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Effect of Feeding Rice Based Distillers Dried Grains Solubles with and without Enzymes on Gut health of Broiler Chickens

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ABSTRACT

A biological experiment was conducted to evaluate the effect of feeding rice based distillers dried grain with solubles (rDDGS) without or with different enzymes on gut health in broiler chickens for 42 days. The experiment was conducted as per 3x4 factorial completely randomized design. A total of 384 broiler chicks were divided into twelve different treatments with 4 replicates for each treatment and each replicate consisted of 8 chicks. Two levels of rDDGS were taken (12.5 and 15%). Protease, xylanase and multienzymes supplementation under different treatments were done. The jejunum histomorphometry in terms of villus height (VH) and their ratio with villus depth (VD) were found significantly (P<0.01) lower at 15% rDDGS level compared to control and 12.5% levels. The xylanase and protease enzyme supplementation significantly (P<0.01) increased the VH and their ratio with VD as compared to control and multienzymes supplemented group. The interaction effect rDDGS and enzyme supplementation was found on VH and VD ratio, where higher ratio was observed at 12.5% rDDGS level with protease enzyme and lower ratio was observed at 15% rDDGS level without enzyme supplementation. The microbiology of the gut in terms of total viable count (TVC) of crop and jejunum significantly (P<0.01) decreased upon increasing rDDGS levels compared to control, whereas reverse effect was observed on *Lactobacillus.* Thus, it may be concluded that enzymes supplementation improved histomorphometry and microbiology of the gut in broiler chickens at inclusion level of 12.5% rDDGS.

HIGHLIGHTS

- The rDDGS inclusion improved the beneficial microbes in the gut.
- Enzymes supplementation improved histomorphometry and microbiology of the gut in broiler chickens at inclusion level of 12.5% rDDGS.

Keywords: Enzyme, Gut health, Histomorphometry, Rice based distillers dried grain with solubles.

Poultry production in India has taken a quantum leap in the last four decades. Feed is the major component in the production of poultry, as it constitutes 65 to 75% of total production cost. Soybean meal is the major proteinic ingredient used in poultry diet. The estimated requirement for soybean meal will be 11.9 million tons (MT) in 2025. However, net deficiency of soybean meal in the country is about 2.5 MT annually (Mandal, 2017). Due to scarcity of soybean at reasonable price, there is need to utilize locally available alternate protein ingredients. However, only a narrow range of feed ingredients are used due to lack of reliable data on their nutritive quality, feeding value and safe or effective level of inclusion.

Rice tops the list of total cereal production in the country. About 92% of total rice production is used for human food and about 8% is used for livestock and poultry feed in form of rice bran, deoiled rice bran, rice polish and broken rice. India is the second largest producers of rice in the world after China, producing approximately 109.7 MT rice in 2016-17 (Agriculture Statistics, 2018). Now days, certain newer rice by products are available in appreciable

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quantities and cheaper rate that can be utilized as protein sources from rice processing industries such as rice based distillers dried grain with solubles (DDGS). The DDGS is co-product of the ethanol industry produced during dry milling process. Its availability is increasing due to higher demand for ethanol as biofuel. Rice as subtract for DDGS is increasing due to relative lower price, increased production and easy availability. Most of the researches were limited to corn, wheat, sorghum, barley DDGS. Scanty researches were done in effect of feeding rice DDGS on gut health in poultry (Gupta, 2016; Rao *et al.,* 2016; Ranjan *et al.* 2017).In the earlier experiment by the author it was found that 12.5% rDDGS can be incorporated for economic broiler production (Dinani *et al.*, 2019). So, in this experiment, 12.5 and 15% rDDGS without or with different enzymes has been tried to study their effect on gut health.

On the other hand, enzyme supplementations in poultry diets are nutritionally, economically and environmentally justified. It leads to increased feeding value of the dietary raw materials, reduction in the variation of nutrient quality of the diet, increased nutrient digestibility and reduction in water content of the excreta, reduced viscosity of intestinal contents and accelerated rate of passage of digest a through the gastrointestinal tract. Different factors such as diet, age as well as health status of the birds influence the establishment of a particular bacterial colony in the gut (Barnes, 1979). However, limited information is available on the appropriate enzyme or the combination of enzymes that are specific for broiler chicken ration based on corn-soya diet and soybean meal partially replaced with rice DDGS.

MATERIALS AND METHODS

Experimental diets

The research work was carried out at the Division of Avian Nutrition and Feed Technology, ICAR-Central Avian Research Institute (CARI), Izatnagar, India. Broilers of CARIBRO-VISHAL (white variety) were used in the study for the period of 42 days. 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).The IAEC/CPCSEA number is 452/01/ab/CPCSEA. Corn-soya meal based basal diets to meet ICAR (2013) standard for broiler chickens were formulated as prestarter (Table 1), starter (Table 2) and finisher (Table 3). The diets along with all the used ingredients including rice based distiller's dried grains with solubles (rDDGS) were analysed by standard procedure. Whole mash type of feed was used for this study. Energy, protein, major minerals and limiting amino acids will be kept constant. *Isonitrogenous* and *isocalorific* diets were used for experiment. The three commercial enzyme preparations protease (P), xylanase (X) and multienzymes (M) were analyzed for different enzyme activities as per standard methods and used as per manufacturer's instruction. The birds were housed in specially designed battery brooder cages with watering and feeding facilities and were reared under standard management conditions.

Experimental design

The experiment was conducted as per 3×4 factorial completely randomized design (CRD). A total of 384 broiler chicks (CARIBRO vishal) of same hatch with uniform weight were used in the experiment. The birds were randomly divided into 48 replicates of eight birds each. There were twelve different treatments with 4 replicates for each treatment. So, each treatment was allocated 32 birds. The allocation of birds in each treatment was based on the similar initial body weight. Two levels of rDDGS were taken, the best inclusion level from earlier experiments as first level (12.5%) and then adding over and above the best level of 2.5 % rDDGS to this level with enzymes. Experimental layout for feeding different level of rDDGS with or without enzymes is presented in Table 4.

Intestinal histomorphology

Samples from jejunum were taken from four birds per treatment at the end of experiment (42 days). Histological slides were prepared by standard procedure. There was one sample for jejunum, two cross-sections per sample and 4 measurements per cross- section (for a total of 32 measurements for each of the jejunum segment per treatment). Four intact villi and crypts of Lieberkuhn were used to give the mean for each chicken. Finally, the mean from four chickens were expressed as the mean villus for one treatment group. Each jejunum segment fixed in 10% neutral buffered formalin embedded in paraffin

Table 1: Ingredients and nutrient composition (%) of pre starter diets with or without enzymes for different level of rDDGS

Ingredients	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Maize	54.42	54.42	54.42	54.42	55.94	55.94	55.94	55.94	56.40	56.40	56.40	56.40
SBM	38.40	38.40	38.40	38.40	25.50	25.50	25.50	25.50	22.90	22.90	22.90	22.90
DDGS	0.00	0.00	0.00	0.00	12.50	12.50	12.50	12.50	15.00	15.00	15.00	15.00
Oil	3.00	3.00	3.00	3.00	1.80	1.80	1.80	1.80	1.52	1.52	1.52	1.52
LSP	1.40	1.40	1.40	1.40	1.30	1.30	1.30	1.30	1.20	1.20	1.20	1.20
DCP	1.82	1.82	1.82	1.82	1.83	1.83	1.83	1.83	1.83	1.83	1.83	1.83
Lysine	0.00	0.00	0.00	0.00	0.23	0.23	0.23	0.23	0.27	0.27	0.27	0.27
Methionine	0.20	0.20	0.20	0.20	0.13	0.13	0.13	0.13	0.11	0.11	0.11	0.11
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
Enzyme	-	+	+	+	-	+	+	+	-	+	+	+
Total	100.01	100.01	100.01	100.01	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient composition												
CP	21.99	21.99	21.99	21.99	22.01	22.01	22.01	22.01	22.02	22.02	22.02	22.02
Lysine	1.19	1.19	1.19	1.19	1.23	1.23	1.23	1.23	1.23	1.23	1.23	1.23
Methionine	0.52	0.52	0.52	0.52	0.53	0.53	0.53	0.53	0.52	0.52	0.52	0.52
Threonine	0.83	0.83	0.83	0.83	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82
Ca	1.03	1.03	1.03	1.03	1.05	1.05	1.05	1.05	1.03	1.03	1.03	1.03
P	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
ME (kcal/kg)**	2998	2998	2998	2998	2998	2998	2998	2998	2999	2999	2999	2999
Cost (₹/ kg)	28.52	29.03	29.13	28.93	26.36	26.86	26.96	26.76	25.86	26.37	26.47	26.27

In prestarter diet *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%. Trace mineral premix supplied mg / kg diet: Mn, 55; I, 1; Fe, 75; Zn, 60; Cu, 10; Se, 0.15 and Cr, 0.2. The vitamin premix supplied per kg diet: Vit. A, 5000 IU; Vit. D_3, 2400 IU; Vit. E,15 and Vit. K, 1mg. Vitamin B complex supplied per kg diet: Vit. B_1, 5 mg; Vit. B_2, 6 mg; Vit. B_6 5 mg; Vit. B_{12}, 15 mcg; nicotinic acid, 35 mg; pantothenic acid, 12 mg; biotin 0.15 mg and folic acid 0.5 mg. Choline chloride supplied per kg diet: choline, 1300 mg. (As per ICAR, 2013) **calculated value.

Table 2: Ingredients and nutrient composition (%) of starter diets with or without enzymes for different level of rDDGS

Ingredients	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Maize	55.63	55.63	55.63	55.63	57.66	57.66	57.66	57.66	58.10	58.10	58.10	58.10
SBM	37.10	37.10	37.10	37.10	24.10	24.10	24.10	24.10	21.40	21.40	21.40	21.40
DDGS	0.00	0.00	0.00	0.00	12.50	12.50	12.50	12.50	15.00	15.00	15.00	15.00
Oil	3.50	3.50	3.50	3.50	2.15	2.15	2.15	2.15	1.90	1.90	1.90	1.90
LSP	1.35	1.35	1.35	1.35	1.20	1.20	1.20	1.20	1.17	1.17	1.17	1.17
DCP	1.55	1.55	1.55	1.55	1.58	1.58	1.58	1.58	1.58	1.58	1.58	1.58
Lysine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.05	0.05	0.05
Methionine	0.10	0.10	0.10	0.10	0.04	0.04	0.04	0.04	0.03	0.03	0.03	0.03
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
Enzyme	-	+	+	+	-	+	+	+	-	+	+	+
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
Nutrient composition												
CP	21.52	21.52	21.52	21.52	21.54	21.54	21.54	21.54	21.50	21.50	21.50	21.50
Lysine	1.38	1.38	1.38	1.38	1.11	1.11	1.11	1.11	1.10	1.10	1.10	1.10
Methionine	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.49	0.49	0.49	0.49
Threonine	0.78	0.78	0.79	0.79	0.81	0.80	0.80	0.81	0.81	0.81	0.81	0.81
Ca	0.95	0.95	0.95	0.95	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
P	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41
ME (kcal/kg)**	3050	3050	3050	3050	3052	3052	3052	3052	3053	3053	3053	3053
Cost (₹/kg)	28.03	28.53	28.63	28.43	25.34	25.85	25.95	25.75	24.92	25.42	25.52	25.32

In starter diet *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%. Trace mineral premix supplied mg / kg diet: Mn, 55; I, 1; Fe, 60; Zn, 60; Cu, 10; Se, 0.15 and Cr, 0.2. The vitamin premix supplied per kg diet: Vit. A, 5000 IU; Vit. D_3, 2400 IU; Vit. E,15 and Vit. K, 1 mg. Vitamin B complex supplied per kg diet: Vit. B_1, 4 mg; Vit. B_2, 6 mg; Vit. B_6 5 mg; Vit. B_{12}, 15 mcg; nicotinic acid, 35 mg; pantothenic acid, 10 mg; biotin 0.15 mg and folic acid 0.5 mg. Choline chloride supplied per kg diet: choline, 1200 mg. (As per ICAR, 2013) **calculated value.

Table 3: Ingredients and nutrient composition (%) of finisher diets for with or without enzymes different level of rDDGS

Ingredients	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Maize	62.00	62.00	62.00	62.00	64.18	64.18	64.18	64.18	64.38	64.38	64.38	64.38
SBM	31.30	31.30	31.30	31.30	18.20	18.20	18.20	18.20	15.70	15.70	15.70	15.70
DDGS	0.00	0.00	0.00	0.00	12.50	12.50	12.50	12.50	15.00	15.00	15.00	15.00
Oil	3.22	3.22	3.22	3.22	1.80	1.80	1.80	1.80	1.60	1.60	1.60	1.60
LSP	1.20	1.20	1.20	1.20	1.00	1.00	1.00	1.00	0.96	0.96	0.96	0.96
DCP	1.45	1.45	1.45	1.45	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Lysine	0.00	0.00	0.00	0.00	0.05	0.05	0.05	0.05	0.10	0.10	0.10	0.10
Methionine	0.06	0.06	0.06	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Enzyme	-	+	+	+	-	+	+	+	-	+	+	+
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
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
Nutrient composition												
CP	19.51	19.51	19.51	19.51	19.50	19.50	19.50	19.50	19.53	19.53	19.53	19.53
Lysine	1.20	1.20	1.20	1.20	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98
Methionine	0.41	0.41	0.41	0.41	0.42	0.42	0.42	0.42	0.43	0.43	0.43	0.43
Threonine	0.68	0.68	0.68	0.68	0.69	0.69	0.69	0.69	0.70	0.70	0.70	0.70
Ca	0.86	0.86	0.86	0.86	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
P	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
ME (kcal/kg)**	3100	3100	3100	3100	3099	3099	3099	3099	3101	3101	3101	3101
Cost (₹/kg)	26.71	27.22	27.32	27.12	24.08	24.58	24.68	24.48	23.78	24.28	24.38	24.18

In finisher diet *Constant 0.77 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%. Trace mineral premix supplied mg / kg diet: Mn, 50; I, 1; Fe, 50; Zn, 60; Cu, 8; Se, 0.15 and Cr, 0.2. The vitamin premix supplied per kg diet: Vit. A, 5000 IU; Vit. D_3, 2400 IU; Vit. E, 15 and Vit. K, 0.8 mg. Vitamin B complex supplied per kg diet: Vit. B_1, 4 mg; Vit. B_2, 6 mg; Vit. B_6 5 mg; Vit. B_{12}, 15 mcg; nicotinic acid, 30 mg; pantothenic acid, 10 mg; biotin 0.15 mg and folic acid 0.5 mg. Choline chloride supplied per kg diet: choline, 900 mg. (As per ICAR, 2013) **calculated value.

and sections of 5-micron thickness of each sample were placed on a glass slide and stained with hematoxyline and eosine for examination (Culling, 1974).

All the light microscopic variables were measured for jejunum of each bird using optical microscope (Motic Inverted microscope, Honkong), at a 10 × magnification, a camera (Motic cam, CMOS, Honkong), and image analysis software (Motic Image 2.0, Honkong). The morphometric indices in each segment evaluation were villus height (height from the tip of the villus to the crypt), crypt depth (crypt depth from the base of the villi to the submucosa), villus width and the villus height to crypt depth ratio.

Microbiological parameters

At the end of trail (42 days), four 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 (1953). Total bacterial count and *Lactobacilli* were grown on nutrient agar and Rogosa SL agar respectively (Deman *et al.,* 1960). One gram crop and jejunum sample were weighed and dissolved in 9 ml sterile normal saline solution (NSS). Diluted 0.5 ml from 10^4 to 10^6 dilutions was taken in sterile Petri dish and 15-20 ml of sterile agar media were poured in each Petri dish. It was mixed gently and allowed to stand until the media solidify. Then plates were incubated in BOD incubator at 37°C for 24 h and *Lactobacillus* sp. was incubated anaerobically at 37°C. Plates were counted between 24 and 48 h after inoculation. Colony forming units (cfu) were defined as distinct colonies measuring at least 1 mm in diameter and expressed in log 10 cfu/g. It was calculated by the formula cfu / g = Total No. of colony counted × Dilution factor / Volume of aliquot taken.

Statistical analysis

Data subjected to test of significance as per completely randomized design (CRD) were analyzed for mean, standard errors and analysis of variance by Snedecor and Cochran (1989) using statistical package for social sciences (SPSS) 16.0 version and comparison of means were done using Tukey's test (1949).

RESULTS AND DISCUSSION

Intestinal histomorphology

Effects of feeding different levels of rDDGS with or without enzymes on intestinal histomorphometry (in µm) are presented in Table 5 and Fig. 1. Feeding different levels of rDDGS (0, 12.5 and 15%) on crypt depth (CD)

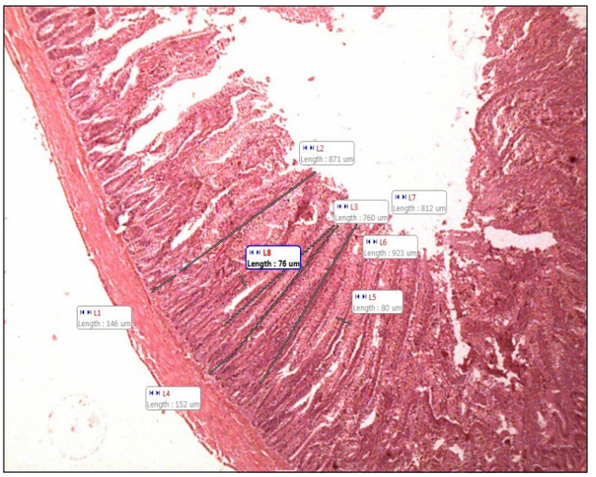
Table 4: Experimental layout for feeding different level of rDDGS with or without enzymes

Experimental design			3×4 factorial CRD		
Treatment	**rDDGS (%)**	**No. of replicates**	**Birds/ replication**	**Total**	**Enzymes**
T1	0.0	4	8	32	—
T2	0.0	4	8	32	Xylanase
T3	0.0	4	8	32	Protease
T4	0.0	4	8	32	Multienzymes
T5	12.5	4	8	32	—
T6	12.5	4	8	32	Xylanase
T7	12.5	4	8	32	Protease
T8	12.5	4	8	32	Multienzymes
T9	15	4	8	32	—
T10	15	4	8	32	Xylanase
T11	15	4	8	32	Protease
T12	15	4	8	32	Multienzymes

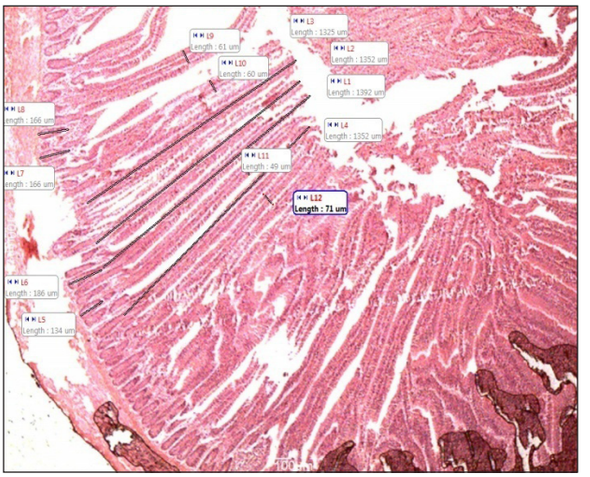
Table 5: Effect of feeding different level of rDDGS with or without enzymes on intestinal histomorphometry (in µm)

Treatment	rDDGS%	Enzyme	Villus height (VH)	Crypt depth (CD)	VH:CD	Villus width
T1	0	—	1204	159	7.62^{ab}	88
T2	0	X	1285	143	9.02^{cd}	97
T3	0	P	1320	159	8.41^{bc}	91
T4	0	M	1203	144	8.40^{bc}	97
T5	12.5	—	1212	147	8.35^{bc}	99
T6	12.5	X	1347	162	8.35^{bc}	98
T7	12.5	P	1392	145	9.72^{d}	98
T8	12.5	M	1238	163	7.65^{ab}	87
T9	15	—	1025	158	6.52^{a}	92
T10	15	X	1252	149	8.37^{bc}	97
T11	15	P	1144	150	7.62^{ab}	99
T12	15	M	1151	146	7.92^{bc}	100
		Pooled SEM	16.57	2.00	0.14	1.31
		rDDGS				
		0	1253^{b}	151	8.36^{b}	93
		12.5	1297^{b}	154	8.51^{b}	95
		15	1142^{a}	151	7.61^{a}	97
		Enzyme				
		—	1146^{a}	154	7.50^{a}	93
		X	1294^{b}	151	8.58^{b}	97
		P	1285^{b}	151	8.58^{b}	96
		M	1197^{a}	151	7.99^{ab}	94
		Significance				
		rDDGS	P<0.01	NS	P<0.01	NS
		Enzyme	P<0.01	NS	P<0.01	NS
		Interaction	NS	NS	P<0.05	NS

Values bearing different superscripts within the column differ significantly *(P<0.01), **(P<0.05) and NS: Non-significant (P>0.05).



T1



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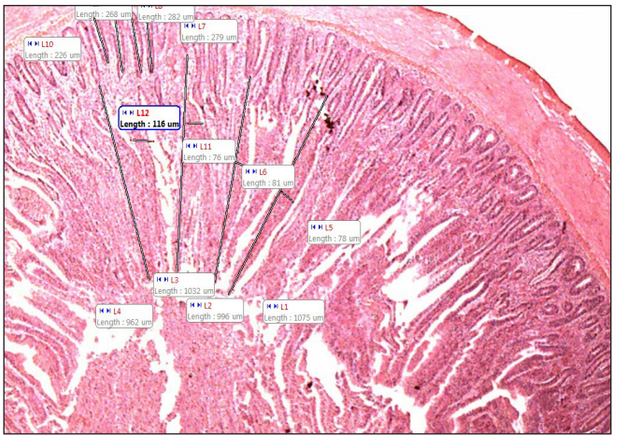

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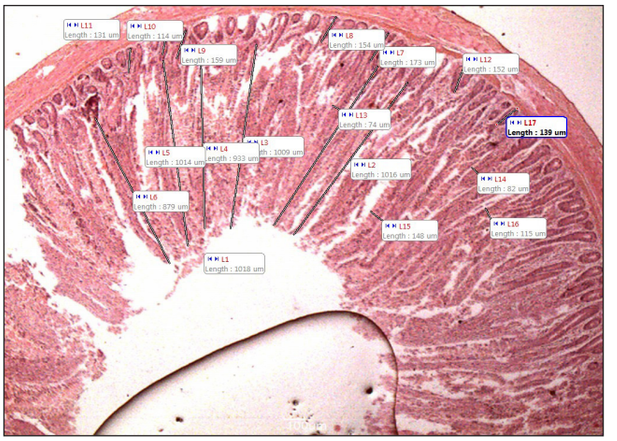

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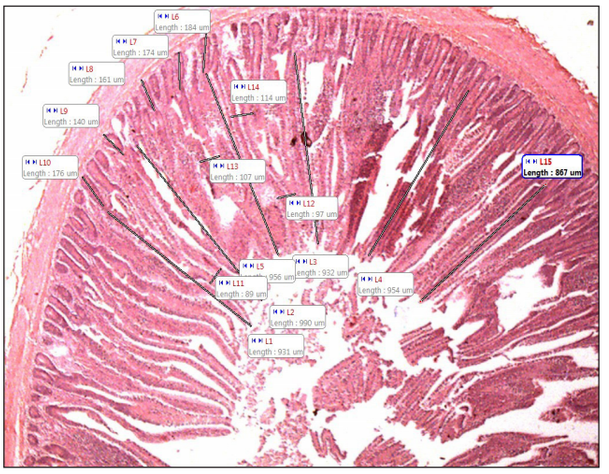
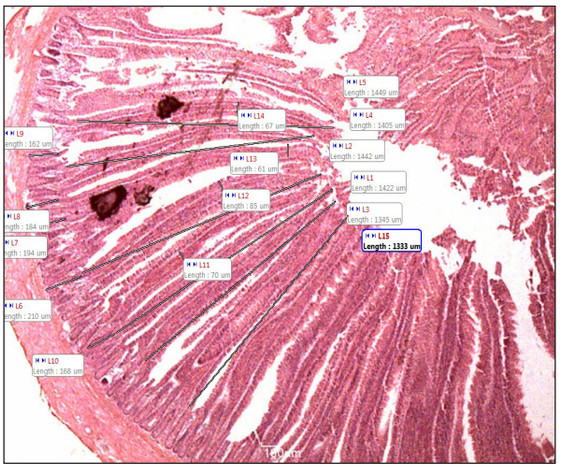


T5



T6

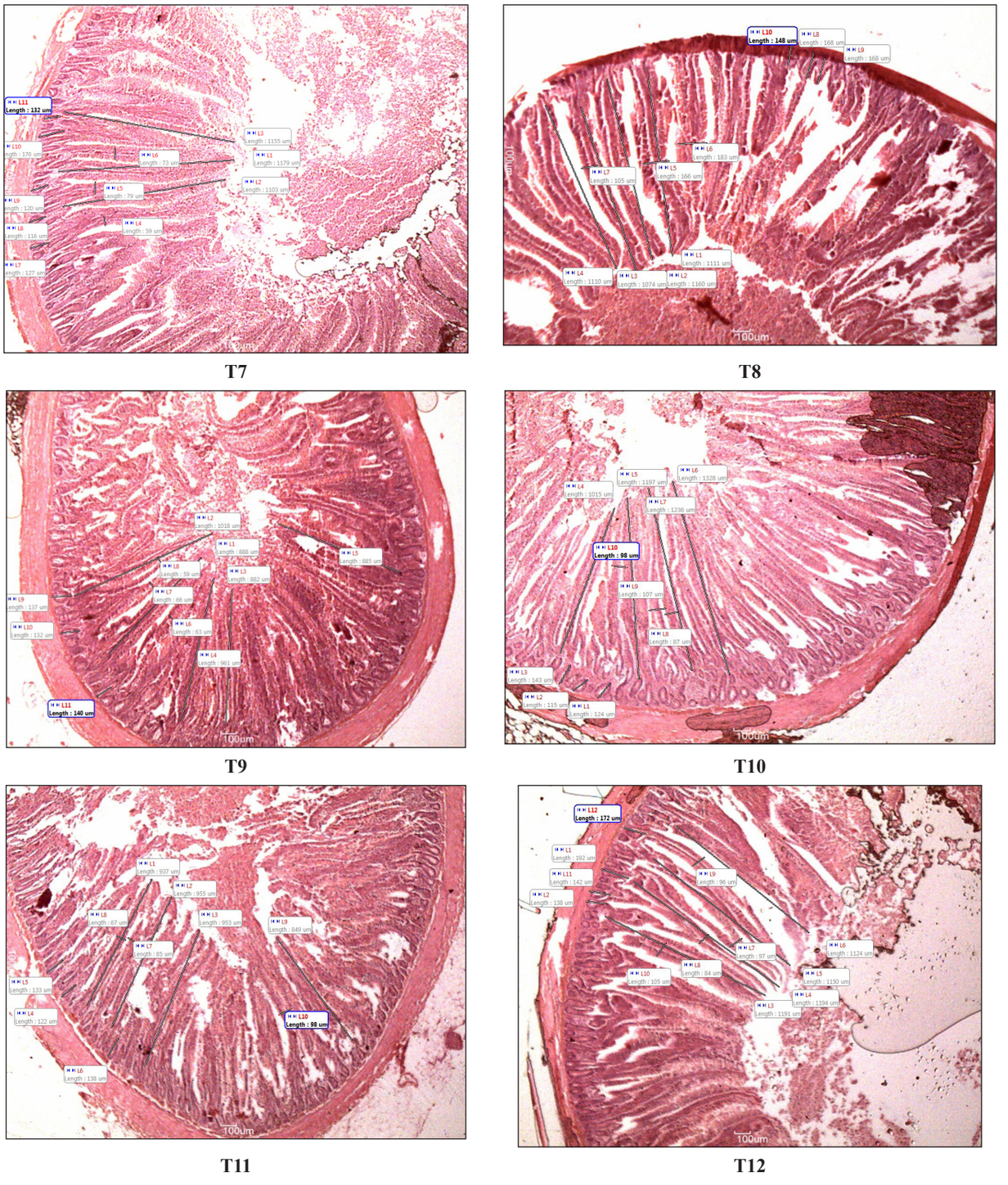


Fig. 1: Effect of feeding different level of rDDGS with or without enzymes on intestinal histomorphometry (in µm)

Table 6: Effect of feeding different level of rDDGS with or without enzymes on microbiological parameters (log 10 cfu/g)

			Crop		Jejunum	
Treatment	**rDDGS%**	**Enzyme**	**TVC**	***Lactobacillus* count**	**TVC**	***Lactobacillus* count**
T1	0	—	6.80	3.37	6.74	3.52
T2	0	X	6.44	3.47	6.50	3.56
T3	0	P	6.63	3.21	6.54	3.75
T4	0	M	6.56	3.36	6.63	3.68
T5	12.5	—	6.20	3.77	6.33	3.82
T6	12.5	X	6.23	4.01	6.31	4.25
T7	12.5	P	6.18	4.00	6.47	4.21
T8	12.5	M	6.38	3.99	6.38	4.22
T9	15	—	6.24	3.86	6.08	4.01
T10	15	X	5.96	3.98	5.96	4.04
T11	15	P	6.07	4.00	6.35	4.16
T12	15	M	6.20	3.84	6.20	4.11
		Pooled SEM	0.04	0.05	0.04	0.04
		rDDGS				
		0	6.60^{c}	3.35^{a}	6.60^{c}	3.62^{a}
		12.5	6.24^{b}	3.94^{b}	6.37^{b}	4.12^{b}
		15	6.11^{a}	3.92^{b}	6.14^{a}	4.07^{b}
		Enzyme				
		—	6.42^{c}	3.67	6.38^{ab}	3.78^{a}
		X	6.19^{a}	3.82	6.25^{a}	3.95^{b}
		P	6.29^{ab}	3.74	6.45^{b}	4.04^{b}
		M	6.38^{c}	3.73	6.40^{b}	4.00^{b}
		Significance				
		rDDGS	P<0.01	P<0.01	P<0.01	P<0.01
		Enzyme	P<0.01	NS	P<0.05	P<0.01
		Interaction	NS	NS	NS	NS

Values bearing different superscripts within the column differ significantly *(P<0.01), ** (P<0.05) and NS: Non-significant (P>0.05).

and villus width (VW) did not exhibit any significant (P>0.05) difference between different dietary treatments and control. Ratio of villus height (VH) with crypt depth (CD) and villus height were significantly (P<0.01) lower in 15% rDDGS level as compared to 0 and 12.5% levels.

Effect of feeding rDDGS without or with enzymes (xylanase, protease and multienzymes) on intestinal histomorphometry did not exhibit any significant (P>0.05) difference on crypt depth and villus width. Xylanase and protease enzyme supplementation significantly (P<0.01) increased villus height compared to multienzymes and without enzyme groups. Xylanase and protease enzyme supplementation also significantly (P<0.01) increased VH: CD ratio as compared to without enzyme groups, but VH: CD ratio did not show any significant (P>0.05) difference as compared to multienzymes supplementation.

Interaction of rDDGS and enzymes did not show any significant (P>0.05) difference in villus height, crypt depth and villus width as compared to control and other dietary treatments except on VH: CD ratio. Ratio of VH and CD significantly (P<0.05) increased in 0% rDDGS with xylanase and 12.5% rDDGS with protease 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 are very scanty in literature. However, our results are in agreement with Loar *et al.* (2010) and Gupta *et al.* (2015), but disagreement with Ranjan *et al.* (2017). Loar *et al.* (2010) reported no adverse effect on intestinal parameters by incorporating DDGS in post hatch 28 days broilers. Gupta (2016) reported no negative effects on intestinal morphology up to 10% inclusion of rDDGS and protease supplementation was beneficial for villus height, width, crypt depth and VH: CD ratio. Contrary to this, Ranjan *et al.* (2017) reported that no significant (P>0.05) difference in villus length and crypt depth in duodenum, jejunum and ileum in duck fed various levels of rice DDGS up to 75% replacement of soybean meal. Decrease in villus length in

15% rDDGS may be associated with poor digestibility at this inclusion level.

Microbiological parameters

The results pertaining to influence of different levels of rDDGS with or without enzymes on total viable count (TVC) and *Lactobacillus* count (log 10 cfu/g) in crop and jejunum are presented in Table 6.

Feeding different levels of rDDGS (0, 12.5 and 15%) on crop and jejunum significantly (P<0.01) decreased TVC at 12.5 and 15% rDDGS levels, but 15% rDDGS level significantly (P<0.01) decreased TVC as compared to 0 and 12.5% rDDGS levels. *Lactobacillus* count on crop and jejunum both significantly (P<0.01) increased at 12.5 and 15% rDDGS levels, but 15% rDDGS level significantly (P<0.01) increased *Lactobacillus* count as compared 12.5% rDDGS level.

Enzymes supplementation (xylanase, protease and multienzymes) did not show any significant (P>0.05) difference in *Lactobacillus* count in crop. Xylanase supplementation significantly (P<0.01) decreased TVC as compared to without or with enzymes (protease and multienzymes) in crop. Xylanase supplementation also significantly (P<0.01) decreased TVC in jejunum as compared to protease and multienzymes supplementation, but it did not show any significant (P>0.05) difference from without enzyme groups. *Lactobacillus* count significantly (P<0.01) increased in jejunum in all enzymes supplemented groups (X, P and M) as compared to without enzyme groups. Interaction of rDDGS and enzymes did not show any significant (P>0.05) difference in microbial population in crop and jejunum.

Our results are coinciding with Hahn (2010), Yang *et al.* (2010) and Gupta *et al.* (2015). Hahn (2010) observed that the diets containing corn DDGS had significantly (P<0.05) higher count of *Lactobacillus* as compared to control diet. Yang *et al.* (2010) reported that diets containing corn DDGS had greater lactic acid containing bacteria than diets containing wheat DDGS. Yan *et al*. (2013) also suggested that addition of NSP degrading enzymes improved gut health in broilers. Gupta *et al.* (2015) 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 which it is more fibrous and offer more nutrients to *Lactobacillus.*

CONCLUSION

Thus, it may be concluded that enzymes supplementation improved histomorphometry and microbiology of the gut by feeding rDDGS in broiler chickens at inclusion level of 12.5% .

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