

Nutritional studies on the seeds of *Pinus Koraisensis seib. et zucc.*(1)

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(Received for publication Dec. 29, 1967)

柏子實成分에 關한 榮養學的研究 (1)

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要 約

柏子實의 Amino 酸, 糖, 脂肪, 無機物, vitamin 을 分析하고 이 結果로 白鼠의 榮養實驗을 한 結果, 蛋白質의 消化率은 87%, T.D.N.은 159.4%며 柏子實中에는 成長促進 及 飼料効率이 增大하는 U. G.F.의 存在를 確認하고 實驗한 白鼠를 解體한 結果 心臟, 腎臟, 脾臟이 若干 肥大하였음을 認定했다.

Introduction

In the previous report (1), the author claimed, that the seed of *pinus Koraisensis Seib. et Zucc.* is an excellent source of nutrition based on a series of analyses. However, little researches have been reported so far on the nutritional value of the seed determined by animal experiments. The writer carried out an experiment, using albino rat, to find out the effective components such as proteins, vitamins, inorganic matters and unknown growth factor (U.G.F.) etc.

Experiments

1. **Analytical Method:** The same methods as reported in the previous report(1), that is, Y. Kume's paper chromatography(2) for the amino acids; Fujida-Aoyama method(3) improved from Kuhn's method for carotins; thiochrome-potassium-ferricyanate method(4) for B; Lumiflavin-Fujida method(5) for B₂ Microbiological assay according

to A.O.A.C. (1955) (6) for niacin; and for the the conventional method(7) general components were employed.

2. **Digestion coefficient:** A certain amount of the test sample was mixed into the basic diet and fed to albino rats to observe the digestibility. Each test animal was put in a metabolism cage and fed each day with a certain amount of basic diet for one week and then the excretions produced during another five days were collected and of the digestibility was determined. The diet then was followed by the sample diet (basic diet mixed with test-sample in 7:8 ratio) and the digestibility was examined by use of the following formula.* The basic diet used for the digestibility test was barley fine bran mixed with wheat bran in 2:1 ratio. Its composition(%) was moisture 8.90, crude protein 17.00, crude fat 5.32, crude fiber 4.80, nitrogen free extract 54.6, and crude ash 9.38.

*—Formula.....Digestion coefficient=

$$\frac{\text{Eaten Quant. of Sample} - (\text{Total Excretion} - \text{Excretion from Basic Diet})}{\text{Eaten Quant. of Sample}} \times 100$$

Table 1. The Composition of Basic Diet

Item	Mixture Ratio
Corn Starch	53
Skim Milk Powder	24
Fish Meal	9
Margarin	8

Soybean Cake	6
Salt	0.5
Vitamin & Minerals*	0.3

* Composition of Vitamins & Minerals.....Vitamin A 325 IU, D 25 IU, B₁ 0.23 mg, Pantothenic Acid 0.33 mg, Niacin 1.5 mg, Folic acid 23 r, Biotin 8.8 r, Choline 130 mg, MnSO₄ 10 mg, Zn SO₄ 10 mg.

Table 2. Amino Acid Composition in the Basic diet

Item	Basic Diet	Minimum Requirement	Ratio (%)
Protein	17	20	85
Arginine	0.4	0.2	200
Histidine	0.3	0.4	75
Lysine	0.9	1.0	90
Tryptophane	0.2	0.2	100
Threonine	0.4	0.5	80
Leucine	0.7	0.8	87
Isoleucine	0.5	0.5	100
Valine	0.6	0.7	85
Glycine	0.3	—	300
Phenylalanine	0.33	0.45	88
Tyrosine	0.37	0.45	
Methionine	0.2	0.5	66
Cystine	0.2	0.1	

Remark: The data of amino acid composition were taken from R.J. Block, K.W. Weiss; Amino Acid Hand Book (1956).

Table 3. Vitamin and Mineral Composition in the Basic Diet

Item	Basic Diet	Minimum Requirement	Ratio (%)
Vitamin A(IU)	362	265	136
Vitamin B ₁ (mg)	0.49	0.19	257
Vitamin B ₂ (mg)	1.16	0.33	351
Pantothenic Acid(mg)	1.93	0.66	392
Niacin (mg)	2.66	2.65	100
Pyridoxin(mg)	0.87	0.29	300
Biotin(mg)	0.03	0.01	300
Folic Acid(mg)	238	200	119
Vitamin B ₁₂ (r)	6.0	1.0	600
Ca(%)	1.63	0.66	246
P(%)	1.19	0.6	191
K(mg)	1.05	0.85	125
Mg(mg)	17.5	59.4	294
Mn(mg)	6.6	6.6	100

Fe(mg)	12.6	2.0	632
Cu(mg)	1.78	0.2	895
NaCl(mg)	0.26	0.13	200
Zn(mg)	0.56	0.2	280
Vitamin D(IU)	375	264	142

Remark: The reference books from which these data were quoted: H. Iwata, General Discussion on Feed and Feeding, Yokendo (1955); H. Iwata, Food Chemistry, Yokendo (1955); Morrison Feed and Feeding Morrison Publishing co. (1950).

3. Growth Experiments: Three male albino rats were put in each plot and fed with the basic diet as shown in Table 1, which is considered nutritionally perfect. The amino acids in the basic diet were compared with the standard of NRC as shown in Table 2, but all other ones proved to be sufficient but histidine, threonine and methionine showing a little shortage. Vitamins and minerals were also examined, as shown in Table 3, but found sufficient compared with those of NRC (8)

The control animals fed with the basic diet vs other animals fed with diets mixed with 3%, 5% and 10% of the sample respectively were observed for 20 days to find out the growth efficiency. The diets were supplied two times a day as the rat's appetite demanded water provided freely.

Results and Discussion

1. Component: The result of analysis of the general components is shown Table 4. As the table indicates, pine tree seed contains more than 17% of protein, very little amount of nitrogen free extract and abundant fat. Vitamins and minerals were also determined and the result is shown in Table 5. Pine tree seed abundantly contains such vitamins as B₁ and B₂ and such minerals as Ca, P, K, etc. proving itself as an excellent source of vitamins and minerals. The analysis of protein amino acid is shown in Table 6. For comparison, the values of soybean and peanut quoted from the literatures (9) were shown in the same table. As Table 6, shows, the protein in pine tree seed is composed of abundant histidine and glycine compared with soybean. The total protein is somewhat less than that of soybean or peanut but pine tree seed is apparently an excellent source of protein.

Table 4. General composition of Pine Tree Seed

	%		%
Moisture	8.4	Crude Fiber	1.2
Crude Protein	17.1	Nitrogen Free Extract	6.0
Crude Fat	66.3	Crude Ash	1.0

Table 5. Vitamin and Mineral contents (%) in Pine tree Seed

Carotin(IU)	2.8	K(mg)	272
Vitamin B ₁ (r)	272	Mn(mg)	1.39
Vitamin B ₂ (r)	99	Mg(mg)	10.05
Vitamin C(mg)	6	Fe(mg)	13
Niacin(mg)	2	Si(mg)	9.3
Ca(mg)	301.2	S(mg)	28.9
P(mg)	361	Al(mg)	5.79

2. Measurement of Digestibility The digestibility of the samples is shown in Table 7. As to the general components, the digestible components and total digestible nutrients were examined. As the table shows the albino rat identifies itself to be an excellent digester of the protein in pine tree seed (87%), considering from 14.9% of the total digestible nutrient.

Table 6. Amino Acid Composition (%) of Pine Tree Seed

Item	In Sample	In Protein	In Soybean Protein	In Peaunt Protein
Arginine	0.6	3.6	7.3	11.3
Histidine	0.9	5.5	2.9	2.1
Lysine	0.3	1.8	6.8	3.0
Asparagine	0.7	4.0	—	—
Tryptophane	+	+	1.4	1.0
Aspartic Acid	0.9	5.3	5.2	—
Threonine	—	—	3.9	1.6
Leucine	0.8	4.6	8.0	6.7
Glutamic Acid	1.0	6.0	18.4	17.4
Isoleucine	—	—	6.0	4.6
Valine	0.6	3.7	5.3	4.4
Glycine	0.1	0.5	0.2	5.0
Alanine	0.1	0.4	3.3	4.2
Phenylalanine	—	—	5.3	5.1
Proline	0.1	0.7	5.0	—
Tyrosine	—	—	4.0	4.4
Methionine	+	+	1.7	1.0
Cystine	—	—	1.9	1.6

Table 7. Digestibility as Observed of Albino Rats

	Crude Protein	Crude Fat	Crude Fiber	Nitrogen Free Extract	Organic Matters
Components of basic diet(%)	17.00	7.32	4.80	54.60	81.72
Digestibility of basic diet (%) (Rat-No. 1)	64.4	78.8	15.7	65.3	63.1
Digestibility of basic diet (%) (Rat-NO. 2)	63.0	75.0	6.9	66.2	62.7
Digestibility of basic diet (%) (Rat-NO. 3)	64.7	77.9	14.1	56.7	56.9

(A) Albino Rat-NO. 1.....

(The amount of mixed diet eaten per day: 2.1 g. of basic diet and 2.4 g. of pine tree seed. total 4.5 g.)

Components of pine tree seed (%)	17.10	66.30	1.20	6.00	90.60
2.4 g of pine tree seed eaten per day (mg)	410	1591	28	144	2174
Excretion (%)	16.35	7.81	10.20	47.04	81.4
1.1 g of daily excretion (mg)	180	86	112	517	895
Non-digestibles out of the excretion from basic diet (mg)	127	24	85	401	633
Non-digestibles of pine tree seed (mg)	53	62	27	116	262
Digested amount of pine tree seed (mg)	357	1592	1	28	1912
Digestibility of pine tree seed (%)	87	96	4	13	88

(B) Albino Rat-NO. 2.....

(The amount of mixed diet per day: 2.0 g. of basic diet and 2.3 g. of pine tree seed, Total 4.3 g.)

Components of pine tree seed(%)	17.10	66.30	1.20	6.00	90.60
2.3 g. of pine tree seed eaten per day (mg)	393	1525	28	138	2084
Excretion (%)	16.35	7.81	10.20	47.04	81.04
1.03 g. of daily excretion (mg)	168	80	105	485	838
Non-digestible out of the excretion from basic diet (mg)	126	26	89	369	610
Non-digestibles of pine tree seed (mg)	42	54	16	116	228
Digested amount of pine tree seed (mg)	351	1471	12	22	1756
Digestibility of pine tree seed (%)	89	96	43	17	84

(c) Albino Rat-NO. 3.....

(The amount of mixed diet eaten per day: 2.2 g. of basic diet and 2.5 g. of pine tree seed, Total 4.7 g.)

Components of pine tree seed (%)	17.10	66.30	1.20	6.00	90.60
2.5 g. of pine tree seed eaten per day (mg)	428	1658	30	150	2265
Excretion (%)	16.35	7.81	10.20	47.04	81.40
1.13 g. of daily excretion (mg)	185	88	115	532	920
Non-digestibles out of the excretion from basic diet (mg)	132	26	91	520	775
Non-digestibles of pine tree seed (mg)	53	62	24	12	145
Digested amount of pine tree seed (mg)	365	1595	6	138	2120
Digestibility of pine tree seed (%)	85	96	20	26	93
Average digestibility of albino Rat-NO. 1, NO. 2 and NO. 3 (%)	87	96	22	19	88
Digestible Component (%)	14.9	63.6	0.3	1.1	159.4 (T.D.N)

3. The Result of growth experiment: 20 days' growth experiment brought the result as shown in Table 8. The 3% sample adding showed 18% of growth increase compared with the control, whereas 5% and 10% sample adding showed no efficiency.

As to the feed efficiency, only the 3% adding showed 13% of growth increase. As to the protein efficiency also the 3% adding exceptionally showed 14% of growth increase. The 5% and 10% adding did not show any growth increase. This might

result, according to the writer's presumption, from the fact that pine tree seed contains sufficient protein and therefore the protein content of mixed diet is more than sufficient.

Judging from the fact that when pine tree seed was added the perfect mixed-diet showed remarkable efficiency in the aspects of growth, feed and protein, it is obvious that the pine tree seed contains U.G.F. beside abundant protein.

Table 8. Growth Efficiency of pine Tree Seed on Albino Rat

	Control	Treatment (3% adding)	Treatment (5% adding)	Treatment (10% adding)
Protein content in feed (%)	17.02	17.53	17.87	18.73
Original body-weight (g)	11.85	13.85	14.15	15.90

Body-weight after 20 days (g)	19.25	24.50	20.02	20.45
Weight increase during 20 days (g)	7.40	10.65	6.05	4.55
Growth rate (%)*	62	77	43	29
Growth per-centage	100	118	69	47
Eaten amount per rat during 20 days	51	62	48	54
Feed efficiency (%)**	15	17	13	8
Feed efficiency per centage	100	113	87	53
Protein efficiency (%)***	85	97	67	46
Protein efficiency per centage	100	114	79	54

Anatomical evaluation.....

Anatomized body-weight (g)	17.80	26.85	20.55	20.50
Lung (g) (Body-weight taken 100 g)	0.898	0.633	0.973	0.634
Heart (g) (Body-weight taken 100 g)	0.449	0.633	0.684	0.732
Liver (g) (Bodyweight taken 100 g)	6.120	7.262	7.786	5.121
Kidney (g) (Bodyweight taken 100 g)	1.292	2.309	1.411	1.463
Pancreas (g) (Body-weight taken 100 g)	0.280	0.372	1.411	0.390

$$* \text{ Growth rate} = \frac{\text{Body-weight increasement}}{\text{Original body-weight}} \times 100$$

$$** \text{ Feed efficiency} = \frac{\text{Body-weight increasement}}{\text{Amount of eaten diet}} \times 100$$

$$*** \text{ Protein efficiency} = \frac{\text{Body-weight increasement}}{\text{Amount of eaten protein}} \times 100$$

Upon the completion of growth experiment, rats were sacrificed and their intestines were examined. The result is shown in Table 8. The slight fattening of heart, Kidney and pancreas can be attributed to the pine tree seed, but intestines showed no change in the sample feeding.

Summary

(1) The general composition, vitamin, amino acid, digestibility and nutrition efficiency of the seeds of *Pinus Koraiensis* Sieb. et Zucc. were examined.

(2) Pine tree seed contains approximately 17% or more protein nearly pure one. The amino acid composition of the protein was analyzed. When compared with the values of soybean and peanut reported in the literature, it contains slightly more histidine and glycine etc., showing that the seeds are of high nutritional value.

(3) The protein digestibility was 87% and the total content of digestible protein was approximately 14.9%, T.D.N. was 159.4%, proving itself excellent.

(4) As to vitamins, it was found out that the

seeds contain 275 r of B₁ and 99 r. As to minerals, Ca (301.2 mg) P (361 mg) and K (272 mg) were found to be contained in the seeds

(5) The basic diet was considered nearly perfect from the view-point of nutrition, and to this were added 3%, 5% and 10% of the test sample respectively and were fed to albino rats. Among the three treatment, the 3% sample adding proved to be the most effective one, showing the most efficient growth. Thus it can be presumed that the pine tree seeds certainly contain U.G.F.

(6) The anatomical examination of the intestines of albino rats showed that the feeding of pine tree seeds had favorable effect on the development of heart, kidney and pancreas.

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