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Effect of Feeding Rice Based Distillers Dried Grains with Solubles (rDDGS) on Gut Health, Immunity and Intestinal Histomorphometry in Broilers

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ABSTRACT

A biological experiment of 160 day old chicks for six weeks was undertaken with completely randomized design (CRD) divided into five treatments with 32 chicks with four replicates per treatment. Five experimental diets as per ICAR (2013) were prepared by incorporating rice distillers dried grains with solubles (rDDGS) at inclusion level of (0, 7.5, 10.0, 12.5 and 15.0%). Total viable count were significantly (P<0.01) decreased in 10, 12.5 and 15% rDDGS groups in crop and jejunum both. Lactobacillus count were significantly (P<0.01) increased in 10,12.5 and 15% rDDGS groups in crop but 12.5 and 15% rDDGS groups in jejunum. Humoral immunity were significantly (P<0.05) better in 12.5 and 15% rDDGS groups but cell mediated immunity did not show any significant (P>0.05) difference as compared to control. Villus height were significantly (P<0.01) decreased in 15% rDDGS group as compared to control and other dietary treatments but villus depth, villus width and villus height/depth did not show any significant (P>0.05) difference as compared to control and other dietary treatments. Thus, it is concluded that rDDGS can safely can be incorporated in broiler diet at the inclusion level of 12.5% with better humoral immunity, gut health and without any adverse effect on intestinal histomorphometry.

Key words: *Rice distillers dried grains with soluble, Gut health, Immunity, Intestinal histomorphometry*

INTRODUCTION

Poultry industry is the fastest growing sector in Indian agriculture. Feed is the major constituent in the poultry production accounts for 65-75% of total recurring expenditure. Feed costs are primarily driven by the cost of protein sources. Substitution of expensive protein sources with lower cost ingredients would potentially reduce the cost of the feed. Soybean meal (SBM) is the major protein source used in poultry diet. Instability in its production, indiscriminate exports and higher demand has resulted in its shortage for the poultry industry leading to its higher price.

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Substitution of SBM at reasonable price will lead to economic broiler production. India is second largest producers of rice in world after China, producing approximately 106.65 MT rice in 2013-14[1]. Now days, certain newer rice by products are available in appreciable quantities and cheaper rate that can be utilized from rice processing industries such as rice based distillers dried grains with solubles (rDDGS). It is relatively a new feedstuff having brownish colour and coarse powdery texture.

Distillers dried grains with soluble (DDGS) is co-product of the ethanol (biofuel) industry produced during dry milling process. Corn, wheat, barley and sorghum cereals are commonly used ingredients for fermentation during bioethanol production. In the present context, rice as subtract for bioethanol production is increasing due to its relative lower price, higher production and easy availability leads to increased availability of co-product rice DDGS (rDDGS). It contains all the nutrients from grain in a concentrated form except for the majority of the starch, which has been utilized in the fermentation process. It contains 65% distiller's grain and 35% its soluble[4]. Addition of rice DDGS up to 10% level did not exert any adverse effect on growth and carcass traits in broiler[14]. Rao *et al.*[18], reported that rDDGS can safely be incorporated in broiler at the inclusion level of 10%. Gupta[12], reported that rDDGS can safely be incorporated in layer diet up to the inclusion level of 10% without affecting egg production and egg quality traits along with improved gut health, immunity and intestinal morphology. Since most of the research work done is limited to corn, wheat, barley and sorghum DDGS. Very scanty researches are available in literature regarding effect of feeding rDDGS in poultry. So, there is need to explore effect of feeding rDDGS on gut health, immunity and intestinal histomorphometry in broilers.

MATERIAL AND METHODS

The research work carried out at the Division of Avian Nutrition and Feed Technology, ICAR-Central Avian Research Institute (CARI), Izatnagar, India in the year 2018. The study was carried out as per the guidelines and approval of Institute Animal Ethical Committee (IAEC) and Committee for the purpose of control and supervision of experiments on animals (CPCSEA).

Experimental design: A biological experiment of six weeks was undertaken with completely randomized design (CRD) on broiler chickens (CARIBRO Vishal) to investigate the effects of rice distiller's dry grain with soluble (DDGS) feeding on gut health, immunity and intestinal histomorphometry in broilers. A total of 160 day old chicks were taken and divided into five groups with 32 birds in each treatment. Each replicate consists of 8 birds housed together in battery cages and 4 replicates allocated for each treatment.

Experimental diets: Five experimental broiler diets *iso-caloric* and *iso-nitrogenous* were prepared by incorporating different levels of rice DDGS (0, 7.5, 10.0, 12.5 and 15.0%) as per ICAR[13], standard. The diets along with all the used ingredients including rice DDGS were analyzed for proximate[2], calcium[22], and fibre fractions[23]. *In vitro* pepsin-pancreatin digestibility of rDDGS and soybean meal was measured according to the method of Gopalkrishnan and Prakash[11]. Mycotoxin (aflatoxin B1 and ochratoxin) screening has been done by thin layer chromatography[2], for rDDGS.

Microbial status: At the end of trial (42 days), eight birds from each dietary treatment were sacrificed by cervical dislocation; crop and jejunum scraping were collected in sterile vials for evaluation of total microbial load colonization. Microbial populations were determined by serial dilution (10^4 to 10^6) of crop and jejunum samples in anaerobic diluents before inoculation onto petri dishes of sterile agar as described by Bryant and Burkey[5]. Total bacterial count and *Lactobacilli* was grown on nutrient agar and rogosa agar respectively[8]. Colony forming units were defined as distinct colonies measuring at least 1 mm in diameter expressed in cfu/g.

Histometry: Samples from jejunum will be taken from four birds per treatment at the end of experiment. All the light microscopic variables will be measured for jejunum of each bird using optical microscope (Motic Inverted microscope, Honkong), at a 10 X magnification, a camera (Motic cam, CMOS, Honkong), and image analysis software (Motic Image 2.0, Honkong). Pieces of 2-3 mm thickness at the midpoint of jejunum was removed, the segment was washed with physiological saline solution and fixed in 10% buffered formalin. Each jejunum segment collected in 1 will be embedded in paraffin and sections of 5-micron thickness of each sample will be placed on a glass slide and stained with hematoxyline and eosine for examination[7].

Immunity: Humoral immune response estimated by method of Siegel and Gross[20], will be followed for slight modified by Saxena *et.al*.[19], assaying the immune response to sheep red blood cells (SRBCs). The *in vivo* cell mediated immune response to PHA-P will be evaluated by the method of Cheng and Lamont[6], on 29th day post hatch. Eight broilers (4 males and 4 females) per treatment were used for assaying immune response.

Statistical analysis: Data was subjected to test of significance as per complete randomized design[21]. Treatments means were separated using Duncan's multiple range test[9]. The SPSS (Statistical Package for the Social Sciences) software program (IBM Corporation, Somers, NY, USA) version 16.0 used for analysis of data.

RESULTS AND DISCUSSION

Chemical composition of test material and diets: Experimental diets ingredients and nutrient composition as prestarter (0-2 wk) and starter (2-3 wk) and finisher diets (3-6 wk) has been given in the table no.1 as per ICAR (2013) feeding standard. Rice DDGS analyzed and reported in this study contained (%) Moisture 8.65, Dry matter (DM) 91.35,Crude protein (CP) 44.68, Ether extract (EE) 6.47,Crude fibre (CF) 9.12, Total ash (TA) 4.01, Acid insoluble ash (AIA) 1.27,Nitrogen free extract (NFE) 35.72, Calcium 0.62, Phosphorus 0.83, Neutral detergent fibre (NDF) 45.60, Acid detergent fibre (ADF) 12.87, Acid detergent soluble (ADS) 32.73, Acid detergent lignin (ADL) 2.14 and Gross energy 4232 kcal/kg on as such basis. *In vitro* pepsin-pancreatin digestibility (IVPPD) of rice DDGS was found 79.81% while IVPPD of soybean meal was reported 88.15%. No detectable aflatoxin B1 and ochratoxin has been found in rDDGS.

Our results are in agreement with Mandal[16], Gupta[12], and Rao *et al.*[18], who reported 45% CP but contrary to this Patil *et al.*[17], reported 30% CP in rDDGS. Furthermore, the drying process can have crucial influence not only on variability of nutrients but also on concentration and availability of nutrients in different samples.

Microbial status: The data pertaining to influence of different levels of rDDGS on total viable count (TVC) and *Lactobacillus* count in crop and jejunum have been presented in Tables 2. Statistical analysis revealed significant ($P<0.01$) changes were observed due to dietary treatment of different levels of DDGS in TVC and *lactobacillus* count. Total viable count were significantly ($P<0.01$) decreased in 10,12.5 and 15% rDDGS groups in crop and jejunum both. Lactobacillus count were significantly ($P<0.01$) increased in 10,12.5 and 15% rDDGS groups in crop but 12.5 and 15% rDDGS groups in jejunum. There were gradual decrease in TVC and increase in *Lactobacillus* count has been reported by increasing rDDGS levels.

Lactobacillus is the major component of the microbial barrier to infection. The earlier reports showed that the effect of DDGS on gut health is beneficial. The corn DDGS diet had a greater ($P < 0.05$) lactic acid concentration than the wheat DDGS diet and lactic acid is implicated to potentially inhibit the growth of pathogens[3]. Hahn[10], observed that the diets containing corn DDGS had a significantly higher count of 1.998 x 10^6 CFU/ml as compared to control diet. Yang *et al.*[24], reported that diets containing corn DDGS had greater lactic acid containing bacteria than diets containing wheat DDGS. Gupta[12], reported rDDGS in layer diet up to the inclusion level of 10% decreased TVC and improved *Lactobacillus* count. This could be because of the composition of DDGS in that it is more fibres offer more nutrients to *Lactobacillus.* Our results are coincide with Hahn[10], Yang *et al.*[24], and Gupta[12], which suggested that the composition of DDGS in that it is more fibrous offers more nutrients to *Lactobacillus.* Decrease in TVC may be associated with antibiotics residue present in DDGS which were used to control fermentation during ethanol production.

Immunity: The data pertaining to influence of different levels of rDDGS on humoral and cell mediated immunity have been presented in Tables 3. Humoral immunity were significantly (P<0.05) better in 12.5 and 15% rDDGS groups but cell mediated immunity did not show any significant (P>0.05) difference as compared to control. Our results are in agreement with Gupta[12], who reported no significant (P>0.05) difference in humoral and cell mediated immunity by feeding up to 10% inclusion of rDDGS. Better humoral immunity in rDDGS diets may be associated with type and composition of amino acids particularly methionine present in higher level in DDGS.

Intestinal histomorphometry: **T**he histometrical changes of villus height, width, crypt depth and villus height/crypt depth of duodenum as influenced by dietary addition of different levels of rice in broiler diets are presented in Table 4 and Fig.1 (a to e). Villus height were significantly (P<0.01) decreased in 15% rDDGS group as compared to control and other dietary treatments but villus depth, villus width and villus height :depth did not show any significant (P>0.05) difference as compared to control and other dietary treatments. Information on effect of feeding diets containing various levels of rice DDGS on histological changes in broiler is very scanty in literature. Our results are in agreement with Loar II *et al.*[15], and Gupta[12]. Loar *et al.* II[15], reported no adverse effect on intestinal parameters by incorporating DDGS in post hatch 28 days broilers. Gupta[12], reported no negative effects on intestinal morphology up to 10% inclusion of rDDGS. Decrease in villus length in 15% rDDGS may be associated with poor digestibility at this inclusion level.

Table 1: Ingredients and nutrient composition of prestarter, starter and finisher diets for different level of rDDGS

	prestarter diet (0-2 wk)					**starter diet (2-3 wk)**					**finisher diet (3-6 wk)**				
Ingredients	**D1**	**D2**	**D3**	**D4**	**D5**	**D1**	**D2**	**D3**	**D4**	**D5**	**D1**	**D2**	**D3**	**D4**	**D5**
Maize	54.42	55.3	55.46	55.94	56.4	55.63	56.94	57.41	57.66	58.1	62	63.41	63.78	64.18	64.37
SBM	38.4	30.7	28.2	25.5	22.9	37.1	29.2	26.6	24.1	21.4	31.3	23.4	20.8	18.2	15.7
oil	3	2.3	2.1	1.8	1.52	3.5	2.7	2.37	2.15	1.9	3.22	2.34	2.1	1.8	1.6
DDGS	0	7.5	10	12.5	15	0	7.5	10	12.5	15	0	7.5	10	12.5	15
LSP	1.4	1.3	1.3	1.3	1.2	1.35	1.27	1.23	1.2	1.17	1.2	1.05	1.03	1.0	0.96
DCP	1.82	1.83	1.84	1.83	1.83	1.55	1.56	1.56	1.58	1.58	1.45	1.5	1.5	1.5	1.5
Lysine	0	0.13	0.19	0.23	0.27	0	0	0	0	0.05	0	0	0.01	0.05	0.1
Methionine	0.2	0.16	0.14	0.13	0.11	0.1	0.07	0.06	0.04	0.03	0.06	0.03	0.01	0	0
Constant*	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient compsition (%)															
CP	21.98	22.01	22.04	22.00	22.01	21.51	21.49	21.50	21.53	21.50	19.50	19.49	19.49	19.50	19.53
ME(kcal/kg)**	2998	2999	3000	2998	2999	3049	3052	3049	3051	3052	3100	3099	3101	3098	3100
Ca	1.03	1.03	1.04	1.05	1.02	0.95	0.95	0.95	0.95	0.95	0.86	0.85	0.85	0.85	0.85
P	0.45	0.45	0.45	0.44	0.44	0.40	0.40	0.40	0.40	0.40	0.38	0.38	0.38	0.38	0.38
Lysine	1.18	1.20	1.22	1.22	1.22	1.15	1.17	1.15	1.10	1.10	1.09	1.03	0.99	0.97	0.97
Methionine	0.52	0.52	0.52	0.52	0.52	0.47	0.48	0.48	0.48	0.48	0.41	0.42	0.41	0.41	0.43
Threonine	0.81	0.83	0.82	0.81	0.81	0.80	0.79	0.77	0.79	0.77	0.68	0.67	0.68	0.69	0.67
Cost (Rs./ kg)	28.53	27.25	26.85	26.36	25.87	28.03	26.45	25.88	25.35	24.92	26.72	25.09	24.55	24.08	23.78

*Constant 0.765 includes salt 0.4%, trace mineral premix 0.1%, vitamin premix 0.15%, vit. B complex 0.015%, choline chloride 0.05% and toxin binder 0.05% (As per ICAR,2013) **calculated value

Table 2: Effect of feeding different levels of rDDGS on microbial count (log10cfu/g)

Treatment	rDDGS (%)	Crop		Jejunum	
		TVC	Lactobacillus count	TVC	Lactobacillus count
T1	**0**	6.54^{d}	3.53^{a}	6.74^{b}	3.04^{a}
T2	**7.5**	6.37^{cd}	3.56^{ab}	6.61^{b}	3.10^{ab}
T3	**10**	6.13^{ab}	3.68^{bc}	6.29^{a}	3.11^{ab}
T4	**12.5**	6.31^{bc}	3.78^{c}	6.31^{a}	3.32^{b}
T5	**15**	6.00^{a}	3.82^{c}	6.28^{a}	3.34^{b}
	Pooled SEM	0.052	0.032	0.053	0.042
	P Value	P<0.01	P<0.01	P<0.01	P<0.01

Values bearing different superscripts within the column differ significantly **(P<0.05) and NS-Non-significant (P>0.05)

Table 3: Effect of feeding different levels of rDDGS on immunological parameters

Treatment	rDDGS (%)	HA (log2)	CMI (mm)
T1	**0**	2.57^{a}	0.58
T2	**7.5**	2.69^{ab}	0.57
T3	**10**	2.63^{ab}	0.58
T4	**12.5**	2.74^{b}	0.58
T5	**15**	2.74^{b}	0.55
	Pooled SEM	0.020	0.006
	P Value	P<0.05	NS

Values bearing different superscripts within the column differ significantly **(P<0.05) and NS-Non-significant (P>0.05)

Table 4: Effect of feeding different levels of rDDGS on intestine morphometry

Treatment	rDDGS (%)	Villus height (VH)	Villus depth (VD)	VH:CD	Villus width
T1	**0**	1269^{bc}	167	7.2	97
T2	**7.5**	1308^{c}	169	7.7	95
T3	**10**	1266^{bc}	172	7.5	100
T4	**12.5**	1194^{b}	212	6.2	100
T5	**15**	1035^{a}	163	6.6	86
	Pooled SEM	25.357	6.943	0.243	1.917
	P Value	P<0.01	NS	NS	NS

Values bearing different superscripts within the column differ significantly **(P<0.05) and NS-Non-significant (P>0.05)

Figure 1: Effect of feeding different levels of rDDGS on intestinal histomorphometry

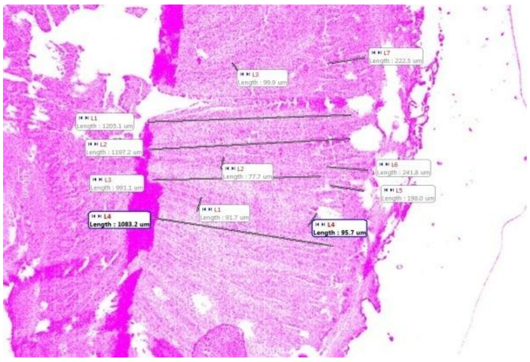

Fig. (a) Control (0% rDDGS)

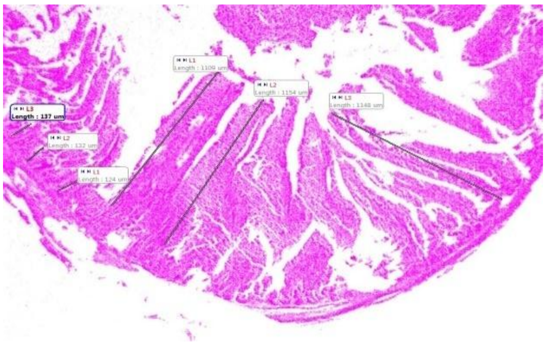

Fig. (b) Control (7.5% rDDGS)

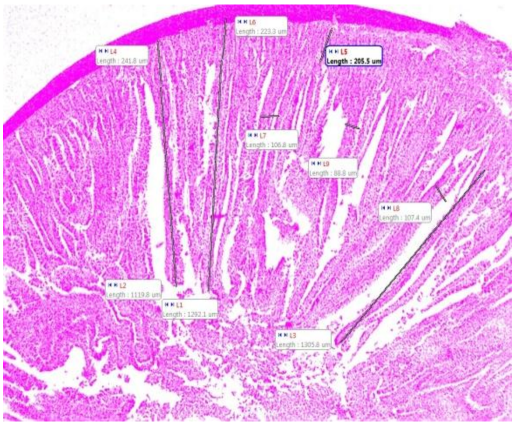

Fig. (c) Control (10% rDDGS)

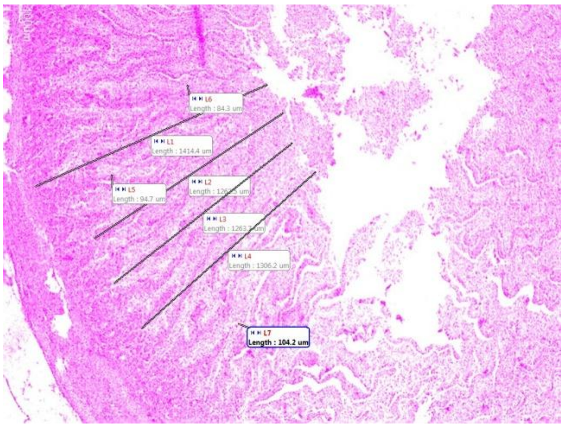

Fig. (d) Control (12.5% rDDGS)

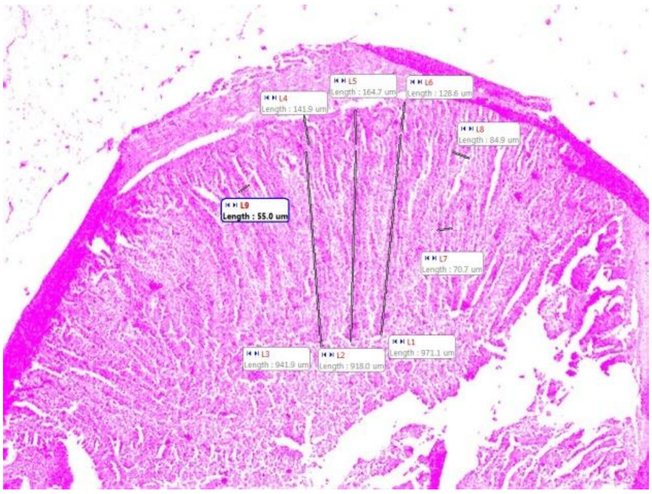

Fig. (e) Control (15% rDDGS

CONCLUSIONS

Thus, it is concluded that rDDGS can be safely incorporated in broiler diet at the inclusion level of 12.5% with better humoral immunity, gut health and without any adverse effect on intestinal histomorphometry.

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REFERENCES

1. Agricultural Statistics., Directorate of Economics & Statistics. Department of Agriculture, Cooperation and Farmers welfare. Government of India, New Delhi (2015-16).
2. AOAC., *Association of Official Analytical Chemists*. Official Methods of Analysis. 17 eds (2000).
3. Axelsson, L., Lactic acid bacteria: Classification and physiology. Pages 1-72 in Lactic Acid Bacteria:Microbiology and Functional Aspects. S. Salminen and A. Von Wright, ed. Marcel Dekker, New York, NY (1998).
4. Babcock, B.A., Hays, D.J. and Lawrence J.D., Using Distillers Grains in the U.S. and international livestock and poultry industry. *Midwest agribusiness Trade Research and Information Center*. First edition, (Ames, Iowa, USA) (2008).
5. Bryant, M.P. and Burkey L.A., Cultural methods and some characteristics of some of the more numerous groups ofbacteria in the bovine rumen. *Journal of Dairy Sci*ence, **36:** 205-17 (1953).
6. Cheng, S. and Lamont, S.J., Breeding and genetics; genetic analysis of immunocompetence in White Leghorn chicken line. Poult. Sci., **67:** 989-995 (1988).
7. Culling, C.F.A., Handbook of Histopathological and Histochemical Techniques. 3rd edn. Butterworths & Co.,London. pp. 343-49 (1974).
8. Deman, J.D., Rogosa, M. and Sharpe, M.E., A medium for the cultivation of *Lactobacilli. Journal of Applied Bacteriology*, **23:** 130-35 (1960).
9. Duncan, D.B., Multiple range and F tests. *Biometrics*. **11:** 1-42. edn., Washington, DC (1955).
10. Hahn, Dana L., The effects of phytase and an enzyme combination in moderate and low nutrient dense diets in laying hens. Theses and Dissertations in Animal Science, Paper 23 (2010).
11. Gopalkrishnan, M.V. and Jamuna, P., Optimum time requirement for enzymatic hydrolysis of food proteins. *J. Food Sci. Tech*. **37:** 319-322 (2000).
12. Gupta, S.L., Tyagi, Pramod K., Tyagi, Praveen K., Mandal, A.B., Mir, N.A. and Sharma, M., Intestinal histomorphometry of laying hens fed diets containing rice based dry distiller's grains with solubles. *Indian Journal of Poultry Science*, **50(3):** 294-299 (2015).
13. ICAR., Nutrient requirements of Animals- Poultry (ICAR-NIANP).pp 13-16 (2013).
14. ICAR-CARI., ICAR- Central Avian Research Institute. Annual report (2014-15).
15. Loar, R.E. II, J. S. Moritz, J.R. Donaldson, and A. Corzo., Effects of feeding distillers dried grains with soluble to broilers from 0 to 28 days posthatch on broiler performance, feed manufacturing efficiency, and selected intestinal characteristics. *Poul. Sci.* **89:**2242-2250 (2010).
16. Mandal, A.B., Alternate poultry feed resources for sustainable poultry meat and egg production, XXXI Annual conference of Indian poultry science association and national symposium on "Poultry production for global trade" Namakkal, pp. 37-45 (2014).
17. Patil B.B., Dhage S.A. and Pachpute S.T., *In Vitro* Evaluation of Different Distiller's Grains with Solubles. *Indian J. Anim. Nutr*. 2015. **32 (2):** 181-186 (2015).
18. Rao *et al.,* The Proceedings of XXV World's Poultry Congress 2016 - Abstracts.pp-52 (2016).
19. Saxena, Ritu, Saxena, V.K., Tripathi, V., Agarwal, R. and Singh, B.P., Effect of dietary protein and energy on intestinal Mucin-2 expression and post-hatch growth in Naked Neck broiler. *Indian Journal of Poult. Sci.*, **48(3):** 286-290 (2013).
20. Siegel, P. B. and Gross W. B., Production and persistence of antibodies in chickens to sheep erythrocytes. I. Directional selection. *Poult. Sci.*. **59:**1-5 (1980).

21. Snedecor, G.W. and Cochran, W.G., Statistical Methods. 7th edn. Oxford and IBH (1989).
22. Talpatra, S.K., Roy, S.C. and Sen, K.C., Estimation of Phosphorus, Chlorine, Calcium, Magnesium, Sodium and Potassium in feed stuffs. Indian Journal of Veterinary Science, **10:** 243-258 (1940).
23. Van Soest, P.J. and Wine, R.H., Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell wall constituents. *J. Asso. Agri. Chem.*, **50:** 50-56 (1967).
24. Yang, Y., Kiarie, E., Slominski, B.A., Brule-Babel, A. and Nyachoti, C.M., Amino acid and fiber digestibility, intestinal bacterial profile, and enzyme activity in growing pigs fed dried distillers grains with solubles-based diets. *Journal of Animal Science,* **88:** 3304-12 (2010).