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Production of Single Cell Protein (SCP) from the Peel Waste of Pea, Potato, and Banana by *Aspergillus Flavus* NRRL 21882 as an Efficient Organic Poultry Supplement

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Cite This: *ACS Omega* 2024, 9, 37763−37770

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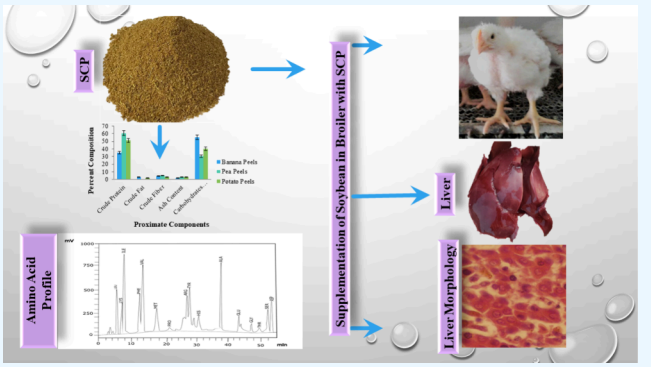
ABSTRACT: Food protein deficit has become a major issue worldwide, particularly in underdeveloped countries. Scientists are searching for a variety of less expensive solutions to this issue. One of these less expensive methods is to create single cell protein as a substrate from leftover fruit and vegetable waste, which is typically thrown away. In this regard, the fungal strain *Aspergillus flavus* (NRRL 21882) was used for the synthesis of SCP (single cell protein) from the waste of banana, potato, and pea. In this manner, 30 samples were collected from the whole substrate with a share of 10 samples each from banana, potato, and pea peels, which were in turn dried and powdered finely. The fermentation process was done by the process of solid state fermentation. *Aspergillus flavus* (NRRL 21882) generated the highest percentage, i.e. 60.67%, of crude protein from the pea peels. The composition of amino acids in crude proteins was also investigated. The findings demonstrated that the highest percentage of aspartic acid (13.34 ± 0.80%) and glutamic acid (14.92 ± 0.69%) was found in *A. flavus* single cell protein produced from pea peels. Soybean was supplemented with single cell protein in the boilers' diet. Compared to all treated groups, there was a substantial ($p \leq 0.05$) increase in the level of antibody titer against the Newcastle disease vaccine. The supplementation of single cell protein with soybean meal had no effect on the levels of liver enzymes. The liver enzymes found in all four groups (A, B, C, and D) were within normal limits. None of the examined groups experienced any change in the feed conversion ratio, with all groups exhibiting an average FCR of 1.6. The current study concludes that broiler health and immunity is increased by supplementing poultry feed with single cell protein.



INTRODUCTION

One of the most important issues, especially in developing nations, is protein deficiency.[1] The primary reasons for this issue are changes in lifestyle, a shortage of agricultural land, and a sharp rise in population growth. As per the available data, 12.5% of the global population is affected by persistent hunger, undernourishment, and insufficient access to nutritious food.[2] Children who suffer from protein-calorie malnutrition (PCM) typically experience impaired immune systems and delayed mental development.[1] Demand for foods high in protein is greater than supply due to the world's expanding population, which causes supply chain inadequacy.[3] Over 25% of people suffer from protein deficiencies, which is a clear illustration of the worldwide protein gap.[4] Therefore, it is imperative to find fresh or different sources of protein to cover the gap.[3]

One of the main protein sources in human beings is poultry products, for which extensive poultry production is necessary.[5] The most crucial and expensive component of customized poultry meals is protein.[6] Poultry feed contains sources of protein from both plants and animals. The rising cost of components for protein feed such as fish meal, soybean meal, and groundnut cake is making it impossible for the chicken industry to increase earnings. The most important sources of protein are derived from animals, because they provide the right amount of protein together with other essential components. Animal sources include fish meal, blood meal, and chicken byproduct meal. Fish meal and soybean meal, which are both scarce and costly due to their use in human and animal nutrition, are said to be the only traditional protein sources for chicken worldwide.[7]

Food waste has become a growing concern on a local and worldwide scale.[8] Agriculture waste is being produced in

Received: April 6, 2024
Revised: August 8, 2024
Accepted: August 12, 2024
Published: August 23, 2024

significant amounts, as food production becomes more intensive globally.[9] When these wastes are not managed properly, it can lead to environmental issues including disease and air pollution as well as risks to public health.[10,11] Food waste is mostly made up of carbohydrate polymers including starch, cellulose, lipids, proteins, and other microelements. This composition qualifies it as a cheap, highly effective second-generation feedstock that can be used as a substrate for microbial fermentation in order to produce valuable products and beneficial substances including animal feeds, biofuels, enzymes, feed additives, single cell protein (SCP), and food grade pigments. This helps in the improvement of safety of foods and the development of a favorable environment.[12−14]

One important initiative to solve these problems is the creation and application of single cell protein (SCP).[15] SCP is produced through fermentation, which is a biological process carried out by microbes such as bacteria, fungi, and yeast that break down complex substrates into simpler molecules for growth.[16] Among the fungal species the *Aspergillus* group is the most important group of fungi, which is used to produce food and pharmaceutical products such as ligninolytic enzymes, pectin, prebiotics, and volatile flavor components.[17−20] They are also beneficial in various other processes like biodegradation in solid state fermentation.[21] In addition, they are also valuable microorganisms for production of SCP.[22] The fungal proteins are in fact a dehydrated cell biomass which contains nucleic acid, carbohydrates, protein, lipids, vitamins, and inorganic compounds.[23] Depending upon the environmental conditions, fungal biomass not only has high protein content but also is a source of rapid and continuous production of biomass. The fungal proteins, having a lower level of nucleic acid content, are more suitable for enrichment of human foodstuff and animal feed in comparison to protein extracted by bacteria.[24] The agricultural sources play a major role in the production of single cell protein (SCP) because it is the agricultural waste which is utilized for this purpose and the product produced is utilized in both food and feeds as a protein supplement.[25]

The goal of single cell protein (SCP) production is to combat the global deficiency of protein.[26] This technique has several advantages over other approaches because it is not reliant on the weather, the properties of the soil, or even the amount of available land.[27]

The main goal of the current research study was to examine the possibility of utilization of different fruit and vegetable wastes for the economical generation of fungal biomass. This work proposed pea, potato, and banana peels as a potential fermentation substrate to generate proteins which can be used as a supplement in animal feeds.

MATERIALS AND METHODS

Collection and Preparation of Substrate. Pea, potato, and banana peels were gathered from Peshawar's local market in Pakistan. The collected substrates were thoroughly washed with clean water. The substrates from the three sources pea, potato, and banana were separated. These substrates were dried at 40−50 °C in a dehydrator for 24 h and then thoroughly chopped into fine particles once they had dried completely. These tiny particles were then filtered through a mesh screen. In order to preserve the samples for further study, Zip-lock polythene bags were used, sealed, and stored at room temperature.

Physiochemical Analysis of Substrates. The substrates prepared from the peels waste of pea, potato, and banana were studied for physiochemical properties such as crude fat, crude fiber, crude protein, moisture, ash and total carbohydrate by specified procedures.[28,29]

Microorganism. In this study, the fungus used for the fermentation of fruit wastes was *Aspergillus flavus* (NRRL 21882), which was acquired from the American Type Culture Collection (ATCC). Fungi were grown on PDA (Potato Dextrose Agar), and every 3 weeks the obtained culture used was subcultured and subsequently incubated at 28 and 32 °C.

Inoculum Preparation. The fungus used in this study, i.e. *Aspergillus flavus* (NRRL 21882), was produced by culturing it on PDA slants at 30 °C incubation for 7 days and subsequently utilized as inoculum. The culture obtained was washed thoroughly with distilled water before being organized. The cleaned spore suspensions were swirled to have a final preparation of 2×10^6 spores/mL. The obtained inoculum were stored at 4 °C in a chiller for later use.[30]

Growth Media Preparation. For each 5 g of substrate, 70% of the moisture content was preserved in a single conical flask. A solution containing 2 mL of inoculum suspension and 10 mL of growth media solution containing 0.5% NH_4NO_3, 0.2% KH_2PO_4, and 0.1% each of NaCl, $CuSO_4{\cdot}5H_2O$, $MgSO_4{\cdot}7H_2O$, $ZnSO_4{\cdot}7S_2O$, and $FeSO_4{\cdot}7H_2O$ was equal to 12 mL at 70% moisture.[30]

Fermentation and Harvesting of Single Cell Protein (SCP). Solid state fermentation was carried out in 250 mL conical flasks. Using 1 N NaOH or 1 N H_2SO_4, the pH was first adjusted to 5.5 in all media. In a 250 mL conical flask, each medium (5 g of pea, potato, and banana peel powder) and 10 mL of growth medium was transferred and sterilized at 121 °C for 15 min. A 2 mL inoculum was put aseptically into each medium from an *Aspergillus flavus* (NRRL 21882) suspension. The medium was fermented at 32 °C under static conditions for 7 days.

Biomass Production Measurement. The required microbial biomass was extracted from the culture broth by vacuum filtration through filter paper after its fermentation and rinsing with clean water. Prior to calculating the biomass weight, all the collected samples (biomass) were placed on an aluminum disc and were dried at 60 °C in an oven for 48 h. The dehydrated biomass was kept desiccated after being crushed into an extremely fine powder in a mortar. Desiccators were cooled to maintain a balance between weight and temperature.[31]

Physiochemical Analysis of Dried Single Cell Protein. Physiochemical properties such as ash, crude fat, crude fiber, crude protein, and total carbohydrate of dried single cell protein generated from pea, potato, and banana peels by *Aspergillus flavus* (NRRL 21882) were determined according to specified methods.[28,29]

Amino Acid Content of Produced Single Cell Protein. Dried single cell proteins were crushed with a pestle and mortar in order to profile the amino acids. Subsequently, an electronic balance was utilized to weigh approximately 5 g of each sample. The samples were acid hydrolyzed by using 0.1 N HCl. Using a vortex mixer, every tube holding a sample was completely vortexed after HCl was added. All of the samples were mixed and then centrifuged for 15 min at 7000 rpm. Until needed, the supernatant was kept at −40 °C after being poured into a fresh tube. A Shimadzu LC-20A analyzer was used to determine the amino acid composition of the single cell

https://doi.org/10.1021/acsomega.4c03317
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protein that was generated. Using a fluorescent substance called OPA (*o*-phthalaldehyde), the concentration of amino acids was determined.[32] The mean data were reported, and each experiment was carried out in triplicate.

Supplementation of Soybean in Broiler Feed with Single Cell Protein. One week old broiler chicks (total 60) were gathered and divided into four groups, designated A, B, C, and D. The groupings were split up into three smaller groups, each with five birds. The pen dimensions were 120 cm × 120 cm, giving each chicken 1200 cm^2 of floor space. Initially the temperature of the house was set at 32 °C, which was decreased gradually to 24 °C at the age of 28 days. For the entire period, a lighting schedule of 24 h illumination with approximately 20 lx was used. In addition to soybean meal, group A−C meals were supplemented with 2, 4, and 6 g/kg of SCP, respectively, as shown in Table 1. The control group was chosen to be group D. Every group received a vaccine against Newcastle disease. After 35 days, blood samples were taken from each group to check for Newcastle disease antibody titer. Liver function tests (LFTs) were also carried out. In groups of birds, mortality and carcass characteristics were also investigated.

Table 1. Composition of Broiler Feed with Varying Quantities of Single Cell Protein[a]

	feed components (g/kg)			
	group A	group B	group C	group D
maize	365	365	365	365
soybean meal	66	64	62	68
SCP	2	4	6	0
wheat	204	204	204	204
broken rice	52	52	52	52
canola meal	54	54	54	54
corn meal	49.1	49.1	49.1	49.1
fish meal	68	68	68	68
guar meal	20	20	20	20
maize gluten feed	25	25	25	25
rice polishing	44	44	44	44
molasses	30	30	30	30
DL-methionine	2	2	2	2
L-lysine	0.9	0.9	0.9	0.9
vit-mineral premix	**9**	**9**	**9**	**9**
DCP	9	9	9	9
total	1000	1000	1000	1000

[a]Calculated from NRC values (1994).

Newcastle Disease (ND) Vaccination. In 10 mL of distilled water, a 1000U vial of Newcastle disease (ND) vaccine was recreated. These vaccinations, which were administered to the birds in the form of eye drops, protected them against Newcastle disease.[33]

LFTs (Liver Function Tests). *Alanine Transaminase (SGPT/ALT) Concentration in Serum.* A biochemical Reactivos kit from Spain was used to estimate the serum alanine ALT (SGPT). A workable solution was made by mixing R1 and R2 in a ratio of 4:1. Then, in a tube with 100 μL of serum sample, 1 mL of working solution was added, gently mixed, and incubated for 1 min at 37 °C. For SGPT analysis, the material was loaded into an Automatic Biochemistry Analyzer (ABA).[33]

Aspartate Transaminase (AST). A biochemical kit containing two reagents (R1 and R2), as well as a standard, was used to estimate the serum AST (SGPT). Reagent 1 was combined with 100 μL of sample in a 500 μL sample tube and incubated for 30 min at 37 °C. After that, 500 μL of reagent 2 was added and incubated for another 20 min at 20−25 °C. After that, 5 L of NaOH was added, and the reading was compared to a blank using a biochemistry analyzer.[33]

Alkaline Phosphatase (ALP). A kit (Biolabo, France) containing three reagents (R1, R2, and R3) as well as a standard was used to estimate serum alkaline phosphatase (ALP). 2 mL of reagent 1 was mixed with 50 μL of serum sample in the sample tube, while 50 μL of standard was mixed in the standard tube and incubated at 37 °C for 15 min. Following that, two reagents named reagents 3 and 4 in a quantity of 0.5 mL each were added. The resultant mixture was incubated in dark room for 10 min. After that, using a biochemistry analyzer, absorbance at 510 nm was compared to a blank.[33]

Liver Histopathology. Liver tissue samples were taken from the dead broilers after their post mortem examination. The collected samples were preserved in 10% formalin according to Clarke's method.[34]

Statistical Analysis. The data, expressed as Mean ± S.E., were checked for statistical Analysis through ANOVA followed by Student *t* tests, considering a P-value <0.05 as statistically significant

RESULTS

Proximate Analysis of Various Agricultural Wastes. Analyzing a variety of agricultural wastes revealed that banana and potato peels had higher carbohydrate contents (48.3% and 35.3%, respectively), whereas pea peels had lower ash contents (5.65%) and higher mineral and vitamin contents (9.50% and 6.67%, respectively). Crude protein content was higher in pea peels (19.79%) compared to potato and banana peels (9.8% and 8.10%, respectively). The crude fat content of banana peels was much higher than those of potato peels (12.1%) and pea peels (2.1%), respectively. The crude fat content of pea peels was lower, at 0.43%, as indicated by Table 2.

Table 2. Proximate Composition of Banana, Pea, and Potato Peels[a]

	moisture contents (%)	ash contents (%)	crude protein (%)	crude fats (%)	carbohydrates (%)
banana peels	21.9	9.60	8.10	12.1	48.3
pea peels	53.41	5.65	19.79	0.43	20.7
potato peels	46.06	6.67	9.84	2.10	35.3

[a]Each value in the table represents the mean standard deviation of three replicates.

Proximate Analysis of Dried *Aspergillus flavus* NRRL (21882) Single Cell Protein. The single cell protein proximate analysis is shown in Figure 1. By cultivation of the necessary organism on various agricultural wastes, the percentage of crude protein in the peels of different wastes varied. Crude protein content was highest in pea peels (60.67%), followed by potato peels (51.10%) and banana peels (35.24%). Banana peels had the highest percentage of carbohydrates (55.10%), followed by potato and pea peels (40.32% and 30.41%, respectively). Potato peels had the greatest ash concentration (3.21%), which was followed by pea peels (3.10%) and banana peels (2.01%). When *Aspergillus*

https://doi.org/10.1021/acsomega.4c03317
ACS Omega 2024, 9, 37763−37770

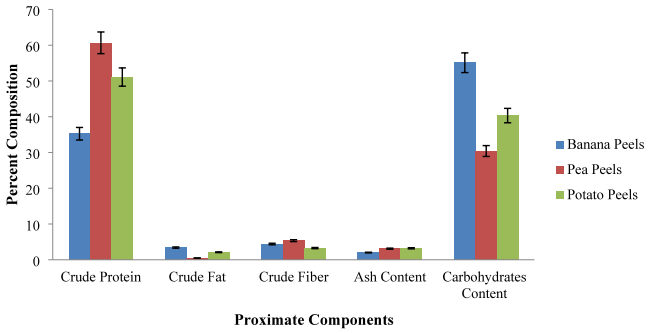

Figure 1. Proximate analysis of dried *Aspergillus flavus* NRRL (21882) single cell protein generated on banana, pea, and potato peels.

flavus was cultivated on these wastes, the crude fiber content of pea peels was greatest (5.34%), followed by potato and banana peels (3.27% and 4.40%). Ultimately, 3.41% of the crude fat content was found in single cell protein generated from banana peels, 2.10% in potato peels, and negligible amounts of 0.48% in pea peels.

Amino Acid Content of Dried Single Cell Protein. Table 3 highlights the total composition of amino acids of *Aspergillus flavus* (NRRL 21882) single cell protein. The amino acid contents of all 16 amino acids including alanine, valine, proline, threonine, isoleucine, glycine, leucine, serine, tyrosine, methionine, arginine, histidine, lysine, phenylalanine, aspartic acid, and glutamic acid were also examined in the single cell protein derived from the peel waste of pea, potato, and banana. The highest amounts of glutamic acid and aspartic acid (14.92 ± 0.69 and 13.34 ± 0.80, respectively) were derived from the peels of pea as compared to potato and banana peels. In contrast, leucine (0.01 ± 0.00) was low in single cell protein made from banana peels.

Supplementation of Soybean in Broiler Feed with Single Cell Protein. Data on liver function tests, antibody titer, and broiler mortality are displayed in Table 4. The antibody titers of treated groups A, B, and C were significantly higher ($p \leq 0.05$) than those of control group D. In comparison to groups B and A, which displayed antibody titers of HI ≥ 1:32 and HI ≥ 1:9, respectively, group C (HI ≥ 1:64) had the highest titer, while group D had the lowest. The liver enzymes were unaffected when soybeans were supplemented with single cell protein, having p values of 0.0628 for ALP, 0.2850 for ALT, and 0.2860 for AST. The liver enzyme levels examined in all four treated groups (A, B, C, and D) were within normal limits. The feed conversion ratio (FCR) in the treated groups stayed constant at 1.6 on average. There were no variations in the vivid pink color of the meat among the A, B, C, and D treated groups. Mortality remained unchanged in each of the treated groups. Group C, in which the supplementation of single cell protein in the soybean meal was at the highest, had a lower death rate than group D (control group).

Morphology of Liver Tissue. The hepatocytes showed no signs of necrosis after being stained with H and E dye. There was no sign of congestion, bleeding, or vacuolation in any of the four treated groups. All four groups of liver hepatocytes appeared to be the same size. No hypertrophy of the bile duct was observed. The treated groups' hepatocytes showed no signs of degeneration. Normal foamy hepatocytes and sinusoidal spaces were identified in all of the treated groups. There were no observed vascular alterations observed. The central vein was properly situated, and the hepatocytes were free of congestion. There was no necrosis noticed, and the architectural detail of the liver hepatocytes was normal, as described in Figure 2.

DISCUSSION

For the purposes of overcoming protein shortage, it is important to generate protein by nonconventional methods. Various microorganisms have been used to make single cell protein. Therefore, to reduce the total expenditure in the synthesis of single cell protein, inexpensive substrates from the agro industry have been reported.[35] Consequently, fungal strains were used to analyze a variety of inexpensive raw materials used in the agro industry in order to produce SCP. Food peels were taken into consideration as a potential substrate for the synthesis of SCP in this investigation. Bioconversion is significantly influenced by the cost and

Table 3. Amino Acid Content of Dried *Aspergillus flavus* (NRRL 21882) Single Cell Protein Generated on Banana, Potato, and Pea Peels[a]

no.	amino acids (%)	symbol	banana peels	pea peels	potato peels
1	lysine	LYS	7.71 ± 0.06	6.81 ± 0.34	8.40 ± 0.72
2	leucine	LEU	0.01 ± 0.00	8.17 ± 0.41	8.93 ± 0.40
3	phenylalanine	PHE	5.98 ± 0.07	5.96 ± 0.36	6.64 ± 2.12
4	isoleucine	ILE	9.06 ± 0.04	4.03 ± 0.24	4.70 ± 0.15
5	methionine	MET	6.79 ± 0.03	1.72 ± 0.10	4.34 ± 0.05
6	valine	VAL	2.52 ± 0.02	5.41 ± 0.27	5.94 ± 0.13
7	arginine	ARG	4.25 ± 0.05	5.37 ± 0.32	6.87 ± 0.67
8	proline	PRO	2.71 ± 0.02	5.20 ± 0.31	5.44 ± 0.19
9	histidine	HIS	4.96 ± 0.01	6.49 ± 0.32	4.84 ± 0.09
10	tyrosine	TYR	6.50 ± 0.03	2.91 ± 0.17	4.57 ± 0.47
11	glutamic acid	GLU	6.31 ± 0.01	14.92 ± 0.69	5.94 ± 0.23
12	alanine	ALA	2.85 ± 0.04	3.78 ± 0.29	7.00 ± 0.20
13	threonine	THR	6.10 ± 0.03	5.42 ± 0.33	5.56 ± 0.25
14	glycine	GLY	18.02 ± 0.82	5.55 ± 0.38	7.56 ± 0.12
15	aspartic acid	ASP	10.64 ± 0.05	13.34 ± 0.80	5.48 ± 0.45
16	serine	SER	6.59 ± 0.04	4.69 ± 0.69	7.79 ± 0.16

[a]Each value in the table represents the mean standard deviation of three replicates.

https://doi.org/10.1021/acsomega.4c03317
ACS Omega 2024, 9, 37763−37770

Table 4. Antibody Titer, LFTs (Liver Function Tests), Mortality, and Feed Conversion Ratio in Broilers[a]

group	ALP (units/L)	AST (units/L)	ALT (units/L)	average antibody titer	mortality	FCR
A	$172.66^{a} \pm 13.6$	144 ± 6.22	28.65 ± 2.6	$4.67^{b} \pm 0.56$ (HI ≥ 1:9)	$0.67^{ab} \pm 0.58$	1.662
B	$144.33^{b} \pm 16.9$	160.34 ± 8.7	32.34 ± 1.09	$6.67^{a} \pm 1.16$ (HI ≥ 1:33)	$0.67^{ab} \pm 1.16$	1.625
C	$177.6^{a} \pm 4.17$	156.33 ± 12.6	29.33 ± 3.06	$7.33^{a} \pm 0.65$ (HI ≥ 1:64)	0^{b}	1.616
D	$168.67^{ab} \pm 19.6$	155 ± 4.17	30.33 ± 2.09	$4.33^{b} \pm 1.53$ (HI ≥ 1:9)	$1.67^{a} \pm 0.58$	1.645
P = 0.05	0.0428	0.2860	0.2850	0.0190	0.027	0.6635

[a]All means were calculated. Means within the same row having different superscripts were significantly different at $P = 0.05$. Group A: supplementing SCP (2 g/kg) for soybean meal. Group B: supplementing SCP (4 g/kg) for soybean meal. Group C: supplementing SCP (6 g/kg) for soybean meal. Group D: control (no supplementation of SCP feed).

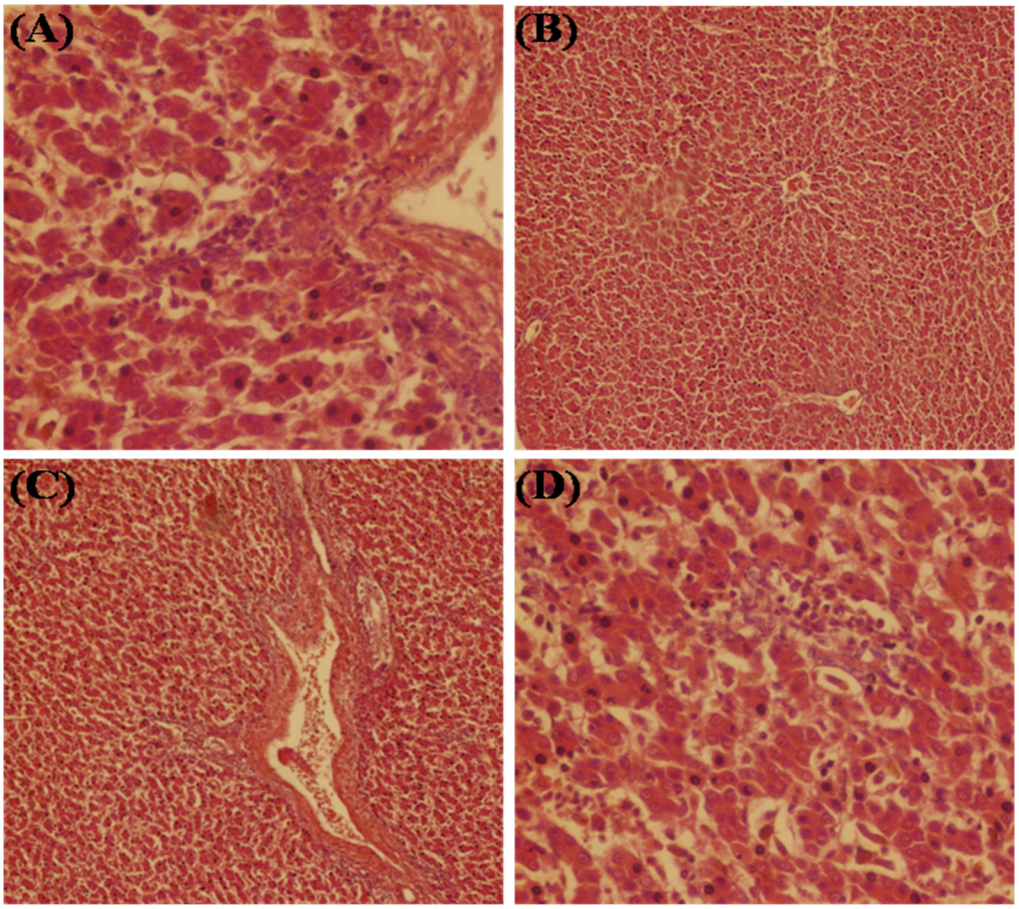

Figure 2. Liver photomicrographs of various groups: (A) supplementing SCP (2 g/kg); (B) supplementing SCP (4 g/kg); (C) supplementing SCP (6 g/kg); (D) control (no supplementation of SCP feed).

availability of these substrates.[36] A readily available agricultural waste with a high organic matter content that can be utilized to fuel fungal growth during the production of SCP is food waste.[37]

The viability of employing potato, banana, and pea peels as substrates for SCP manufacture was investigated in this work. The wastes were converted to fermentable sugar by heat treatment. Different fruit peels have been used by researchers as a good substrate for the production of a single cell protein. Tropea et al. employed fish waste and pineapple, banana, apple, and citrus peels as a good substrate for microbial growth in order to produce SCP.[38] The production of SCP utilizing orange peels as a substrate was also documented by Milala et al.[39] The impact of different parameters on the generation of SCP was examined using a single variable optimization.

The proximate composition of the single cell protein made using different substrates (pea, potato, and banana peels) was examined. The analysis conclusions demonstrated that the single cell protein had a high crude protein content. The maximum protein content in the dry cell biomass generated by *A. flavus* (NRRL 21882) was calculated to be 60.67% using optimal culture composition. The findings are similar to the study conducted by Ardestani et al., which showed the dried cell biomass protein content of *A. flavus* PTCC5004 to be 55.75%.[40]

In contrast, the study conducted by Jaganmohan et al. showed a total protein content in the dried cell biomass of *Aspergillus terreus* to be 35%, which was lower than *A. flavus* (NRRL 21882) SCP. Using *Aspergillus terreus* and several raw materials, they carried out solid state fermentation.[41] Additionally, *A. flavus* (NRRL 21882) cell biomass had substantially higher protein content than *Aspergillus niger*, i.e. 18.9%, as reported by Said et al.[42]

https://doi.org/10.1021/acsomega.4c03317
ACS Omega 2024, 9, 37763−37770

Additionally, the single cell protein made from the three main sources (banana, potato, and pea peels) was examined for its amino acid composition. The results showed that the varied agricultural wastes created various amounts of amino acids in the dried SCP. Banana waste derived SCP had a low leucine content, whereas pea peels yielded single cell protein with a high aspartic acid content, followed by potato and banana peels. The conclusions of Pęksa et al., Tsado et al., and Mousa et al. are supported by these data.[43−45] The highest quantity of amino acid makes single cell protein a better option for utilization in poultry, animal, and human foods.[46,47]

Using single cell protein had no adverse impacts on any of the body's functions, including the liver's ability to work in boilers, as compared to the control group. In poultry and livestock production, natural growth boosters such β-1-, β-3-, and β-1,6-glucan along with mannan oligosaccharide are commonly utilized.[10,48] The most potent microbial protein is thought to be that of *Aspergillus*. It increases health when introduced to a poultry diet.[49]

As per the field reports, broiler feed supplementation with fungi proved to be very advantageous. By controlling gut microbiota, probiotics support the body's defenses naturally.[50,51] The body weight increases in broilers because the oligosaccharide component accounts for approximately half of the total carbohydrate load. Villus height increased in the first week following treatment, indicating that these compounds are helpful to the intestinal mucosa.[52,53]

The antibody titer levels achieved by supplementing soybean with single cell protein in broiler feed were elevated, and this higher protein content was noticed more in group B and C as compared to group A and control group D. As a metric for quantifying immunological responses to antigens, antibody response is usually employed.[54] Antibody-mediated immunity increased in the mannan oligosaccharide group, proving that MOS might be used to increase immunity, as suggested by the experiments conducted by Chacher et al,[55] Although the role of β-glucans in immune system regulation is well established, it is still unclear how exactly MOS affects the humoral immune system. However, studies done on broilers by Salianeh et al., Lilburn et al., and Aravind et al. discovered that MOS addition to feed did not raise antibody titers against Newcastle disease and IBDV.[56−58]

No fatalities were detected in group C when soybean meal was supplemented with *Aspergillus flavus* (NRRL 21882) single cell protein (6 g/kg). Mannan-oligosaccharides, which cattle ingest to stop dangerous bacteria from developing, may be responsible for the aforementioned findings.[59,60] Mannan-oligosaccharides prevent dangerous bacteria from adhering to and colonizing the intestine of birds, therefore offering possible protection against a variety of illnesses. Additionally, mannan-oligosaccharides improve the health of birds by providing nourishment for other common microbes.

Single cell microbial protein enhances broiler immunity and increases birds' resistance to infections, improving birds' general health.[60] According to Teng et al. and Dudkiewicz et al., mannose inhibited *Salmonella typhimurium*'s ability to adhere to the chicks' small intestine. Additionally, it was found that addition of mannose to the chicks' drinking water prevents the cecum from colonization of *S. typhimurium*.[61,62]

CONCLUSION

As a result of this research study, it was concluded that SCP can be produced from the peel waste of pea, potato, and banana by the fungi *Aspergillus flavus* (NRRL 21882) through solid state fermentation. The process is less expensive, and the resultant product is a cheap source of poultry feeds. Furthermore, the study concludes that chicken feed containing protein can be supplemented with produced single cell protein which promises good broiler health and meat by enhancing the gut microbiota, ultimately strengthening broiler immunity.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c03317.

Amino acid chromatograms of pea peel single cell protein generated by *Aspergillus flavus* (NRRL 21882), potato peel single cell protein generated by *Aspergillus flavus* (NRRL 21882), and banana peel single cell protein generated by *Aspergillus flavus* (NRRL 21882) (PDF)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors are very much indebted to Center of Biotechnology and Microbiology, University of Peshawar and PCSIR laboratories complex, Peshawar for their full cooperation and extension of required lab facilities, financial support and expertise. We also very much acknowledge the help and full support of Department of Animal Nutrition, Faculty of Animal Husbandry and Veterinary Sciences, University of Agriculture, Peshawar.

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