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Use of Silkworm (*Bombyx mori L.*) Pupae as a Protein Supplement in Poultry Rations

M. S. WIJAYASINGHE AND A. S. B. RAJAGURU
Department of Animal Husbandry, Faculty of Agriculture, University of Sri Lanka, Peradeniya Campus, Peradeniya, Sri Lanka.

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Abstract : An investigation was conducted to find the effect of various levels of replacement of local fishmeal with silkworm pupae (SWP) on the performance of broiler starters, broiler finishers and laying hens. The results indicated that SWP could successfully replace local fish-meal in poultry rations. The presence of an unidentified growth factor in SWP for chicks was also observed. Improvement in reproductive performance in terms of hatchability of eggs and weight of chicks at hatching time were observed when SWP was included in layers rations. A favourable alteration of the sex ratio in chicks towards feminity was observed.

1. Introduction

Little work has been done in Sri Lanka, to replace the traditional animal protein supplements in animal feed with by-products of agro-industrial origin. In silk manufacture, silkworm pupae (SWP) are discarded as a by-product during the reeling operation. At this stage, however, they are surrounded by a tough, chitinous covering of a gluco-proteinous material which must be removed before utilization for animal feeding.[6] With the rapid expansion of the sericulture industry in Sri Lanka it is expected that a large quantity of SWP would be available as a by-product. Both dry and wet methods have been employed for the removal of this chitinous covering. In the dry method, mechanical cutting, crushing and sifting is carried out in order to separate the chitin from pupal matter. In the wet methods, boiling is done either in water or in a dilute solution of caustic soda to soften the chitin, which is then peeled off mechanically or manually.[2]

The pupal matter that remains after the separation of the chitinous covering contains a high percentage of protein and oil. The pupal oil contains nearly 75% unsaturated fatty acids which has valuable industrial uses.[2,6] Both SWP and pupal residue after oil extraction have been used as animal feeds for chicken, pigs, rabbits and cattle and also as a food for freshwater fish.[2] The presence of large quantities of unsaturated fatty acids imparts a peculiar smell to the SWP and it is generally believed that flesh or eggs of animals fed on undefatted SWP have an unpleasant odour for the same reason.[1,2] However, this phenomenon was not observed in the investigations carried out by Habe *et al.*[1] and Yoshimura,[5] using undefatted SWP. Today, there are a number of patented biological and chemical methods for the deodourization of SWP. No attempt was made to deodourize or defat the pupal matter used in the investigation reported here. It was attempted in this study to evaluate the possibility of replacing local fishmeal with silkworm pupae in broiler starters, broiler finishers and layer rations.

2. Materials and Methods

2.1. Materials

A consignment of dried silkworm pupae with their chitinous coverings was obtained from the Central Sericulture Station, Pallekelle, Sri Lanka. This product was compared with Grade I fish meal produced locally.

2.2. Preparation of SWP

The pupae were first ground (in a feed grinder) to tear open the chitinous coverings and to release the enclosed pupal matter that was powdered in the process. The chitinous matter was then manually separated from the pupal powder. The turn out of the latter from this process was about 46%. This method is simple and involves no loss of soluble nutrients as in the wet methods. In order to obtain the maximum recovery, manual separation, although cumbersome, needs to be carried out carefully.

2.3. Chemical Analysis

The pupal material and the fish meal were analysed for major nutrients according to the methods recommended by the Association of Official Agricultural Chemists.[4] These analytical values are reported in the Table 1. The calcium and phosphorus levels were analysed spectrophotometrically. Amino acid analysis was done with the assistance of Dr. T. Kurose of Japan, using JLC-AH, Nihon Denshi (JEOL) automatic amino acid analyser.

TABLE 1. Chemical composition of Silkworm Pupae and Fish meal (%).

Constituents	Silkworm Pupae	Fish meal
Moisture	8.50	17.40
Ether Extract	19.47	2.77
Crude Protein	63.30	40.10
Crude Fibre	3.10	2.80
Ash	4.50	35.90
Nitrogen free extract	1.13	1.03
P_2O_5	2.03	—
$CaCO_3$	0.545	—

2.4. Feeding Trials

Three feeding trials, viz. I, II and III were conducted using broiler starters, finishers and layers, respectively. The experimental rations were balanced to carry nutrients according to the recommendations of the National Research Council of USA.[3] These rations were compared with the control rations in each trial (Table 3), containing local fish meal as the main source of animal protein.

2.5. Feeding Trial I

Experiment I, consisting of six treatments, was designed to test SWP in broiler starter rations (Table 2). The first four diets were formulated to be iso-caloric and iso-nitrogenous. The control ration (1) carried 15% local fish meal. The experimental rations 2 and 3 carried 10% and 12% SWP, respectively. SWP was the only source of animal protein in the diet 3. Ration 4 was the same as Ration 3 except that the former carried 60 grams (2 ounces) of lysine per 50 kg of the ration. Rations 5 and 6 were formulated to determine the possible presence of an unidentified growth factor in SWP. Ration 5 was balanced above the recommended nutrient requirements for starter chicks. Ration 6 was made by mixing 100 parts of Ration 5 and 5 parts of SWP.

The above rations were fed in mash form to one week old, Cornish × White Rock broiler starter chicks for a period of five weeks. Every treatment was replicated twice and carried 10 chicks in each. The replicates were arranged in a randomized block design. All chick groups were equalized for weight initially and were raised in a Multiplo Battery Brooder during the entire experimental period. Feed and water were provided *ad libitum*. Observations were made on weekly weight gains (G) and feed consumption (F). From the data thus obtained, the average of the weekly feed efficiency $\left(\frac{F}{G}\right)$ was calculated.

TABLE 2. Amino acids in protein extracted from Silkworm Pupae.

Amino Acid	Mass g/100g protein
1. Alanine	3.89
2. Arginine	4.62*
3. Aspartic acid	8.65
4. Cystine	0.35
5. Glutamic acid	8.65*
6. Glycine	3.46*
7. Histidine	2.25
8. Isoleucine	3.77*
9. Leucine	6.02*
10. Lysine	5.31*
11. Methionine	1.75*
12. Phenylalanine	4.25*
13. Proline	1.60*
14. Serine	3.68
15. Threonine	3.83*
16. Tyrosine	4.80
17. Valine	4.59*
18. Ammonia	1.35
Total Recovery	73.29

*Amino acids essential for chicken.

TABLE 3. Percentage composition of rations used.

	EXPERIMENT I						EXPERIMENT II				EXPERIMENT III	
Ingredients		Control	Experimental	Diets			Control	Experimental Diets			Control	Exp. Diets
	1	2	3	4	5	6	1	2	3	4	1	2
Corn	49.00	50.00	50.50	50.50	46.00	46.00	60.00	62.00	62.00	62.00	56.5	59.5
Rice Bran	—	1.50	4.00	4.00	—	—	—	1.00	1.00	1.00	—	—
Gingelly poonac	12.00	12.00	12.00	12.00	12.00	12.00	15.00	15.00	15.00	15.00	8.00	8.00
Coconut poonac	18.00	18.00	18.00	18.00	12.00	12.00	10.00	10.00	10.00	10.00	20.00	20.00
Fish meal	15.00	—	—	—	15.00	15.00	10.00	—	—	—	10.00	10.00
Skim milk powder	5.00	5.00	—	—	15.00	15.00	5.00	5.00	2.50	—	2.00	—
Silkworm pupae	—	10.00	12.00	12.00	—	5.00	—	5.00	7.50	10.00	—	7.5
Bone meal	1.00	3.00	3.00	—	—	1.00	1.00	1.00	1.00	1.00	1.10	2.5
Shell powder	—	0.50	0.50	0.50	—	—	—	1.00	1.00	1.00	2.5	3.5
Lysine	—	—	56 gm	56 gm	28 gm	28 gm	—	—	—	—	2.5	3.5
Methionine	28 gm	—	—	—	28 gm	28 gm	—	—	—	—	—	—
Vitamin Premix	100 gm	100 gm	100 gm	100 gm	100 gm	100 gm	100 gm	100 gm	100 gm	100 gm	100 gm	100 gm
Coccidostat	100 gm	100 gm	100 gm	100 gm	100 gm	100 gm	100 gm	100 gm	100 gm	100 gm	100 gm	—

2.6. Feeding Trial 2

Experiment II included four treatments and was designed to test SWP in broiler finisher rations (Table 2). The control ration (1) carrying local fish meal at 10% level was compared with Rations 2, 3, and 4, carrying 5%, 7.5%, and 10% SWP respectively.

The above rations were fed to six weeks old Cornish × White Rock, broiler finisher chicks for a period of five weeks. The experimental design followed in this experiment was similar to that of Experiment I. Here the birds were housed in colony cages during the entire experimental period. Weekly weight gains and feed consumption were recorded. Feed efficiency was calculated from the data thus obtained. At the conclusion of the experiment, 3 birds from each replicate were slaughtered and dressed to estimate the carcass recovery percentage.

2.7. Feeding Trial 3

Experiment III was designed to test SWP in layer rations and to study its effects on egg production, egg weight, egg quality, shell thickness, egg palatability, fertility, hatchability, weight and percentage of normal chicks at hatching time and their sex ratio. This experiment consisted of two treatments. The Treatment 1 which was the control ration carried 10% local fish meal and the Treatment 2 which was the test ration 7.5% SWP, without any fish meal (Table 2). The treatments were replicated twice and each replicate carried eight, one year old White Leghorn hens which were housed in individual battery cages. The hens were artificially inseminated twice a week, using pooled semen collected from cock birds maintained on a commercial diet. The experiment was conducted over a period of six weeks. During this period egg production and individual egg weight were recorded daily and the feed consumption on a weekly basis. Feed efficency was then calculated to express the amount of feed consumed per dozen eggs produced. Except during the first week of the experimental period, shell thickness and albumen height were measured in six eggs taken at random from each replicate, every week. Using egg weight and albumen height, Haugh Index was calculated as a measure of internal quality of eggs. In order to find out any differences in palatability, flavour and odour, a representative number of eggs from both treatments were hard-boiled, halved and served to a taste panel. Eggs were hatched weekly after the first week from the commencement of the trial and for five weeks. Candling of incubated eggs was carried out every 7th and 14th day of each incubation and fertility was calculated :

$$\% \text{ Fertility} = \frac{\text{Number of fertile eggs} \times 100}{\text{Number of eggs set}}$$

At the end of each incubation, the number of chicks hatched out was estimated to calculate the hatchability =

$$\frac{\text{Number of chicks hatched} \times 100}{\text{Number of fertile eggs}}$$

The percentage of normal chicks at hatching time was also recorded. The chicks were sexed by the vent sexing method and the sex ratio for each treatment was recorded.

3. Results

3.1. Chemical Analysis

Table 1 presents the proximate analysis of SWP and fish meal. The amino acid composition of SWP is given in Table 2. SWP was higher in dry matter, protein and fat content when compared to the locally produced fish meal, but relatively low in total ash and in calcium and phosphorus.

3.2. Feeding Trial 1

The total and weekly weight gains, total feed consumption and the average feed efficiency of Experiment I are given in Table 4. Here the weekly as well as the total weight gains of chicks on Ration 6 were superior to those on all other rations throughout the entire experimental period. ($P = 0.01$). Rations 3 and 4 were not significantly different from the control. Also the Rations 2, 3, 4, and 5 were not significantly different from each other.

TABLE 4. Weight gains, feed consumption, and feed efficiency of Experiment I

Treatments		Total Weight Gains (g)	Total Consumption (g)	Average Feed Efficiency
Control	1	1071.00	4323.5	4.00
	2	2050.50	5379.4	2.55
	3	1484.75	4024.7	3.05
	4	1561.75	4553.2	2.85
	5	1960.25	4984.9	2.67
	6	3776.50	7772.9	2.22
Least Significant Difference	5%	808.02	1585.15	0.81
	1%	1223.57	2400.37	1.24

Feed consumption of birds on Ration 6 was significantly superior to those on Ration 2 at (P = 0.05) level and to all other groups at (P = 0.01). There were no significant differences among Rations 1 to 5.

Ration 6 had the highest feed efficiency. The feed efficiency of Rations 2 to 5 were significantly (P = 0.05) superior to the control, but there was no significant difference between them.

3.3. Feeding Trial 2

In Experiment II, Rations 2, 3 and 4 were significantly (P = 0.05) superior to the control in terms of total weight gains, feed consumption and average feed efficiency for the entire experimental period (Table 5). Rations 3 and 4 did not differ significantly in both weight gains and feed consumption. Feed efficiency of birds on Rations 2 and 3 and birds on Rations 3 and 4 did not differ significantly. Ration 2 however was significantly (P = 0.05) superior to Ration 4 in feed efficiency. Carcass recovery percentages have been increased with the increase in the SWP in rations, except for the Treatment 2. Ration 4 containing 10% SWP gave the highest increase in recovery percentage, which was superior to the rest of the rations at a statistical significance of 1% level.

TABLE 5. Weight gains, feed consumption and feed efficiency and carcass recovery percentages—Experiment II.

Treatments		Total Weight Gain (g)	Total Feed Consumption (g)	Average Feed Efficiency	Carcass Recovery percentages
Control	1	3773.00	13,588	3.97	68.80
	2	5096.00	15,442	2.90	67.55
	3	5796.00	17,976	3.03	69.75
	4	5936.00	18,452	3.13	70.85
	5	—	—	—	—
	6	—	—	—	—
Least significant Difference	5%	187.57	1581,60	0.153	1.56
	1%	310.27	2617,03	0.253	2.58

3.4. Feeding Trial 3

In Experiment II, differences were observed between the control and the test ration, in terms of total egg production, feed consumption, feed efficiency, average egg weight and egg quality, and shell thickness (Table 6). On the other hand most of the measurements of character associated with reproductive performance, obtained for the test ration were significantly superior to those on the control ration (Table 7).

Significant (P = 0.05) differences were seen in percentage hatchability and in average weight of chick at hatching time. There were no significant differences in percentage fertility and percentage normal chicks at hatching time. Assuming a null hypothesis of 1 : 1, the "Chi-Square" test was applied on the total number of male and female chicks obtained for each treatment. The deviation observed in the control ration did not differ significantly from the expected values. However the deviation observed in the test ration differed significantly (P = 0.01) from the expected values. The sex ratio in chicks was altered favourably by the inclusion of SWP in a layers ration, with an increase in the number of female chicks over the number of males.

TABLE 6. Effects of SWP on the performance of laying hens, during 6 weeks of experimental period.

Treatment		Total egg production (No.)	Total feed consumption (kg)	Feed Efficiency for a dozen of eggs	Average egg weight (kg)	Egg quality (Haugh Units)	Shell Thickness (× 0.0025cm)
Control	I	156	31.38	2.76	58.85	86.88	0.034
	II	175	33.64	2.41	57.4	86.19	0.032

Statistically not significant.

TABLE 7. Effects of SWP on hatchability, fertility, hatch weight, vigour and sex ratio at hatching time.

Treatment		Average hatchability	Average fertility	Vigour at hatching time	Hatch weight of chickens	Sex Ratio	
						Male	Female
Control	I	65.63	84.7	86.80	44.05	48±	52±
	II	78.57	85.7	94.07	40.92	64*	36*
Least Significant Difference	5%	9.80			2.37		
	1%	22.62			5.46		

* Statistically significant
± Statistically not significant.

4. Discussion

The results of Experiment I suggest that local fish meal could be successfully replaced by SWP upto 12% level. Weight gains of birds on Ration 2 over those on the control were superior by about 48%. This was probably due to the higher biological value of the mixture of proteins in Ration 2 which contained both SWP and skim milk powder. Lack of significant differences between Rations 3 and 4 shows that lysine supplementation of SWP containing rations when the SWP is used as the only source of animal protein, is not essential. The lack of significant differences between Rations 3 and 4 and between the control ration indicates that SWP could successfully replace fish meal and skim milk powder in chick ration. This finding is strengthened by the fact that the nutritive value of SWP protein when compared with casein is 134%.[6] The extremely superior weight gains, feed consumption and feed efficiency of birds on Ration 6 over those on Ration 5 during the entire experimental period, resulting in an increase in total weight gains by about 48%, indicates very clearly that there is an unidentified growth factor for chicks in SWP.

Results of Experiment II point out that SWP could successfully replace local meal in broiler finisher rations either in combination with skim milk powder or when used as the only source of animal protein. The superior performance of birds on Ration 2 over those on Ration 4 confirms the complementary combining effect of SWP and skim milk powder, which was observed in Experiment I.

In Experiment III, the absence of significant differences in egg production, egg weight, egg quality may be because of the fact that SWP when used at 7.5% level in the test ration supplied adequate amounts of amino acids and protein equivalent to the 10% local fish meal plus 2% skim milk powder. However, Sakia *et al.*[5] report that inclusion of SWP in layer rations improved feed efficiency and egg weight, and reduced mortality and increased egg laying.[7] The maintenance of adequate levels of calcium in SWP containing ration is the reason for the lack of significant differences in the shell thickness. The participants of the taste panel were unable to detect any differences in flavour, odour or palatability in eggs from the two treatments. Similar results have been reported by Habe *et al.*[1] and Yoshimura.[7] It is likely that the influence of SWP on the flavour, odour and palatability of animal products depend on the level of inclusion of this material in rations. The improvement of reproduction performance in birds fed on SWP containing diets have also been reported by Yoshimura.[7] These findings were evident in the present investigation in which the inclusion of SWP in a layers ration improved hatchability, average weight of chick at hatching time and femininity in chicks hatched out.

5. Conclusions

The results of the three trials undertaken in this investigation to test the feasibility of using SWP in poultry rations indicate that it can be used in place of local fish meal, as a source of animal protein in chick starter, broiler finisher and layer rations. A higher level of calcium and phosphorus needs to be maintained in rations carrying SWP. In view of the improvement in reproductive performance observed in this study and in studies carried out elsewhere, it would be particularly useful to include SWP in breeders rations. In order to enable poultry farmers to use this material as a protein supplement in feeds, the price of SWP needs to be more realistic than at present.

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