

Single-cell protein by-product derived from lysine fermentation as a sustainable soybean meal alternative in broiler nutrition

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ABSTRACT. This study evaluated the effects of dietary supplementation with single-cell protein (SCP) by-product derived from a lysine fermentation on growth performance, nutrient digestibility, organ development, excreta characteristics, and meat quality in broiler chickens. A total of 630 one-day-old Ross 308 broilers were randomly assigned to diets containing 0, 1, 3, 5, or 7% SCP for 35 days. Growth performance indicators, including body weight gain, feed intake, and feed conversion ratio, were not significantly affected by SCP inclusion throughout the experimental period ($P > 0.05$). Similarly, the apparent digestibility of dry matter, nitrogen, and energy, as well as the relative weights of the heart, crop, gizzard, proventriculus, liver, kidney, spleen, and bursa of Fabricius remained unchanged ($P > 0.05$). No differences were observed in excreta gas emissions, excreta consistency, or breast meat quality parameters such as pH, colour, water-holding capacity, cooking loss, and drip loss. Overall, these results demonstrate that SCP can successfully replace conventional soybean meal in broiler diets without negative effects on performance, nutrient utilisation, physiological development, or meat quality. Collectively, the findings highlight the potential of SCP as a sustainable and efficient alternative protein source in poultry production.

Introduction

Poultry production represents one of the most dynamic and rapidly expanding sectors of the global livestock industry, driven by the increasing demand for affordable, high-quality animal protein (Mottet and Tempio, 2017). As the broiler industry expands, efficient feed utilisation becomes essential, since feed accounts for more than 60–70% of total production costs (Ravindran, 2013).

Traditionally, soybean meal has been the main protein source in poultry due to its favourable amino acid composition and high availability (Karr-Lilienthal et al., 2005). However, its long-term sustainability is increasingly affected by

fluctuating market prices, competition with human food supplies, and environmental concerns associated with large-scale soybean cultivation (Elferink et al., 2008; Samtiya et al., 2020). Therefore, there is a need to identify alternative, cost-effective, and sustainable protein sources that can replace soybean meal without compromising bird performance or product quality.

In this context, single-cell proteins (SCPs) derived from microorganisms such as bacteria, yeasts, and algae have recently emerged as promising alternatives to traditional plant-based proteins (Anupama and Ravindra, 2000; Nasseri et al., 2011; Wang et al., 2013). SCPs are protein-rich microbial biomass obtained from the cultivation of

microorganisms and characterized by high crude protein content, a balanced profile of essential amino acids, and functional properties that may improve nutrient utilisation and gut health (Øverland et al., 2010; Glencross et al., 2020).

Among bacterial SCPs, those produced as by-products of industrial L-lysine fermentation by *Corynebacterium glutamicum* represent a distinct category of microbial protein. Unlike previously studied SCP sources, including methanotrophic bacteria (e.g., *Methylococcus capsulatus*; Hombegowda et al., 2021), yeast (Øverland et al., 2010), and other bacterial strains such as *Corynebacterium ammoniagenes* (Wang et al., 2013; An et al., 2018), the lysine-fermentation by-product evaluated in the present study has a markedly higher crude protein content (approximately 76 vs. 48–50% in soybean meal) and standardised ileal digestibility (SID) of lysine (4.86 vs. 2.88%). Despite these favourable nutritional characteristics, previous studies in broilers have typically evaluated bacterial SCP at relatively low inclusion levels (2–5%) (An et al., 2018; Hombegowda et al., 2021; Bhunia et al., 2023). This limitation is partly due to the high nucleic acid content of bacterial SCP, which restricts its dietary inclusion. As a result, most previous studies have tested SCP at relatively low doses. In the present study, inclusion levels of 1, 3, 5, and 7% were applied to represent progressively higher degrees of soybean meal replacement while remaining within nutritionally and physiologically acceptable ranges. At the highest level, SCP replaced approximately 13–15% of soybean meal in the diet. To date, no study has comprehensively assessed this SCP derived from lysine fermentation at such inclusion levels while simultaneously analysing growth performance, nutrient digestibility, excreta gas emissions as indicators of environmental impact, and meat quality. Such an integrated, multi-parameter evaluation is necessary to determine the safety, efficacy, and environmental profile of SCP as a practical alternative to soybean meal, yet it has not been addressed in the existing literature.

Therefore, this study characterised the effects of graded dietary inclusion of lysine-fermentation by-product SCP (0, 1, 3, 5, and 7%) on growth performance, apparent nutrient digestibility, excreta gas emissions, organ development, and breast meat quality traits in broiler chickens, to provide a comprehensive assessment of its potential as a sustainable alternative to conventional soybean meal.

Material and methods

All experimental procedures involving animals were reviewed and approved by the Institutional

Animal Care and Use Committee of Dankook University, Korea (Approval No. DK-1-2424).

Experimental design, animals and managements

A total of 630 one-day-old mixed-sex Ross 308 broiler chicks (initial body weight: 47.22 ± 0.50 g) were used in a 35-day feeding trial. Dietary treatments consisted of a basal diet supplemented with 0, 1, 3, 5, or 7% SCP. As SCP inclusion increased from 0 to 7%, soybean meal was proportionally reduced: in the starter phase from 23.30 to 8.41%, in the grower phase from 26.77 to 13.39%, and in the finisher phase from 21.31 to 7.96%. At the highest inclusion level (7% SCP), this corresponded to a replacement of approximately 13–15% of soybean meal relative to the control diet. To maintain consistent amino acid profiles in all dietary treatments, crystalline amino acids, including L-methionine (99%), L-lysine (78%), L-threonine (99%), L-isoleucine (90%), L-valine (98%), and L-arginine (98.5%), were adjusted accordingly to ensure nutritional equivalence. Thus, SCP was evaluated not only as a direct substitute for soybean meal, but as a functional protein under amino acid-balanced dietary conditions.

The SCP preparation (Prosin™, CJ Cheiljedang Bio, Seoul, Korea) contained fermentation by-products and *Corynebacterium glutamicum* bacterial cells during industrial L-lysine fermentation. The nutrient composition, including SCP crude protein and amino acid contents, is presented in Table 1.

Table 1. Comparison of standardized ileal digestibility of amino acids between single-cell protein (SCP) and soybean meal

Analysed value, %	SCP	Soybean meal
SID arginine	2.43	3.46
SID histidine	1.10	1.22
SID isoleucine	1.53	2.09
SID leucine	2.74	3.49
SID lysine	4.86	2.88
SID methionine	0.64	0.68
SID phenylalanine	1.86	2.11
SID threonine	2.16	1.67
SID tryptophan	0.45	0.65
SID valine	2.23	2.18
SID alanine	4.41	1.75
SID asparagine	4.52	4.71
SID cystine	0.20	0.62
SID glutamine	7.10	7.60
SID glycine	2.12	1.72
SID proline	1.71	2.86
SID serine	1.96	2.10
SID tyrosine	1.22	1.40
Crude protein	76.03	48.50
Crude fat	5.33	1.80
Crude fibre	2.70	6.80
ME, kcal/kg	2 878	2 560

SID – standardized ileal digestibility, ME – metabolizable energy

Table 2. Feed composition of broiler (starter)

Item	Experimental diet				
	CON	1% SCP	3% SCP	5% SCP	7% SCP
Ingredients, %					
corn	54.38	54.88	55.73	57.41	58.09
soybean meal (48%)	23.30	20.70	16.78	12.27	8.41
corn gluten meal	2.00	2.00	2.00	2.00	2.00
palm kernel meal	5.00	5.00	6.00	6.00	7.00
rapeseed meal	5.00	6.00	6.00	7.00	7.00
wheat bran	5.00	5.00	5.00	5.00	5.00
tallow	1.36	1.39	1.35	1.03	1.07
MDCP	1.02	1.03	1.04	1.08	1.14
limestone	1.33	1.34	1.34	1.34	1.33
salt	0.20	0.20	0.20	0.20	0.20
L-methionine (99%)	0.21	0.22	0.23	0.25	0.27
lysine (78%)	0.47	0.45	0.42	0.38	0.34
threonine (99%)	0.20	0.21	0.22	0.23	0.24
isoleucine (90%)	0.08	0.09	0.13	0.16	0.20
valine (98%)	0.12	0.12	0.14	0.16	0.17
arginine (98.5%)	0.08	0.12	0.17	0.24	0.29
mineral mix ¹	0.10	0.10	0.10	0.10	0.10
vitamin mix ²	0.10	0.10	0.10	0.10	0.10
choline (25%)	0.05	0.05	0.05	0.05	0.05
single-cell protein	-	1.00	3.00	5.00	7.00
Total	100.00	100.00	100.00	100.00	100.00
Calculated value, %					
crude protein	22.00	22.00	22.00	22.00	22.00
Ca	0.95	0.95	0.95	0.95	0.95
available P	0.50	0.50	0.50	0.50	0.50
SID lysine	1.32	1.32	1.32	1.32	1.32
SID methionine+cysteine	1.00	1.00	1.00	1.00	1.00
SID methionine	0.55	0.56	0.57	0.58	0.60
SID threonine	0.88	0.88	0.88	0.88	0.88
SID valine	1.04	1.03	1.04	1.04	1.03
SID isoleucine	0.88	0.87	0.88	0.87	0.88
SID arginine	1.40	1.40	1.40	1.40	1.40
SID tryptophan	0.22	0.21	0.19	0.18	0.17
SID leucine	1.66	1.63	1.62	1.60	1.58
ME, kcal/kg	3000	3000	3000	3000	3000
fat	4.66	4.41	4.80	4.61	4.74
fibre	5.32	5.23	5.29	5.18	5.16
ash	6.00	5.95	5.80	5.68	5.58

CON – control diet, SCP – single-cell protein, MDCP – monocalcium phosphate, SID – standardized ileal digestibility, ME – metabolizable energy; ¹ provided per kg of complete diet: mg: Zn (as ZnSO₄) 37.5, Mn (as MnO₂) 37.5, Fe (as FeSO₄·7H₂O) 37.5, Cu (as CuSO₄·5H₂O) 3.75, I (as KI) 0.83, Se (as Na₂SeO₃·5H₂O) 0.23; ² provided per kg of complete diet: IU: vit. A 15 000, vit. D₃ 3 750, vit. E 37.5; mg: vit. K₃ 2.55, thiamin 3, riboflavin 7.5, vit. B₆ 4.5, niacin 51, folic acid 1.5, biotin 0.2, Ca-pantothenate 13.5; ug: vit. B₁₂ 24

The experiment followed a completely randomised design with five dietary treatments, each with seven replicates of 18 birds. The feeding programme was divided into three phases: starter (days 1–7; Table 2), grower (days 8–21; Table 3), and finisher (days 22–35; Table 4).

Table 3. Feed composition of broiler (grower)

Item	Experimental diet				
	CON	1% SCP	3% SCP	5% SCP	7% SCP
Ingredients, %					
corn	57.87	58.23	59.73	60.35	61.05
soybean meal (48%)	26.77	24.85	21.02	17.24	13.39
palm kernel meal	6.00	6.50	7.00	8.00	9.00
wheat bran	5.00	5.00	5.00	5.00	5.00
tallow	1.52	1.53	1.23	1.29	1.31
MDCP	0.67	0.67	0.71	0.77	0.83
limestone	1.09	1.10	1.10	1.09	1.07
salt	0.20	0.20	0.20	0.20	0.20
L-methionine (99%)	0.20	0.21	0.23	0.24	0.26
lysine (78%)	0.27	0.25	0.21	0.16	0.12
threonine (99%)	0.13	0.14	0.15	0.16	0.17
isoleucine (90%)	-	0.01	0.04	0.07	0.10
valine (98%)	0.03	0.04	0.05	0.06	0.08
arginine (98.5%)	-	0.02	0.08	0.12	0.17
mineral mix ¹	0.10	0.10	0.10	0.10	0.10
vitamin mix ²	0.10	0.10	0.10	0.10	0.10
choline (25%)	0.05	0.05	0.05	0.05	0.05
single-cell protein	-	1.00	3.00	5.00	7.00
Total	100.00	100.00	100.00	100.00	100.00
Calculated value, %					
crude protein, %	20.50	20.50	20.50	20.50	20.50
Ca, %	0.75	0.75	0.75	0.75	0.75
available P	0.42	0.42	0.42	0.42	0.42
SID lysine	1.18	1.18	1.18	1.18	1.18
SID methionine+cysteine	0.92	0.92	0.92	0.92	0.92
SID methionine	0.51	0.52	0.54	0.54	0.56
SID threonine	0.79	0.79	0.79	0.79	0.79
SID valine	0.93	0.93	0.92	0.92	0.92
SID isoleucine	0.79	0.79	0.79	0.79	0.78
SID arginine	1.35	1.35	1.35	1.35	1.35
SID tryptophan	0.22	0.21	0.20	0.19	0.17
SID leucine	1.53	1.51	1.48	1.46	1.43
ME, kcal/kg	3050	3050	3050	3050	3050
fat	4.80	4.86	4.68	4.84	4.95
fibre	5.11	5.10	5.00	4.98	4.96
ash	5.33	5.26	5.14	5.04	4.92

CON – control diet, SCP – single-cell protein, MDCP – monocalcium phosphate, SID – standardized ileal digestibility, ME – metabolizable energy; ¹ provided per kg of complete diet: mg: Zn (as ZnSO₄) 37.5, Mn (as MnO₂) 37.5, Fe (as FeSO₄·7H₂O) 37.5, Cu (as CuSO₄·5H₂O) 3.75, I (as KI) 0.83, Se (as Na₂SeO₃·5H₂O) 0.23; ² provided per kg of complete diet: IU: vit. A 15 000, vit. D₃ 3 750, vit. E 37.5; mg: vit. K₃ 2.55, thiamin 3, riboflavin 7.5, vit. B₆ 4.5, niacin 51, folic acid 1.5, biotin 0.2, Ca-pantothenate 13.5; ug: vit. B₁₂ 24

Birds were housed in three-tier battery cages equipped with feeders and nipple drinkers, with *ad libitum* access to feed and water. Experimental diets were formulated in mash form according to the nutritional recommendations of the NRC (1994) and the Korean Feeding Standard

Table 4. Feed composition of broiler (finisher)

Item	Experimental diet				
	CON	1% SCP	3% SCP	5% SCP	7% SCP
Ingredients, %					
corn	63.27	63.67	64.33	64.94	65.56
soybean meal (48%)	21.31	19.36	15.55	11.74	7.96
palm kernel meal	6.50	7.00	8.00	9.00	10.00
wheat bran	5.00	5.00	5.00	5.00	5.00
tallow	1.33	1.33	1.37	1.44	1.50
MDCP	0.38	0.38	0.42	0.48	0.54
limestone	1.03	1.04	1.04	1.04	1.02
salt	0.20	0.20	0.20	0.20	0.20
L-methionine (99%)	0.19	0.20	0.21	0.23	0.25
lysine (78%)	0.32	0.30	0.26	0.22	0.17
threonine (99%)	0.14	0.14	0.15	0.16	0.17
isoleucine (90%)	0.03	0.05	0.08	0.10	0.13
valine (98%)	0.05	0.05	0.07	0.08	0.09
arginine (98.5%)	-	0.03	0.07	0.12	0.16
mineral mix ¹	0.10	0.10	0.10	0.10	0.10
vitamin mix ²	0.10	0.10	0.10	0.10	0.10
choline (25%)	0.05	0.05	0.05	0.05	0.05
single-cell protein	-	1.00	3.00	5.00	7.00
Total	100.00	100.00	100.00	100.00	100.00
Calculated value, %					
crude protein, %	18.50	18.50	18.50	18.50	18.50
Ca, %	0.65	0.65	0.65	0.65	0.65
available P	0.36	0.36	0.36	0.36	0.36
SID lysine	1.08	1.08	1.08	1.08	1.08
SID methionine+cysteine	0.86	0.86	0.86	0.86	0.86
SID methionine	0.48	0.49	0.49	0.51	0.53
SID threonine	0.72	0.72	0.72	0.72	0.72
SID valine	0.85	0.85	0.85	0.85	0.84
SID isoleucine	0.73	0.73	0.73	0.72	0.72
SID arginine	1.20	1.20	1.20	1.20	1.20
SID tryptophan	0.19	0.18	0.17	0.15	0.14
SID leucine	1.46	1.44	1.41	1.39	1.36
ME, kcal/kg	3100	3100	3100	3100	3100
fat	4.75	4.80	4.94	5.10	5.26
fibre	4.98	4.97	4.95	4.93	4.92
ash	4.69	4.62	4.50	4.41	4.31

CON – control diet, SCP – single-cell protein, MDCP – monocalcium phosphate, SID – standardized ileal digestibility, ME – metabolizable energy; ¹ provided per kg of complete diet: mg: Zn (as ZnSO₄) 37.5, Mn (as MnO₂) 37.5, Fe (as FeSO₄·7H₂O) 37.5, Cu (as CuSO₄·5H₂O) 3.75, I (as KI) 0.83, Se (as Na₂SeO₃·5H₂O) 0.23; ² provided per kg of complete diet: IU: vit. A 15 000, vit. D₃ 3 750, vit. E 37.5; mg: vit. K₃ 2.55, thiamin 3, riboflavin 7.5, vit. B₆ 4.5, niacin 51, folic acid 1.5, biotin 0.2, Ca-pantothenate 13.5; ug: vit. B₁₂ 24

for Poultry (2022), ensuring that all nutrient requirements were met. Environmental conditions, including temperature and humidity, were controlled according to the Ross Broiler Management Manual.

Sampling and measurements

Growth performance

Body weight was recorded on days 1, 7, 21, and 35. Feed intake (FI) was calculated as the difference between feed offered and feed residue. Feed conversion ratio (FCR) was calculated as feed intake divided by body weight gain (BWG).

Nutrient digestibility

Chromium oxide (0.2%) was added to the diet from day 28 to 35 as an indigestible marker for the determination of apparent total tract digestibility (ATTD) of dry matter, nitrogen, and energy. On day 35, excreta were collected from ten pens per treatment using stainless steel trays. Samples were oven-dried at 105 °C for 24 h and ground to pass through a 1 mm screen. Analyses were conducted according to AOAC International (2001) procedures. Chromium concentration was measured using a UV-1201 spectrophotometer (Shimadzu, Kyoto, Japan); gross energy using a Parr 6400 oxygen bomb calorimeter (Parr Instrument Co., USA); and nitrogen using a Kjeltac™ 8400 analyser (Foss, Höganäs, Sweden). ATTD (%) was calculated as:

$$\text{ATTD} = 100 - [(\text{NF}/\text{ND}) \times (\text{CrD}/\text{CrF}) \times 100],$$

where: NF – nutrient concentration in faeces, ND – nutrient concentration in the diet, CrD – chromium in the diet, and CrF – chromium in faeces.

Excreta score

Excreta consistency was scored on day 35 using a four-point scale: 1 – firm, well-formed faeces with minimal moisture; 2 – slightly irregular faeces with small amounts of undigested feed; 3 – poorly formed faeces with higher moisture; 4 – watery, unformed faeces with a high proportion of undigested feed.

Gas emission

Approximately 300 g of fresh excreta per replicate was placed in a 2.6 l sealed container and fermented at room temperature for 7 days. After fermentation, the concentrations of ammonia (NH₃), hydrogen sulphide (H₂S), methyl mercaptan, acetic acid, and carbon dioxide (CO₂) were measured using a multi-gas meter (MultiRAE Lite, model PGM-6208; RAE Systems, USA).

Organ weight and meat quality

On day 35, ten birds per treatment were randomly selected and euthanized by CO₂ inhalation. The heart, crop, gizzard, proventriculus, liver, kidney, spleen, and bursa of Fabricius were excised and weighed to calculate relative organ weight (organ weight / live body weight × 100).

At 24 h postmortem, breast muscle pH was measured using a digital pH meter (Testo 205, Lenzkirch, Germany) after homogenising 10 g of tissue with 90 ml of double-distilled water. Meat colour (CIE L*, a*, b*) was assessed in triplicate using a Minolta colorimeter (CR-300, Tokyo, Japan). Cooking loss was determined by sealing samples in Cryovac® Cook-In Bags and heating in a 100 °C water bath for 30 min. After cooling, samples were reweighed, and cooking loss (%) was expressed as the percentage weight reduction. Water-holding capacity (WHC) was measured according to Kristensen and Purslow (2001): 5 g of muscle was heated at 70 °C for 30 min, cooled on ice, and centrifuged at $1\,000 \times g$ for 10 min at 4 °C. WHC (%) = (fluid retained / initial water content) \times 100. Drip loss was measured in 5×5 cm fillets stored at 4 °C for 1, 3, 5, and 7 days, with loss calculated as the percentage weight difference before and after storage.

significance was set at $P < 0.05$, and all analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, USA).

Results

Growth performance

The effects of dietary SCP supplementation (0, 1, 3, 5, and 7%) on broiler growth performance during the 35-day trial are summarised in Table 5. From days 1 to 7, no significant differences ($P > 0.05$) were observed in BWG, FI, or FCR between the treatments. Similar results were obtained during the grower (days 8–21) and finisher (days 22–35) phases. Over the entire experimental period (days 1–35), dietary SCP inclusion exerted no significant effect ($P > 0.05$) on BWG, FI, or FCR.

Table 5. The effect of dietary single-cell protein (SCP) supplementation on growth performance in broilers

Items	Experimental diet					-SEM	P-value
	CON	1% SCP	3% SCP	5% SCP	7% SCP		
days 1 to 7							
BWG, g	172	176	175	173	170	5	0.8759
FI, g	181	184	184	182	179	6	0.9722
FCR	1.054	1.045	1.051	1.054	1.058	0.008	0.8132
days 8 to 21							
BWG, g	690	706	699	694	685	11	0.6638
FI, g	937	947	943	940	935	15	0.9839
FCR	1.360	1.340	1.349	1.356	1.367	0.012	0.5873
days 22 to 35							
BWG, g	1.024	1.050	1.041	1.032	1.007	18	0.4981
FI, g	1.750	1.772	1.766	1.757	1.738	27	0.9052
FCR	1.713	1.686	1.698	1.706	1.726	0.021	0.7268
days 1 to 35							
BW, g	1.932	1.980	1.963	1.946	1.908	28	0.4331
BWG, g	1.885	1.932	1.916	1.898	1.861	28	0.4357
FI, g	2.868	2.903	2.893	2.880	2.853	39	0.9003
FCR	1.523	1.501	1.511	1.518	1.533	0.012	0.4513
mortality	3.17	2.38	3.17	2.38	2.45	0.20	0.9629

CON – control, basal diet; 1% SCP – CON diet supplemented with 1% single-cell protein; 3% SCP – CON diet supplemented with 3% single-cell protein; 5% SCP – CON diet supplemented with 5% single-cell protein; 7% SCP – CON diet supplemented with 7% single-cell protein; BWG – body weight gain; FI – feed intake; FCR – feed conversion ratio; SEM – standard error of the mean; $P > 0.05$ (not statistically significant)

Statistical analyses

Data were analysed using a completely randomised design with one-way ANOVA to evaluate treatment effects. The pen was considered the experimental unit for growth performance and nutrient digestibility, whereas individual birds were used as the experimental unit for organ weight and meat quality analyses. When significant differences were detected, means were compared using Tukey's honestly significant difference (HSD) test. Statistical

Nutrient digestibility

The ATTD of dry matter, nitrogen, and energy did not differ significantly ($P > 0.05$) between dietary treatments (Table 6).

Organ weights

The relative weights of the heart, crop, gizzard, proventriculus, liver, kidney, spleen, and bursa of Fabricius were not significantly affected ($P > 0.05$) by SCP incorporation (Table 7).

Table 6. The effect of dietary single-cell protein (SCP) supplementation on nutrient digestibility in broilers, %

Items	Experimental diet					SEM	P-value
	CON	1% SCP	3% SCP	5% SCP	7% SCP		
Dry matter	69.49	70.81	70.45	69.80	69.17	0.85	0.6505
Nitrogen	68.04	69.17	68.83	68.39	67.85	0.63	0.5734
Energy	69.34	70.15	69.87	69.52	69.12	0.68	0.8275

CON – control, basal diet; 1% SCP – CON diet supplemented with 1% single-cell protein; 3% SCP – CON diet supplemented with 3% single-cell protein; 5% SCP – CON diet supplemented with 5% single-cell protein; 7% SCP – CON diet supplemented with 7% single-cell protein; SEM – standard error of the mean; $P > 0.05$ (not statistically significant)

Table 7. The effect of dietary single-cell protein (SCP) supplementation on organ weight in broilers, %

Items, %	Experimental diet					SEM	P-value
	CON	1% SCP	3% SCP	5% SCP	7% SCP		
Heart	0.49	0.52	0.51	0.50	0.49	0.01	0.4541
Crop	0.79	0.84	0.81	0.79	0.78	0.03	0.7797
Gizzard	1.97	2.02	2.00	1.98	1.95	0.03	0.3910
Proventriculus	0.38	0.40	0.40	0.39	0.38	0.01	0.5941
Liver	2.48	2.55	2.53	2.50	2.46	0.11	0.9831
Kidney	0.61	0.64	0.62	0.61	0.59	0.04	0.9298
Spleen	0.09	0.12	0.11	0.10	0.09	0.01	0.5633
Bursa of Fabricius	0.20	0.22	0.21	0.20	0.20	0.03	0.9875

CON – control, basal diet; 1% SCP – CON diet supplemented with 1% single-cell protein; 3% SCP – CON diet supplemented with 3% single-cell protein; 5% SCP – CON diet supplemented with 5% single-cell protein; 7% SCP – CON diet supplemented with 7% single-cell protein; SEM – standard error of the mean; $P > 0.05$ (not statistically significant)

Excreta gas emission and excreta score

Dietary SCP supplementation did not significantly influence ($P > 0.05$) the concentrations of excreta gases, including NH_3 , H_2S , methyl mercaptan, acetic acid, or CO_2 . Likewise, excreta scores remained unchanged in all treatments (Table 8).

Table 8. The effect of dietary single-cell protein (SCP) supplementation on excreta gas emission and faecal score in broilers

Items	Experimental diet					SEM	P-value
	CON	1% SCP	3% SCP	5% SCP	7% SCP		
NH_3	15.00	14.38	14.63	14.75	15.25	1.49	0.9944
H_2S	1.83	1.55	1.63	1.75	1.90	0.30	0.9189
Methyl mercaptans	5.00	4.38	4.50	4.75	5.13	0.53	0.8329
Acetic acid	3.25	2.75	3.13	2.63	3.13	0.42	0.7924
CO_2	1 425	1 450	1 475	1 450	1 425	90	0.9937
Faecal score	1.50	1.36	1.43	1.50	1.57	0.18	0.9367

CON – control, basal diet; 1% SCP – CON diet supplemented with 1% single-cell protein; 3% SCP – CON diet supplemented with 3% single-cell protein; 5% SCP – CON diet supplemented with 5% single-cell protein; 7% SCP – CON diet supplemented with 7% single-cell protein; NH_3 – ammonia; H_2S – hydrogen sulphide; CO_2 – carbon dioxide; SEM – standard error of the mean; $P > 0.05$ (not statistically significant)

Meat quality

There were no significant differences ($P > 0.05$) in breast meat pH, colour parameters (lightness, redness, and yellowness), water-holding capacity, cooking loss, or drip loss between the treatments. Drip loss measured on days 1, 3, 5, and 7 post-storage also did not differ between the groups (Table 9).

Table 9. The effect of dietary single-cell protein (SCP) supplementation on meat quality in broilers

Items	Experimental diet					SEM	P-value
	CON	1% SCP	3% SCP	5% SCP	7% SCP		
pH	5.71	5.68	5.64	5.76	5.73	0.15	0.9822
WHC, %	60.08	59.42	60.21	59.81	60.52	1.59	0.9900
Meat colour							
L*	58.83	58.49	59.27	59.71	60.03	2.96	0.9857
a*	5.33	5.17	5.41	5.40	5.42	0.67	0.9986
b*	15.04	15.25	15.19	15.20	15.13	1.05	0.9999
Cook loss, %	20.25	21.02	20.88	21.59	21.61	1.07	0.8873
Drip loss, %							
day 1	1.89	1.93	1.90	1.86	1.80	0.15	0.9778
day 3	3.18	3.21	3.15	3.13	3.20	0.26	0.9993
day 5	4.20	4.19	4.21	4.24	4.23	0.28	0.9999
day 7	5.28	5.18	5.26	5.30	5.32	0.22	0.9917

CON – control, basal diet; 1% SCP – CON diet supplemented with 1% single-cell protein; 3% SCP – CON diet supplemented with 3% single-cell protein; 5% SCP – CON diet supplemented with 5% single-cell protein; 7% SCP – CON diet supplemented with 7% single-cell protein; WHC – water holding capacity; L* – lightness; a* – redness; b* – yellowness; SEM – standard error of the mean; $P > 0.05$ (not statistically significant)

Discussion

The present study demonstrated that dietary supplementation with SCP at inclusion levels up to 7% had no adverse effects on broiler growth performance. In all growth phases (days 1–7, 8–21, 22–35, and overall), body weight gain, feed intake, and feed conversion ratio did not differ significantly between the treatments. These results indicate that SCP can effectively replace conventional soybean meal without compromising broiler productivity.

Soybean meal is widely recognised as a high-quality protein source in poultry nutrition; however, its limited availability and price volatility have increased interest in alternative protein ingredients. Various SCPs, such as those derived from *Methylococcus*, yeast, and bacterial strains, have shown potential as sustainable feed components in poultry diets. For instance, SCP produced from *Methylococcus* spp., used to replace soybean meal and fishmeal at 2.5–10% in broiler diets, resulted in comparable body weight gain and improved feed

efficiency, while higher levels ($\geq 7.5\%$) reduced growth performance (Hombegowda et al., 2021). Similarly, *Corynebacterium ammoniagenes* SCP supplementation at 1–5% improved early-phase growth at 1% inclusion but reduced feed intake at higher percentages, without affecting blood parameters, bone characteristics, or meat quality (An et al., 2018). Bhunia et al. (2023) also reported that replacing soybean meal with SCP maintained body weight at 2% inclusion but reduced growth at higher levels. Collectively, these studies suggest that bacterial SCP can maintain or improve broiler performance at moderate dietary levels, whereas excessive supplementation may impair nutrient utilisation.

In the current study, SCP showed a higher standardised ileal digestibility (SID) of lysine than soybean meal (Table 1). Lysine plays a critical role in protein synthesis and muscle development and is commonly used as a reference amino acid in broiler diet formulation. Although SCP had higher SID of lysine and crude protein compared with soybean meal, this advantage did not translate into improved growth performance. This can be explained by the fact that all experimental diets were formulated to be iso-nitrogenous and balanced for essential amino acids through the inclusion of crystalline amino acids. According to the concept of the first-limiting amino acid, growth responses occur only when the deficiency of the most limiting amino acid in the diet is corrected. In the present study, the amino acid profile of all diets was already optimised to meet the birds' nutritional requirements; therefore, additional lysine supplied by SCP would not necessarily result in further improvements in growth performance. In this trial, a diet containing 7% SCP replaced approximately 13–15% of soybean meal in all feeding phases while maintaining comparable performance. This outcome indicates that SCP provides an adequate amino acid profile and digestibility to meet the nutritional requirements of broilers, confirming its suitability as a sustainable and nutritionally efficient protein source.

Consistent with the growth data, nutrient digestibility was not significantly affected by SCP inclusion. Previous studies have shown variable results depending on SCP type and its dietary proportion. Schøyen et al. (2007) observed that bacterial SCP did not adversely affect ileal amino acid digestibility in broilers at moderate levels, although autolysed forms slightly reduced the digestibility of lysine, methionine, and arginine. Likewise, Zhang et al. (2013) reported that replacing 2.5% fishmeal with SCP in weaned pig

diets maintained digestibility, but 5% substitution led to a slight reduction in protein digestibility. The absence of such effects in this study suggests that SCP inclusion at up to 7% provides a well-balanced nutrient composition that supports efficient digestion and absorption.

It should be noted that interpreting the present results requires careful consideration of the diet formulation strategy. As SCP progressively replaced soybean meal (up to 7%), all experimental diets were formulated to be iso-nitrogenous and balanced for essential amino acids through supplementation with crystalline amino acids. Consequently, the absence of significant differences in growth performance and nutrient digestibility reflects the biological equivalence of SCP to soybean meal as a protein source under nutritionally standardised conditions. This formulation strategy is consistent with current commercial poultry production practices, where crystalline amino acids are routinely used to optimise amino acid profiles and reduce feed costs (Emmert and Baker, 1997). Under such conditions, SCP functions effectively as a protein component within a balanced diet rather than as a direct standalone substitute for conventional protein sources. Organ development and relative organ weights were also unaffected by dietary SCP, suggesting that it does not interfere with physiological development or metabolic health. These findings are consistent with reports by Hombegowda et al. (2021) and An et al. (2018), who found no significant changes in liver, spleen, or bursa weights when SCP was added as a partial soybean meal replacement. Thus, SCP appears to be physiologically safe for broilers even at inclusion levels as high as 7%.

Moreover, excreta gas emissions and consistency were not influenced by SCP supplementation, indicating that intestinal fermentation and microbial activity in the intestine were not adversely affected. The stability of ammonia, hydrogen sulphide, methyl mercaptan, and volatile acid emissions indicated that nitrogen and sulphur metabolism remained stable across treatments. This is likely because protein degradation and amino acid utilisation from SCP were comparable to those from soybean meal, leading to similar nitrogen excretion profiles. Ammonia emission from poultry manure is closely associated with nitrogen utilisation efficiency in birds. The absence of increased NH_3 emissions in the present study suggests that the replacement of soybean meal with SCP did not impair nitrogen utilisation or increase nitrogen losses in manure. From a practical perspective, maintaining similar levels of ammonia and sulphur-containing gas emissions demonstrates

that the addition of SCP does not introduce additional challenges for manure management or environmental control in broiler production systems. Therefore, these results support the use of SCP as a sustainable alternative protein source that can be incorporated into poultry diets without increasing manure-related environmental emissions.

Finally, no detrimental effects were observed on breast meat quality parameters, including pH, colour, water-holding capacity, cooking loss, and drip loss. These findings are consistent with previous reports (An et al., 2018), indicating that partial substitution of soybean meal with SCP does not alter carcass and meat characteristics obtained with conventional diets. This is an important outcome for commercial production, as meat quality directly affects consumer acceptance and marketability. Although many measured parameters were not significantly different among treatments, the results indicate a clear biological equivalence between diets containing SCP and conventional soybean meal. Retaining comparable growth performance, nutrient digestibility, organ development, and meat quality, despite a substantial reduction in soybean meal, demonstrates that SCP can effectively function as an alternative protein ingredient in broiler diets and supports its practical application in commercial feed formulation.

Conclusions

Dietary supplementation with single-cell protein (SCP) at levels up to 7% effectively replaced soybean meal in broiler diets without adverse effects on growth performance, nutrient digestibility, organ development, excreta characteristics, or breast meat quality. These findings demonstrate that SCP is a safe and nutritionally viable alternative protein source for broilers. Moreover, its incorporation into feed formulations can contribute to more sustainable, resource-efficient, and environmentally responsible poultry production systems.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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