EVALUATION OF RICE BASED DRY DISTILLERS GRAINS WITH SOLUBLE AND ITS IMPACT ON EGG CHOLESTEROL, SENSORY ATTRIBUTES, SERUM BIOCHEMICAL AND HEMATOLOGICAL PROFILE IN LAYING HENS

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ABSTRACT

A biological experiment was undertaken with (4x2) factorial CRD in 26 wk old CARI Sonali layers (n=120) assigned to eight treatments (i.e. 2 control + 6 test diets) having 15 replicates with one layer in each replicate for ten weeks (26th-35th week of age). Eight experimental diets (D1 to D8) were prepared by incorporating rice dry distiller grain with soluble (DDGS) without and with enzyme at 0, 5, 7.5 and 10% level. Weighed amount of respective diet was offered to individual birds daily. All diets had been kept isocaloric and isonitrogenous in nature. Findings through experiment I revealed that rice DDGS employed in this study contained CP 45.0% which is almost at par of fish meal protein and slightly lower to SBM. However, the gross energy is higher than FM and SBM but lower than maize. *In vitro* pepsin-pancreatin digestibility and available carbohydrate contents (ACHO) of rice DDGS was found 78.05 and 23.25% respectively. Phytate phosphorus content in rice DDGS was 0.44%. However non-phytate phosphorus was 0.33% which is 57.14% of total phosphorus. Different levels of rice DDGS either sole or in combination with enzyme did not have any significant (P>0.05) effect on egg cholesterol, sensory attributes except appearance and colour of boiled egg, however significantly (P<0.01) increased the haematological, serum protein profile and have lowering effect on serum lipid profile, which was found better effect after dietary addition of enzyme during II experiment.

Key words: Egg cholesterol, pepsin-pancreatin digestibility, rice DDGS, sensory attributes, serum protein profile

Introduction

With growing demand for food and scarce resources, the poultry industry doesn't have the luxury of letting anything go to waste. Dry distiller grains with solubles (DDGS) from the ethanol industry are considered to be waste, but can still have a use as a feed ingredient because of very cheap source of crude protein, available phosphorus, unsaturated fatty acids and essential amino acids. DDGS is a low cost alternative energy and protein source (Adam, 2008). High variability among DDGS is due to the several factors sources such as differences in the protein content of the grains grown in various geographical locations that are used to produce DDGS, percentage of grain vs. soluble during the production process as well as the differences in esidual starch content caused by differences in fermentation efficiency and processing techniques (Belyea *et al*., 2004; Martinez-Amezcua *et a*l., 2007; Babcock *et al.,* 2008).

Rice DDGS is a byproduct of distilleries in which rice is used in ethanol industry as a raw material to produce ethanol. A large quantity of different grains is spoiled (which are unfit for human consumption) every year in India because of unfavourable climatic conditions and inadequate transport and storage facilities. The damage includes discoloured, broken, cracked, attacked by fungi, insect damaged, chalky, partially softened by being damp, dirty and bad smell, etc. damaged but sound grains has been selected for ethanol production because they are cheaper than fresh grains and are available in large quantities. Rice DDGS is an alternative feed ingredient that continues to be produced in large quantities by the dry grind fuel ethanol industry. Rice DDGS also have yeast enzyme (probiotic factor) which increases the level of production. It helps to enrich the egg yolk and the color of the skin i.e., xanthophylls. Because of its high energy and protein content rice DDGS becomes an attractive substitute of expensive source of energy (corn) and protein (soybean meal) ingredients of poultry feed. There has been a significant amount of recent research conducted on the use of high quality DDGS in layer diets confirming that it is an excellent partial replacement for corn, soybean meal and inorganic phosphate and supports excellent layer performance and egg quality.

Materials and Methods

Experimental birds, housing and design

A biological experiment was undertaken in a 4x2 factorial completely randomized design (CRD) with twenty six weeks old CARI Sonali layer birds (RIR M x WLH F) (n =120) which were randomly assigned into eight groups (D1 to D8) in such a way that each treatment have 15 birds per treatment. All the layer birds were reared in battery cages fitted with individual feeder, waterers and dropping trays and reared under standard management conditions. The allocation of birds in each treatment was based on egg production at the start of the experiment. The experiment was conducted for 10 weeks (26th to 35th weeks of age).

Experimental diet

Feed ingredients and supplements for formulation of control and test diets were procured from the feed storage and processing unit of the institute. All the feed ingredients were analyzed for proximate constituents, phosphorus (AOAC, 2000) and calcium (Talpatra *et al.*, 1940) contents prior to formulation of experimental diets. Gross energy of rice DDGS was evaluated by using an adiabatic bomb calorimeter. From the basal diet as maintained above, eight experimental diets (D1= 0.0% DDGS without protease, D2= 0.0% DDGS with protease, D3= 5.0% DDGS without protease, D4= 5.0% DDGS with

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protease, D5= 7.5% DDGS without protease, D6= 7.5% DDGS with protease, D7= 10.0% DDGS without protease, D8= 10.0% DDGS with protease) was formulated. Every attempt was made to minimize feed spillage/wastage.

In vitro pepsin pancreatin digestibility (IVPPD)

In vitro pepsin-pancreatin digestibility of DDGS sample was measured according to the method of Gopalkrishnan and Prakash (2000). For the determination of IVPPD, 1 g of finely ground DDGS was heated over a water bath with 5 ml of water for 10 min., suspended in 15 ml of 0.1 N HCl, containing 1.5 mg pepsin in 100 ml conical flask. The mixture was incubated at 37°C for 3 h. The suspension was then neutralized with 0.5 N NaOH and treated with 4 mg pancreatin in 7.5 ml of 0.2 M phosphate buffer (pH 8.0), containing 0.005 M sodium azide (NaN_3). The mixture was incubated for different time intervals (30-50 min.). Ten millilitres of 10% tri-chloroacetic acid (TCA) was added to the mixture to stop the reaction. The mixture was then centrifuged at 3000 rpm for 20 min. at 27°C. Nitrogen in the supernatant was estimated by micro-Kjeldahl method. A blank was also prepared in the same manner without the sample and the value was subtracted from total digestibility of each sample (Akeson and Stahmann, 1964).

Available carbohydrates

The available carbohydrate contents (ACHO) of DDGS sample was determined by the method of Clegg (1956) by using anthrone reagent.

Non-phytate (available) phosphorus

For the determination of available phosphorus, the total phosphorus content in the DDGS sample was analyzed following standard techniques (AOAC, 1990). The phytate phosphorus content was analyzed as per Haugh and Lantzsch (1983).

Egg yolk cholesterol and sensory evaluation

Ten eggs per dietary treatment were randomly collected at monthly basis for analyzing the total egg cholesterol content (Wybenga and Pileggi, 1970) and at the end of experiment the organoleptic evaluation of eggs (Larmond and Elizabeth, 1977) was also carried out during ten weeks of experiment. For each of the sensory parameters (appearance, texture, flavour, juiciness and tenderness) of hard-boiled eggs, panelists were asked to rate the difference between each sample and the control using a 7-point intensity scale where 1= extremely poor, 2 = very poor, 3 = poor, 4 = fair, 5 = good, 6 = very good and 7 = excellent.

Serum biochemical and haematological profile

At the end of the feeding experiment blood samples from ten birds per dietary treatment were randomly collected into sterile glass test tubes with anticoagulant EDTA for haematology and without addition of any anticoagulant to facilitate separation of serum. Serum was separated by centrifugation at 3000 rpm for 10 minutes and serum was decanted into ependorff tubes and then stored at -20°C for estimation of biochemical parameters. Estimation of haemoglobin was done spectrophotometrically at 540 nm wavelength by cyanmet-hemoglobin (Drabkin's) method using of Drabkin's solution (Fudge, 2000). PCV was estimated using micro haematocrit method as described by Sharma and Singh (2000). Estimation of glucose was done by glucose oxidase-peroxidase (GOD-POD) end point assay (Kaplan and Pesce, 1984). The total cholesterol, HDL (high density lipoprotein) concentration in serum according to Wybenga and Pileggi (1970) and serum triglyceride (Fossati and Lorenzo, 1982), low density lipo-protein (LDL) concentration (Friedewald *et al.,* 1972) were also estimated spectrophotometrically using Span Diagnostic Kit. For Serum protein profile, total protein concentration in serum was estimated by modified biuret end point assay (Doumas *et al.*, 1981) and albumin concentration by bromocresol green end point assay method (Johnson *et al*., 1999). Globulin and albumin-globulin ratio was calculated with the help of albumin and total protein.

Results and Discussion

Experiment 1

In this experiment, the rice dry distiller grain with soluble (DDGS) was evaluated for chemical composition, gross energy and available nutrients in term of *in vitro* pepsin pancreatin digestibility, available carbohydrates and available phosphorus. The results are as follows:

Composition of rice DDGS and feed ingredients used in feeding trial

The chemical composition (% DM) of various feedstuffs viz., maize, soybean meal, fishmeal and rice based dry distiller grain with soluble (DDGS) employed in this study are presented in Table 1. The composition of DDGS is highly variable, depending on many factors such as the age of the manufacturing plant, the base grain used, the distillation process, and the preparation of the final product, especially drying and packaging (Cozannet *et al.,* 2009; Meyer *et al*., 2010; Cozannet *et al.,* 2010). However, researchers reported higher crude fat, crude fibre, ADF, NDF and lower CP content as compared to our values (Manh *et al.,* 2000; Xue, 2012; Patil *et al.* 2015). Belyea *et al.* (2004) postulated possible contribution of proportional rate of components to variation in protein content of DDGS and Kingsly *et al.* (2010) observed variable nutritive value of DDGS when different ratio of wet distiller grains and solubles were blended together. Additionally, yeast protein and its amino acid composition may also have an effect on protein content and composition of DDGS since yeast protein constitutes approximately 50 % of the protein in DDGS (Belyea *et al.* 2004). Rice DDGS employed in present experiment contained 4.28% AIA, which is almost similar to the report of Patil *et al.* (2015). The total phosphorus present in rice based DDGS is very much variable. Xue (2012) and Patil *et al. (*2015) reported 0.42% total phosphorus present in rice based DDGS. The rate of addition of solubles to the wet grains prior to drying could also affect the P content, as the solubles have more than three times as much P as do the wet grains (Martinez-Amezcua *et al*., 2007; Noll *et al.,* 2001). There is so much variability in Ca content in DDGS likewise Patil *et al.* (2015) found 0.55 and 0.09%, respectively. Ca content in rice based DDGS against to our finding (0.73% Ca). It is expected that the rate of all the nutrients that have not been utilized in the fermentation process will increase 3 fold, because about 2/3 of the weight of grain is converted into ethanol and CO_2 during fermentation process (Batal and Dale, 2003). Rice DDGS was also analyzed for gross energy, which was found to be 4097 kcal/kg. In contrary to our result, Xeu (2012) reported 4513 kcal/kg gross energy value of rice DDGS, which is very high than our value.

Rice dry distiller grain with soluble employed in this study contained CP 45.0% which is almost at par of fish meal protein and slightly lower to SBM. However, the gross energy is higher than FM and SBM but lower than maize. These variability in chemical composition and nutritive value of rice DDGS is due to quality of grains, technology used in their manufacture, efficiency in the ethanol manufacture process, mixing ratio of the final components obtained and drying time and temperature.

In vitro pepsin-pancreatic digestibility (IVPPD)

In present experiment *in vitro* pepsin-pancreatin digestibility of DDGS sample was calculated and it was found 78.05% (Table 1). No study has been conducted to evaluate the *in vitro* pepsin-pancreatin digestibility of rice DDGS. In general, the concentrations of crude protein and amino acids in DDGS are close to three times that of grains. Various processing treatments are known to improve the digestibility and nutritive value of grains (Alka-Sharma and Kapoor, 1996). Fermentation (Chavan *et al*., 1988; Usha *et al*., 1996) has been also reported to increase the protein digestibility of millet. El Hag *et al*. (2002) found that fermentation of millet seeds improved its *in vitro* protein digestibility.

Available carbohydrates (ACHO)

Carbohydrates are quantitatively the most important constituents, forming about 80% of the total dry matter of cereals. The carbohydrates present in cereal grains include more than 90% of starch. The remaining portion is cellulose, hemicellulose, pentosans, dextrins and sugars. The available carbohydrate contents (ACHO) of rice DDGS sample was determined and it was found 23.25% (Table 1). In our knowledge, there are no published data to estimate the *in vitro* available carbohydrates in rice based DDGS.

Available phosphorus

About 85 per cent of the total phosphorus found in feedstuffs of vegetable origin, particularly the cereals, cereal by-products and oil cakes is present in form of phytic acid (inositol 1, 2, 3, 4, 5, 6-hexa-phosphate). Phytate reduces the bioavailability of minerals, and the solubility, functionality and digestibility of proteins and carbohydrates. Phytic acid has strong chelating potential and forms a variety of complexes with cations and proteins, rendering these nutrients biologically unavailable. When phytic acid is hydrolysed by microbial phytase, all minerals bound to it are released. Findings of the present experiment for determination of available phosphorus, the total phosphorus content in the rice DDGS sample analyzed by following standard techniques (AOAC, 1990) was found 0.77%. The phytate phosphorus content was observed 0.44%. However non-phytate phosphorus was 0.33% which is 57.14% of total phosphorus (Table 1). In agreement with our results Manwar and Mandal, (2008) reported that phytate was decreased significantly due to fermentation of cereal grains. Therefore, fermentation would be an effective process for increasing availability of P.

Experiment II

The rice dry distiller grain with soluble (DDGS) was evaluated for egg cholesterol, egg sensory attributes, serum-biochemical and hematological profile under this experiment. The results are as follows:

Egg cholesterol

Cholesterol content in yolk is relatively resistant to change, and only slightly differ by cholesterol levels in the feed (Fenton and Sim, 1991). Increased cholesterol content in yolk was observed when high cholesterol diets were fed (Harris and Wilcox, 1963; Weiss *et al.,* 1967), especially when total fat in a diet was high. Several studies found that feeding polyunsaturated fatty acids would increase yolk cholesterol level (Summers *et al.,* 1966; Weiss *et al.,* 1964). Furthermore, dietary fiber has been found to have cholesterol-lowering effect (Kirby *et al.,* 1981), and soluble fiber is the main component to have this effect (Glassman *et al.,* 1990; Williams *et al*., 1991). Bruckert and Rosenbaum (2011) found that increased fiber intake can significantly lower cholesterol concentration of serum. However, high fiber content in DDGS diets did not show any cholesterol lowering effect, which would be due to the low amount of soluble fiber present in the DDGS diets. Because multiple factors would influence the cholesterol content in egg yolk, the effect of DDGS diet on cholesterol content would be compromised, and did not show a significant difference. Statistical analysis of data in present experiment revealed that different levels of rice DDGS and enzyme either sole or in combination with each other did not have any significant ($P>0.05$) effect on egg cholesterol. However, consistent decreased values of egg cholesterol were observed at higher levels of DDGS in combination with enzyme (Table 2). In agreement to our results, Sun *et al.* (2013) found that cholesterol level in egg yolk from hens fed with high level of corn DDGS diet was continuously higher.

Egg sensory attributes

The organoleptic evaluation i.e. appearance, texture, flavour, the distinctive aroma and test of the eggs, juiciness, tenderness and overall acceptability of hard boiling eggs were carried out from both control and test groups. Findings of present experiment shows that the appearance score of egg was significantly ($P<0.05$) highest at 10% inclusion level of DDGS (Table 2.) and other levels were comparable. Dietary addition of enzyme was significantly ($P<0.01$) beneficial in enhancing the appearance score of eggs. No significant effect of interaction was observed on appearance of egg. The flavour, texture and juiciness score of chicken egg under various levels of DDGS either singly or in combination with enzyme remained statistically similar ($P>0.05$) for all the samples. The overall acceptability score showed more or less same pattern. Limited work has so far been carried out on the effect of dietary inclusion of rice DDGS with and without enzyme on sensory attributes of egg. In this regard, Swiatkiewicz and Korelski (2006) observed the effect of inclusion of corn DDGS up to 20% in diet on sensory attributes of egg. There was no significant difference in properties of boiled eggs in 1st (26th to 35th weeks of age) phase of production.

Table 1: Chemical composition (% DM basis) and gross energy (kcal/kg) of dietary ingredients and rice DDGS

Moisture	DM	CP	EE	CF	TA	NFE	AIA	Ca	P	GE (Kcal/kg)
10	90	9	3.2	2.1	1.3	74.4	0.43	0.12	0.21	4608
10.15	89.85	48.7	1.8	6.9	8.1	24.35	3.12	0.33	0.67	3302
10.82	89.18	45.8	6.5	1.4	22.4	13.08	4.85	3.92	1.87	2728
8.28	91.72	45	4.49	4.89	10.22	27.12	4.28	0.73	0.77	4097
Available nutrients in dry distiller grain with solubles (DDGS) (%)										
IVPPD	ACHOS	Total P	Phytate P	NPP	NPP (%, TP)					
78.05	23.25	0.77	0.44	0.33	57.14					

TP- Total phosphorus, NPP- Non phytate Phosphorus

Table 2: Effect of feeding rice DDGS with and without enzyme supplementation on egg sensory attributes and egg cholesterol (mg/g yolk)

Diet	DDGS (%)	Enz.	Appearance & Colour	Flavour	Texture	Juiciness	Acceptability	Egg Cholesterol	
								4th week of trail	8th week of trail
D1	0	-	6.63	6.67	6.58	6.58	6.96	16.65	16.96
D2	0	+	7.38	6.83	6.67	6.67	7.17	16.58	16.43
D3	5	-	6.67	6.67	6.50	6.58	6.96	16.66	16.38
D4	5	+	7.46	6.92	6.67	6.67	7.08	16.56	16.25
D5	7.5	-	6.83	6.75	6.58	6.42	6.92	16.50	16.31
D6	7.5	+	7.54	6.92	6.67	6.58	7.21	16.39	16.22
D7	10	-	6.96	6.92	6.58	6.50	7.17	16.44	16.00
D8	10	+	7.88	7.08	6.83	6.75	7.21	16.39	15.90
DDGS (%)									
0			7.00^{a}	6.75	6.63	6.63	7.06	16.62	16.69
5			7.06^{a}	6.79	6.58	6.63	7.02	16.61	16.31
7.5			7.19^{ab}	6.83	6.63	6.50	7.06	16.45	16.27
10			7.42^{b}	7.00	6.71	6.63	7.19	16.42	15.95
Enzyme									
-			6.77^{a}	6.75	6.56	6.52	7.00	16.56	16.41
+			7.56^{b}	6.94	6.71	6.67	7.17	16.48	16.20
Pooled SEM			0.070	0.056	0.052	0.053	0.064	0.195	0.322
Probability									
DDGS			P<0.05	NS	NS	NS	NS	NS	NS
Enzyme			P<0.01	NS	NS	NS	NS	NS	NS
Interaction			NS	NS	NS	NS	NS	NS	NS

NS- Non-significant (P>0.05)

Serum biochemical and haematological profile

Serum-biochemical and haematological profile are related to the health status and vital indicators of physiological and nutritional status of birds. The results pertaining to influence of different levels of rice DDGS with and without enzyme on serum-biochemical profile revealed that different levels of rice DDGS had significant higher effect on albumin, total protein (P<0.01), A/G ratio (P<0.05) and significantly (P<0.01) lowering effect on serum lipid profile (Total cholesterol, triglycerides, LDL and VLDL) (Table 3). Enzyme addition in layer diet has been significantly (P<0.01) beneficial lowering in terms of total cholesterol, triglycerides, LDL and VLDL. Interaction effect of different levels of DDGS with and without enzyme in layer diet had significant (P<0.01) influence only on triglycerides and VLDL cholesterol. Similarly, Abd El-Hack (2015) noticed that replacing soybean meal by DDGS insignificantly increased serum total protein, albumin and globulin; with increased DDGS replacement of soybean meal with DDGS depressed total protein and globulin values. Whereas, Bor-Ling *et al*. (2011) postulated that DDGS levels (0, 6, 12 and 18%) did not influence plasma total protein of laying hens diet while plasma cholesterol was significantly (P<0.01) increased when 12 or 18% DDGS diets were used. The same authors added that serum triglycerides were not influenced by DDGS levels. Ghazalah *et al*. (2011) stated that DDGS incorporation level at 75% instead of soybean meal significantly depressed total protein compared to the control group. Moreover, Abd El-Hack (2015) indicated that increasing DDGS level significantly (P<0.01) increased serum triglycerides, cholesterol and LDL for hens fed diet contained DDGS. Pertaining to haematology there was a significant (P<0.01) improvement in PCV and Hb values at different levels of DDGS inclusion in hens diet and enzyme addition enhances these values (Table 3). Dietary addition of enzyme either singly or in combination with different levels of DDGS (interaction effect) did not have any significant (P<0.05) effect on PCV and Hb. Contrary to present findings, Ghazalah *et al*. (2011) reported that DDGS at higher level of substitution for SBM significantly increased Hb % with enzyme in layers, while, in broiler, DDGS up to 15% insignificantly affected the haematological parameter (Youssef *et al*., 2013).

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Table 3: Effect of feeding rice DDGS with and without enzyme supplementation on serum-biochemical and haematological profile

Diet	DDGS (%)	Enz.	Albumin (g/dl)	Total Protein (g/dl)	Globulin (g/dl)	A:G Ratio	Glucose (mg/dl)	Serum Cholesterol (mg/dl)	Serum Triglyceride (mg/dl)	Serum HDL (mg/dl)	Serum LDL (mg/dl)	Serum VLDL (mg/dl)	Hematology	
													PCV	PCV
D_1	0	-	1.73	4.86	3.13	0.56	184.74	186.36	575.93 ^{d}	39.08	43.75	115.19^{d}	23.10	8.08
D_2	0	+	1.81	4.87	3.06	0.60	183.39	178.69	532.24^{bc}	41.71	41.07	106.45^{bc}	23.10	8.05
D_3	5	-	1.86	5.10	3.24	0.58	183.05	184.11	572.07^{d}	39.36	43.01	114.41^{d}	24.00	8.66
D_4	5	+	1.87	5.14	3.27	0.59	183.04	177.57	544.43^{c}	42.35	40.04	108.89^{c}	24.10	8.82
D_5	7.5	-	1.89	5.11	3.22	0.59	183.01	174.21	526.00 ^{bc}	42.70	35.60	105.20^{bc}	24.20	8.71
D_6	7.5	+	1.88	5.17	3.28	0.59	180.68	170.65	512.93^{b}	41.07	31.30	102.59^{b}	24.10	8.84
D_7	10	-	2.08	5.23	3.15	0.67	181.02	171.78	492.42^{a}	44.98	37.21	98.48 ^{a}	24.20	9.14
D_8	10	+	2.10	5.37	3.27	0.65	180.68	167.29	492.02^{a}	46.33	35.66	98.40 ^{a}	24.80	9.25
DDGS														
0			1.77^{a}	4.86 ^{a}	3.10	0.58 ^{a}	184.07	182.52 ^{b}	554.09 ^{c}	40.39 ^{a}	42.41 ^{c}	110.82 ^{c}	23.10^{a}	8.065 ^{a}
5			1.87^{b}	5.12 ^{b}	3.26	0.58 ^{a}	183.05	180.84 ^{b}	558.25^{c}	40.86 ^{a}	41.53 ^{c}	111.65 ^{c}	24.05 ^{b}	8.74 ^{b}
7.5			1.89^{b}	5.14 ^{b}	3.25	0.59 ^{a}	181.86	172.43 ^{a}	519.46 ^{b}	41.89 ^{a}	33.45^{a}	103.89 ^{b}	24.15 ^{b}	8.78 ^{b}
10			2.09^{c}	5.30 ^{b}	3.21	0.66 ^{b}	180.85	169.53 ^{a}	492.72^{a}	45.65 ^{b}	36.44^{b}	98.54 ^{a}	24.50 ^{b}	9.20^{c}
Enzyme														
-			1.89	5.08	3.19	0.60	182.97	179.11 ^{b}	541.61^{b}	41.53	39.89^{b}	108.32^{b}	23.88	8.65
+			1.91	5.14	3.22	0.61	181.95	173.55 ^{a}	520.65 ^{a}	42.86	37.02^{a}	104.13^{a}	24.03	8.74
Pooled SEM			0.02	0.04	0.04	0.01	0.63	1.41	4.06	0.61	0.54	0.81	0.168	0.078
Probability														
DDGS			P<0.01	P<0.01	NS	P<0.05	NS	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.05	P<0.01
Enzyme			NS	NS	NS	NS	NS	P<0.05	P<0.01	NS	P<0.01	P<0.01	NS	NS
Interaction			NS	NS	NS	NS	NS	NS	P<0.01	NS	NS	P<0.01	NS	NS

NS- Non-significant (P>0.05)

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