IMPACT OF REPLACEMENT OF DIETARY SOYBEAN MEAL WITH DRIED DISTILLERY YEAST SLUDGE ON PERFORMANCE AND HEALTH OF LAYER CHICKS

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KEYWORDS	ABSTRACT
Chicks, Distillery Yeast Sludge, Feed Conversion Ratio, Immunity & Gut Health	The 600 layer chicks (day-old) were distributed into 5 groups having 4 replicates comprising 30 chicks each to evaluate impact of replacement of dietary soybean meal with different level of dried distillery yeast sludge (DYS) on performance, immunity, gut health and histopathology. The DYS was dried in a dryer and ground to a mesh size of 2 mm in a hammer mill. Five isocaloric and isonitrogeneous C (control), DYS5, DYS10, DYS15 and DYS20 layer starter diets were formulated to contain 0, 5, 10, 15 and 20% DYS replacing soybean meal. Chicks fed ad libitum for the eight weeks. Water was available round the clock. The findings revealed significantly high ($P<0.05$) feed intake and weight gain in the chicks fed diet C than chicks fed DYS15 and DYS20 diets, whereas, it was same to chicks fed diet DYS10 and DYS5. The feed conversion ratio was similar among the chicks fed DYS5, DYS10, DYS15 and C diets, however, it was better ($P<0.05$) than chicks fed DYS20 diet. The lactobacillus count was different ($P<0.05$) across all diets. In conclusion, DYS can be included in the diet of layer chicks up to 10% without any adversarial impact on health as well as performance of chicks.

INTRODUCTION

The soybean meal and corn are considered standard feed ingredients to feed commercial poultry as well as large animals (Yin, Fatufe & Blachier, 2011). Recently, it looks that no possible substitute occurs internationally for these feedstuffs (Yegani & korver, 2008). The imposed ban by European countries on the animal protein base feedstuffs in poultry feed has increased poultry feeding dependency on vegetable protein feed ingredients especially soybean meal (Yin et al., 2011). Although, soybean meal is considered as the gold standard in poultry nutrition, however, its increasing cost and unstable availability limit its extensive feeding in the poultry (Chand & Khan, 2014). Single cell protein is quality protein extracted from bacteria, algae, yeast and fungi. The use of the alternative protein sources instead of high price conventional protein feed ingredients can help to compensate the protein deficiency (Jalasutram, Kataram, Gandu & Anupoju, 2013; Attia, Harthi & Deek, 2003). The Yeast and soybean meal have almost the parallel amino acids pattern (Adedayo, Ajiboye, Akintunde & Odaibo, 2011).

The use of yeast products as feed additives in poultry feeding is very common now a day (Chen, Chen & Wang, 2017; Yasar & Yegen, 2017). The yeast, particularly Saccharomyces cerevisiae (SC) is an important SCP source and can be economically harvested with less nucleic acid than bacteria (Bacha, Nasir, Khalique, Anjum & Jabbar, 2011) havening true protein approximately 20% (Sharif, Shoaib, Ahmad & Javaid, 2016). The distillery yeast sludge (DYS) is a byproduct of the distillery industry which primarily comprises SC as a protein source. Existing reports on DYS demonstrate that this source of SCP contains substantial nutritional worth and has crude protein 27 to 29 percent (Ali, 2004). Additionally, it contains some essential amino acids which result to improve growth as well as health of birds (Rameshwari & Karthikeyan, 2005; Sharif et al., 2016; Mujahid, Hashmi, Anjum, Waris & Tipu, 2012). Therefore, present study designed to determine impact of replacing of dietary soybean meal with graded level of dried DYS on performance, intestinal health, immunity and histopathology of chicks.

MATERIALS AND METHODS

Experimental Birds

The 600 layer chicks (one day old) were bought from the local hatchery after ensuring standard weight and physical health. The chicks were reared under standard managemental practices of Hy-line throughout the experimental period. All chicks were individually weighed and were divided randomly into 5 experimental groups having 120 birds each which were subsequently divided into four replicates (30 chicks per replicate). The chicks were vaccinated against infectious bronchitis, newcastle disease, hydropericardium, infectious bursal disease and fowl pox during the experiment.

Experimental Diets

The DYS was collected from Sugar Mill and dried in a locally manufactured steam jacket dryer. The procured DYS was ground to a mesh size of 2mm in a hammer mill. Five *iso-nitrogenous* (CP 21%) and *iso-caloric* (ME 2800 kcal/kg) layer starter chicks' diets control (C), DYS5, DYS10, DYS15 and DYS20 were formulated giving to NRC (1994) having varying levels of DYS (0, 5 ,10, 15 & 20%, respectively) by replacing soybean meal (Table 1). The chicks fed *ad libitum* for 8 weeks and fresh water was provided round the clock.

Table 1 Ingredient and Chemical Composition of Experimental Diets

	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
Rice tips	11.00	10.00	8.00	9.00	7.00
Yellow corn	39.58	38.56	39.04	37.62	38.50
Sunflower meal	5.00	4.50	4.00	4.00	4.00
Cotton seed meal	5.00	4.00	4.00	4.00	3.50
Canola meal	5.00	5.00	5.00	4.00	4.00
Rice polish	5.00	5.00	5.00	5.00	4.00
Corn gluten 60%	3.00	4.00	4.50	4.90	6.00
Fish meal	3.00	3.00	3.00	3.00	3.00
Guar meal	4.00	4.50	4.50	5.00	4.50
Molasses	5.00	5.00	4.50	3.00	3.00
Dicalcium phosphate	1.00	1.00	1.00	1.00	1.00
Limestone	1.00	1.00	1.00	1.00	1.00
Vitamin-mineral premix	0.50	0.50	0.50	0.50	0.50
Chemical composition %					
ME, Kcal/kg	2852	2848	2848	2851	2849
Crude protein	21.01	21.06	21.04	21.01	21.01
Crude fiber	5.40	5.35	5.42	5.25	5.22
Ether extract	3.96	3.90	3.92	3.93	3.90
Calcium	0.99	1.02	1.03	0.98	1.01
Available phosphorus	0.45	0.49	0.48	0.42	0.44
Lysine	1.15	1.13	1.09	1.11	1.13
Methionine	0.64	0.61	0.62	0.63	0.60

[1]The percentage of distillery yeast sludge is 0, 5, 10, 15 and 20 in C, DYS5, DYS10, DYS15 and DYS20 diet, respectively.

Data Collection

Chicks weighed in the start of the experiment and then repeated weekly. The feed intake was recorded and feed conversion ratio (FCR) was calculated every week during trial. Samples of blood were collected (3 birds/replicate) at 21st and 42nd days of age for the estimation of antibody titer against Avian influenza virus (AIV) and Newcastle disease virus (NDV) by Haemagglutination Inhibition method (Allan & Gaugh, 1974). Three chicks per replicate were slaughtered for pathological lesions (on liver, kidney, intestine) and for gut health at the termination of experiment. Data were statistically analyzed in a CRD using analyses of variance technique. Means were separated by Tukey's test (Steel, Torrie & Dickey, 1997).

RESULTS OF STUDY

Growth performance

Feed intake in chicks fed diet C was higher ($P<0.05$) than chicks fed diet DYS20, while, it was same to the chicks fed DYS5, DYS10 and DYS15 diets (Table 2). Similar trend was found regarding weight gain in chicks. Feed conversion ratio was similar among the chicks fed DYS15, DYS10 and DYS5 and C diets, however, it was better ($P<0.05$) than those fed DYS20.

Table 2 Impact of Different Levels of Dietary DYS on Performance of Layer Chicks

	Diets[1]				
Parameters	**C**	**DYS5**	**DYS10**	**DYS15**	**DYS20**
Feed intake (g)	$1701^{a}\pm 37.67$	$1685^{a}\pm 68.56$	$1657^{ab}\pm 48.81$	$1605^{ab}\pm 39.73$	$1570^{b}\pm 18.25$
Weight gain (g)	$614^{a}\pm 13.17$	$603^{a}\pm 12.97$	$588^{ab}\pm 39.69$	$536^{ab}\pm 30.38$	$501^{b}\pm 7.39$
Feed conversion ratio	$2.77^{a}\pm 0.01$	$2.79^{a}\pm 0.14$	$2.82^{a}\pm 0.11$	$3.00^{ab}\pm 0.15$	$3.13^{b}\pm 0.07$

[1]The percentage of distillery yeast sludge was 0, 5, 10, 15 and 20 in C, DYS5, DYS10, DYS15 and DYS20 diet, respectively.
Means having dissimilar super superscripts differ significantly ($P<0.05$).

Immune Responses

Immune response was higher in the chicks fed C, DYS5 and DYS10 diets compared with chicks fed DYS20 and DYS15 diets (Table 3). The antibody titer against NDV and AI in the layer chicks has been presented in Table 3 and 4. Non-significant difference was recorded cumulative mean titer against ND among chicks fed C, DYS5, DYS10, DYS15 and DYS20 diets (Table 3). Similar results were observed regarding cumulative mean titer against AI in chicks fed different diets (Table 4).

Table 3: Impact of different levels of dietary DYS on post-vaccine geometric mean titer and cumulative mean titers of layer chicks against Newcastle disease virus

	Diets[1]				
Weeks	**C**	**DYS5**	**DYS10**	**DYS15**	**DYS20**
			Geometric Mean Titer		
2nd	60.41	57.02	50.80	40.31	40.31
4th	120.82	114.04	107.63	85.43	71.83
6th	181.02	181.02	170.86	143.68	120.81
8th	241.63	228.07	215.27	181.02	170.86
			Cumulative Mean Titer		
	146.87	143.44	141.84	112.61	100.95

[1]The percentage of distillery yeast sludge is 0, 5, 10, 15 and 20 in C, DYS5, DYS10, DYS15 and DYS20 diet, respectively.

Table 4 Impact of Different Levels of Dietary DYS on the Post-Vaccine Geometric Mean Titer and cumulative mean titers of layer chicks against Avian Influenza virus

	Diets[1]				
Weeks	**C**	**DYS5**	**DYS10**	**DYS15**	**DYS20**
Geometric Mean Titer					
2nd	30.20	30.20	30.20	23.97	22.63
4th	64.00	64.08	60.41	42.71	33.90
6th	95.89	85.43	76.11	67.81	67.81
8th	143.68	135.69	135.61	107.63	101.59
Cumulative Mean Titer					
	82.54	78.85	75.58	61.64	53.21

[1]The percentage of distillery yeast sludge is 0, 5, 10, 15 and 20 in C, DYS5, DYS10, DYS15 and DYS20 diet, respectively.

Gut Lactobacilli Count

The lactobacillus count was different ($P<0.05$) across all diets and gradually decreased with increasing dietary level of DYS (Table 5).

Table 5 Impact of different levels of dietary DYS on layer chicks *Lactobacilli* count (10^5 cfu/gram) of layer chicks

Parameter	**Diet[1]**				
Lactobacilli Count	C	DYS5	DYS10	DYS15	DYS20
	$13.39^{a}\pm0.08$	$12.37^{b}\pm0.22$	$12.20^{b}\pm0.16$	$9.27^{c}\pm0.09$	$6.67^{d}\pm0.05$

[1]The percentage of distillery yeast sludge is 0, 5, 10, 15 and 20 in C, DYS5, DYS10, DYS15 and DYS20 diet, respectively.
Means having dissimilar super superscripts differ significantly ($P<0.05$).

Histopathology

Hepatocytes showed no histologic lesions at microscopic level in the liver of chicks fed C and DYS5 diets (Figure 1). Congested as well as dilated (white stars) sinusoidal spaces and fatty changes in hepatocyte cytoplasm were eminent in chicks fed DYS10 diet (Figure 2). These changes were more intense in chicks fed DYS15 and DYS20 diets, respectively, in a dose dependent manner.

Figure 1 Photomirograph of Control and DYS5 Group Liver of Starter Layer Chicks, No HistoLogical Abnormalities Were Found (H&E; 400x)

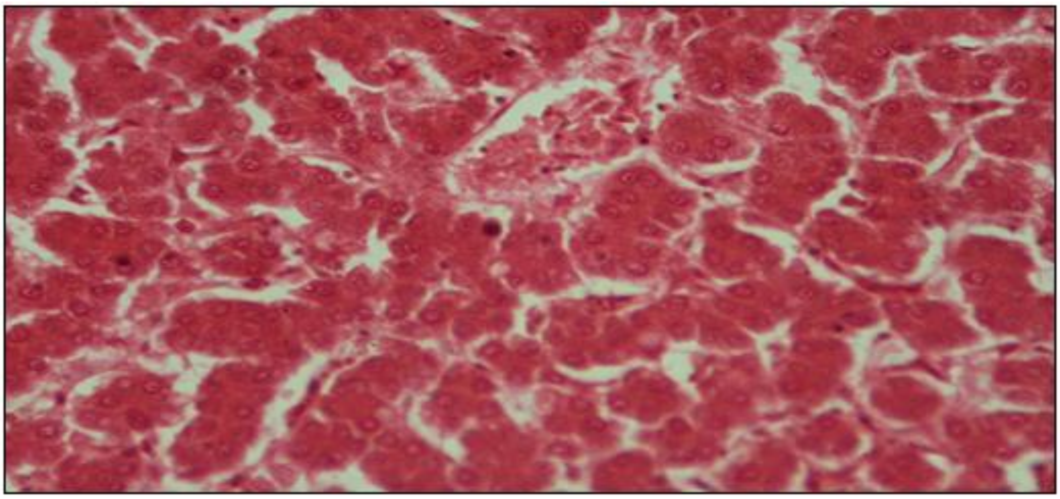
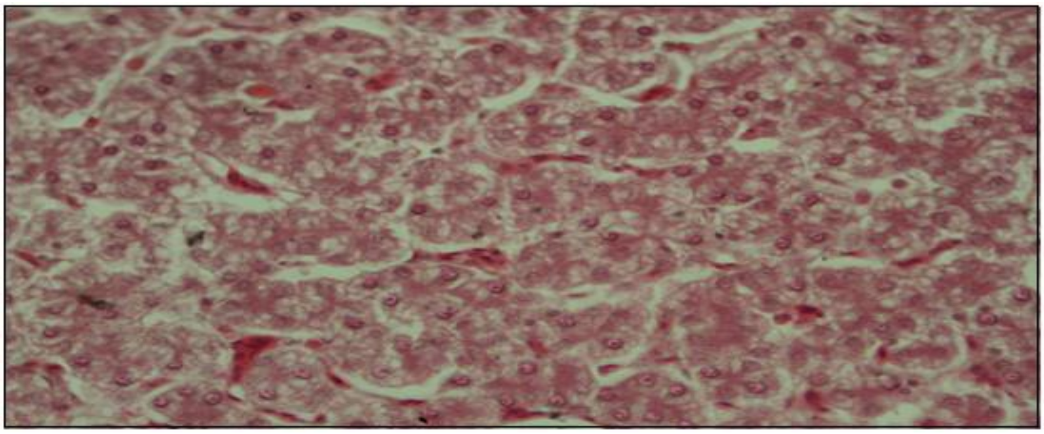


Figure 2 Photomicrograph of Liver of Starter Layer Chicks fed DYS10 Diet. Sinusoid was seen Congested and Dilated (White stars). Fat vacuoles were seen inside hepatocyte cytoplasm indicating fatty change (Thin black arrows). (H&E; 400x).



In case of microscopic examination of kidneys, there was no prominent change in chicks fed with C and DYS5 diets (Figure 3). Conversely, tubular epithelial cells showed hydropic degeneration with lumen of tubules filled with proteinious *eosinophillic* masses in chicks fed DYS10 diet (Figure 4).

Figure 3 Photomicrograph of Kidney of Chicks Fed Control and DYS5 Diets. There were No histological alterations

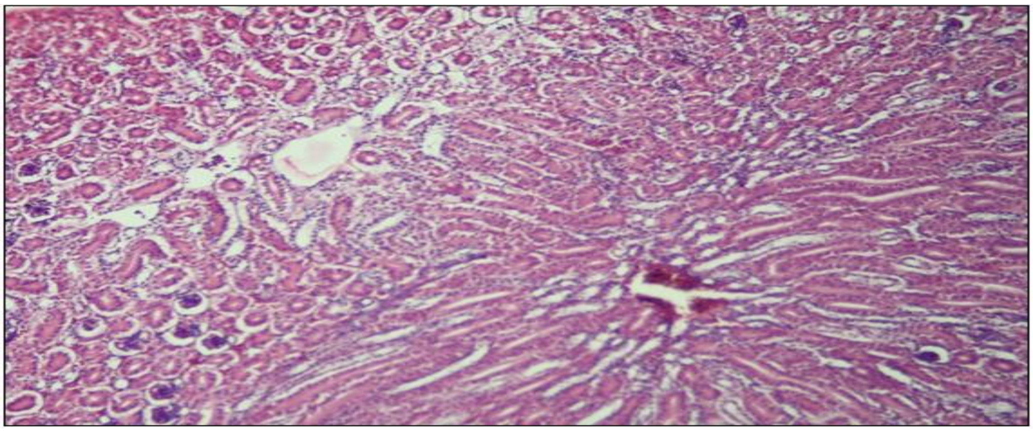
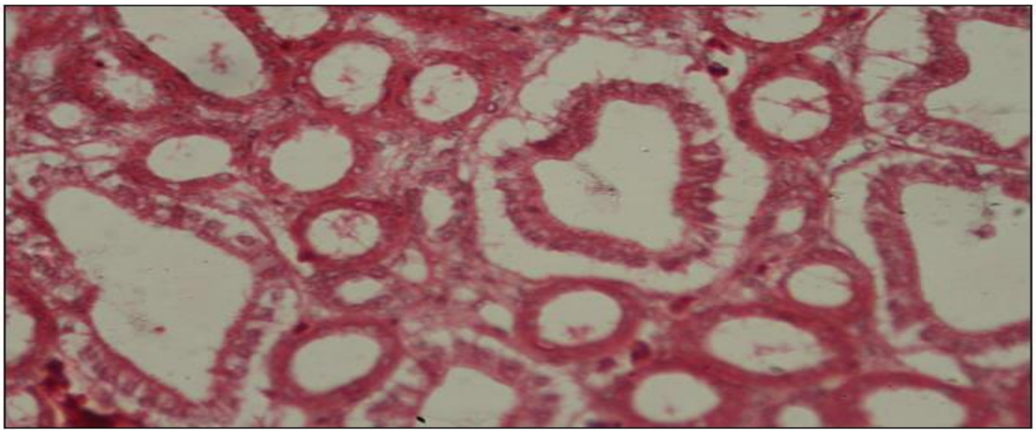


Figure 4 Microphotograph layer chick kidney of DYS10 group. The curved arrows are showing degeneration in epithelial cells of kidney. White stars are showing sloughed eosinophillic material in dilated tubules lumen.



The intensity of these lesions was more in chicks fed DYS15 and DYS20 diet. Intestines of birds fed on C and DYS5 diet showed no histological changes (Figure 5). Whereas, degenerative changes in mucosal layer showing broken villous tips, were noted in chicks fed DYS10 diet. There was apparent necrotic change in villi layer of intestine with leukocytic infiltration (Figure 6). Lesions were more prominent in chicks fed DYS20 and DYS15 diet. Heart showed no histological changes in chicks receiving any treatment.

Figure 5 Microphotograph repents that there were no histological changes in the intestine of chicks fed DYS5 and control diets.

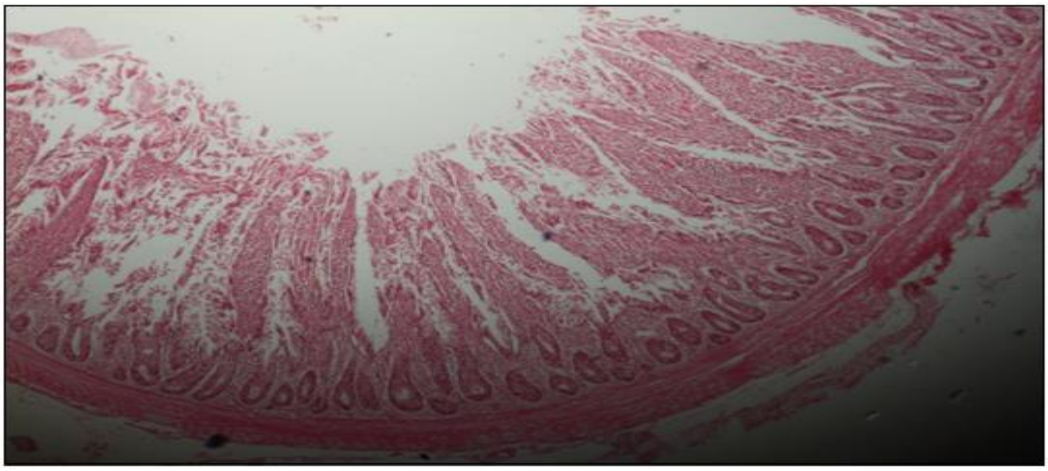
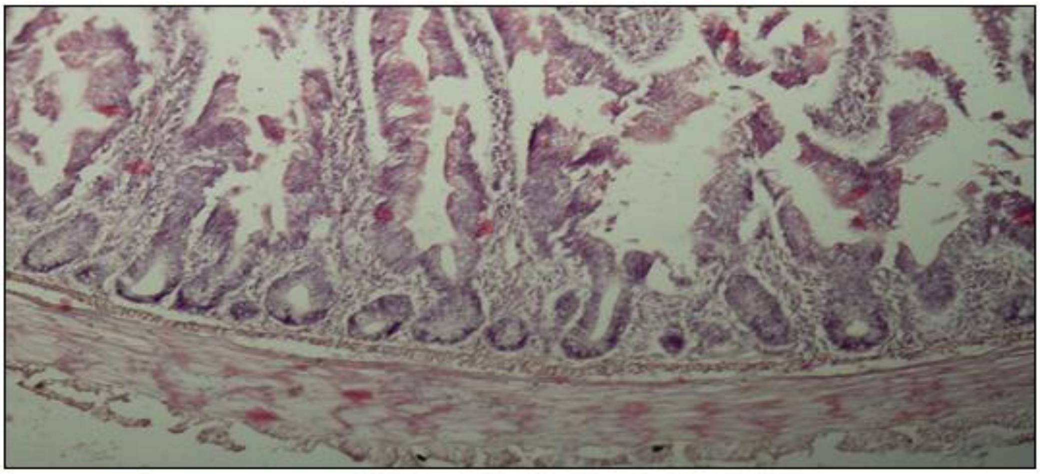


Figure 6 Microphotograph of represents broken villi tips of mucosa in the intestine of layer chicks fed DYS10 diet.



DISCUSSION

Growth Performance

In present study, the results regarding feed intake were similar to control replacing DYS up to 15% with soybean meal and beyond this level there was decline in feed intake in chicks. Likewise, Bilal, Sarwar and Sultan (2016) stated that soybean meal can be replaced up to 10% with DYS in the layers' diet without affecting intake of feed. Findings of this experiment support the results of Herria and Garcia (1983) who observed increased feed intake in broilers by replacing 7% of soybean meal with yeast in diet. Khan (2001) noted enhanced feed intake in chicks fed raw DYS (up to 6%) but he found decreased feed intake when fed DYS beyond this level. Koc, Samli, Ozduven, Akyurek & Senkoylu (2010) observed increased feed intake by supplementing yeast birds and this rise may be due to balance of the gastrointestinal tract microbial population. Likewise, Rameshwari and Karthikeyan (2005) noted low feed intake in group of birds fed diets introduced with higher concentrations of DYS. This negative effect in feed intake might be because of increase in the pulverization of the feed comprising DYS. In agreement with this research, Rodriguez, Valdivie and Lezcano (2014) reported less intake of feed in birds fed high (30%) DYS than those fed low (10 and 20%) DYS. They further stated that this decrease in feed intake might be due to high nucleic acid of DYS. The findings of our research have supported the results of Gao et al. (2008) who documented high weight gain in broilers fed diet containing low yeast (2.5g/kg of diet) than those fed high doses of yeast. Additionally, they stated that this change in weight gain might partially be related to change in partitioning of energy toward turnover of tissues.

Likewise, Rameshwari and Karthikeyan (2005) documented less weight gain in broilers fed diet containing more DYS. Calcium and phenol are high in yeast sludge which may adversely affect the absorption of phosphorus in intestine. Rodrigiez et al. (2014) also documented less gain in body weight of layers fed more (30%) yeast sludge. Similarly, reduced gain in weight was noted in birds fed diet high (500ppm) in yeast cell wall than those fed diet low (125, 250 and 375 ppm) in yeast cell wall (Flower et al., 2015). The results regarding FCR in the present study are supported by Rameshwari and Karthikeyan (2005) who documented that FCR was poor in broilers fed diet comprising high DYS. The FCR values of current experiment are also in line to those recorded by Mansour et al. (2011) who stated improved efficiency of feed in broilers fed diet comprising low yeast culture (1.25g/kg of feed) than those fed diet comprising high yeast culture (1.5g/kg of feed). Likewise, Ghally and Latif (2007) noted improved efficiency of feed in Japanese quail fed diets comprising 2% yeast. A study was done by Mujahid et al. (2012) to assess the yeast efficiency on the performance of broilers. They reported that feed efficiency was better when broilers fed diet comprising 2% yeast sludge.

Immune Responses

The immune response decreased replacing soybean meal with DYS, however, chicks fed DYS5 and DYS10 showed comparable results to control regarding immune response against NDV and AIV. High response of immunity was observed in chicks fed lower level of DYS as compared to fed higher level of DYS. Similar finding was reported by Bilal et al. (2016) in laying hens. Yalcin et al. (2014) reported enhanced response of immunity in layers supplemented with yeast cell wall. An et al. (2008) have also found an effective immune response in broilers when raised on β-glucans. Similarly, 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 possible reason for reduced immunity in the birds fed DYD15 and DYS20 diet might be that high concentration of nucleic acid had served an anti-nutrient agent (Ozorio, Portz, Borghesi & Cyrino, 2012).

Gut Lactobacilli Count

The lactobacilli count was decreased replacing soybean meal with DYS, however, *lactobacilli* count was high in chicks fed diet containing low DYS than those fed diet containing high level of DYS. Similarly, Hussnain and Soliman (2010) documented high lactobacilli count in layers fed low dose rate of live yeast (0.8%) than those fed high live yeast. The *lactobacilli* bacteria cause fermentation of feed and subsequently produce lactic acid resulting acidic intestinal medium and ultimately limit putrefactive bacterial growth as well as harmful bacteria and consequently save birds from prevalent diseases (Servin, 2004; Li et al., 2014). The decrease in Lactobacilli in chicks fed DYS20 and DYS15 diets might be due to high contents of ash as well as nucleic acid resulting unbalance of favorable microbiota causing poor nutrient absorption and subsequently decrease performance of chicks (Rodriguez et al., 2014). Bilal et al. (2016) also reported decreased lactobacilli count replacing soybean meal with DYS in laying hens.

Histopathology

Findings of present experiment are in line with findings of Ansar, Khan, Chaudhary, Mian, Tipu & Rai (2004) who stated degenerative changes on the size, color, texture and shape with haemorrhages in the group of birds fed a basal diet containing high calcium and low phosphorus. The probable reason for the more pronounced histological abnormalities in layer chick's liver, kidney and lungs fed with DYS10, DYS15 and DYS20 diets than birds fed C and DYS5 diets that heavy doses of DYS's contain more mineral content which might have, caused detrimental effects in the structure and function of visceral organs.

CONCLUSION

Inclusion of DYS in layer chick diet up to 10% revealed comparable findings regarding growth performance, immunity and gut health to control. This study reveals that DYS can effectively replace soybean meal in layer chick starter diet up to 10% and has no adversarial effects on the health and performance of layer chicks. This work was supported by Pakistan Agriculture Research Board. We acknowledge to Institute of Animal Sciences, University of Agriculture, Faisalabad, for their assistance.

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