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Evaluation of single cell protein replacement (pl 68) by soybean meal in broiler chickens

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This experiment was conducted to investigate the effects of different levels of yeast single cell protein on functional traits, blood biochemical parameters, immune titer, population of caecum bacteria and morphology of broilers intestine in the form of a completely randomized design with 6 treatments, each treatment 5 replication and each replication 15 one-day pieces of broilers. The experimental treatments were included in: 1) control group; 2) the receiving group of single cell protein 0.03 percent; 3) the receiving group of single cell protein 0.05 percent; 4) the receiving group of single cell protein 0.07 percent; 5) the receiving group of protein Single cell 0.09 percent, 6) the receiving group of single cell protein 0.1 percent. The results of the experiment showed that the treatments receiving different levels of yeast single cell protein showed a higher weight gain compared to feed intake control treatment and as a result, their conversion factor was less than that of control treatment. The use of different levels of single cell protein improved blood biochemical parameters and increased immune titer against Newcastle and influenza disease compared to control treatment. In this study, it was observed decreasing population of Salmonella and E. coli, as well as the increasing population of caecum lactobacillus in chicks receiving single cell protein in comparison to control. Also, it was observed increasing Willy's height, decreasing crypt depth, and reducing the number of goblet cells in chicks receiving different level of single cell protein.

Keywords: Single cell protein, Broiler, Biochemical blood parameters, Intestinal morphology

INTRODUCTION

In the poultry production industry, feed costs typically account for 65-75% of breeding total costs, which it is increasing day by day (Chand et al., 2014). Expensive food in the poultry breeding industry can be found in soybeans and fish powder, which can reduced breeding costs by using alternative and inexpensive materials (Chand et al., 2014). Given that increasing population in the world and increasing food consumption, make people to thinking of new food sources that are inexpensive and affordable. One of the sources of inexpensive for supplying the protein to the international community is the use of single-cell proteins, which are microbial-based products. The term "single cell" proteins refers to dried cells of microorganisms such as bacteria, yeasts, molds, algae and organic fungus that are grown on large scale and used as a source of protein for human or animals. Given the rapid growth of these microbes, the use of single-cell protein has been used as a source of superseded protein in human and livestock and poultry diets (Akman, 1980). One of the advantages of using single-cell proteins is that these single cells can use inexpensive materials and even garbage as a feedstock and deliver high-nutritional protein to humans, livestock and poultry (Nasseri et al., 2011). Considering the usefulness of using single cell proteins, interest in using this protein source has been considered in various studies (Najib et al.,). Yeasts are one of the best microorganisms that can be used to produce microbial proteins because yeasts have high nutritional value and can compete with animal proteins (Adedayo et al., 2011). Yeasts are well in terms of the amino acid balance as well as the presence of vitamins of B group, which can therefore be used in livestock and poultry feeds (Amata, 2013). The use of yeast can be used as a substitute for soybean in the poultry diet due to the balancing of amino acids and presence of different vitamins (Gohl, 1981). In Various studies have shown that the use of yeast in poultry diet has improved the weight gain and feed conversion factor in broilers (Naila et al.,2014). In a study by Lee and Chen (1983), the addition of single-cell protein (SCP) to the diet of laying hens showed an increase in weight gain and increased egg production compared to the control group Lee and Chen (1983). The aim of this study was to use different levels of single-cell protein derived from yeast in broilers diet and its effect on biochemical parameters of blood, microbiology and morphology of the intestine and immune titer performance.

MATERIALS AND METHODS

This experiment was conducted in a completely randomized design on Ross 308 commercial strain one-day broilers. The temperature of the salon before entering broilers was 33 ° C. After weighing the broilers were randomly transferred to each experimental unit. The breeding was carried out on the bed and the access of broilers to water and feed was free throughout the breeding period. The exposure was set as 23 hours of lighting and 1 hour of blackout until the end of the period. Broilers feeding from the first day was based on corn-soybean and in accordance with Ross's proposal for commercial strain 308 and WUFFDA rationing software were set at an initial diet level of 1-10 days of age, a growth period of 11-28 days of age, and a final period of 29-42 days of age (Table 1). This study was conducted in the form of two experiments.

Table 1: Used Diets during breeding

feed	Beginning period (1-10 days of age)	Growth period (11-28 days of age)	Final period (29-35 days of age)
Corn	49/30	59/6	65/99
Wheat	5/58	5	5
Soy	26/86	16/05	10/12
Corn gluten	10	11/48	11/5
soy oil	3/50	3/34	3/09
Limestone	1/45	1/23	1
Di-calcium phosphate	1/95	1/8	1/83
Salt	0/36	0/36	0/36
Vitamin Supplement [1]	0/25	0/25	0/25
Mineral Supplement [2]	0/25	0/25	0/25
Di L methionine	0/52	0.58	0/57
Lysine	0/25	0.06	0/04
Chemical analysis of the used diets during breeding			
feed	Beginning period (1-10 days of age)	Growth period (11-28 days of age)	Final period (29-35 days of age)
Metabolisable energy (Kcal / Kg)	3010	3150	3200
Crude protein (%)	23	20	18
Calcium (%)	1	0/9	0/9
Usable phosphorus (%)	0/5	0/45	0/45
(%) Lysine	1/41	1/16	1/05
(%) Methionine+ Cysteine	1/09	0/81	0/78

[1]. Each kilogram of diet provides the following vitamins: Vitamin A 3200 International Unit, Vitamin D3 1150 International Unit, Vitamin K3 1 mg, Vitamin B1 1 mg, Vitamin B2 3 mg, Vitamin B3 20 mg, Vitamin B5 2.5 mg, vitamin B6 1 mg, vitamin B9 0/36 mg, vitamin B12 5 mg and vitamin H2 5 mg.

[2]. Each kilogram of diet provided the following minerals: manganese 35, iron 20, zinc 5, copper 40, iodine 0.4 and choline 100 mg.

In this study, 450 pieces of broilers were done completely as randomized design in the form of 6 treatments and each treatments 5 replication and each replication 15 one-day pieces of broilers. The experimental treatments were included: 1) control group; 2) the recipient group single cell protein 0.03 percent; 3) the recipient group single cell protein 0.05 percent; 4) recipient group single cell protein 0.07 percent; 5) recipient group single cell protein 0.09 percent, 6) recipient group single cell protein 0.1 percent. In this experiment it was evaluated feed intake, body weight, and feed conversion factor rates. At the end of the breeding period, from each experimental unit, two birds were selected and after slaughter by euthanizing method, the sampling was done for intestinal morphological tests of the ileum area at the rate of 5 cm. These specimens were sent to a pathology laboratory in containers containing 10% formalin. In the laboratory, after fixing the ileum tissue with paraffin and cutting by microtome, staining of hematoxin and eosin (H & E) and also staining PAS, which respectively are used to check the villi height, crypt depth and also the count of the goblet cells and studied by optical microscope with object lens 40 (**Xu et al.,2003**). For counting the amount of intestinal bacteria in the caecum area after slaughter by euthanizing method, sterile samples were taken from the caecum area and placed in sterile containers next to the ice sheet sent to the laboratory and then counting was performed by in a sterilized phosphate buffer medium (PBS) pH 7.2 serial dilution method. Then, from each test tube, 1 cc were cultured on selected agar media containing: Eosin methylene blue (EMB), De Man, Rogosa and Sharpe agar, and Salmonella Shigella Agar (SS) agar, for cultivation of Escherichia coli, Lactobacillus and Salmonella respectively and it was incubated at 37 ° C for 24 hours, and counting was carried out after growth of bacteria in the medium on the basis of the fact that each clone is a monoclonal symbol Miller and Wolin (1974). To study Blood parameters and immune titers from each experimental unit in a completely randomized method were also taken two blood samples at a dose of 2 ml and was sent to the biochemical laboratory to analyze the blood biochemical parameters including cholesterol, triglycerides, HDL, LDL, albumin and total protein and immune titer against Newcastle and influenza disease (KEC et al., 1998). Data obtained from this study were analyzed by statistical software SAS 1/9 (2003) and the comparison of the meanings was performed using Duncan's (1995) multi-domain test and a significant level ($P \leq 0.05$) was considered (SAS. 1990).

RESULTS AND DISCUSSION

Table 2 shows the effect of experimental treatments on broilers functional traits. The use of different levels of single cell protein has improved feed intake and increasing broilers weight gain of different ages which ultimately resulted in a reduction in the feed conversion factor. In this experiment, the highest and lowest feed intake and weight gain were related to the receiving treatment 0.01% SCP and control treatment. By increasing the concentration of SCP in broilers diet, it has increased feed intake and weight gain, and ultimately improved the conversion factor.

Table 3 shows the effect of experimental treatments on broilers blood biochemical parameters at 42 days of age. According to the obtained results, the use of different levels of single-cell protein in broilers diet decreased cholesterol concentration, triglyceride and LDL serum in comparison with control group that the lowest concentration was observed in broilers receiving 0.1% single cell protein Also, the treatments receiving single levels of mono-cellular protein increased the concentration of HDL, albumin and total protein in serum compared to the control group, and the highest concentration was observed in the treatment receiving 0.1% single cell protein.

Table 4 shows the effect of experimental treatments on the broilers immune titers at the end of the experiment period. According to the obtained results, by increasing in mono-cellular protein concentration in broilers diet was increased in immune titer different treatment against Newcastle and influenza disease compared to the control group. The lowest immune titer was observed in control treatment broilers and the highest immune titer was observed in the receiving treatment broilers 0.1% single cell protein.

Table 5 shows the effect of experimental treatments on the growth of caecum bacterial in broilers at 42 days of age. According to the obtained results of this experiment it can be found that the receiving treatments different levels of single cell protein of Lactobacillus caecum population increased and the highest population of lactobacillus was observed in broilers receiving 0.1% single cell protein and the lowest Lactobacillus population was related to the control treatment broilers. Reducing the population of Escherichia coli and Salmonella was observed in broilers receiving treatments different levels of single cell protein in comparison to control treatment. The receiving treatment broilers 0.1% of single-cell protein showed the lowest population of Escherichia coli and Salmonella in comparison with other experimental treatments. The highest population of these bacteria was observed in control treatment broilers.

Table 2: the effect of experimental treatments on broilers functional traits

	21 days of age			42 days of age		
	feed intake (g)	Weight gain (g)	Conversion factor	feed intake (g)	Weight gain (g)	Conversion factor
control	1152.12	770.21	1.50	4388.29	2243.24	1.96
0.03 % SCP	1161.39	779.21	1.49	4393.54	2251.63	1.95
0.05 % SCP	1167.32	786.23	1.48	4397.29	2259.31	1.95
0.07 % SCP	1174.28	793.36	1.48	4406.41	2267.20	1.94
0.09 % SCP	1180.54	804.39	1.47	4412.37	2273.37	1.94
0.1 % SCP	1186.72	812.73	1.46	4421.39	2286.19	1.93
MES	***0.819***	***0.780***	***0.002***	***0.439***	***0.705***	***0.001***

SCP =Single cell protein

Table 3: the effect of experimental treatments on broilers blood biochemical parameters at 42 days of age

	Cholesterol (mg/d)	Tri glyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Albumin (mg/dl)	Total protein (mg/dl)
control	129.28	78	77	78	1.39	3.13
0.03 % SCP	128.49	77	77	78	1.38	3.14
0.05 % SCP	128.89	77	77	78	1.39	3.17
0.07 % SCP	128.30	76	79	77	1.41	3.19
0.09 % SCP	127.47	74	80	75	1.41	3.22
0.1 % SCP	125.79	74	80	73	1.43	3.26
MES	***0.240***	***0.577***	***0.353***	***0.540***	***0.0026***	***0.0088***
SCP =Single cell protein						

Table 4: the effect of experimental treatments on the broilers immune titers at the end of the experiment period

	Newcastle	Flu
control	3/06	3.14
0.03 % SCP	3.11	3.23
0.05 % SCP	3.19	3.49
0.07 % SCP	3.46	3.69
0.09 % SCP	3.86	3.71
0.1 % SCP	4.10	3.93
MES	0.003	0.004

SCP =Single cell protein

Immunity titers are based on the logarithm of 10

Table 5: the effect of experimental treatments on the growth of caecum bacterial in broilers at 42 days of age

	Lactobacillus	Escherichia coli	Salmonella
control	6.93	8.45	8.12
0.03 % SCP	6.98	8.41	8.03
0.05 % SCP	7.06	8.32	7.56
0.07 % SCP	7.14	7.37	7.16
0.09 % SCP	7.26	7.14	6.81
0.1 % SCP	7.38	7.05	6.47
MES	***0.081***	***0.0135***	***0.068***

SCP =Single cell protein

Table 6: the effect of experimental treatments on the morphology of small intestinal ileum in broilers at the end of breeding period

	Willey height (Micrometer)	crypt depth (Micrometer)	Willey height-to-crypt depth ratio	number of goblet cells
control	589.28	124	4.75	8.31
0.03 % SCP	593.49	121	4.90	8.20
0.05 % SCP	601.57	119	5.06	8.06
0.07 % SCP	609.24	116	5.25	7.74
0.09 % SCP	617.36	115	5.37	7.34
0.1 % SCP	626.39	113	5.54	7.12
MES	***0535***	***0479***	***0.016***	***0.042***

SCP =Single cell protein

Table 6 shows the effect of experimental treatments on the morphology of small intestinal ileum in broilers at the end of breeding period. According to the obtained results, the receiving treatments different levels of yeast single cell protein in comparison to control treatment show increasing Willy's height and reduction in crypt depth. The highest Willey height and the lowest crypt depth were observed in the receiving treatment of single-cell protein 0.1%. Among the experimental treatments, the lowest Willey height-to-crypt depth ratio was observed in control treatment broilers. The use of single cell protein in broilers reduced the number of goblet cells in single-cell protein broilers receiving. The lowest number of goblet cells was observed in broilers receiving 0,1% yeast single cell protein and control treatment broilers showed the largest number of goblet cells.

The use of yeasts in humans, livestock and poultry feed, as well as the use of yeasts from substrates such as neptose, hexoses, hydrocarbons, and the production of high nutritious materials are among the reasons why yeasts are used to produce SCP (Abou Hamed, 1993). One of the commercial factors necessary for the use of single cell proteins is the determination of the protein yield ratio, which is in the form of weight gain and reduction in the feed conversion factor per unit protein consumed by livestock and poultry (Rivire, 1977). In this experiment, the use of different levels of single-cell protein improved feed intake and weight gain in broilers, which ultimately resulted in a reduction in feed conversion factor. The positive effects of the use of yeasts in the broilers diet depend on several factors. Initially, it can be said that the yeast single-cell protein can act as a growth stimulator due to having protein and balanced amino acids, vitamins, and minerals (Amata, 2013) as well as the cell wall of yeasts due to having Glucans and mannan oligosaccharides. To the positive effects of the use of yeasts on mucus and intestinal wall, it improves weight gain in poultry which increases the Willy's height, provides intestine conditions for the growth and activity of beneficial bacteria in the intestine and improves intestine pH to increase digestion and absorption of nutrients, which ultimately results in weight gain and reduction in feed conversion factor (Abdel-azeem, 2002). In a study by Naila et al., (2014), the use of yeast single-cell protein as a substitute for soybean in broiler diets did not have a significant effect on broilers feed intake. But it increased the weight gain of broilers compared to control treatment and finally, the broilers receiving single-cell protein showed less conversion factor compared to control treatment. According to the report, broilers receiving SCP compared to control treatment their mortality rate was lower than the control group. Also, the weight of the internal organs of the liver and viscera increased compared to the control group, but it was not statistically significant (Naila et al., 2014). The study by Manal and Abou (2012) suggests that adding yeast to broiler diets will result in weight gain and feed intake (Manal and Abou el-Nagha, 2012), which is consistent with the results of this study.

Waldroup and Payne (1973) used a single-cell bacterial protein in broiler diets and reported that the use of this single-cell bacterial protein compared with the base diet receiving group improved weight gain, feed intake, and feed conversion factor (Waldroupandj, 1974). In a study by Panda et al., (2005), the effect of cultured yeast on poultry performance was investigated and it was reported that the use of cultured yeast had no significant effect on the blood biochemical parameters of broilers (Panda et al., 2005) which contrasts with the results of the present experiment that the use of single cell yeast protein has a significant effect on biochemical parameters of blood. In a report by Stanley et al., (2004), they studied the addition of cultured yeast to the diet of laying hens and reported that serum albumin concentration in laying hens serum has increased compared to control treatment (Stanley et al.,2004) which it was consistent with the results of this study. In 2011, Yalcin and Ozsoy investigated the effect of yeast use on the performance and biochemical parameters of the serum and Immune System of broilers, and reported that the use of cultured yeast in broiler diets had no significant effect on the blood biochemical parameters, including total protein, cholesterol, triglyceride and alanine Ozsoy and Yalcin (2011).

, which had contrast with the results of the present study. In this study, the use of yeast single-cell protein in broilers reduced the population of Salmonella and E. coli bacteria. The presence of Mannan and Beta-Glucan in the cell wall of the yeast by binding to the pathogenic bacteria in the gastrointestinal tract prevents them from sticking to the intestinal wall, thereby increased the rate of excretion Loddi et al., 2002.

As evidenced in the present experiment, the use of yeast single-cell protein in broiler diets reduced the population of Salmonella and E. coli bacteria and provided conditions for the growth of beneficial bacteria such as Lactobacillus, which, according to the obtained results, increasing the population of Lactobacillus bacteria was observed in broilers receiving yeast single-cell protein. Improving broilers feed intake and weight gain in yeast single-cell protein receiving treatments can be due to a reduction in the number of harmful bacteria and an increase in the number of beneficial bacteria due to the use of yeast single cell protein. Researchers believe that the use of yeast in broilers diet can develop the digestive tract of broilers and maintain the integrity of the mucous membrane of the intestine (Iji et al., 2001) .In this experiment, the use of yeast single-cell protein increased Willy's height and reduced crypt depth in recipient broilers. Increasing the Willy's height and reducing the crypt depth increases the level of nutrients absorption and improves the function of the gastrointestinal tract and, as a result, will improve weight gain and will decrease the feed conversion factor (Pirgozliev et al.,2008)**.**The mucine is secreted from goblet cells containing oligosaccharide, which is a good place to connect the bacterial lectin, so the bacteria will be able to locate and invade the underlying layers of the intestinal mucosa by attaching to these oligosaccharides. The cell wall of yeast, due to having mannan and mannan oligosaccharide, causes the attaching of harmful bacteria and increases their excretion Oyofo et al., 1989**.** Lactobacillus also prevents growth with pathogenic bacteria by secreting antibacterial agents such as bactericides. Also, existed bacteria in the digestive tract compete with each other for occupancy of binding sites nutrition consumption (Corrier et al.,1990)**.**As a result, harmful bacteria such as E.coli and Salmonella can be reduced by the use of yeast single cell protein to stabilize beneficial bacteria such as lactobacillus Ohimain and Ofongo (2012) and as the results of this study showed, the improvement of intestinal conditions such as increased Willie's height will reduce crypt depth and the number of goblet cells and ultimately will increase feed intake and weight gain, which is evident in this study. One of the reasons for the increase in albumin and total protein in broilers blood serum that receive yeast single cell protein can be increased and improved digestion and absorption of nutrients. Another reason to increase the concentration of albumin and total protein in broilers blood serum that receive yeast single cell protein compare with the control treatment can be referred to increase the concentration of dietary protein by using single cell protein and the balanced amino acid in yeast single cell protein. Broilers receiving SCP increased and serum total protein concentration will increased, since the balanced amino acid of the yeast single cell protein is desirable and as a result of digestion and absorption of nutrients, especially proteins and amino acids. Given the high levels of amino acids in the structure of immunoglobulins (29), therefore an increase in feed protein concentration and an increase in absorption of proteins and amino acids will causes amino acids and proteins be sufficient to produce immunoglobulins.

CONCLUSION

In summary, the improvement of intestinal conditions such as increased Willie's height will reduce crypt depth and the number of goblet cells and ultimately will increase feed intake and weight gain, Increasing the immune titer in broilers receiving different levels of yeast single cell protein can be due to increased digestion and absorption of amino acids, which is associated with an increase in total protein concentrations and serum albumin, which results in sufficient proteins and amino acids to produce immunoglobulins and eventually compared to the control treatment increased the immune titer

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All the authors contributed equally in this paper.

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