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Evaluation of Single Cell Protein as a Non-Conventional Feedstuff in Broilers Feeding

Research Article

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ABSTRACT

Single cell protein (SCP) is the definition for dried cells originated from single-celled organisms intended to be used as a protein source in human foods or animal feeds. An experiment has been conducted to evaluate the various levels of single cell protein on fecal microflora and productive traits of broiler chickens. A total of 192 day-old Ross 308 male chicks were randomly divided into 4 treatments. Each treatment consists of 48 birds and 4 replicates of 12 birds each. From day one, birds were fed either a corn-soybean meal basal diet with different levels of SCP (0, 5, 10 and 15 percent of as fed basis diet). Four birds from each treatment were randomly selected for slaughter and microbial study at day 47. Total counts of aerobic bacteria, *Lactobacillus* and *Coliforms* in the gut were not significantly affected by dietary treatments. The SCP at the levels of 10 and 15% caused to severe mortality. Also, SCP had adverse impacts on body weight gain, feed intake and feed efficiency. In conclusion, although SCP reduced feed cost, but it had some adverse effects on birds' performance.

KEY WORDS broiler, gut microflora, performance, single cell protein.

INTRODUCTION

World population is growing fast and subsequently the meat demand also will increase. Chicken meat is an important source of nutritious food and protein for many of the world's humans. Soybean meal, the major protein source in poultry production, cannot be grown in sufficient quantities to meet high needs of common feed ingredients. Also, use of other sources of protein, for example, fishmeal, is decreasing. The use of fishmeal is decreasing partially due to reduction in the supplies of caught fish due to tighter quotas, stricter control of unregulated fishing, and enlarged utilization of more low-cost dietary substitutes (FAO, 2012). As a consequence, there is an increasing demand for alternative high-quality protein sources for poultry production industry.

Finding suitable non-conventional feedstuffs is increasingly of utmost importance (Leeson and Summers, 2005). However, each new product has its disadvantages such as anti-nutritional factors (Leeson and Summers, 2001). One of the possible alternative protein sources is single cell protein (SCP). The SCP consists of microorganisms such as filamentous fungi, yeast, algae, and bacteria that are rich in protein. It is a very fast way of producing protein compared to the production of protein through cultivation of agricultural crops or animal farming. The SCP can be produced from residual streams from different industries giving the possibility of a cheap production (Nasseri *et al.* 2011). In addition; the SCP production can be performed in bioreactors and does not tie up agricultural land. Thus, SCP as a replacement for traditional protein sources has been evaluated in this trial.

MATERIALS AND METHODS

Birds, diets and experimental design

A total number of 192 day-old male chicks (Ross 308) were purchased form a local hatchery. All the chicks were weighed individually (mean=46 g) and randomly were divided into 4 treatments. Each treatment consists of 48 birds and three replicates of 12 birds per replicate. Pen dimensions was 120 × 120 cm, so that each chicken had 1200 cm^2 floor space. The initial house temperature was set at 32 ˚C and gradually decreased to reach 24 ˚C at 28 day of age. A lighting schedule of 24 h illumination with approximately 20 lx was used for the entire period. Chicks were vaccinated for Infectious Bronchitis and Newcastle Lasota on d 4, and Avian Influenza + New Castle on d 14 of age.

From d one, birds were fed either a corn-soybean meal basal diet with different levels of SCP; 0, 5, 10, and 15% as T1, T2, T3 and T4, respectively.

Nutrient analysis of SCP which was used in this trial is shown in Table 1. All diets (Tables 2 and 4) were formulated to meet the nutrient requirements of the broiler chickens as recommended by Ross 308 broiler management guide (Aviagen, 2009). Feed and water were offered *ad libitum* throughout the trial.

Growth performance

Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were measured as starter (0-10 d), grower (11-24 d), finisher (25-42 d) phases, and overall as recommended by Ross 308 catalogue.

Table 1 Nutrient analysis of single cell protein (SCP) used in this trial (as-fed %)

ME (kcal/kg)	2674.32
Dry matter	91.5
Crude protein	40
Total volatile nitrogen (TVN) (mg/100)	121.4
Calcium	3.07
Available phosphorus	2.02
Lysine	1.156
Methionine	0.295
Methionine + cystine	0.837
Threonine	1.397

Table 2 The composition of starter (0-10 d) diets

Item	Treatment 1 (control)	Treatment 2	Treatment 3	Treatment 4
Ingredients (%)				
Single cell protein	0	5	10	15
Corn	39.82	39.25	47.55	48
Soybean meal	36.22	34.62	31.37	29.58
Corn gluten germ	15.42	13.20	3.49	0.38
Vegetable oil	3.50	3.50	3.50	3.50
Oyster shell	0.99	0.94	0.88	0.82
Dicalcium phosphate	2.06	1.50	1.03	0.48
Common salt	0.30	0.31	0.35	0.36
L-lysine HCl	0.35	0.35	0.40	0.45
DL-methionine	0.32	0.32	0.39	0.40
L-threonine	0.02	0.01	0.04	0.03
Vitamin and mineral premix[1]	0.50	0.50	0.50	0.50
Sodium bentonite	0.50	0.50	0.50	0.50
Calculated contents (%)				
Metabolizable energy (ME) (kcal/kg)	3025	3025	3025	3025
Crude protein	23.26	24	23.26	23.87
Calcium	1.05	1.05	1.05	1.05
Available phosphorus	0.50	0.50	0.50	0.50
Sodium	0.16	0.16	0.16	0.16
Lysine	1.43	1.43	1.40	1.43
Methionine	0.68	0.69	0.73	0.74
Methionine + cystine	1.07	1.07	1.07	1.07
Threonine	0.94	0.94	0.94	0.94

[1] Vitamin and mineral premix supplied per kilogram of diet: vitamin A: 10000 IU; vitamin D_3: 9800 IU; vitamin E: 121 IU; B_{12}: 20 µg; Riboflavin: 4.4 mg; Calcium pantothenate: 40 mg; Niacin: 22 mg; Choline: 840 mg; Biotin: 30 µg; Thiamin: 4 mg; Zinc sulfate: 60 mg and Manganese oxide: 60 mg.

Table 3 The composition of grower (11-24 d) diets

Item	Treatment 1 (control)	Treatment 2	Treatment 3	Treatment 4
Ingredients (%)				
Single cell protein	0	5	10	15
Corn	43.64	47.69	47.10	51.73
Soybean meal	28.23	26.99	25.39	25.09
Corn gluten germ	20	12.36	10.16	0
Vegetable oil	3.69	4	4	5.1
Oyster shell	0.78	0.72	0.67	0.68
Dicalcium phosphate	1.8	1.29	0.73	0.33
Common salt	0.29	0.32	0.33	0.39
L-lysine HCl	0.33	0.35	0.35	0.35
DL-methionine	0.24	0.28	0.28	0.33
Vitamin and mineral premix[1]	0.5	0.5	0.5	0.5
Sodium bentonite	0.5	0.5	0.5	0.5
Calculated contents (%)				
Metabolizable energy (ME) (kcal/kg)	3150	3150	3150	3170
Crude protein	21	21.15	21.91	21.97
Calcium	0.90	0.90	0.90	0.90
Available phosphorus	0.45	0.45	0.45	0.45
Sodium	0.16	0.16	0.16	0.17
Lysine	1.24	1.24	1.24	1.24
Methionine	0.58	0.61	0.62	0.65
Methionine + cystine	0.95	0.95	0.95	0.95
Threonine	0.83	0.83	0.85	0.84

[1] Vitamin and mineral premix supplied per kilogram of diet: vitamin A: 10000 IU; vitamin D_3: 9800 IU; vitamin E: 121 IU; B_{12}: 20 µg; Riboflavin: 4.4 mg; Calcium pantothenate: 40 mg; Niacin: 22 mg; Choline: 840 mg; Biotin: 30 µg; Thiamin: 4 mg; Zinc sulfate: 60 mg and Manganese oxide: 60 mg.

Table 4 The composition of finisher (25-42 d) diets

Item	Treatment 1 (control)	Treatment 2	Treatment 3	Treatment 4
Ingredients (%)				
Single cell protein	0	5	10	15
Corn	47.93	49.90	56.10	59.3
Soybean meal	24.22	19.86	19.66	18.84
Corn gluten germ	20	18.43	7.23	0
Vegetable oil	3.69	3.11	3.78	4.16
Oyster shell	0.76	0.71	0.64	0.58
Dicalcium phosphate	1.66	1.13	0.64	0.12
Common salt	0.29	0.30	0.34	0.36
L-lysine HCl	0.27	0.21	035	0.35
DL-methionine	0.18	0.35	0.29	0.29
Vitamin and mineral premix[1]	0.5	0.5	0.5	0.5
Sodium bentonite	0.5	0.5	0.5	0.5
Calculated contents (%)				
Metabolizable energy (ME) (kcal/kg)	3200	3200	3200	3200
Crude protein	19.5	19.5	19.5	19.5
Calcium	0.85	0.85	0.85	0.85
Available phosphorus	0.42	0.42	0.42	0.42
Sodium	0.16	0.16	0.16	0.16
Lysine	1.09	1.09	1.09	1.09
Methionine	0.51	0.53	0.56	0.58
Methionine + cystine	0.86	0.86	0.86	0.86
Threonine	0.78	0.77	0.76	0.76

[1] Vitamin and mineral premix supplied per kilogram of diet: vitamin A: 10000 IU; vitamin D_3: 9800 IU; vitamin E: 121 IU; B_{12}: 20 µg; Riboflavin: 4.4 mg; Calcium pantothenate: 40 mg; Niacin: 22 mg; Choline: 840 mg; Biotin: 30 µg; Thiamin: 4 mg; Zinc sulfate: 60 mg and Manganese oxide: 60 mg.

The FI was determined from the difference between supplied and residual feed in each pen. The FCR was calculated from the ratio between FI and weight gain of each chick, in each pen and was adjusted for mortality. Mortality was weighed and recorded daily.

Internal organs

At the end of the experiment, the weights of thighs, breast, heart, bursa of fabricious, spleen, and gizzard were measured. Relative organ weights were calculated as:

[organ weight (g) / live body weight (g)] × 100

Microbial population

Microbial population was determined by serial dilution (10^{-4} to 10^{-7}) of ileal samples before inoculation onto sterile agar. For total aerobic enumeration samples were cultured from serial dilutions on brain heart infusion agar (BHI). In addition, *Escherichia coli* and *Lactobacilli* were grown on eosin methylene blue agar (EMB) and MRS agar, respectively. Plates for *E. coli* and aerobic bacteria were incubated aerobically at 37 ˚C. Plates for *Lactobacillus* were incubated anaerobically at 37 ˚C. Colony forming units (CFU) were defined as being distinct colonies measuring at least 1 mm in diameter (Alzawqari *et al.* 2013).

Statistical analysis

Primary data of microflora number were converted to $\log_{10}$ per ml (CFU) before the analysis. All data were analyzed using the general linear model procedure of the statistical analysis system (SAS, 2004). Duncan's multiple range test was used to compare the means (Duncan, 1955). All statements of significance were based on probability of $P < 0.05$.

RESULTS AND DISCUSSION

The results of this trial are presented in Tables 5-12. High inclusion rates of SCP (10 and 15%) significantly decreased feed intake and body weight gain of broilers and resulted in sever mortality. As are shown in Figures 1-3, the SCP caused to black discoloration of excreta, dark hepatic discoloration, and death. Moreover, the SCP at the level of 15% decreased relative weight of breast and caused hypertrophy of heart. Relative weights of other internal organs were not influenced by treatments.

There was a positive relationship between levels of dietary SCP and mortality rate (Table 5). Chicken fed diet containing no SCP and 5% SCP, showed 1.19% mortality, while, 40% and 100% mortality were seen in chickens fed diet containing 10% and 15% SCP, respectively.

Postmortem examination showed that a high proportion of the deaths was the result of fatal liver rupture. Although the relative incidence of fatal liver rupture was similar for all diet treatments, the number of fatal liver ruptures increased with increments of SCP level in diet. In contrary to our study, some researchers showed mortality decreased with increasing level of yeast single cell protein in broiler's diet (Gao *et al.* 2008; Yalcin *et al.* 2008). Al-Awadhi *et al.* (1995) found no significant abnormalities in the weights or histopathological appearances of the organs in rats fed SCP in diet.

They concluded that the KISRI-TM 1A SCP could serve as a potential dietary ingredient at levels of up to 30% for poultry, young lambs and calves. In line with our results, Ashraf (1981) showed that chicken mortality increased with increments of dietary SCP. He postulated that it was the result of selenium deficiency.

The difference in response may be due to SCP product formulation and SCP origins. The black box of the SCP might be related to lysinoalanine, N6-(DL-2-amino-2-carboxyethyl)-L-lysine which is an unusual amino acid.

Sternberg *et al.* (1975) mentioned that lysinoalanine as a renal toxic factor in rats, has been found in proteins of home-cooked and commercial foods and ingredients.

Although it has been reported to occur in both edible and nonfood proteins only after alkali treatment, it has now been identified in food proteins that had not been subjected to alkali. Lysinoalanine is generated in a variety of proteins when heated under nonalkaline conditions. Moreover, White and Balloun (1977) evaluated the SCP replacing soybean meal up to 15% of the broilers diet. The powdery consistency of SCP tended to reduce BWG and feed conversion efficiency when fed at 9% or more of the mash form diet. Interestingly, pelleting diets containing SCP eliminated these adverse effects. When diets were pelleted, substitution of SCP for soybean meal, on a weight-for-weight basis at levels up to 15% of the diet produced greater and more efficient gains. Anonymous (1989) mentioned that free lysinoalanine is more toxic than covalently lysinoalanine in food proteins. This effect may be due to differences in absorption and copper-binding affinity between dietary LL and DL isomers of lysinoalanine.

Feed intake was significantly decreased by SCP. Chicken fed diet containing 10% SCP consumed 19% less feed than those fed diets without SCP in overall (Table 6). It was postulated that the adverse effect of dietary SCP on feed intake was the underlying cause of reduced performance of the chicken. Body weight gain was significantly lower for chicken fed diets containing SCP than for chicken fed 0% SCP. Birds in groups 10% SCP gained an average of 47% less than control group in overall (Tables 7 and 8).

Table 5 Effects of different levels of single cell protein on mortality percent of broilers

Treatments (single cell protein %)	0-10 d	11-24 d	25-42 d	Overall
0% (Control)	0[c]	0[b]	1.19[b]	1.19[c]
5%	0[c]	0[b]	1.19[b]	1.19[c]
10%	6[b]	11[b]	30.87[a]	40[b]
15%	32[a]	78[a]	-	100[a]
SEM	1.5	6.5	2.043	1.75
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Effects of different levels of single cell protein on feed intake of broilers

Treatments (single cell protein %)	0-10 d	11-24 d	25-42 d	Overall
0% (Control)	280[a]	1303[a]	2842[a]	4427[a]
5%	301[a]	1323[a]	2781[a]	4406[a]
10%	290[a]	1099[b]	2213[b]	3603[b]
15%	217[b]	-	-	-
SEM	7.3	61.8	100	152.6
P-value	< 0.0001	0.046	0.003	0.0006

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 7 Effects of different levels of single cell protein on body weight of broilers at various ages

Treatments (single cell protein %)	d 11	d 25	d 42
0% (Control)	240[b]	813[b]	2050[a]
5%	267[a]	864[a]	1835[a]
10%	242[b]	609[c]	1123[b]
15%	185[c]	-	-
SEM	4.5	11.2	69.1
P-value	< 0.0001	< 0.0001	< 0.0001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 8 Effects of different levels of single cell protein on body weight gain of broilers during various phases

Treatments (single cell protein %)	0-10 d	11-24 d	25-42 d	Overall
0% (Control)	240[b]	572[a]	1218[a]	2004[a]
5%	267[a]	597[a]	951[a]	1789[a]
10%	242[b]	366[b]	-165[b]	1077[b]
15%	185[c]	-	-	-
SEM	4.5	9.44	87.7	69.1
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Figure 1 Black excreta in treatments containing 10 or 15% SCP

Figure 2 Dead chick in treatments with 10 or 15% SCP

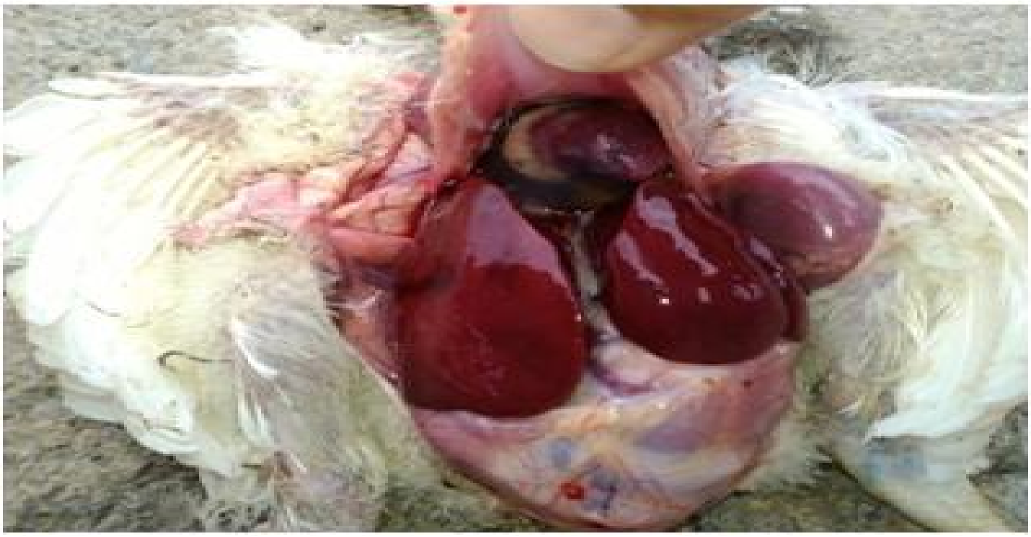

Figure 3 Liver discoloration in treatments containing 10 or 15% SCP

The FCR was significantly lower for chicken fed diets containing 10% SCP compared with 0 and 5% SCP (Table 9).

These results are in agreement with reports of Pirmohammadi *et al.* (1999) which mentioned three types of SCP up to 6% of broilers diet had no adverse impacts on performance. Also, Zohari *et al.* (1386) did not recommend more than 6% inclusion rate of SCP in chicken's ration.

It seems that high doses of SCP witch used in this study (10% and 15%), caused these results. It can be said that a decrease in broiler chicken weight gain, FCR, and feed intake was happened with the increase in the dietary level of SCP. Manal and Abou El Nagha (2012), reported higher body weight gain in broilers fed SCP at the level of 0.3, 0.5 and 0.7%. Shareef and Al-Dabbagh (2009) also reported improved body weight gain using yeast as SCP at the level of 2% in broiler diet.

There were no differences among dietary treatments with respect to the absolute weights of the liver, spleen, bursa of fabricious, and gizzard (Tables 10 and 11). These results are in line to the findings of Naila Chand and Khan (2014) along with Ozsoy and Yalcin (2011) who both reported no effect of yeast based SCP on weight of gizzard, liver and heart in broiler chicks fed diet having 1 to 3% yeast. The results of this study showed no effect of different levels of SCP on gut microbial population (Table 12).

Table 9 Effects of different levels of single cell protein on feed conversion ratio of broilers during various phases

Treatments (single cell protein %)	0-10 d	11-24 d	25-42 d	Overall
0% (Control)	1.43[ab]	2.27[b]	2.35[b]	2.21[b]
5%	1.36[b]	2.22[b]	2.95[a]	2.46[b]
10%	1.48[ab]	2.99[a]	-	3.39[a]
15%	1.56[a]	-	-	-
SEM	0.041	0.130	0.140	0.152
P-value	0.03	0.003	0.047	0.001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 10 Effects of different levels of single cell protein on relative weight of liver, pancreas, spleen, and small intestine of broiler chickens at 47 d of age[1]

Treatments (single cell protein %)	Liver	Heart	Spleen	Bursa of fabricious	Gizzard
0% (Control)	2.23	0.41[b]	0.11	0.15	1.81
5%	2.75	0.53[b]	0.14	0.11	1.66
10%	3.49	0.86[a]	0.14	0.18	1.68
SEM	0.490	0.066	0.038	0.54	0.150
P-value	0.243	0.003	0.786	0.602	0.786

[1] Relative weight of organ to live body weight as percentage (g/g).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 11 Effects of different levels of single cell protein on relative weight of breast, thighs, and carcass[1]

Treatments (single cell protein %)	Breast	Thighs	Carcass
0% (Control)	24.5[a]	18.6	66.4
5%	22.4[ab]	18.4	63.4
10%	18.8[b]	19.2	62.6
SEM	1.347	1.09	2.77
P-value	0.042	0.856	0.617

[1] Carcass percentage is sum of breast, thighs, wings, neck and back without skin relative to live body weight as percentage (g/g).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 12 Effects of different levels of single cell protein on gut microbial population (Log 10 cfu) of broiler chickens at 47 d of age

Treatments (single cell protein %)	Total aerobic bacteria	*Lactobacillus*	*Coliforms*
0% (Control)	9.71	7.49	8.22
5%	9.56	6.67	8.65
10%	9.73	6.83	8.69
SEM	0.120	0.550	0.250
P-value	0.60	0.55	0.36

SEM: standard error of the means.

CONCLUSION

The results indicate that supplementing the broiler diets with up to 5% SCP may produce no harmful effect to the growth performance of chickens and may be included in their diets. However, it is concluded that higher levels of SCP inclusion (10% and higher) might be harmful and even lethal to the broilers in terms of productive performance.

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