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Replacement of Soyabean Meal in Poultry Feed with De-Oiled Silkworm Pupa Cake obtained through Pilot-Scale Supercritical Fluid Extraction Technology

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ABSTRACT

Surge in price of soyabean in the domestic and international market has led to a hunt for potential alternative for protein sources in poultry feed. Silkworm pupa is a rich nutrient reservoir of fats, protein, minerals, essential amino acids etc., which is underutilized for its potential nutritional applications. Despite its rich nutritional value, it is considered as a waste and discarded unscientifically resulting in serious environmental concerns. Therefore, in the present study De-oiled Silkworm Pupa Cake (DOSPC) rich in protein (64.88%), obtained from defatting the discarded silkworm pupa through a pilot scale Supercritical Fluid Extraction technology, was used as a sustainable alternative to soyabean meal in poultry feed. For the present study, 1500 numbers of broiler chicks (day old chicks) of COBB variety were selected and divided into 5 experimental groups and 10 replicates per group with 30 birds per replicate, which were fed in amounts Control Diet comprising 100% Soyabean meal and 0% DOSPC(Group 1), Diet with 25% DOSPC and 75% Soyabean meal (Group 2), Diet with 50% DOSPC and 50% Soyabean meal (Group 3), Diet with 75% DOSPC and 25% Soyabean meal (Group 4) and Diet with 100% DOSPC and 0% Soyabean meal (Group 5). Experimental trials were conducted for 6 weeks and details of average body weight gain, feed consumption and FCR was collected for each week. The results from this study suggested that Soyabean meal can be replaced with DOSPC upto 50% and/or 75% for optimum FCR and Body weight gain in the birds. Both group 3 and 4 reported similar output with no significant variation. Body weight gain of 2610 g/bird was observed in the treatment group 3 compared to the control group which was 2324 g/bird, FCR of 1.36 was observed in Group 3 which was significantly better than the FCR of the control group which was 1.52. The immunological assay against Newcastle Disease (ND) and Infectious Bursal Disease (IBD) revealed improved immune response in treatment groups supplemented with DOSPC in their feed. The organometry, histopathological, gut health and overall growth performance of the treatment group was on par with the control group and this suggested that replacement of Soyabean Meal with 50% and/or 75% is beneficial for poultry farming in terms of better nutritional intake for the birds and profitability for the poultry farmers.

Keywords: *De-oiled silkworm pupa cake, Supercritical fluid extraction, Poultry, Broiler, Feed, FCR, Growth, Omega 3 Fatty Acid, ELISA, Immunological Assay and Carcass traits etc.,*

1. INTRODUCTION

Poultry industry is highly sensitive to price fluctuations of the feed ingredients. Feed contributes to 60%-70% of the cost in poultry rearing (TNAU Agri portal Animal Husbandry, 2015), wherein maize comprises 55-60% of the feed and soyabean meal comprises 30-35% approximately. In India, poultry meat contributes to 37% of the total animal protein consumption (Ahmed and Islam, 1990), but the poultry industry has been facing setbacks due to the prices and availability of key ingredients of the feed. Soyabean meal contributes to 30% of the poultry feed composition. COVID19 related logjams have resulted in a sudden jump in the prices of soyabean meal. The prices of soyabean meal was INR 30/kg in March, 2020 which plunged to a record high of INR 92.5/kg in July, 2021. This has gravely impacted the poultry sector.

Most common alternative to soyabean meal protein in poultry feed is the fishmeal. However, it is expensive, usually contaminated and loaded with pesticides which has lethal impact on the poultry farms (Khatun *et al.,* 2003). Focus is being slowly shifting towards insects as the future protein resources and one of the most promising protein source from the insects is the silkworm pupa, most underutilized material produced in the sericulture industry.

In the Sericulture industry, only 20% of the silkworm cocoon is converted into yarn and fabric. The remaining 80% comprises underutilized materials like silkworm pupa which is discarded as waste. The annual silk cocoon production in India is 1,35,080 Metric Tonnes (Central Silk Board Annual Report, 2019-20) and silkworm pupa produced is 1,01,310 Metric Tonnes. It is highly underutilized material produced in the sericulture industry, which when discarded unscientifically results in environmental hazards. In many Asiatic countries, silkworm pupa, is considered as a folk medicine, dietary supplement and a delicacy due to its high nutritional and therapeutic importance. The silkworm pupa is rich in Omega 3 Fatty Acids, protein, antioxidants, essential Amino acids, minerals, vitamins and other therapeutic phytochemicals. Silkworm pupa is one of the most novel sources of Omega 3 Fatty Acids and it has been estimated that if the entire 1,01,310 Metric Tonnes of silkworm pupa is completely utilized to produce Omega 3 Fatty Acids, then it can cater to the Omega 3 Fatty Acid requirement of 29 million infants for the entire year with an RDA of 0.5g/day.

However, it is not effectively used and as per the review of literatures, it was reported that silkworm pupa powder as such was being used in the feed for aqua and poultry, but, issues pertaining to odour and infestation eventually result in affecting the quality of the feed. Kongsup *et al*., (2022), Khatun *et al*., (2005) and Das *et al*., (1972) have also reported that, silkworm pupa powder as such can be used in the poultry feed. DOSPC has superior amino acid profile when compared to soyabean meal as it has higher content of cysteine, methionine, tyrosine and lysine which are essential in poultry growth performance (Bandlamori *et al*., 2012 and Banaszkiewicz T 2011)

As per the literature reviews, it was found that, conventional techniques were used for production of silkworm pupa meal. However, in the present study pilot scale supercritical fluid extraction technology has been used for the extraction of silkworm pupa oil enriched with Omega 3 Fatty Acid and remnant after extraction, which is protein rich DOSPC powder that is odorless and suitable for replacement of soyabean meal in the poultry feed. The amino acid profile of the DOSPC is on par with the fish meal and superior to soya meal. DOSPC is a sustainable alternative to cut down the surging costs and decline in the availability of the soyabean meal. Keeping the above scenario in mind, the present study was undertaken with the aim to utilize the DOSPC for partial replacement of soyabean meal in the poultry feed to reduce the cost of production and improve the economic parameters in the poultry industry.

2. MATERIAL AND METHODOLOGY

The spent silkworm pupa of CSR2 x CSR4 were collected from the silk reelers of Chikkaballapur district. Silkworm pupa collected with initial moisture content of 82.88% was subjected to standardized process of tray drying at 60℃ to obtain dried silkworm pupa with moisture content of less than 10%. Following this, fine silkworm pupa powder was obtained by pulverizing and sieving the dried material.

The silkworm pupa powder was subjected to the established protocol of supercritical fluid extraction process at 280 bar pressure, 35℃ temperature and 26 g/min CO_2 flow rate to obtain silkworm pupa oil enriched with Omega 3 Fatty Acids. The pharmaceutical grade silkworm pupa oil rich in Omega 3 Fatty Acids was converted into various nutraceutical products for human, poultry and aqua feed application.

In the present study, the remnant after the extraction of the oil known as the DOSPC was taken for the replacement of soyabean meal in the poultry feed industry.

Proximate analysis: Moisture, protein, fat and fiber contents were assayed by AOAC, 1984 and 2019 standard methods.

Application of silkworm pupa cake in poultry feed: Poultry feed trial comprising DOSPC was carried out in Bangalore. Soyabean meal was replaced in the trials to study the effectiveness of silkworm pupa cake as an alternative to soyabean meal for poultry's growth, overall performance, feed intake etc.,

A total of 1500 numbers of unsexed day-old commercial broiler chisks (COBB Variety) were distributed randomly into 5 groups/formula. A completely randomized design with 5 groups and 10 replicates in each group were used. Each replicate had 30 birds for the trial.

Group 1 (100% Soyabean meal+0% DOSPC), Group 2 (75% Soybean meal + 25% DOSPC), Group 3 (50% Soybean meal + 50% DOSPC), Group 4 (25% Soybean meal + 75% DOSPC) and Group 5 (0% Soybean meal + 100% DOSPC). Birds were fed with separate diet for pre-starter, starter and finisher stages as per the BIS 2007, Indian Standard POULTRY FEEDS-SPECIFICATION, fifth Revision, during the experimental period. In the experimental trials, the soyabean meal was replaced with DOSPC in different percentages for pre-starter, starter and finisher stages. Feed Concentrate for pre-starter, starter and finisher comprising of vitamins, minerals etc., was procured from M/s Aspartika Biotech Pvt. Ltd., Bangalore, India. The formulations are tabulated in Table 1, Table 2 and Table 3.

Table-1: Formulation of Feed for Pre-starter Poultry birds

Pre-starter	Control	Treatment			
	Group 1	Group 2	Group 3	Group 4	Group 5

Sl No	Ingredient	Qty (kg)	Qty (kg)	Qty (kg)	Qty (kg)	Qty (kg)
1	Maize	570	570	570	570	570
2	Soyabean meal	365	273.75	182.5	91.25	0
3	Vegetable Oil	15	15	15	15	15
4	DOSPC	0	91.25	182.5	273.75	365
5	Aspartika's Feed Concentrate- Pre-starter	50	50	50	50	50
	Total	1000	1000	1000	1000	1000

Table-2: Formulation of Feed for Starter Poultry birds

Starter		Control	Treatment			
		Group 1	Group 2	Group 3	Group 4	Goup 5
Sl No	Ingredient	Qty (kg)	Qty (kg)	Qty (kg)	Qty (kg)	Qty (kg)
1	Maize	625	625	625	625	625
2	Soyabean meal	300	225	150	75	0
3	Vegetable Oil	25	25	25	25	25
4	DOSPC	0	75	150	225	300
5	Aspartika's Feed Concentrate-Starter	50	50	50	50	50
	Total	1000	1000	1000	1000	1000

Table-3: Formulation of Feed for Finisher Poultry birds

Finisher		Control	Treatment			
		Group 1	Group 2	Group 3	Group 4	Goup 5
Sl No	Ingredient	Qty (kg)	Qty (kg)	Qty (kg)	Qty (kg)	Qty (kg)
1	Maize	640	640	640	640	640
2	Soyabean meal	270	202.5	135	67.5	0
3	Vegetable Oil	40	40	40	40	40
4	DOSPC	0	67.5	135	202.5	270
5	Aspartika's Feed Concentrate-Finisher	50	50	50	50	50
	Total	1000	1000	1000	1000	1000

Mortality of the birds were recorded every week from 1st week to 6th week in all the groups. The mortality percentage was calculated by recording the total dead birds throughout the experimental period and dividing by the total number of birds used in the trial.

Immunological assay: About 2 ml of blood was collected in non-heparinized tubes from 10 birds in each group at weekly interval by puncturing the brachial vein. Serum was collected after 8 to 10 hours as per standard procedure (Calneck *et al*., 1992) and was stored at –200°C for subsequent analysis. The serum samples were assayed for antibody titer against Newcastle disease (ND) by HI test and Infectious Bursal disease (IBD) using ELISA technique to assay the immune response in the birds.

Gut pH value was evaluated for the trial groups. Blood samples were obtained weekly in heparinized tubes from the brachial vein of birds (6 birds per replicate). Blood samples were centrifuged at 3000 RPM for 15 minutes to separate clear plasma which was stored at-20°C. pH values in plasma were determined by Electric pH meter.

Biochemical parameters like blood constituents, serum enzymes, protein fractions and minerals were evaluated in all the 5 groups of birds. The Ebra Biochemical analyzer XL-1000 model was used for serum protein analysis by poultry pathology lab. Random samples were collected from 10 birds in each study (replicates of each study groups).

Intestinal histopathology: From each bird, 1 cm of the jejunum proximal to the vittelline diverticulum was sampled and fixed in 4% neutral buffered formalin. The sample was then embedded in paraffin, sliced in 4 µm-thick slices, and haematoxylin and eosin (H&E) stained. General histopathological examination was performed to check presence of crypt abscesses, erosion of villus tips and mixed infiltrate. Histopathology of caecal parts were done by paraffin wax (58-600) embedding method.

Organometry: The samples were collected from all trial groups of broiler study conducted at development of vital organs like liver, gizzard, heart, spleen, thymus and bursa weight were studied.

Birds of each replicate were housed in an independent pen in an open sided deep litter house. Brooding temperature as per the standard requirement was maintained till three weeks of age by using incandescent bulbs. Birds were provided with continuous light throughout the trial. Each pen was fitted with a semi-automatic bell type drinker and a hanging circular feeder. Standard vaccination schedule was followed for immunizing the birds. All the birds were reared under uniform standard conditions throughout the study as per poultry farm manual, a reference guide for central & state poultry farms 2014-15 by the Department of Animal Husbandry Dairying and Fisheries, Ministry of Agriculture and Farmers Welfare, Government of India.

The feed intake, average weight gain, FCR, mortality, overall performance like immune response against New Castle Disease and Infectious Bursal Disease was evaluated in the present study. The report was obtained from 10 birds from each replicate of each group.

Statistical analysis: The statistical analysis of data was carried out according to the methods described by Snedecor and Cochran (1968).

3. RESULTS

Wet silkworm pupa collected from Chickballapura district having average moisture content of 10 trials was 82.88%. After drying process, the final average moisture content in the dried silkworm pupa was 9.22%.

The supercritical fluid extraction of the dried, powdered and sieved silkworm pupa yielded 30% silkworm pupa oil rich in Omega 3 Fatty acids ($\geq$ 45%) and 70% of DOSPC rich in protein (64.88%).

Proximate analysis of the DOSPC: The protein %, moisture %, fat% and fiber% of the DOSPC was recorded to be 64.88%, 6.57%, 1.52% and 2.68% respectively (Table-4).

Table-4: Proximate Analysis of DOSPC

Parameters	Result Average of 6 samples (%)
Moisture %	6.57 ± 0.14
Protein %	64.88 ± 0.01
Fat %	1.52 ± 0.01
Fiber %	2.68 ± 0.06

Replacement of Soyabean meal with DOSPC in poultry feed: The feed formulation in the group 3 with 50% of the soyabean meal replaced by 50% DOSPC as protein source, resulted in optimum body weight gain in the poultry birds which was recorded as 2610 g/bird as compared to the control group which was 2324 g/bird, thus showing significant improvement in the body weight gain.

There was no significant variation in the average body weight gain in the birds of Group 3 comprising 50% DOSPC as protein source and Group 4 comprising 75 % DOSPC as protein source.

The average body weight gain/ bird is tabulated in Table-5.

Table-5: Average Weight Gain (g) studies of poultry birds

Duration	CONTROL GROUP	TREATMENT GROUPS			
	Weight Gain in Group 1 (Average of 10 replicates) (g)	Weight Gain in Group 2 (Average of 10 replicates) (g)	Weight Gain in Group 3 (Average of 10 replicates) (g)	Weight Gain in Group 4 (Average of 10 replicates) (g)	Weight Gain in Group 5 (Average of 10 replicates) (g)
I Week	154.2 ± 1.46	164.6 ± 1.12	165.3 ± 1.02	165.9 ± 1.14	166.4 ± 1.21
II Week	220.06 ± 9.91	241 ± 10.91	247.6 ± 7.69	243 ± 6.84	248.2 ± 7.31
III Week	614 ± 5.15	629 ± 7.00	631 ± 6.80	630 ± 5.41	630 ± 7.02
IV Week	1166 ± 2.53	1259 ± 3.61	1274 ± 12.02	1273 ± 7.97	1272 ± 6.68
V Week	1762 ± 4.05	1955 ± 6.55	1957 ± 7.15	1951 ± 11.12	1950 ± 8.43
VI Week	2324 ± 10.11	2437 ± 6.97	2610 ± 9.41	2603 ± 12.86	2605 ± 12.09

The cumulative feed consumption of birds in group 3 was recorded as 3548 g/bird (for 42 days) as compared to control group with cumulative feed consumption recorded as 3536 g/bird. There was no significant variation in the cumulative feed consumption between group 3 and group 4. The cumulative feed consumption per bird is tabulated in Table-6

Table-6: Average feed consumption (g) studies of poultry birds

Duration	CONTROL GROUP	TREATMENT GROUPS

	Feed consumption in Group 1 (Average of 10 replicates) (g)	Feed consumption in Group 2 (Average of 10 replicates) (g)	Feed consumption in Group 3 (Average of 10 replicates) (g)	Feed consumption in Group 4 (Average of 10 replicates) (g)	Feed consumption in Group 5 (Average of 10 replicates) (g)
I Week	188.6 ± 0.37	192.8 ± 1.48	191.6 ± 1.39	191.7 ± 1.44	191.3 ± 1.41
II Week	364.6 ± 3.75	365.6 ± 4.46	367.9 ± 4.50	366.9 ± 4.35	367.9 ± 4.50
III Week	949 ± 7.66	992 ± 7.02	991 ± 6.97	987 ± 7.71	991 ± 6.98
IV Week	1662 ± 7.46	1641 ± 10.94	1655 ± 6.03	1653 ± 4.06	1656 ± 6.12
V Week	2120 ± 2.57	2199 ± 12	2209 ± 9.85	2212 ± 8.95	2209 ± 9.48
VI Week	3536 ± 15.03	3584 ± 31.98	3548 ± 14.45	3548 ± 15.10	3551 ± 13.01

Optimum FCR of 1.36 was observed in birds of group 3 which was significantly better than the FCR of the control group which was 1.52.

There was no significant variation in the FCR between group 3 and group 4.

The FCR is tabulated in Table-7.

Table-7: FCR studies of poultry birds

Duration	CONTROL GROUP	TREATMENT GROUPS			
	FCR Group 1 (Average of 10 replicates)	FCR in Group 2 (Average of 10 replicates)	FCR in Group 4 (Average of 10 replicates)	FCR in Group 4 (Average of 10 replicates)	FCR in Group 5 (Average of 10 replicates)
I Week	1.22	1.17	1.16	1.16	1.15
II Week	1.68	1.55	1.50	1.52	1.49
III Week	1.55	1.58	1.57	1.57	1.57
IV Week	1.43	1.30	1.30	1.31	1.30
V Week	1.20	1.12	1.13	1.13	1.13
VI Week	1.52	1.47	1.36	1.36	1.37

Mortality: The Mortality percentage is presented in the Table-8 and the data recorded 1.67% to 2% mortality. There is no significant difference in the mortality of birds in all the groups.

Table-8: Mortality studies of poultry birds

Duration	Group 1	Group 2	Group 3	Group 4	Group 5
Total Mortality (1st Week to 6th Week)	6	6	5	5	6
Mortality %	2.00	2.00	1.67	1.67	2.00

Immunological assay: The average titer value of all the replicates of each group is presented below. The antibody titer data presented below is the average value of the titer of all the 10 replicates of each group, where 10 birds were picked up randomly for the study. The status of antibody titer in birds against Newcastle disease measured at weekly intervals in various groups was assayed. The mean HI titer values were significantly higher in the treatment groups 3, 4 and 5 as compared to the Control group and the treatment group 2 during all the weeks (Table-9).

Table-9: Average HI titer values of antibodies against Newcastle Disease (ND) of birds

Interval	Average of Group 1 (Average of 10 replicates)	Average of Group 2 (Average of 10 replicates)	Average of Group 3 (Average of 10 replicates)	Average of Group 4 (Average of 10 replicates)	Average of Group 5 (Average of 10 replicates)
14th day	1882 ± 28.2	2226 ± 52.6	3188 ± 11.9	3128 ± 12.2	2987 ± 91.9
21st day	1645 ± 35.6	3421 ± 63.3	3510 ± 51.1	3150 ± 16.1	3369 ± 55.1
28th day	2428 ± 70.9	2783 ± 5.7	2792 ± 17.6	3096 ± 8.7	3972 ± 12.8
35th day	2003 ± 14.3	2402 ± 48.3	3127 ± 12.3	3117 ± 7.2	3271 ± 62.3
42nd day	1690 ± 41.3	2276 ± 55.1	2371 ± 12.4	2471 ± 2.2	3268 ± 22.2

Similar to the HI titer, week wise serum collected from different birds was subjected to determination antibody titer of IBD by ELISA method. The mean values of ELISA titer under different treatment groups during the 7-day intervals are presented in the table below. As a general trend, the immune response against IBD was more Groups 3, 4 and 5 as compared to the Control group and the treatment group-2 during all the weeks (Table-10).

Table-10: Average HI titer values of antibodies against Infectious Bursal Disease (IBD) of birds

Interval	Average of Group 1 (Average of 10 replicates)	Average of Group 2 (Average of 10 replicates)	Average of Group 3 (Average of 10 replicates)	Average of Group 4 (Average of 10 replicates)	Average of Group 5 (Average of 10 replicates)
14th day	2042 ± 47.7	2131 ± 63.5	2544 ± 32.7	2784 ± 11.2	2654 ± 17.5
21st day	1922 ± 16.5	1930 ± 18.1	2487 ± 81.1	2997 ± 25.2	2597 ± 12.6
28th day	1857 ± 82.8	2429 ± 85.3	2197 ± 11.9	3097 ± 91.9	2017 ± 16.3
35th day	2275 ± 87.5	2509 ± 35.9	2546 ± 70.6	2256 ± 20.6	2526 ± 18.2
42nd day	2219 ± 68.9	2125 ± 29.1	2631 ± 22.5	2622 ± 20.5	2631 ± 19.3

Gut pH of the group 1 to group 5 are tabulated in Table-11

Table-11: Gut pH levels from 1st day to 40th day

Average pH Level for 6 birds	1 to 10 Days	11 to 20 Days	21 to 30 Days	31 to 40 Days
Average of Group 1	7.26 ± 0.05	7.30 ± 0.06	7.66 ± 0.11	6.64 ± 0.11
Average of Group 2	7.59 ± 0.08	7.27 ± 0.10	7.67 ± 0.26	7.17 ± 0.07
Average of Group 3	7.32 ± 0.03	7.78 ± 0.05	6.95 ± 0.03	7.08 ± 0.10
Average of Group 4	7.21 ± 0.05	7.21 ± 0.05	6.43 ± 0.28	7.04 ± 0.13
Average of Group 5	7.36 ± 0.12	7.80 ± 0.06	6.55 ± 0.13	7.21 ± 0.05

The total protein and albumin remains on par in all the study groups. However, there was a significant increase in the serum globulin level in the treatment groups when compared to the Control group. This depicts the total antibodies in the serum. The observation is reported in the Table-12.

Table-12: Average serum protein value:

Parameter	**Average of Group 1 (Average of 10 replicates)**	**Average of Group 2 (Average of 10 replicates)**	**Average of Group 3 (Average of 10 replicates)**	**Average of Group 4 (Average of 10 replicates)**	**Average of Group 5 (Average of 10 replicates)**
Serum Total Protein	7.25 ± 0.17	7.48 ± 0.13	7.45 ± 0.12	7.37 ± 0.04	7.39 ± 0.09
Serum Albumin	1.28 ± 0.06	1.34 ± 0.06	1.37 ± 0.08	1.38 ± 0.08	1.38 ± 0.06
Serum Globulin	1.28 ± 0.06	6.11 ± 0.19	6.04 ± 0.25	6.18 ± 0.20	6.08 ± 0.25

The prevalence of lesions in the intestine is minimal in all the treatment and control groups. There was no significant level of enteric pathogens observed in the treatment groups. However, there were minimal pathogenic load observed in the control group.
The vital organ weights and dressing % was found to be better in the treatment groups compared to the control group Table-13.

Table-13: Visceral and lymphoid organ weights (g)

Group	**LIVER (g)**	**GIZZARD (g)**	**HEART (g)**	**SPLEEN (g)**	**THYMUS (g)**	**BURSA (g)**
Group 1 (Average of 10 replicates)	40.39 ± 0.14	39.45 ± 1.80	10.77 ± 0.22	1.65 ± 0.05	12.52 ± 0.31	1.19 ± 0.10
Group 2 (Average of 10 replicates)	43.90 ± 0.85	43.89 ± 0.85	10.79 ± 0.22	2.61 ± 0.09	13.83 ± 0.19	1.71 ± 0.11
Group 3 (Average of 10 replicates)	42.09 ± 0.70	41.90 ± 0.75	10.95 ± 0.20	2.29 ± 0.15	13.23 ± 0.16	1.56 ± 0.12
Group 4 (Average of 10 replicates)	42.37 ± 1.66	42.49 ± 1.66	10.94 ± 0.20	2.09 ± 0.19	13.45 ± 0.10	1.54 ± 0.13

Group 5 (Average of 10 replicates)	41.61 ± 2.12	41.56 ± 2.06	11.15 ± 0.23	2.29 ± 0.10	13.52 ± 0.11	1.57 ± 0.13

The carcass traits like dressing %, breast weight % and thigh weight % are tabulated in Table-14

Table-14: Carcass traits (g%) at the end of trial

Group	Dressing %	Breast Weight %	Thigh Weight %
Group 1 (Average of 10 replicates)	71.95 + 0.81	13.80 + 0.19	3.95 + 0.31
Group 2 (Average of 10 replicates)	72.07 + 0.78	15.20 + 0.51	5.20 + 0.25
Group 3 (Average of 10 replicates)	72.30 + 0.86	16.48 + 0.67	5.90 + 0.23
Group 4 (Average of 10 replicates)	72.66 + 0.92	14.77 + 0.30	5.98 + 0.16
Group 5 (Average of 10 replicates)	72.51 + 0.76	15.13 + 0.49	5.84 + 0.17

4. DISCUSSION

Usub *et al.,* (2008), conducted the experiment for drying of fresh spent silkworm pupae which is rapidly spoilt due to high moisture content. In their studies, the solar tunnel dryer (STD) which was used to dry silkworm pupae under tropical weather conditions of Thailand which is expensive process compared to tray drying and sun drying methods. The spent pupa are generally sun-dried on ground (Usub *et al.,* 2008; Wijayasinghe *et al.,* 1977; Jintasataporn, 2012) due to high water content. In the present study, tray drier was used for drying of waste silkworm pupa collected from the silk reeling industries with final moisture reported as 9.22% before extraction of oil and 6.57% in the DOSPC.

Sheikh *et al.,* (2018) and Finke *et al.,* 2002 have reported the crude protein content 50%-80% in the de-fatted meal from different insects. The present study reported 64.88% protein in the DOSPC which was obtained as remnant after extraction through sophisticated technique of supercritical fluid extraction technology. Dutta *et al.,* 2012, reported that silkworm pupa meal is the potential and cheapest protein source from insect as an alternative to contaminated fish meal used in poultry feed. Karthick *et al.,* (2019) has also reported dry silkworm as source for high quality insect protein with crude protein of 50–70%. The present has also reported the crude protein content of DOSPC to be in the same range.

Khatun *et al.,* (2005), has done the experiment for growth and egg production of Rhode Island Red (RIR) poultry birds, they have used sundried silkworm pupa powder as feed for layer poultry birds. It was reported that the birds body weight was increased by increasing levels of dietary silkworm protein powder which was also supported by Das and Saikia (1972); Panda (1968), Horie and Watanabe (1980). The same trend was observed in the present study, however, the aim of the study was to replace the major protein source in poultry feed soyabean meal which has fluctuating prices based on the season. In the present study, growth studies were done on broiler birds using DOSPC which was obtained after the removal of oil through sophisticated technology called supercritical fluid extraction in pilot plant facility. Seema Langer *et al.,* (2011) studied the effect of low cost locally available materials like silkworm pupa, earth worm meal, fish meal and soyabean meal as a replacement of fishmeal for growth and survival rate of poultry birds. As per their report, they have replaced the fish meal with locally available materials. The results of their studied showed the higher rate of growth, survival and feed conversion ratio (FCR) in prawns fed with earth worm meal than silkworm pupa meal and soya meal. The feed conversion ratio (FCR) between 0.8633 and 2.2433 were recorded and the lowest and best FCR value was obtained in earth worm meal (EWM). As per the studies by Seema *et al.,* (2011), the growth rate was more in fish meal than EWM. The purpose of the Seema *et al.,* (2011) study was completely different from the present study which is focused on the poultry industry. In the present study, the silkworm de-oiled cake was used for the partial replacement of Soya meal as a potential protein source for poultry industry. Sheikh *et al.,* (2018) has reviewed the silkworm pupa meal can be used to replace soyabean and fishmeal in livestock and poultry industry (Khan *et al.,* 2018, Elahi *et al.,* 2022)and it has no adverse effects on the production performances of livestock and poultry. The observations of the present study also conforms to the review reported by Sheikh *et al.,* (2018). Kongsup *et al.,* (2022) has reported the usage of Eri silkworm pupae at 10% as an alternative protein from insect source in feed of broiler chickens. The present study has evaluated the effectiveness of replacing 50% of soyabean meal with DOSPC. The DOSPC, in the present study was obtained after the removal of Silkworm pupa oil through the supercritical fluid extraction technology (SFE). Ullah *et al.,* (2017) has reported silkworm pupa meal as a potential alternative for soybean meal in layer birds for overall performance and egg production, where replacement at 50% achieved better result. The present study was done to evaluate average body weight gain, feed consumption and FCR in broiler birds where optimum result was reported in 50% and 75% replacement with no significant difference in both the groups.

Goodhope *et al*., 1991, evaluated that less mortality in the first week of broiler's life cycle means good quality feed and broiler birds. In the present study, the first week mortality in the treatment and control groups was negligible. The mortality percentage throughout the experimental period was only 1.67% to 2% and there was no significant difference in the control and treatment batches. Heier *et al*., (2002), confirmed the average weekly cumulative mortality during the first week was 1.54%. The present study also confirms the same.

Infectious Bursal Disease (IBD) is commonly known as Gumboro disease caused by the IBD virus. It results in immunity

suppression in the birds and is responsible for huge economic loss in the poultry farms (Sohini *et al*., 2019). In the present study, there was good immune response against Newcastle and Infectious Bursal Disease in the treatment batches. There was no significance difference reported in the same between the Control and the Treatment batches.

The serum protein values depend and vary with the age of the broiler birds. Serum protein is an important factor in ascertaining the health status of the bird. It varies with age and production parameters (Csilla *et al*., 2019). Serum profile is also influenced by physiological and pathological factors. It is an important study to evaluate dehydration, infections, immune response and inflammatory response in the birds (Silva *et al*., 2007). The present study reports the serum profile of the treatment group to be similar to the control group even at 100% replacement of the soyabean meal with DOSPC. It suggests that replacement of soyabean meal with DOSPC has no negative impact on the biochemical profile of the blood in the study groups.

In the intestinal histopathological studies there were minimum prevalence of lesions and no significant level of enteric pathogens observed in the treatment groups. However, there were minimal pathogenic load observed in the control group.

Nastain *et al*., 2021, evaluated the effect of different levels of protein and protease enzyme supplementation on the visceral organ weight in the broiler birds. In the present study, enzyme was not used in the trials, however, protein source was varied at varying concentration in the poultry feed. It was observed that there is no significant difference in the visceral organ weight in the treatment group and control groups. The supplementation of the diet with DOSPC did not have any negative impact on the weight of the visceral organs like liver, gizzard, heart, spleen, thymus and bursa.

Talpur *et al*., 2012, supplemented poultry feed with Calcium and inferred that there is no significant difference in the Carcass traits and dressing percentage. In the present study, variation in the protein source with 64.88% protein content did not have adverse effect on the carcass trait, dressing percentage, breast and thigh weight. This supplementation reported economic benefit in partially replacing expensive soyabean meal with cheaper alternative which is DOSPC.

5. CONCLUSION

The DOSPC processed through the pilot scale supercritical fluid extraction technology is a highly nutritious and protein rich substitute for soyabean meal in poultry feed. The DOSPC contains 64.88% protein, which can be effectively used to replace upto 75% of Soyabean meal in the poultry feed with positive impact on the body weight gain, FCR, feed consumption and immune response against Infectious Bursal Disease and Newcastle Disease in the poultry birds. There were no significant difference between the control and treatment groups observed in the biochemical parameters like serum protein, gut pH, weight of the visceral organs like liver, thymus, spleen, gizzard, heart and bursa and the carcass traits (dressing percentage, breast and thigh weight). In conclusion it is suggested that 50% of the Soyabean meal can be replaced with DOSPC in the poultry feed, considering the availability of silkworm pupa in the sericulture industry.

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