EFFECT OF REPLACEMENT OF SOYBEAN MEAL WITH DRIED DISTILLERY YEAST SLUDGE ON PERFORMANCE, IMMUNITY AND GUT HEALTH OF LAYER PULLETS

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KEYWORDS	ABSTRACT
Pullets, Distillery Yeast Sludge, Performance, Immunity & Gut Health	Present study was conducted to determine impact of dried distillery yeast sludge (DYS) on performance, immunity and gut health of layer pullets with replacement of soybean meal. Eight weeks old 500 layer pullets were selected randomly and divided into 5 major treatment groups, each group comprising 4 replicates having 25 pullets in each replicate. The DYS dried and ground to mesh size of 2 mm in hammer mill. Five isocaloric and isonitrogeneous diets viz., control, DYS5, DYS10, DYS15, and DYS20 were formulated to contain DYS 0, 5, 10, 15, 20 %, respectively, replacing soybean meal. The pullets fed ad libitum for 8 weeks. Feed intake was highest ($P<0.05$) in pullets fed control diet and lowest ($P<0.05$) in pullets fed DYS20 diet. The weight gain was higher ($P<0.05$) in the pullets fed DYS5, DYS10 and control diet than those fed DYS20 diet. Feed conversion ratio was better in pullets fed control, DYS5 and DYS10 diet than those fed DYS20. There was decrease ($P<0.05$) in lactobacillus count with the increasing dietary level of DYS. Highest immune response was observed in pullets fed control diet followed by pullets fed DYS5, DYS10, DYS15 and DYS20 diet. The study revealed that DYS can be included in the diet of layer pullets up to 10% having no ill effect.

INTRODUCTION

Poultry is second largest industry with 10-12% growth rate in the country. It is playing important role in producing animal protein and generating income. Poultry industry is progressing at faster rate and about 1.5 million people are engaged with this potential industry (Economic Survey, 2016-2017). There is rapid increase in poultry production and human population resulting competition of foodstuffs between poultry and human beings. Consequently, a rise in cost and shortage of feedstuffs negatively impacted the poultry industry due to rise in input costs (Ncube, Roberts & Zengeni, 2016). Likewise, price hike of compound feed and inadequate conventional feed ingredients has required to search existing inexpensive alternate feed resources, mainly those that do not compete in consumption between livestock and human (Esonu, Okoli, Opara, Obikaonu, Udedibie & Iheshiulor, 2006; Bilal, Sarwar & Sultan, 2016). Use of single cell protein (SCP) can be an appropriate strategy to resolve matter. The SCP is high crude protein (CP) biomass obtained from bacteria, fungi, yeast and algae.

The alternative use of CP sources replacing expensive CP ingredients like soybean meal can lead to reimburse the scarcity of CP (Attia, Al-harthi & El-deek, 2003; Jalasutram, Kataram, Gandu & Anupoju, 2013). Somewhat, yeast and soybean meal have parallel profile of the amino acids (Adedayo, Ajiboye, Akintunde & Odaibo, 2011). The yeast, primarily Saccharomyces cerevisiae (SC), is a good source of SCP. The yeast has low nucleic acid than bacteria and can easily be harvested from cost effective raw material (Wolf, Bindraban, Luijten & Vleeshouwers, 2003; Bacha, Nasir, Khalique, Anjum & Jabbar, 2011). Currently, the commercial products of yeast have attracted substantial consideration as a feed additive in poultry feeding (Yalcın, Uzunoglu, Duyum & Eltan, 2012; Chen, Chen & Wang, 2017; Yasar & Yegen 2017). The distillery yeast sludge (DYS) is the byproduct of distillery industry and primarily comprises SC as a source of protein. Existing data on DYS as SCP source in the country represent its important inherent nutritional potential as it contains 27-29 % CP (Ali , 2004; Sharif, Shoaib, Rahman, Ahmad & Rehman, 2018). This study was conducted to explore the effect of partial replacement of the dietary soybean meal with varying levels of DYS on the performance, immunity and gut health of layer pullets.

MATERIALS AND METHODS

This study was accompanied at Poultry Research Centre, University of Agriculture Faisalabad, to determine the influence of replacement of dietary soybean meal with dried DYS on performance, immunity and gut health of layer pullets. The DYS was collected from Shakarganj Sugar Mills district Jhang and dried in steam dryer. The dried DYS was ground to a mesh size of 2 mm in a hammer mill. Five *iso-caloric* (ME 2700 Kcal/kg) and *iso-nitrogenous* (CP 16%) layer pullet diets were formulated according to NRC (1994) using DYS (0, 5, 10, 15 and 20%) with replacement of soybean meal (Table 1). Eight weeks old 500 healthy Hy-line (CV-22) pullets (average weight 620 g) were used in a completely randomized design (CRD). The pullets were randomly distributed into 5 groups, each having 4 replicates and 25 birds per replicate. The pullets were treated with standard management practices of Hy-line (CV-22).

The pullets were vaccinated for Avian Influenza H9 (AI), Fowl pox, New castle disease (ND), Egg Drop Syndrome and Infectious bronchitis. The pullets fed *ad libitum* for 8 weeks. The data regarding weight gain, feed intake and feed conversion ratio (FCR) were recorded. Three birds per replicate were selected to collect the blood samples at age of 10^{th}, 12^{th}, 14^{th} and at 16^{th} weeks to find out post-vaccine antibody titers against AI and ND by haemagglutination inhibition test (Allan & Gough 1974). Three pullets from each replicate were slaughtered at end of 8^{th} week of experiment to calculate gut *lactobacillus* count. Cecal digesta samples were collected and total bacterial counts were measured by colony counter (Strompfova, Marcinakova, Gancarcikova, Jonecova, Scirankova, Guba, Koscova, Boldizarova & Laukova, 2005). The data recorded for various parameters were analysed by analysis of variance techniques in a CRD layout. Means were separated by Tukey's test (Steel, Torrie & Dickey, 1997).

Table 1: Ingredients & Nutrient Composition of Layer Pullet Diets

Description	Diets[1]				
Ingredients %	**C**	**DYS5**	**DYS10**	**DYS15**	**DYS20**
Soybean meal	11.92	8.94	5.96	2.98	0.00
Distillery yeast sludge	0.00	5.00	10.00	15.00	20.00
Rice tips	31.00	30.00	24.00	25.00	22.00
Yellow corn	26.08	26.06	29.04	30.02	31.00
sunflower meal	4.00	5.00	4.00	3.00	3.00
Cotton seed meal	0.00	0.00	1.00	1.00	1.00
Canola meal	2.00	3.00	3.00	1.00	1.00
Rice polish	6.00	6.00	7.00	2.40	3.00
Corn Gluten 30%	8.00	6.00	6.00	6.00	6.00
Fish meal	1.00	1.00	1.00	1.00	1.00
Guar meal	0.00	0.00	0.00	3.60	4.00
Molasses	6.00	5.00	5.00	5.00	4.00
Dicalcium phosphate	2.00	2.00	2.00	2.00	2.00
Limestone	1.00	1.00	1.00	1.00	1.00
[2]Premix	1.00	1.00	1.00	1.00	1.00

Nutrient Composition %					
ME, Kcal/kg	2702	2699	2701	2705	2702
Crude protein	16.04	16.05	15.99	15.98	15.99
Crude fibre	6.17	6.15	5.98	5.97	5.90
Ether extract	3.40	3.42	3.40	3.38	3.35
Calcium	1.70	1.69	1.67	1.70	1.65
Available phosphorus	0.55	0.56	0.54	0.51	0.49
Lysine	1.03	1.05	1.08	1.07	1.02
Methionine	0.47	0.5	0.45	0.46	0.48

[1]C, DYS5, DYS10, DYS15 and DYS20 diets contain 0, 5, 10, 15 and 20% distillery yeast sludge, respectively.
[2]Provided per kilogram of diet: vitamin A 9900 IU; vitamin D3 3300 IU; 25-hydroxy vitamin D3 0.055 IU; vitamin E 22.1 IU; vitamin K (menadion) g 0.0033; thiamin g 0.0022; riboflavin g 0.0066; niacin g 0.033; pantothenic acid g 0.011; pyridoxine 0.0044; biotin g 0.055; folic acid g 0.0009; cobalamine g 0.0221; choline g 0.11; manganese g 0.088; zinc 0.088; iron g 0.055; copper g 0.011; iodine g 0.0017; selenium g 0.0003.

RESULTS OF STUDY

Growth Performance

Feed consumption was highest (P<0.05) in pullets fed control diet and it was lowest in pullets fed DYS20 diet (Table 2). Feed consumption was decreased with increasing level of DYS. However, feed intake did not change in pullets fed DYS5 and control diets. Highest (P<0.05) body weight gain was noted in pullets fed control diet and lowest in pullets fed DYS20 diet. The increase in dietary level of DYS reduced the weight gain of pullets. However, weight gain did not change in pullets fed DYS5, DYS10 and control diets. The FCR was better (P<0.05) in pullets fed DYS5, DYS10 and control diets than that of pullets fed DYS20 diet. However, non-significant difference (P>0.05) was noted for FCR in pullets fed DYS5, DYS10 and control diets.

Table 2: Effect of Various Levels of Dietary Distillery Yeast Sludge on Pullet's Performance

Parameters	Diet[1]					
	C	DYS5	DYS10	DYS15	DYS20	SE[2]
Feed intake (g)	3248[a]	3211[ab]	3183[ab]	3163[ab]	3108[b]	40.03
Weight gain (g)	535[a]	525[a]	517[a]	502[ab]	481[b]	10.72
Feed conversion ratio	6.07[a]	6.12[a]	6.16[a]	6.30[ab]	6.46[b]	0.07

[1]C, DYS5, DYS10, DYS15 and DYS20 diets contain 0, 5, 10, 15 and 20% distillery yeast sludge, respectively.
Treatment means in a row with different superscripts differ significantly (p<0.05).
[2]Standard error

Gut Lactobacilli Count

Greatest (P<0.05) *Lactobacillus* count was noted in pullets fed DYS5 and control diet, while, lowest in pullets fed DYS20 diet (Table 3). The increase in dietary level of DYS resulted in reduced *Lactobacillus* count.

Table 3: Effect of Various Dietary Distillery Yeast Sludge Levels on Lactobacillus Count (10^5cfu/gram) of Pullets

Parameters	Diet[1]					
	C	DYS5	DYS10	DYS15	DYS20	SE[2]
Lactobacillus Count	13.40[a]	13.37[a]	12.32[b]	8.57[c]	6.50[d]	0.14

[1]C, DYS5, DYS10, DYS15 and DYS20 diets contain 0, 5, 10, 15 and 20% distillery yeast sludge, respectively.
Treatment means in a row with different super superscripts differ significantly ($p<0.05$).
[2]Standard error

Immune Responses

Highest cumulative mean antibody titers against ND were observed in pullets fed control diet, followed by the pullets fed DYS5, DYS10, DYS15 and DYS20 diet (Table 4). The gradual increase in dietary inclusion of DYS was resulted to reduce antibody titers against ND in pullets. Similar results were observed regarding cumulative mean antibody titers against AI in layer pullets (Table 5).

Table 4: Effect of varying dietary distillery yeast sludge levels on post-vaccinal geometric mean antibody (GMT) titers against Newcastle disease (ND) at various stages of production of pullets

	Diet[1]				
Age (weeks)	C	DYS5	DYS10	DYS15	DYS20
10	271.22	256.00	215.26	203.18	170.86
12	287.35	271.22	256.00	228.07	181.01
14	322.54	304.43	322.54	256.00	203.18
16	430.53	430.53	406.37	383.56	271.22
CMT[2]	327.91	315.55	300.04	267.70	206.57

[1]C, DYS5, DYS10, DYS15 and DYS20 diets contain 0, 5, 10, 15 and 20% distillery yeast sludge, respectively.
[2]Cumulative mean titer

Table 5: Effect of varying dietary distillery yeast sludge levels on the post-vaccinal geometric mean antibody (GMT) titers against Avian Influenza (AI) at various stages of production of layer pullets

	Diet[1]				
Age (weeks)	C	DYS5	DYS10	DYS15	DYS20
10	191.78	181.01	161.27	143.67	135.61
12	241.63	215.26	215.26	161.26	152.21
14	271.22	256.00	241.63	181.01	170.85
16	304.43	304.43	287.35	271.22	203.18
CMT	252.26	239.18	226.38	189.29	165.46

[1]C, DYS5, DYS10, DYS15 and DYS20 diets contain 0, 5, 10, 15 and 20% distillery yeast sludge, respectively.
CMT stands for cumulative mean titer

DISCUSSION

Growth performance

The results of this study are in line with the findings of Bilal et al. (2016) who observed comparable results regarding feed intake in layers when SBM was replaced up to 10% with DYS and they further explained that there was decline in feed intake when SBM was replaced beyond 10%. Likewise, Manal and Nagha (2012) also observed the best results regarding feed intake in broilers fed 0.5% dry yeast diet as compared to fed higher levels of yeast (0.7%). Similarly, Khan (2001) noted increased feed intake in broilers fed low level of DYS (6%) and indicated reduced feed intake in broilers fed DYS exceeding this level. Findings of present study are in line with Herria and Garcia (1983) reported higher feed intake in broilers when fed with yeast up to 7% by substituting SBM. Paryad and Mahmoudi (2008) also observed increase in feed intake of broilers during a 42 trial period when diet enriched with 1.5% yeast relative to 2% yeast. The decline in feed intake with gradually increased DYS inclusion may be due to the fact that higher yeast inclusion has raised nucleic acids contents which displayed anti-nutritional properties (Ozorio et al. 2012).

Rodrigiez et al. (2014) reported adverse effects on feed intake when level of yeast sludge exceeded 30%. Rameshwari and Karthikeyan (2005) also reported reduced feed intake in birds raised on high DYS level. This adverse effect on feed intake may be due to many reasons, firstly higher levels of DYS pulverized the experimental diet (Rodrigiez et al., 2014) and smaller feed particle might have shown negative effect on intake and nutrient digestibility. Secondly, low feed intake may be related with intestinal hypertrophy due to bacterial fermentation which suppressed the appetite of birds indirectly (Pacheco et al., 2013). The findings regarding weight gain in present study are in line with the results of Gao et al. (2008) who indicated higher weight gain in broilers fed diet comprising 2.5g yeast/kg compared with those fed high concentration (5.0 and 7.5 g/kg) of the yeast. Similarly, Manal & Nagha (2012) achieved best feed efficiency by adding 0.5% dry yeast as compared to other dietary treatments. The cells of yeast contain B-complex vitamins, proteins and minerals while cell wall possesses mannan and glucan which shows positive effects on birds' intestinal mucosa (Amata, 2013).

Besides, it increases population of cellulytic and anaerobic bacteria and villus height, which encourage lactate utilization and regulates gut pH, thereby improved growth performance by increasing nutrients digestibility (Hassanein & Soliman, 2010). Flower et al. (2015) recorded low weight gain in birds fed yeast cell wall (500ppm) as compared to those fed yeast cell walls 125, 250 and 375 ppm. Rameshwari and Karthikeyan (2005) observed reduction in body weight when the level of DYS increased gradually. Rodrigiez et al. (2014) also reported similar results in White Leghorn chicks.. In agreement with our study Al-Mansour et al. (2011) find good results regarding feed efficiency of birds fed 1.25g yeast culture/kg diet as compared to those fed 1.5g yeast culture/kg of diet. Manal and Abou-El-Nagha (2012) achieved best feed efficiency by adding 0.5% dry yeast as compared to higher levels. The better performance of birds fed yeast might be due to fact that more nutrients are partitioned towards growth. Mujahid et al. (2012) reported diet having 2% yeast sludge resulted positive effect on feed efficiency in broilers. Still, some reported poor FCR when dietary DYS level increased in broilers. Also, Bilal et al. (2016) reported poor FCR replacing SBM with DYS in layers.

Gut Lactobacilli Count

The lactobacilli count in pullets fed control diet was similar to pullets fed 5% DYS. However, decline in the lactobacilli count was noted in birds fed DYS beyond 5%. Similar findings were observed by Bilal et al. (2016) in layers. Hassanein and Soliman (2010) noted optimum growth of Lactobacilli in layers fed 0.8% live yeast as compared to those fed 1.2 and 1.6% live yeast. The Lactobacilli enhances the host immune status and by positively modulating the gut microflora communities defend the birds from invaders and pathogenic organism (Servin 2004; Li et al. 2014). Trials with old layers indicated that dietary live yeast can rapidly raise ideal Lactobacilli count (Hussnain & Soliman, 2010). In the same context, Gao et al. (2008) stated that a dietary addition of 2.5 g/kg yeast cell exhibited optimum beneficial effect on intestinal morphology i.e. increase in villi height and depth of villus to crypt at 21 and 42 days of trial. The increased DYS levels leads to low Lactobacilli count which might be due to high yeast inclusion that hampered the nutrient absorption possibly by increasing gut viscosity (Olvera et al., 2002).

Immune Responses

The immune system protects the body against diseases whilst the production of anti-bodies is considered as the best indicator of bird immune level (Yalcın et al., 2013). The existance of a better immune level in diets DYS5 and DYS10 against NDV and AIV indicates that yeast can trigger immune system. The best antibody titer by incorporating (2 g /kg feed) yeast autosylate in broilers. Furthermore, it has been observed that yeast based products exert anti-inflammatory response in combination with stimulation of the natural killer cells and B lymphocytes. Similarly, An et al. (2008) have also found an effective immune response in broilers when raised on β-glucans. Mehdi and Hassan (2012) reported a high antibody titer against NDV when birds were assigned a diet containing 0.2% mannan-oligosaccharides as compared to 0.3 %. Yeast is rich in nucleic acid and plausible reason for reduced immunity in the birds fed DYD15 and DYS20 diet might be the high concentration of nucleic acid that acted as an anti-nutrient agent (Ozario et al. 2012).

CONCLUSION

In conclusion, SBM can be replaced with DYS in the layer grower ration up to 10% without adverse effects on the health and performance of layer pullets. Highest immune response was observed in pullets fed control diet followed by pullets fed DYS5, DYS10, DYS15 and DYS20 diet. The study revealed that DYS can be included in the diet of layer pullets up to 10% having no ill effect.

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