Original Article

Allied Medical Research Journal

Vol:4 No.4: 03-11

© The Author (s) 2026

Single Cell Protein from Agro-Industrial Waste: A Sustainable Alternative for the Efficient Growth of the Broiler Chickens

Aqsa Maqsood[1], Fariha Ibrahim[2], Iffat Imran[1]

[1]*Department of Biomedical Engineering, Ziauddin University, Karachi, Pakistan*
[2]*Department of Biotechnology, Bahria University, College of Allied Health Sciences, Karachi, Pakistan*

ABSTRACT

Background: Single cell protein derived from food waste by *Aspergillus niger* serves as a cost-efficient and environmentally sustainable alternative to protein source in chicken feed. Microbial fermentation of agricultural byproducts generates high-quality, protein and reduces waste. These substrates, such as orange, sugarcane and pomegranate, generate single-cell protein, enhancing feed efficiency and improving protein utilization in poultry feed formulation. This method enhances chicken production, reduces feed expenses, and increases the availability of protein-rich meals and food security.

Methods: Three agro-based substrates sugarcane, pomegranate and orange wastes, were tested to produce single cell protein by *Aspergillus Niger*. Biomass yields were measured, and it was found that orange waste supported greatest fungal biomass, and was therefore chosen for subsequent large scale production of *Aspergillus Niger* single cell protein. The 120 chicks were allocated at random into treatment group, each with 3 replicates of ten birds. Four diets with equal nitrogen and energy content were prepared containing SCP 0% (Control T1), 5% 10%, and 15% SCP, which replaced with soybean meal (T2 to T4). Separate starter and finisher diet were prepared for and fed to every replicate. At the end of the experimental time, record the growth performance as well as the feed conversion ratio. Liver histopathology were observed at 42 day experiment last day.

Results: The findings indicate that among the substrates tested, orange waste yielded more SCP biomass then the other substrates. Body weight gain in the groups 5% and 10% SCP diets was equivalent to the control within each feeding and the whole trial; however, it was significantly lower ($p<0.05$) in the group 15% SCP diets. SCP showed appropriate stability at 5%and 10% levels, highlighting the necessity for controlled incorporation at high doses.

Conclusion: SCP inclusion from 5% to 10% of broiler diets to enhance feed efficiency and promote sustainable production of proteins from waste resources.

Keywords: *Agricultural-Waste, Animal Feed, Aspergillus Niger, Chickens, Single-Cell Protein.*

Received: February 9th, 2026; **Revised:** July 10th, 2026; **Accepted:** August 27th, 2026

Corresponding Email: farihaibrahim.buhsck@bahria.edu.pk

DOI: https://doi.org/10.59564/amrj/04.04/001

INTRODUCTION

Feed availability poses a significant challenge to the poultry industry because feed expenses constitute nearly around 60 to 75% of overall broiler chicken production costs. Within the different feed components, protein is one of the most highest-cost inputs and considered the second most important nutritional component of broiler feed. High-growth-rate broiler required protein-enriched diets with well-balanced amino acid composition. Proteins and amino acids are essential for the synthesizing of biomolecules, such as hormones, biocatalyst, and immune protein, which are essential for normal physiological functions[1]. The demand for protein exceeds its availability, underscoring the necessity for sustainable protein sources[2]. Proteins are generated by plants, animals, fungi, and microbes. The world's protein supply is 60% plant-based and 40% animal-based, including meat, poultry, dairy, and fish. Animal-derived protein diets give energy, essential amino acids, and micronutrient such as iron, zinc, and vitamin B12 that are difficult to obtain through plants[3]. Oilseed-derived protein meals, soybean meal (SBM) is the most preferred protein resource as it provides 80% of protein and essential amino acids, including lysine. Increasing costs and restricted supply of these proteins threaten economic viability and require other sources[4-6]. Edible protein (SCP) obtained through microbes such as: bacteria, fungi, algae, and yeast in the form of either a microbial biomass, protein extract, or fermented mass. Offering sustainability and efficiency in feed or food production, SCP serves as a perspective alternative to traditional protein resources[7-11]. SCP production from agro-wastes can improve

This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0), which permits others to share, copy, redistribute, and adapt the work for non-commercial purposes, provided the original author(s) and source are credited appropriately. Further details are available on the official AMRJ Open Access policy page: https://ojs.amrj.net/index.php/1/14.

poultry nutrition, reduce dependence on traditional proteins, and increase environmental sustainability. Renewable protein resources, such as plant derived by-product, insects, and microbes, have generated significant scientific interest due to their ability to be produced without the need for land for agriculture[12]. These sources can be cultivated using renewable substrates, specifically residual and by-product material obtained from various agro-food waste material. These non-traditional resources, microorganism's exhibit increased protein content. Biomass is known as 'single cell protein' (SCP) or microbial-cell origin[13].

Numerous studies have examined various types of microorganisms, including methanotrophic bacteria, yeast[14,15]. Fungal proteins consist of dried cellular biomass that includes nucleic acids, carbohydrates, proteins, lipids, vitamins, and inorganic compounds. Fungal biomass exhibits a significant protein content and demonstrates rapid and continuous biomass production, depending upon environmental conditions. Fungal proteins are superior for enhancing food and feed compared to bacterial protein due to their lower nucleic acid content[16].

In recent years, various studies conducted on the synthesis of SCP through different microbes with diverse agro-waste substrates and utilize in food sector industries. A recent trial revealed that a moderate inclusion of SCP (about 2%) yielded growth performance similar to control diets, whereas higher inclusion levels diminished performance, so indicating optimal SCP integration thresholds for productivity. The incorporation of single-cell protein derived from *methylococcus* species, substituting up to 5% of soybean meal enhanced growth and feed conversion output without altering carcass features or gut architecture. However, larger inclusion levels adversely impacted performance indicating the necessity for cautious integration[17].

In other research, it was showed that the SCP can be generated from the peas, potatoes, and bananas wastes by using the fungal specie *Aspergillus flavus* and add 2kg, 4kg, and 6kg in the replacement of soybean meal. The process requires lower costs, resulting in an economical source of poultry feeds[18]. Previous research showed that yeast single-cell protein may replace large volumes of soybean meal in broiler diets, boosting growth and feed efficiency[19].

These findings suggest that SCP could replace soybean meal in chicken feeds, promoting more sustainable protein nutrition strategies and demonstrating the need for species and dose-specific dietary assessment.

Therefore, the object of this study is to investigate the prospective use of various agro-wastes for the cost-effective production of single cell protein and to assess the graded levels of proteins on the physiological performance of the broiler chickens.

METHODOLOGY

Group Division and Experimental Diet

The experimental diets were formulated to be iso-nitrogenous and iso-caloric, by including SCP at levels of 0%, 5%, 10%, and 15%, replacing Soybean meal in accordance with the nutrient requirements set by the Pakistan standards and quality control authority (PSQCA). The feed were prepared in mash form and formulated for each feeding phase, starter phase (0 to 21 days), and the finisher phase (22 to 42 days). The composition of ingredients and nutrients of the respective diets is given in (Table1). A total of 120 one-day-old, straight-run commercial chicks of the Ross 308 strain were obtained from K&N Hatchery, wing banded, and transferred to the experimental site at Raja farmhouse. The chicks were randomly allocated to four treatment groups, with three replicates of 10 chicks per treatment. Each of the diets was offered ad libitum to all group chicks in completely randomized way. The chicks were raised in a deep litter system within a adequate environment. Brooding was carried out during the two weeks of age according to standard management protocols. All birds were vaccinated against Newcastle disease on days 7 and 21. For growth parameter all birds were weighed as individuals and feed intake was measured per replicate as they were weighed on a last day of experimental trial.

Collection and Preparation of Substrate

Agro-waste from sugarcane, oranges, and pomegranates were collected by the local market of Karachi, Pakistan. The obtained substrates were thoroughly rinsed with distilled water to eliminate impurities and contaminates. Each substrates were been chopped into portions. The substrates were then cut into small pieces and oven-dried at 40-50°C for 24 hours. The dried materials were subsequently pulverized into fine powder and sieved using a mesh screen. The processed substrates were placed in polythene bags, securely sealed, and

stored at room temperature until further analysis.

Microorganism and Inoculum Preparation

Aspergillus niger was used as the fungus strain in this study. It was cultivated on Potato Dextrose Agar and sub-cultured every three weeks, with incubation maintained at 28–32°C. Spores were harvested from 7-day-old PDA slants using sterilized distilled water, and the resulting suspension was adjusted to concentration of 2×10^6 spores/mL. The prepared inoculum was subsequently preserved at 4°C until required for analysis.

Production of Single Cell Protein (SCP)

A 200mL fermentation medium was prepared by using yeast extract (2g), peptone (2g), KH_2PO_4 (1g), $MgSO_4 .7H_2O$ (0.05g), sucrose (2g), and fine powder of agro waste (sugarcane, orange, and pomegranate; 10 g), with each substrate added to a separate flask. A weight balance instrument was used to weigh the desired products carefully and dissolve them using around 180 mL of distilled water which is about 90% of the total volume needed in a 250 mL flask that contained enough space to allow aeration to occur. The last volume was brought to 200mL and the pH adjusted to 6.5. Sterilization of the medium was done at 121ºC at 15 minutes. Each medium was aseptically inoculated with 2mL of an *Aspergillus Niger* suspension and the flasks were left in incubator at 28-30ºC during 7 days. Fermentation was carried out under static conditions at 32ºCover the 7 days. Fungal biomass after fermentation was collected at day 7 by filtration through Whatman No. 1 filter paper.

Biomass Measurement

Once the fermentation is complete, culture broth washed with clean water, the necessary microbial biomass was harvested through vacuum filtration using filter paper. All of the gathered samples were spread on an aluminum disc and oven-dried for 48 hours at 60°C before the biomass weight was determined. After grinding to a very fine consistency in a mortar, the dried biomass was maintained desiccated. To keep weight and temperature in balance, desiccators were cooled.

Physiochemical Analysis

Fat content, fiber, total protein, Ash and total carbohydrate of SCP produced by *Aspergillus niger* from enhanced biomass derived from orange, pomegranate and sugarcane were measured by following of standard AOAC producers. Ash determination, sample were incinerated in muffle furnace until a constant weight archived. Crude fat was extracted using the soxhlet method petroleum ether. Crude fiber was estimated by sequential acid and alkali digestion. Total protein was calculated by using the kjeldahl method, based on nitrogen level converted using standard factor. Total carbohydrate was determined by difference, subtracting the combined percentage of moisture, ash, crude fat, crude fiber, and crude protein from 100[20].

Composition of Amino Acid in Produced Single Cell Protein

Prior to amino acids analysis, SCP were ground using a pestle and mortar. About 5g from each sample was measure out using an electronic balance. The samples were hydrolyzed with 0.1 N HCl. After adding HCl, each sample was thoroughly vortex-mixed. The samples were then centrifuged at 7,000 rpm for 15 minutes. The supernatant was transferred to a new tube and stored at −40°C until further analysis. The amino acid composition of the single-cell protein was determined using an HPLC system (Shimadzu Corporation, Kyoto, Japan). During substrate screening, orange waste, which produced the maximum fungal biomass, was selected for scale-up production. Fermentation was conducted under optimized conditions to achieve maximum biomass and protein yield.

Incorporation of Single Cell Protein with Soybean Meal in Broiler Chickens

A total of 120 ross 308 one-day-old chicks, which were collected from the hatchery and divided into four groups, T1, T2, T3 and T4. Each group consisted of 3 replicates of 10 birds each, housed in pens measuring 120cm × 120cm providing1200 cm^2 of floor per chicken. At the age of 28 days, the house's temperature was gradually lowered from its initial setting of 32°C to 24°C. A 24-hour lighting cycle at approximately 20 lx was maintained throughout the study period. Alongside soybean meal, groups T2-T4 were supplemented with 5%, 10% and 15% of SCP, as shown in Table1. Group T1 were served as the control group. All groups were administered a vaccine against Newcastle disease.

Vaccination against Newcastle Disease

All birds were vaccinated against Newcastle disease, administered with eye drops. Mortality, feed intake were recorded every weekly and FCR during both phases.

Liver Histopathology of Broiler Chickens

On the final day of experiment birds from each replicate of every group were chosen and sacrificed to collect liver samples for

histopathological evaluation. The collected samples were immersed in 10% formalin following Clarke's protocol[21].

Table 1. Feed Composition of the Broiler Chickens.

Ingredients	Feed Composition % of diet (Starter and Finisher phase)							
	T1 (Control)		T2 (5% SCP)		T3 (10% SCP)		T4 (15% SCP)	
	S	F	S	F	S	F	S	F
Maize	62.4	63.1	62.4	63.1	62.4	63.1	62.4	63.1
Vegetable Oil	0.80	2.5	0.80	2.5	0.80	2.5	0.80	2.5
Fish meal	5	6	5	6	5	6	5	6
Soybean meal	22	20	20.9	19	19.8	18	18.7	17
Single Cell protein	0	0	1.1	1	2.2	2	3.3	3
Canola meal	7	6	7	6	7	6	7	6
Limestone	0.90	0.55	0.90	0.55	0.90	0.55	0.90	0.90
DCP	0.65	0.60	0.65	0.60	0.65	0.60	0.65	0.60
Pre-Mix	0.185	0.185	0.185	0.185	0.185	0.185	0.185	0.185
D-L Methionine	0.250	0.255	0.250	0.255	0.250	0.255	0.250	0.255
Sodium bicarbonate	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Salt NaCl	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-threonine 98.5%	0.09	0.10	0.09	0.10	0.09	0.10	0.09	0.10
Phytase 10K FTU	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
LYS-Sulphate 70%	0.37	0.375	0.370	0.375	0.370	0.375	0.370	0.375
Choline Chloride	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Total	100		100		100		100	

Premix contains vitamins A (20,000,000 IU/kg), D3 (6,000,000 IU/kg), E (60,000 IU/kg), K (4,000 mg/kg), B1 (4,000 mg/kg), B2 (12,000 mg/kg), B6 (8,000 mg/kg), B12 (30 mg/kg), niacin (80,000 mg/kg), pantothenic acid (22,000 mg/kg), folic acid (2,000 mg/kg) and biotin (250 mg/kg).

Statistical Analysis

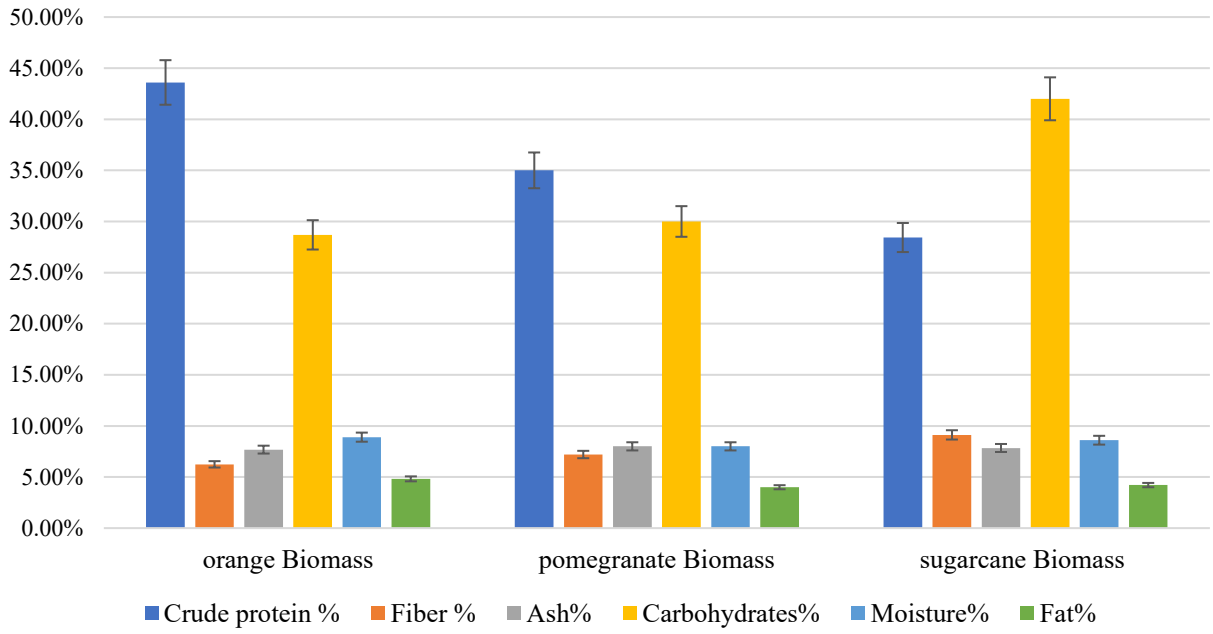
Data were evaluated through one-way ANOVA in SPSS v.26.0, with the experiment arranged as a completely randomized design (CRD). The treatment means were compared byTurkey HSD post-hoc test, and results were given as mean ± SD. Differences were regarded as significant at *p-values <0.05*.

RESULTS

Prominent Analysis Measurements

Study of several agro-waste demonstrated that orange waste had larger dried biomass as compare the pomegranate waste dried biomass (7.3%, respectively). While, pomegranate waste dried biomass and sugarcane waste dried biomass have equivalent quantity (3.3% and 3.4% respectively). Figure 1 shows orange waste (43.6%) had higher crude protein content, whereas pomegranate waste (35%) and sugarcane (28.43%) respectively lower compared to orange waste. Crude content fiber lower in orange waste (6.24%) as compared to pomegranate waste (7.2%) and sugarcane waste (9.12% respectively). Ash content in orange waste (7.68%) was lower than the sugarcane (7.8%) and pomegranate waste and 8% respectively). Content of carbohydrate was higher in sugarcane (40%) than orange, (30%) pomegranate waste 28.69% respectively. While, moisture content in orange waste (8.9%) was higher as compared to sugarcane waste (8.6%), pomegranate (8.0% respectively). Whereas fat content slightly same in each waste biomass. (Orange waste 4.8%, pomegranate waste 4% and sugarcane waste 4.21% respectively).

Figure 1: Proximate analysis of biomass



Amino Acid Constitution in Single Cell Protein

Amino acid analysis of the SCP derived from orange by *Aspergillus niger* contain 17 amino acid including Lysine, Methionine, Leucine, Isoleucine, Phenylalanine, Valine, Arginine, Proline, Histidine, Tyrosine, Glutamic acid, Alanine, Threonine, Glycine, Aspartic acid, Serine Cysteine. Methionine highly present in single cell protein in comparison with soybean meal while cysteine was marginally lower in SCP in contrast to soybean meal represented in (Table 2).

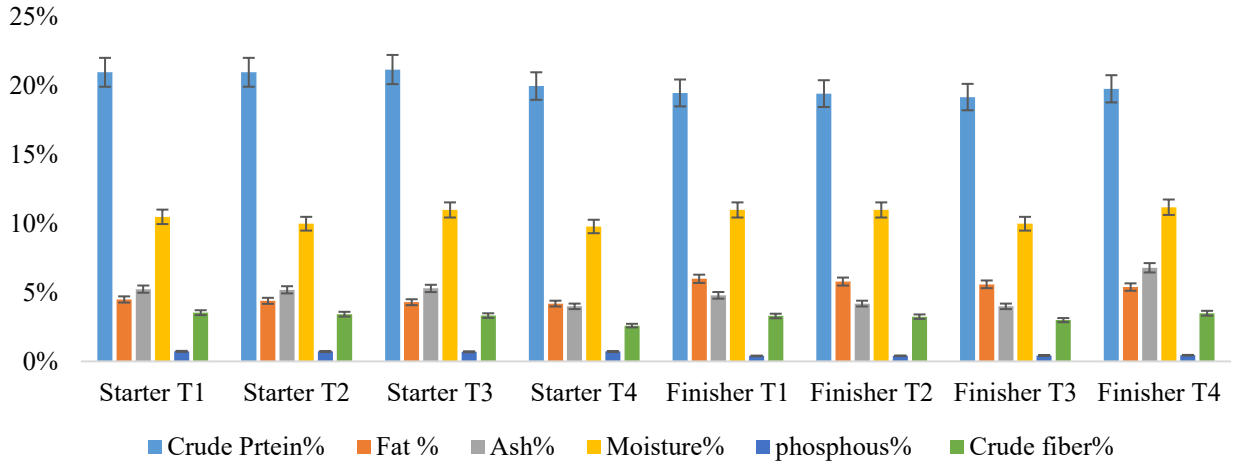
Table 2. Comparative analysis of amino acid content of Single cell protein and soybean meal.

Amino Acids	Symbol	Single Cell Protein (SCP) %	Soybean meal (SBM) %
Lysine	LYS	2.44	3.3
Methionine	MET	0.98	0.7
Leucine	LEU	4.08	4.0
Isoleucine	ILE	2.66	2.55
Phenylalanine	PHE	2.10	2.7
Valine	VAL	2.99	2.6
Arginine	ARG	3.09	3.8
Proline	PRO	2.38	2.0
Histidine	HIS	1.10	1.3
Tyrosine	TYR	1.52	1.6
Glutamic acid	GLU	10.67	6.0
Alanine	ALA	4.86	1.7
Threonine	THR	3.38	2.1
Glycine	GLY	2.59	1.6
Aspartic acid	ASP	5.32	3.0
Serine	SER	2.15	2.0
Cysteine	CYT	0.50	0.7

Feed Proximate Analysis

In the starter phase, Group T1–T4 revealed that the crude protein levels between 20% and 21.2%, with metabolizable energy varying from 2900 to 2962 kcal/kg. Ash percentage ranged from 4% to 5.3%, moisture from 9.8% to 11%, crude fiber from 2.6% to 3.54%, phosphorus from 0.70% to 0.73%, and fat from 3.8% to 4.5%. During the finisher phase, crude protein levels ranged from 19.2 to 19.8%, while energy content varied between 3021 and 3099 kcal/kg. Ash percentage ranged from 4 to 6.8%, moisture content from 10 to 11 %, crude fiber from 3.01 to 3.5%, phosphorus from 0.40 to 0.45%, and fat from 5.4 to 6% (Figure 2).

Figure 2: Feed proximent analysis



Analysis of Growth Parameter

The average body weight gains (g/kg) of broiler chickens across different groups were differed significantly in all phases and over the whole trial (*p=0.001*). Chickens fed 5 and 10% SCP showed higher body weight as compared to the control group in both phase starter and finisher while 15% SCP group lower body weight compared to other groups and control group. No significant variation in feed intake was observed across the groups (*p=0.388*). Although the 15% SCP lower feed intake in both phases relatively to control and other treatment groups. FCR differed significantly among groups (*p=0.002*); the 5% and 10% was similar to the control, while FCR increased with higher SCP inclusion livability also differed significantly among groups (*p=0.025*). In this study, enhanced FCR was recorded at 5% and 10% incorporation of SCP feed formulation shown in (Table 3). Dietary single-cell protein (SCP) had a substantial impact on feed conversion ratio and overall growth performance T2 moderate and T3 had larger ultimate body weights, while T4 had the lowest weight and feed conversion ratio (2.1 ± 01).

Table 3. Analysis of Growth Parameters of Broiler Chickens.

Groups	Total Weight Gain (g/bird)	Total Intake Weight (g/bird)	FCR	Livability (%)
One day old	41.4±58			
T1	2705 ± 13^{b}	4283.5 ± 510.34^{a}	1.5 ± 0.02^{a}	100
T2	2646 ± 75^{b}	4129.5 ± 60.30^{a}	1.5 ± 0.04^{a}	100
T3	2919 ± 11^{c}	4499 ± 72.19^{a}	1.5 ± 0.03^{a}	100
T4	2073 ± 40^{a}	4418.73 ± 74.96^{a}	2.1 ± 0.05^{b}	90
P-value	*0.001*	*0.388*	*0.002*	*0.025*

Values represented as mean ± S.D, within the same column, means followed by different superscript letters (a,b,c) differ significantly (P≤0.05), whereas means sharing the same superscript letter do not significantly different (P>0.05), regardless of small numerical differences between them. Superscript letters are assigned in ascending order of mean value, where as "a" denotes the lowest mean, and "b" and "c" are progressively higher. One-way ANOVA was perform on data and applied Tukey's HSD post-hoc test.

This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0), which permits others to share, copy, redistribute, and adapt the work for non-commercial purposes, provided the original author(s) and source are credited appropriately. Further details are available on the official AMRJ Open Access policy page: https://ojs.amrj.net/index.php/1/14.

Liver Histopathology

The hematoxylin and eosin (H&E)-stained section of chicken liver from the control group demonstrated typical hepatic architecture upon histological examination, featuring well-organized hepatocyte cords radiating from the central vein, intact sinusoids, and unremarkable portal regions (Figure 3a). Chickens consuming a 5% SCP diet exhibited liver morphology similar to the control group, characterized by preserved hepatocellular architecture and the absence of inflammatory or degenerative changes (Figure 3b). The 10% SCP group had negligible histological alterations, including slight sinusoidal dilation, moderate vascular congestion, and mild hepatocellular hypertrophy (Figure 3c). The overall lobular architecture remained intact with no evidence of necrosis or fibrosis. On the other hand, liver sections from chickens administered 15% SCP exhibited considerable disruption of hepatic cords, marked sinusoidal congestion and dilation hepatocellular vacuolation, and rare instances of nuclear pyknosis indicating hepatic stress at elevated SCP inclusion (Figure 3d).

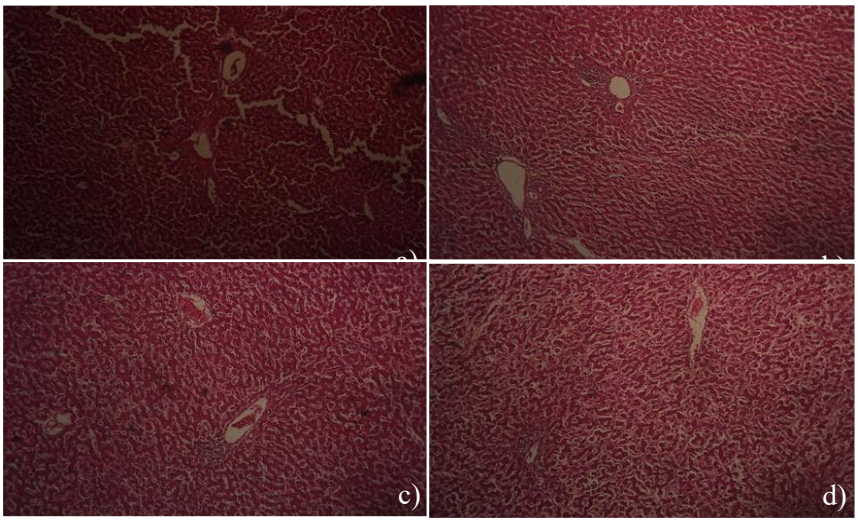


Figure 3: Representation of histopathology of liver samples of the different treatment groups of Boiler Chicken a) Control; b) 5% SCP; c) 10% SCP; d) 15% SCP

DISCUSSION

The search for renewable and exceptional-quality alternative protein resources in poultry nutrition holds led to heightened interest in single-cell protein (SCP), specifically microbial biomass generated from agro-wastes through fermentation. SCP presents a viable alternative to traditional protein resources such as soybean meal and fishmeal, which are costly and vulnerable to supply fluctuations. This study indicates that broiler diets containing fungal SCP significantly impact growth performance and feed efficiency, aligning with published findings.

The proximate analysis of orange waste biomass revealed that the content of dry matter, organic matter 7.3%, crude protein 43.6%, crude fiber 6.24%, was high, and on as-is basis, respectively and is comparable to the results of the proximate analysis[21]. SCP profile of constituents of amino acid Table 2 was closely resemble to amino acid profile of soybean meal all the essential amino acids are found in similar percentage in the SCP compared to soymeal bean. Two of the amino acids that included sulfur containing cysteine and proline were slightly less in SCP comparison to SBM.

Analysis of the SCP sample revealed the amino acid composition of the was align to the amounts of the amino acids reported by Felix N., et al.[22] In this research, birds fed on 5% and 10% SCP attained a similar gained body weight comparable to the control group showing that this amount of inclusion was the best in our experimental condition. In comparison, the growth performance in the study reported by Hombegowda et al[17] was optimized at lower SCP inclusion level of 2.5% and higher levels led to lower weight gain. Although the optimum inclusion percentage differs slightly between the two studies (5% in the our study and up to 5% in Hombegowda et al) both showed consistent results indicating that low-level SCP supplementation maintains growth performance, While higher inclusion level

reduce weight gain and worsen FCR which is supported by the earlier report of bhunia. et al[23], whom similarly recorded the reduce weight gain, feed intake and performance index, along with higher FCR, at higher SCP inclusion levels (up to 10% replacement of soymeal), with results comparable to only control at the lowest tested level 2%. Taken together, these studies support the conclusion that excessive inclusion can lead to decreased growth efficiency in broiler chicken. These findings indicate that low to moderate levels of SCP inclusion (approximately 5%) is safe and efficient in broilers rations whereas the optimal levels could vary based on SCP source and diet composition. 5% (SCP) inclusion is well tolerated according to the current research since it did not cause any histological changes in the hepatic tissue. 10% concentration level of SCP adverse effect which was not observed at all. At 15% SCP concentration, the hepatic cord disruption, congestion and vacuolation at hepatocellular level were observed. The results are agreeable with reported a study by Mousa MA. et al[24] on SCP, which have revealed that hepatic tolerance only has a threshold with regard to including SCP below a certain level. Low inclusions in the diet did not lead to any liver pathology whereas higher levels led to necrosis and inflammatory infiltration. Another pervious study reported by Rashedi Dehsahraee[25] revealed similar results. Very high SCP inclusion lowered the productivity of laying hens. It can be a sign of metabolic stress brought on by elevated SCP levels. As such, studies are pointing to the same direction with one proving that over-inclusion of SCP harms the hepatic structure and function and moderate supplementation will maintain liver integrity.

CONCLUSION

Waste derived SCP presents a viable alternative protein source for broiler chicken diets. The inclusion of SCP notably enhanced the FCR in the 5% inclusion and 10% inclusion, showing an optimistic development in body weight gain while maintaining no negative impact on feed intake. SCP exhibited sustained growth performance; however, it did not improve feed efficiency, suggesting a diminished responsiveness in older chickens. It is advisable to include SCP at levels of 5–10% no more than 10% diets to achieve optimal performance and sustainability. The appearance of the hepatocellular vacuolation is an important sign of a focal cellular injury with despite the high survival rate of 90%. Further studies are therefore justified to clarify the long-term metabolic consequences and also to determine the reversibility of these changes in the liver after exposure is stopped. SCP demonstrated good tolerance at lower to moderate inclusion levels; however, findings at high doses suggest a necessity for optimizing inclusion thresholds.

Author Contributions

AM: Data Collection, Data Analysis & Interpretation, Manuscript Writing, and Final Approval.
FI: Conception & Design, Data Analysis & Interpretation, Critical Revision and Final Approval.
II: Data Collection, Data Analysis & Interpretation, and Final Approval.

Conflict of Interest

The authors declare no conflict of interests.

Funding Source

This research did not receive any funding.

Data Availability

Data will be available on request.

Ethics approval

The study was reviewed and approved by the Ethical Review Committee (ERC) of Ziauddin University, Karachi, Pakistan (Reference No.: ERC/ZUFESTM/0020).

REFERENCES

1. Falowo AB, Oloruntola OD, Atiba OI, Ayodele OA, Olarotimi OJ, Gbore FA. Growth performance, carcass quality, immune response, and production economics of broiler chickens fed avocado seed meal under feed restriction. Translational Animal Science. 2025;9:txaf047. https://doi.org/doi:10.1093/tas/txaf047.
2. Smith K, Watson AW, Lonnie M, Peeters WM, Oonincx D, Tsoutsoura N, et al. Meeting the global protein supply requirements of a growing and ageing population. European journal of nutrition. 2024;63(5):1425-33. https://doi.org/doi:10.1007/s00394-024-03358-2
3. Drewnowski A, Hooker K. The protein transition: animal-to-plant ratios in global diets. Front Nutr. 2025. https://doi.org/doi:10.3389/fnut.2025.1518793.
4. Maynard C, Maynard C, Jackson A, Kidd M, Rochell S, Owens C. Characterization of growth patterns and carcass characteristics of male and female broilers from four commercial strains fed high- or low-density diets. Poultry Science.2023;102(3):102435. https://doi.org/10.1016/j.psj.2022.102435
5. Zhang A, Wei M, Yan L, Zhou G, Li Y, Wang H, et al. Effects of feeding solid-state fermented wheat bran on growth performance and nutrient digestibility in broiler chickens. Poultry Science. 2022;101(1):101402. https://doi.org/doi:10.1016/j.psj.2021.101402
6. Bharathi P, Dayana R, Rangaraju M, Varsha V, Subathra M, Gayathri, et al. Biogas production from food waste using nanocatalyst. Journal of Nanomaterials. 2022;2022(1):7529036. https://doi.org/10.1155/2022/7529036
7. Tokarchuk D, Pryshliak N, Shynkovych A, Mazur K. Strategic potential of agricultural waste as a feedstock for biofuels production in Ukraine. Rural Sustainability research 2021 Vol 46 (341) P 1-12. 2021. https://doi.org/10.2478/plua-2021-0012
8. Fatima A, Zahid A, Ali S, Anwar W, Zia R, Arshad A, et al. Microbial consortia-mediated rice residue decompositon for eco-friendly management. Scientific Reports. 2025;15(1):32381. https://doi.org/10.1038/s41598-025-99613-5
9. Kieliszek M, Piwowarek K, Kot AM, Pobiega K. The aspects of microbial biomass use in the utilization of

selected waste from the agro-food industry. Open Life Sciences. 2020;15(1):787-96. https://doi.org/10.1515/biol-2020-0099
10. Abdeen MA, Hussein SM, Hassane AM. Single cell protein production from some food wastes using yeasts. Archives of Agriculture Sciences Journal. 2024 Dec 1;7(3):10-22. doi:10.21608/aasj.2024.399216. https://doi.org/doi:10.1016/j.biortech.2021.125364
11. Spanoghe J, Vermeir P, Vlaeminck SE. Microbial food from light, carbon dioxide and hydrogen gas: kinetic, stoichiometric and nutritional potential of three purple bacteria. Bioresource Technology. 2021;337:125364. https://doi.org/doi:10.1016/j.biortech.2021.125364
12. Najib H. Feeding value of single cell protein produced from date palm (Phoenix dactylifera) fruits for broiler chickens. Annu Res Rev Biol. 2014;4(14):2406-13. https://doi.org/doi:10.9734/ARRB/2014/9524
13. Dunuweera AN, Nikagolla DN, Kapilan R. Fruit waste substrates to produce single-cell proteins as alternative human food supplements and animal feeds using baker's yeast (Saccharomyces cerevisiae). J Food Qual. 2021;2021:9932762. https://doi.org/doi:10.1155/2021/9932762
14. Zha X, Tsapekos P, Zhu X, Khoshnevisan B, Lu X, Angelidaki I. Bioconversion of wastewater to single cell protein by methanotrophic bacteria. Bioresource Technology. 2021;320:124351. https://doi.org/doi:10.1016/j.biortech.2020.124351
15. Razzaq ZU, Khan MKI, Maan AA, Rahman Su. Characterization of single cell protein from Saccharomyces cerevisiae for nutritional, functional and antioxidant properties. Journal of food measurement and Characterization. 2020;14(5):2520-8. https://doi.org/10.1007/s11694-020-00498-x
16. Ahmed MG, Gouda SA, Donia S, Hassanein NM. Production of single cell protein by fungi from different food wastes. Biomass Conversion and Biorefinery. 2025 Feb;15(4):5447-62. https://doi.org/10.1007/s13399-024-05478-5.
17. Hombegowda GP, Suresh BN, Shivakumar MC, Ravikumar P, Girish BC, Rudrappa SM, et al. Growth performance, carcass traits and gut health of broiler chickens fed diets incorporated with single cell protein. Animal bioscience. 2021;34(12):1951. https://doi:10.5713/ab.20.0844
18. Khan AR, Ahmad B, Khan M, Khan MA, Sultan S, Sultana K, et al. 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. ACS Omega. 2024;9(36):37763-70. https://doi:10.1021/acsomega.4c03317
19. Alqhtani AH, Al Sulaiman AR, Alharthi AS, Abudabos AE. Dietary supplementation of prebiotic yeast Saccharomyces cerevisiae cell wall promotes growth performance and intestinal health in broiler chickens challenged with Clostridium perfringens. Br Poult Sci. 2024;65(2):129-136. https://doi:10.1080/00071668.2023.2296938.
20. AOAC INTERNATIONAL. Official Methods of Analysis of AOAC International. 22nd ed. Gaithersburg, MD: AOAC INTERNATIONAL; 2019. https://doi:10.1093/9780197610145.003.1403.
21. Clarke G, Eidt S, Sun L, Mawdsley G, Zubovits J, Yaffe M. Whole-specimen histopathology: a method to produce whole-mount breast serial sections for 3-D digital histopathology imaging. Histopathology. 2007;50(2):232-42. https://doi.org/10.1111/j.1365-2559.2006.02561.x
22. Felix N, et al. Proximate and amino acid composition of fishmeal and single-cell protein for broiler diets. Anim Feed SciTechnol.2023;296:115549. https://doi.org/10.1016/j.anifeedsci.2023.11554.
23. Bhunia TAR, Roy B, Das TK. Growth performance of broiler chicken fed diet with single cell protein. Indian J Anim Sci. 2023;93(5):501-504. https://doi.org/10.56093/ijans.v93i5.131043.
24. Mousa MA, Asman AS, Ali RMJ, Sayed RKA, Majrashi KA, Fakiha KG, Selim S.Impacts of dietary lysine and crude protein on performance, hepatic and renal functions, biochemical parameters, and histomorphology of small intestine, liver, and kidney in broiler chickens. Vet Sci. 2023;10(2):98. https://doi.org/10.3390/vetsci10020098.
25. Rashedi Dehsahraee R, Mahdavi AH, Sedghi M, Saleh H. Effect of different levels of single cell protein and probiotic microorganisms on performance, immunologicalresponses, and intestinal histology in laying hens. J Anim Physiol Anim Nutr (Berl).2024;108(5):1177-1188. https://doi.org/10.1111/jpn.13963