watched until their weights were within the range 
of 260-65 gm. when they were started on dietary regimen. The rats were 
distributed equally with regard to sex and were housed in cylindrical cages. 
Water was also supplied ad libitum.
TABLE I
Composition in gm. per Kilogram of Diet

<table>
<thead>
<tr>
<th>Diets</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prawn Protein (Pulp)</td>
<td>75</td>
<td>100</td>
<td>125</td>
<td>150</td>
<td>175</td>
<td>200</td>
</tr>
<tr>
<td>Sucrose</td>
<td>425</td>
<td>400</td>
<td>375</td>
<td>350</td>
<td>325</td>
<td>300</td>
</tr>
<tr>
<td>Sucrose and Vitamins*</td>
<td>280</td>
<td>280</td>
<td>280</td>
<td>280</td>
<td>280</td>
<td>280</td>
</tr>
<tr>
<td>Sweet Oil</td>
<td>169</td>
<td>169</td>
<td>169</td>
<td>169</td>
<td>169</td>
<td>169</td>
</tr>
<tr>
<td>Salt Mixture (Osborne and Mendel)</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Yeast</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* Each 1,000 gm. of diet contained 2 mg. thiamine hydrochloride, 2 mg. pyridoxine hydrochloride 3 mg. riboflavin, and 4 mg. calcium pantothenate.

TABLE II

Stock Diet

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat flour</td>
<td>60 gm.</td>
<td>Sodium Chloride</td>
<td>0.50 gm.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram flour</td>
<td>20 &quot;</td>
<td>Calcium Carbonate</td>
<td>1.50 &quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ankoria Baby Food</td>
<td>5 &quot;</td>
<td>Milk</td>
<td>60 cc.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweet Oil</td>
<td>5 &quot;</td>
<td>Sodium Phosphate</td>
<td>3.60 gm.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat Paste</td>
<td>2 &quot;</td>
<td>Ascorbic Acid</td>
<td>6 mgm.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The six diets, viz., 5, 10, 12.5, 15, 17.5 and 20% diets of Sode (Parapæniopsis sculpitius) were fed to the correspondingly numbered groups of animals, each group containing 10 animals, for a period of 30 days. The animals were sacrificed and after prolonged boiling of the fore-limbs in water, the humeri were dissected free of soft tissue. The bones were dried and rendered fat-free by continuous extraction in a Soxhlet apparatus for 48 hours with a mixture of equal parts of alcohol and ether. Again the
bones were dried and weighed, the length of the bones was determined to
the nearest 0.1 mm. with a vernier micrometer. Finally they were ashed
to constant weight at 700°C. and their weight recorded. The ash of the
humeri of the individual animal was dissolved in hydrochloric acid and
analysed for calcium and phosphorus. The calcium determinations were
made by the method of Clark and Collip (1925) and the phosphorus analyses
by the method of Fiske and Subbarow (1925).

**Table III**

*Observations pertaining to the Animals and Results with regard to the Humeri†*

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Protein content of diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5%</td>
</tr>
<tr>
<td>Initial Body Weight (g.)</td>
<td>258</td>
</tr>
<tr>
<td>Final Body Weight (g.)</td>
<td>246</td>
</tr>
<tr>
<td>Daily food Intake (g.)</td>
<td>11.80</td>
</tr>
<tr>
<td>Dry, Fat-free weight of Humeri (g.)</td>
<td>0.209</td>
</tr>
<tr>
<td>Ash weight of Humeri (g.)</td>
<td>0.140</td>
</tr>
<tr>
<td>Calculated Ash content (%)</td>
<td>67.07</td>
</tr>
<tr>
<td>Length of Humeri (mm.)</td>
<td>25.15</td>
</tr>
<tr>
<td>Ca/P Ratio of Ash:</td>
<td>0.029</td>
</tr>
</tbody>
</table>

† The data are shown as averages.

**Results and Discussion**

In Table III are given the length, weight and ash content of the humeri
of the rats at 5, 10, 12.5, 15, 17.5 and 20% protein intake respectively.
All the data related to the humeri are the means of the average obtained
with the two bones from the individual animal in each group. It is seen
from Table III that the gain in weight of the animals varied in the same
manner as the protein content of the food. The animals receiving 20%
protein gained on an average 5 gm. while those receiving 5% protein lost on
an average 16 gm. The humeri of the groups receiving 5 and 10% protein
diet suffered a significant reduction in weight of the fat-free dry bone and
its ash weight. Increasing the protein from 12.5 to 20% in the diet effected a gradual increase in the skeletal growth and also in the weight of the ash. Maximum skeletal growth and ash weight were found at 15% protein level. Further with an increase in the prawn protein in the diet from 10 to 12.5% there is an increase in the skeletal growth as well as the ash content. It is seen that 12.5% protein level is the minimum level for normal skeletal growth. The 5 and 10% protein diets are inadequate to support normal skeletal constitution in mature rats equal to that of a diet containing 15% prawn protein.

### TABLE IV

<table>
<thead>
<tr>
<th>Variations due to</th>
<th>Groups</th>
<th>Treatment</th>
<th>Error</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of Squares</td>
<td>4.7102013</td>
<td>78.8669453</td>
<td>21.1676687</td>
<td>104.7528183</td>
</tr>
<tr>
<td>Degrees of freedom</td>
<td>9</td>
<td>5</td>
<td>45</td>
<td>59</td>
</tr>
<tr>
<td>Variance of Mean square</td>
<td>0.524245</td>
<td>15.77338966</td>
<td>0.47039264</td>
<td>..</td>
</tr>
</tbody>
</table>

\[ F = 33.53239043. \]
\[ 1\% = 3.45 \]
\[ t_{45} \text{ at } 1\% \text{ value } = 2.69. \]

Difference of two means greater than 0.82508218 will be significant.

Statistical data obtained for these results confirmed the superiority of 15% prawn protein diet over the other diets, with regard to skeletal growth and body weight. The optimum level protein for normal skeletal growth was found to be 12.5% prawn protein.

The effect on body weight produced by feeding the mature rats for 30 days with diets containing 5, 10, 12.5, 15, 17.5 and 20% prawn protein was also determined. It is seen from Table III that 5% prawn protein did not support the body weight, of mature rats while diets containing 10, 12.5, 15, 17.5 and 20% permitted an increase in body weight in approximate proportion to the protein contents.

The composition of the bone ash in all the groups was not affected by protein deficient diets, as can be seen from the Ca:P ratio in Table III,
SUMMARY AND CONCLUSION

The body weight and humeri of mature rats fed for 30 days with diets containing 5, 10, 12.5, 15, 17.5 and 20% prawn protein have been determined. The following conclusions are drawn:

1. A diet containing 5% prawn protein will not support the body weight of mature rats, while diets containing 10, 12.5, 15, 17.5 and 20% protein permit an increase in body weight in approximate proportions.

2. A diet containing 12.5% prawn protein supports a degree of skeletal constitution in mature rats equal to that of a diet containing 15% prawn protein.

3. 5 and 10% prawn protein diets are inadequate to support normal skeletal constitution.

4. The compositions of the bone ash is not affected by feeding prawn protein deficient diets to mature rats.

LITERATURE CITED

9. ——— and Sherman H. C. . . . Ibid., 1938, 126, 381.