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Nutritional evaluation of sunflower-seed protein products

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With 6 figures and 4 tables

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Oilseeds constitute one of the largest potential sources of protein in the world. At present an estimate of about 135 million tons of oilseed, corresponding to 50 million tons of proteinaceous food, are produced annually. The actual contribution of oilseeds as sources of proteins used by humans is about 11.3 million tons, that is about 8 % of the world protein supplies (17.

Sunflower *(Helianthus annuus)* is by now one of the major oilseed crop, occupying the fourth place after soybeans, peanuts and cottonseed, and with a total annual production of 11.1 million M.T. of seed. The crop is being cultivated for oil production; however the seeds contain substantial amounts of protein averaging 26 %, with an estimate of about 300 lbs protein per acre (2). Unlike other oilseed proteins where the limiting amino acids are methionine and isoleucine, the limiting amino acid in sunflower is lysine (3). Also, sunflower seed contains substantial quantities of yellow-green chlorogenic acid and associated tannin-like compounds which cause colour problems in food applications.

The present study has been designed to investigate the nutritional value of sunflower-seed meal, protein concentrate and protein isolate. Chicks are used as experimental animals.

Materials and methods

Seeds of Helianthus annus, Peredovic were obtained from local breeders. Defatted meal was prepared by extracting ground seed kernels with cold hexane, followed by desolventization at room temperature. The meal was finely ground to pass 0.2 mm screen.

Sunflower-seed protein isolate was prepared according to the countercurrent extraction procedure proposed by *Taha* et al. (3).

Sunflower-seed protein concentrate was prepared by similar countercurrent extraction procedure of the defatted meal while adjusting the pH at each extraction step to 4.0.

Table 1 gives the analysis of sunflower seed, defatted meal, protein concentrate, and protein isolate.

Analytical procedures

Moisture, lipids, crude fiber, and ash were determined according to standard procedures (4). Total nitrogen was determined by semimicro Kjeldahl procedure,

Table 1. Analysis of sunflower seed, defatted meal, protein concentrate, and protein isolate.[1])

	Seed	Meal	Protein concentrate	Protein isolate
Protein %	25.4	59.1	90.8	100.5
Oil %	27.4	0.5	0.5	0.0
Ash %	14.6	8.8	1.4	0.1
Fiber %	24.0	8.2	6.8	0.1
Nitrogen-free extract	8.3	23.4	0.4	0.0
Available lysine		2.86	2.80	2.97

[1]) Values are given on moisture-free basis.

and protein was calculated as N × 6.25. Available lysine was estimated as described by *Rao* et al. (5) and *El-Nockrashy* (6).

Feeding experiments

The procedure used of the evaluation of the nutritive value of sunflower-seed meal, sunflower-seed protein concentrate and protein isolate is basically *Heiman* et al. (7) technique.

Male Dokki-4 chicks (a breed between white *Leghorn* and *Fayoumi*, 1 day old. were kept in batteries with raised screen floors and given food and water *ad libitum*, throughout the experiment.

First, the chicks were depleted of embryonic protein reserves by placing them on an standardization ration composed of yellow corn meal fortified with vitamins and a salt mixture. This diet was fed for seven days after which the chicks were weighed individually and divided into groups of ten animals each. The total weight of the various groups, which weighed 349 g, did not differ by more than ± 2.5 g.

Following the depletion period, the chicks received test diets which were formulated to contain a total of 12 % protein, 6 % from yellow corn meal, and 6 % from sunflower meal, sunflower protein concentrate or sunflower protein isolate. Vitamins and salt mixtures were added, and equal amounts of starch and sugar to make the ration up to 100 parts. Chicks were fed test rations for a period of two weeks. Every other day the total feed consumption and weight gain of each group were determined.

Table 2 gives the constitution of the salt mixture which was added as 5 % of all rations, and the vitamin mixture (values being given as mg/kg ration.

Table 2. Composition of the salt mixture and vitamin mixture.

Salt	g/kg ration
$CaCO_3$	15.000
K_2HPO_4	16.125
$CaHPO_4 \cdot 2\ H_2O$	3.750
$MgSO_4 \cdot 7\ H_2O$	5.100
NaCl	5.000
$Fe(C_6H_5O_7)_2 \cdot 5\ H_2O$	1.375
KI	0.040
$MnSO_4 \cdot 4\ H_2O$	0.250
$ZnCl_2$	0.0125
$CuSO_4 \cdot 5\ H_2O$	0.0150

Table 2 (continued).

Vitamin	g/kg ration
Riboflavin	6.0
Ca. pantothenate	15.0
Niacin	100.0
Pyrodoxine B_6	3.0
Thiamine B_1	4.0
Biotin	0.2
Folic acid	2.0
Inositol	1000.0
p-aminobenzoic acid	100.0
Choline chloride	2000.0
Cyanocobalamine B_{12}	50.0 μg
Vitamin A acetate	10.0
Vitamin E	6.0
Vitamin K menadione	0.5
Vitamin D_3	0.015

Table 3. Protein source in test rations.[1])

Ration No.	Protein source	Amino acid added[2])
I	Casein	None
II	Sunflower seed meal	None
III	Sunflower protein concentrate	None
IV	Sunflower protein isolate	None
V	Sunflower seed meal (autoclaved)[3])	None
VI	Sunflower seed meal	L-Lysine
VII	Sunflower seed meal	L-Methionine
VIII	Sunflower seed meal	L-Lysine + L-Methionine
IX	Sunflower protein isolate	L-Lysine
X	Sunflower protein isolate	L-Methionine
XI	Sunflower protein isolate	L-Lysine + L-Methionine

[1]) Chick were fed rations constituting 12 protein (6 % from above-given protein sources and 6 % from yellow corn meal).

[2]) Amino acids were added at a level of 0.3% of the diet.

[3]) The calculated amount of sunflower seed meal to be used in the diet was weighed in a cotton plugged wide-mouth conical flask. The sample was placed into an autoclave and heated by passing steam (at atmospheric pressure) for 30 minutes.

A total of eleven experimental diets were tested. Table 3 gives the protein source included in each test diet.

Results and discussion

Sunflower-seed defatted meal, protein concentrate and protein isolate prepared therefrom, and casein were fed to week-old chicks after being depleted from their embryonic protein reserves. The effect of enrichment of meal and of protein isolate with lysine, methionine or a mixture of both on the nutritive value was investigated. Throughout the feeding experi-

Table 4. Nutritional value of sunflower seed meal, sunflower seed protein concentrate and sunflower seed protein isolate.

		Wt. gain (g)	Food consumed (g)	PER	Q. I.
I	Casein	434.7	1228.0	2.81	
II	Meal (M)	211.5	936.0	1.88	0.67
III	Protein concentrate (PC)	240.5	1060.0	1.90	0.68
IV	Protein isolate (PI)	276.5	1044.0	2.21	0.79
V	M-autoclaved	198.5	1076.0	1.54	0.55
VI	M + Lysine	440.5	1502.9	2.44	0.87
VII	M + Methionine	164.6	972.0	1.41	0.50
VIII	M + Lysine + Methionine	478.0	1396.0	2.85	1.01
IX	PI + Lysine	423.3	1212.0	2.91	1.04
X	PI + Methionine	100.0	930.0	0.81	0.29
XI	PI + Lysine + Methionine	395.7	1360.0	2.42	0.86

ments, which extended for two weeks, as proposed by *Heiman* et al. (7), three nutritional criteria were evaluated: First, the weight gain "total weight gain of the group of ten chicks"; second, the protein/efficiency ratio (PER) "ratio between the gain in weight and the protein consumed"; and third, the quality index (Q.I) "ratio of PER of test protein and PER of casein". The results are given in Table 4.

Seed meal, protein concentrate and protein isolate

The data obtained on food consumption, weight gain and protein/efficiency ratio throughout the two-weeks period are diagrammatically represented in Figure 1.

It is evident that casein supports better growth for the chicks than the three investigated sunflower-seed protein products. Throughout the experimental period, casein diet showed more food consumption and resulted in more weight gain and gave higher PER compared to the other three sunflower-seed protein diets. By the end of the two-weeks period, the gain in weight of the chicks fed meal, concentrate and isolate was ca. 49, 55 and 64 %, respectively, the weight gain resulting from feeding casein-supplemented diet. The PER values by the end of the two-weeks period were in the following order: 2.81, 2.21, 1.90, and 1.80 for casein, isolate, concentrate, and meal, respectively; (Q.I.: 0.79, 0.68, 0.67 for isolate, concentrate, and meal, respectively). The results also indicate that sunflower-seed protein isolate has better nutritional quality than the meal from which it is isolated.

The isolate resulted in weight gain 30.7 % higher than the meal in the two-weeks feeding period. Amino-acid analysis (g/16 g N) of meal and isolate indicates the following: Lysine, 3.9 and 4.0; available lysine, 2.86 and 2.97; methionine 2.6 and 3.0, respectively, compared to the reported requirements of chicks 4.5 and 2.25 for lysine and methionine, respectively (3). The data reveal that sunflower-seed protein products contain all essential amino acids in proportions that meet chick requirements with the exception of lysine.

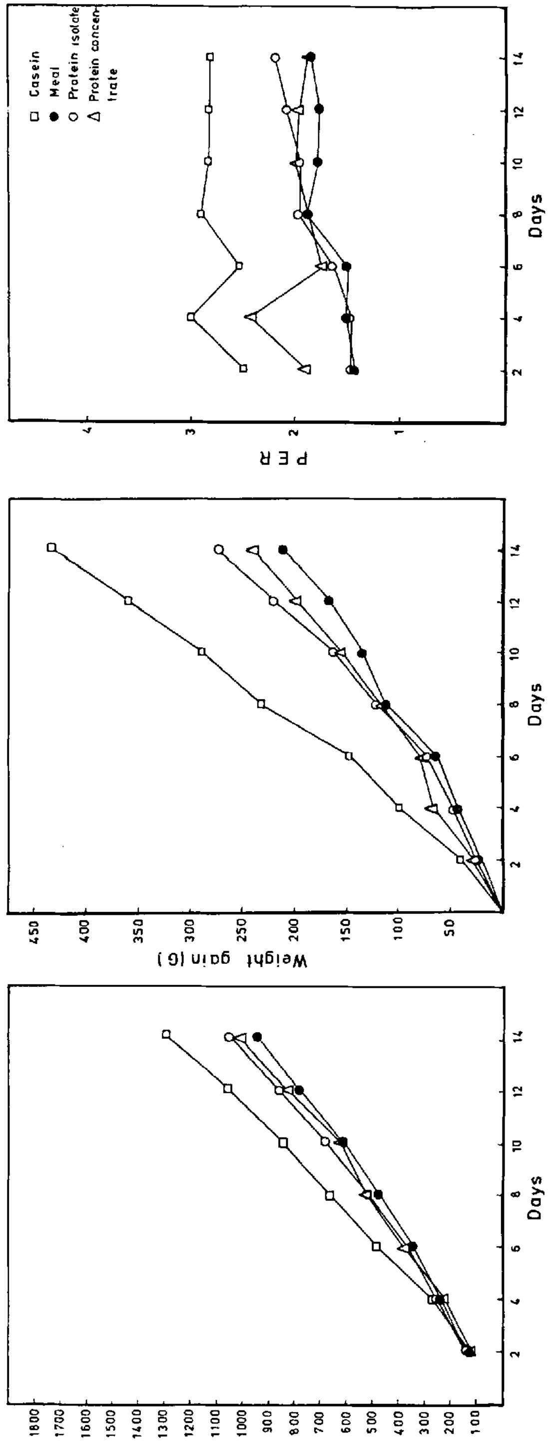

Fig. 1. Nutritional value of casein, sunflower-seed meal, sunflower-seed protein concentrate and sunflower-seed protein isolate.

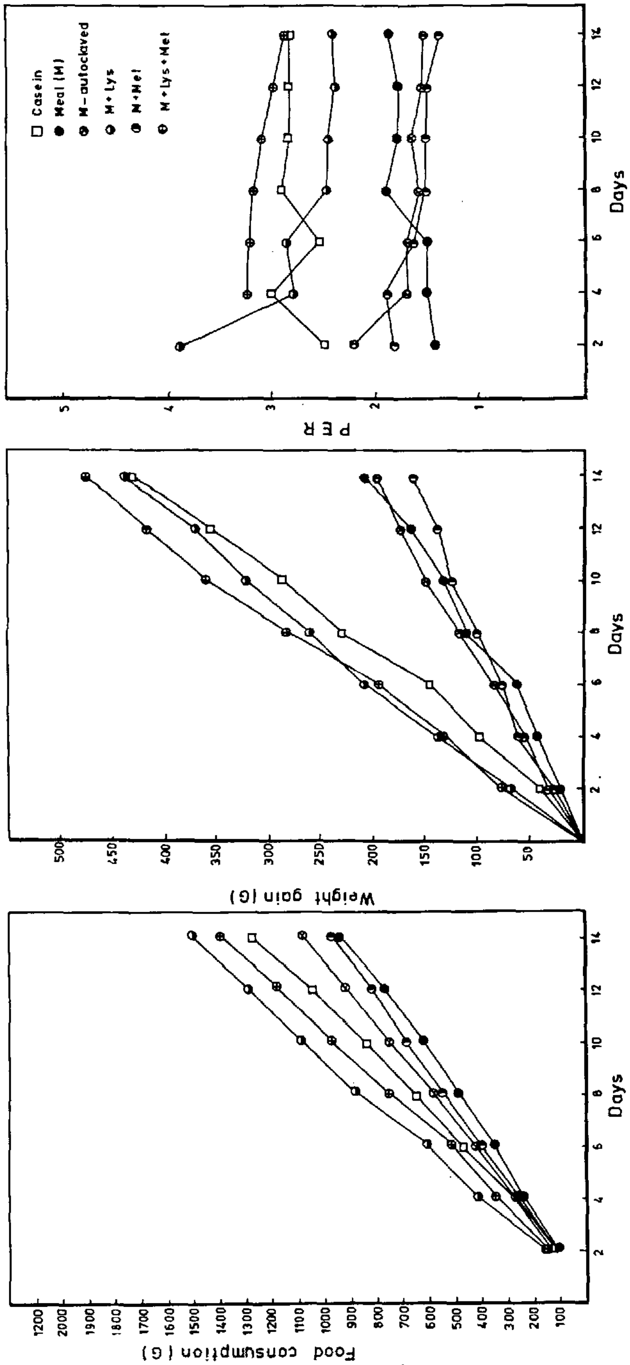

Fig. 2. Nutritional value of sunflower-seed meal, enriched with lysine, methionine or mixture of both.

Enrichment of sunflower-seed meal with lysine, methionine, or mixture of both

The amino acids were added at a level of 0.3 % of the diet. A fourth group of chicks were fed sunflower-seed meal that has been steamed in an autoclave for 30 minutes. Figure 2 shows the nutritive value of meal samples.

The steam-heated sunflower-seed meal resulted in an increase in the weight gain of the chicks, compared to unsteamed throughout the first twelve days of feeding. In the fourteenth day of feeding, the chicks' weight was slightly higher with the unheated meal. After two weeks, total food consumption and PER were lower for the steam-heated meal. *Piroska* (8, 9) reported on the effect of heat on sunflower-seed meal and found that dry- or moist-heat treatment at 120 °C for 10–20 minutes decreased protein solubility, increased denaturation of protein and causes loss in methionine, cystine, leucine, tyrosine, and serine. The nutritive value on *in vitro* digestability was also reduced. The presence of an antitrypsin factor in sunflower seed, which is known to be deactivated by heat, has been a subject of investigation. While *Shpirko* (10) found that sunflower cake inhibits trypsin activity, *Roy* and *Bhatt* (11) reported that some sunflower varieties were free from trypsin inhibitor.

After two weeks, the group fed sunflower-seed meal enriched with lysine gained 440 g, compared to 212 g gained by the group fed non-enriched meal, i.e. 108 % higher. This was also coincident with 60 % increase in food consumption, and the PER was highly improved (2.44 and 1.88, respectively, for meal with and without lysine). It is quite evident that sunflower-seed meal, which was found to result in a weight gain almost half that of casein, supported better growth after enrichment with lysine throughout the experimental period. The food consumption was higher, while the PER was lower (2.88 and 2.44, respectively, for casein- and lysine-enriched meal).

Surprisingly, addition of methionine to sunflower-seed meal did not improve growth and even resulted in lower gain in weight and PER values during the last eight days of the experiment. However, enrichment of sunflower-seed meal with both lysine and methionine resulted in superior nutritional criteria. This group of chicks showed weight gain 126 % higher than that fed non-enriched meal, which is also higher than the casein-fed group. The PER values were in the following increased order:

meal + methionine < meal < meal + lysine < casein < meal + lysine + metionine (1.41, 1.88, 2.44, 2.81 and 2.85, respectively). *Pokrovskii* et al. (12) came to a similar conclusion when feeding sunflower-protein isolates to rats. No improvement in the weight gain of rats was obtained by the addition of methionine, while the addition of both lysine and methionine, or only lysine resulted in great increase in the weight gain.

Amino-acid analysis of sunflower-seed meal supports the fact that lysine is the limiting amino acid (3).

Enrichment of sunflower-seed protein isolates with lysine, methionine, or mixture of both

Figure 3 is a diagrammatic representation of results of feeding sunflower seed protein isolate after enrichment with lysine, methionine, or both. Amino acids were added at a level of 0.3 % of the diet.

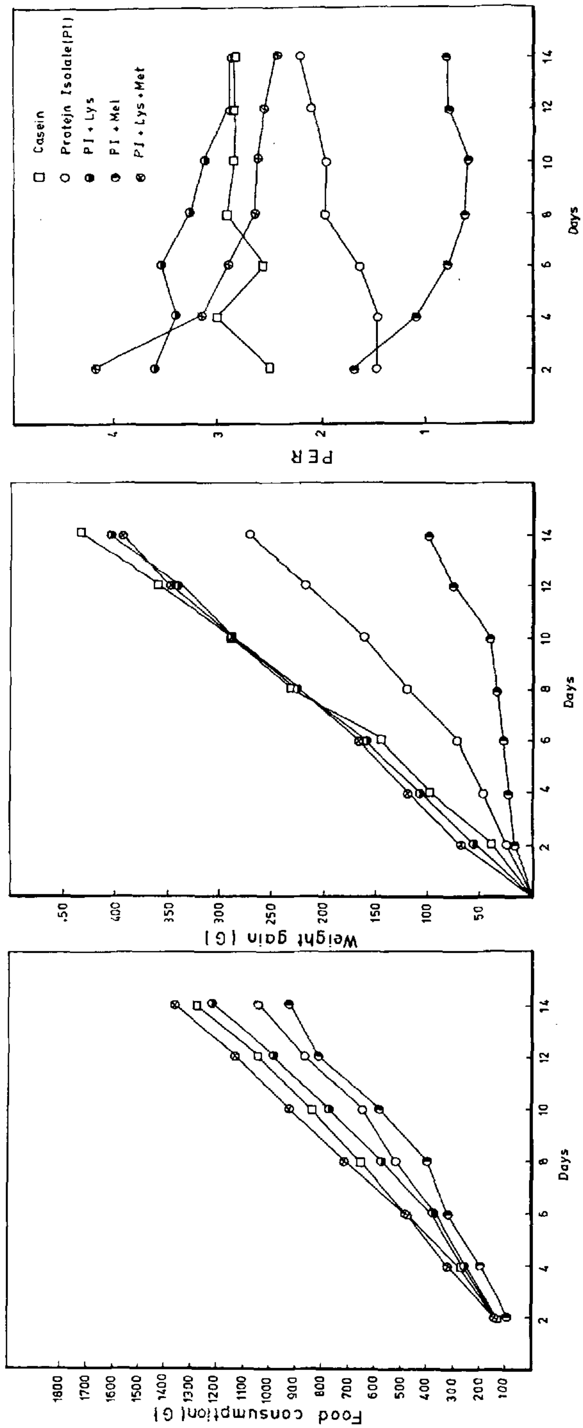

Fig. 3. Nutritional value of sunflower-seed protein isolate, enriched with lysine, methionine or mixture of both.

Similar to that found with sunflower-seed meal, enrichment of the protein isolate with lysine, or lysine + methionine resulted in great improvement of its nutritional quality, while enrichment with methionine resulted in a decrease in the nutritional quality of the isolate.

After two weeks of feeding, values for weight gain and PER were in the following order: non-enriched isolate 277, 2.21; lysine-enriched isolate 423, 2.91; lysine- and methionine-enriched isolate 396, 2.42; methionine-enriched isolate 100, 0.81.

From the overall findings of feeding sunflower-seed protein products the following conclusions could be drawn:

Lysine is the first limiting amino acid in sunflower-seed protein. Nutritional studies using chicks (13–16), rats (17–22), mice (23) and pigs (24–27) as experimental animals proved beyond any doubt the limitation of this amino acid in sunflower-seed meal and protein isolate.

Methionine enrichment of sunflower-seed products does not inprove the nutritional quality and even results in decreased growth rate of chicks, while enrichment with both lysine and methionine results in considerable improvement of the nutritive value. There has been a controversy in regard to methionine. While *Schiller* (18), *Robert* (21) and *Afifi* (13) reported difficiency; *Pokrovskii* et al. (12), *Nehring* et al. (28), *Gyorgy* (29) and *Taha* et al. (3) revealed adequacy.

Feeding sunflower-seed meal + lysine + methionine, or protein isolate + lysine results in weight gain and PER similar to or higher than casein. This is illustrated diagrammatically in Figure 4 where casein is given a value of 100.

The gain in weight of chicks fed non-enriched protein isolate, though far lower than those fed casein- or amino-acid-enriched isolate at early stages of chick growth. It is believed that the isolate might support comparable growth rate at later stages even without amino-acid enrichment.

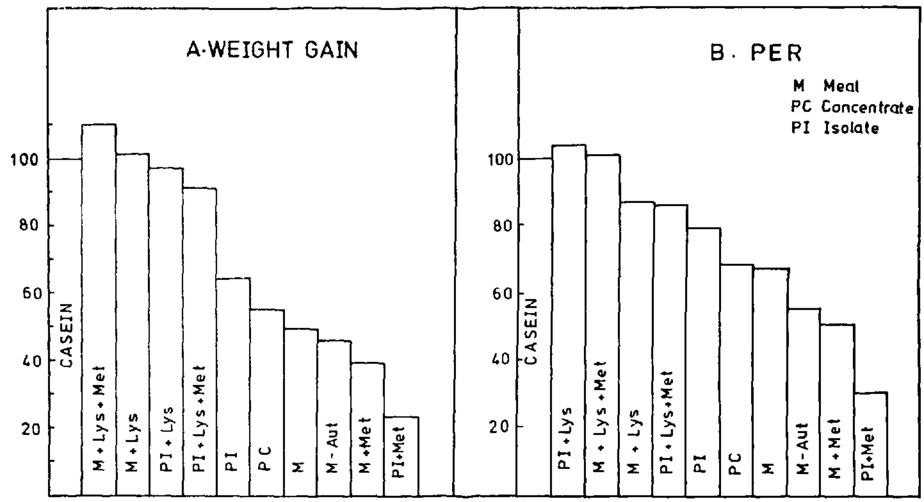

Fig. 4. Weight gain and PER compared to casein.

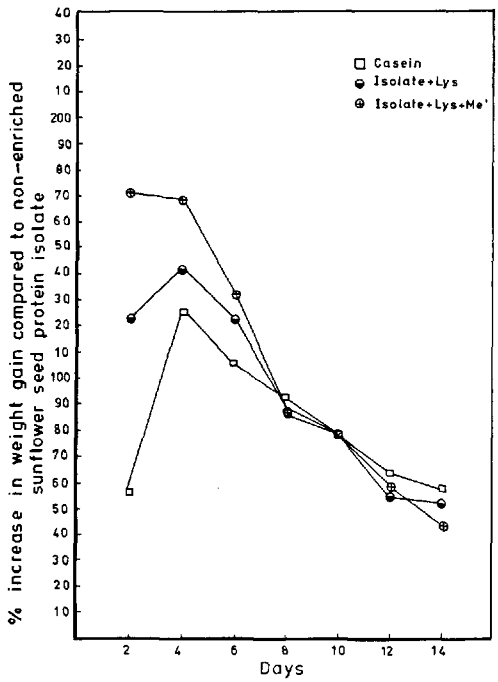

Fig. 5. Increase in weight gain of chicks fed casein, protein isolate enriched with lysine and protein isolate enriched with lysine and methionine compared to non-enriched protein isolate.

Figure 5 represents the percentages increase in the weight gain (Wt.G.) of chicks fed casein (CAS), sunflower-seed protein isolate enriched with lysine (PI+Lys), or lysine and methionine (PI+Lys+Met), compared to non-enriched protein isolate (PI), throughout the experimental period.

$$\frac{[\text{Wt.G. CAS (or) PI+Lys (or) PI+Lys+Met}] - [\text{Wt.G. PI}]}{[\text{Wt.G. PI}]} \times 100$$

The Figure illustrates that though the weight gains by the fourth day of feeding were 125, 141 and 168 % higher when feeding CAS, PI+Lys, PI+Lys+Met, respectively, than when feeding non-enriched protein isolate, yet the percentage decrease gradually to become 57, 59 and 43 %, respectively, by the fourteenth day.

Non-enriched sunflower-seed protein isolate supports better growth than non-enriched meal at later stages of chick growth. Figure 6 illustrates the percentage increase in the weight gain of chicks fed casein and enriched sunflower-seed meal compared to non-enriched meal throughout the experimental period. The Figure is similar to Figure 5, but with sunflower-seed meal.

The Figure shows that though there is a decrease in the percentage weight gain as the chicks grow, yet it is not as pronounced as in the case of the isolate. After the fourteenth day of feeding casein, meal + lysine, and

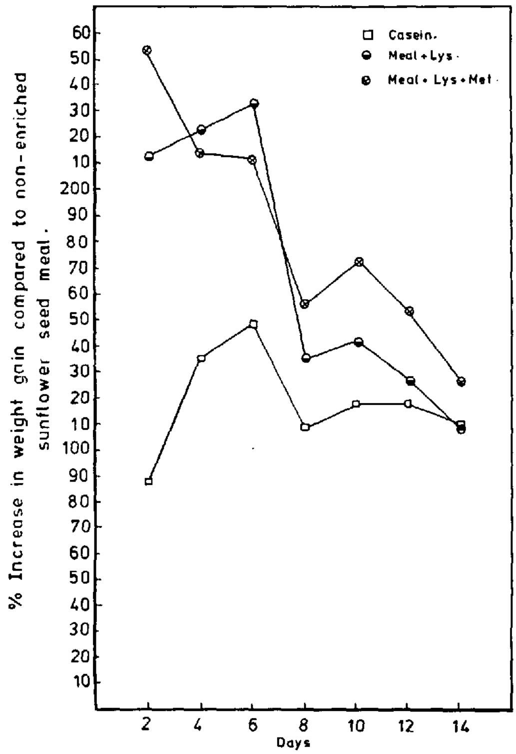

Fig. 6. Increase in weight gain of chicks fed casein, meal enriched with lysine and meal enriched with lysine and methionine compared to non-enriched meal.

meal + lysine + methionine, values were still 106, 108 and 126 %, respectively, higher than the non-enriched meal.

Summary

Defatted sunflower-seed meal, sunflower-seed protein concentrate and protein isolate were fed to protein-depleted chicks. The effect of the enrichment of meal and of protein isolate with lysine, methionine, or a mixture of both on the nutritional value was investigated. Sunflower-seed protein products are primarily limited in lysine. Food consumption, weight gain and protein/efficiency ratio reveal superior performance of lysine- or lysine- and methionine-enriched meal or protein isolate, as compared to the correponding non-enriched products. Methionine enrichment does not improve the nutritional quality and even result in inferior performance. Non-enriched protein isolate supports better growth than non-enriched meal specially at later stages of chick growth.

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