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Feeding Effect of Rice Based Dry Distillers Grains With Soluble on Hemato-Biochemical and Egg Sensory Attributes During 45th to 54th Week of Laying

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ABSTRACT

A biological experiment was undertaken with (4x2) factorial CRD in 45 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 (45th-54th 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 of this experiment revealed that consistent decreased values of egg cholesterol were observed at higher inclusion levels of DDGS in combination with enzyme, however significantly (P<0.01) increased the hematological, serum protein profile and have lowering effect on serum lipid profile, which was found better effect after dietary addition of enzyme. There was no detrimental effect on sensory attributes of egg.

Key words: *Egg cholesterol, Rice DDGS, Sensory attributes, Serum protein profile.*

INTRODUCTION

The conventional diet used in poultry is maize and soybean meal, but the ever increasing cost of conventional feedstuffs for livestock and poultry has led to increase in cost of production. As there is scarcity of both maize and soybean meal at reasonable price and constant price hike of the available feed resources, there is need to utilize locally available feed ingredients. Thus, it is a major means of modulating the production cost. In India, there is production of huge quantity of raw materials for livestock feeding but out of them only a narrow range of the raw materials are used for poultry feed formulation due to lack of reliable data on their nutrient composition, presence of toxic / anti nutritional factor, feeding value and effective level of inclusion. Thus, there is an urgent need to do research work on these aspects. Rice DDGS is a byproduct of distilleries in which rice is used in ethanol industry as a raw material to produce ethanol.

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A large quantity of different grains is spoiled (which are unfit for human consumption) every year in India because of unfavorable climatic conditions and inadequate transport and storage facilities. The damage includes discolored, 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. When ethanol plants produce ethanol, they use starch only from input grains like rice, corn, millets or wheat. In the process of ethanol production, the sugars from starch in cereal grain are fermented by yeast to produce ethanol, releasing carbon dioxide. The remaining "wetcake" is distillers' grains, to which the condensed solubles are added back at drying ending up with DDGS for feeding to livestock and poultry. Cereal grain yields about one-third ethanol, one-third CO_2, and one third DDGS[19]. 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 & Design

A biological experiment was undertaken in a 4x2 factorial completely randomized design (CRD) with forty fifth week 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 (45^{th} to 54^{th} 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[2] and calcium[24] contents prior to formulation of experimental diets. 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 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.

Egg yolk cholesterol and Sensory evaluation

Ten eggs per dietary treatment were randomly collected at monthly basis for analyzing the total egg cholesterol content[28] and at the end of experiment the organoleptic evaluation of eggs[18] was also carried out during ten weeks of experiment. For each of the sensory parameters (appearance, texture, flavor, 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 hematological profile

Blood samples from ten birds per dietary treatment were randomly collected into sterile glass test tubes with anticoagulant EDTA (for hematology) and without addition of anticoagulant to facilitate separation of serum at the end of the feeding trial. Serum was separated by centrifugation at 3000 rpm for 10 minutes and decanted into ependorff tubes and then stored at -20^{o}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[10]. PCV was estimated using micro haematocrit method as described by Sharma and Singh[20]. Estimation of glucose was done by glucose oxidase-peroxidase (GOD-POD) end point assay[16]. The total cholesterol, HDL (high density lipoprotein) concentration in serum according to Wybenga and Pileggi[28] and serum triglyceride[8], low density lipo-protein (LDL) concentration[9] 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[6] and albumin concentration by bromocresol green end point assay method[15].

RESULT AND DISCUSSION

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[7]. Increased cholesterol content in yolk was observed when high cholesterol diets were fed[13,26] especially when total fat in a diet was high. Several studies found that feeding polyunsaturated fatty acids would increase yolk cholesterol level[21,25] . Furthermore, dietary fiber has been found to have cholesterol-lowering effect[17] and soluble fiber is the main component to have this effect[12,27]. Bruckert and Rosenbaum[4] 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. It may be due to statistically similar egg weight during experimental period. However, consistent numerically decreased values of egg cholesterol were observed at higher inclusion levels of rice DDGS in combination with enzyme. In agreement to our results, Sun[22] found that cholesterol level in egg yolk from hens fed with high level of corn DDGS diet was continuously higher.

Egg sensory attributes

During this phase of laying 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. The sensory attributes was observed unaffected significantly ($P>0.05$) with the dietary inclusion of different levels of DDGS. Dietary addition of enzyme was significantly ($P<0.01$) beneficial in enhancing the appearance (colour) score of eggs. The appearance, colour, 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[23] 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 2^{nd} phase of production (44 to 68 weeks of age).

Serum-biochemical profile

Serum-biochemical and hematological profile are related to the health status and vital indicators of physiological and nutritional status of birds. Statistical analysis of data revealed that different levels of rice DDGS had significantly ($P<0.01$) higher effect on serum albumin, total serum protein, serum A/G ratio, serum glucose value and significantly ($P<0.01$) lowering effect on serum lipid profile (triglycerides, LDL and VLDL). Glucose level was decreased and HDL level increased significantly ($P<0.01$) in rice DDGS supplemented groups but unaffected between the levels of DDGS. Rice DDGS had significant (P<0.01) lowering effect on serum triglycerides (492.72mg/dl) and VLDL (98.54mg/dl) at 10% inclusion level of rice DDGS in layer diet whereas, LDL cholesterol was significantly (P<0.01) lower at all levels of DDGS. Enzyme addition in layer diet has been significantly (P<0.01) beneficial effect in terms of serum albumin, serum A/G ratio and LDL cholesterol. Abd El-Hack[1] noticed that replacing soybean meal by 25% corn DDGS insignificantly increased serum total protein and serum albumin; with increased corn DDGS replacement of soybean meal with DDGS at 50 or 75 % depressed total protein values and Youssef *et al*[29] reported that glucose concentration decreased significantly (P < 0.05) at 15% inclusion level of corn DDGS and total lipid was also decreased but at higher level of DDGS, however Bor-Ling *et al*[3] postulated that corn DDGS levels (0, 6, 12 and 18%) did not influence plasma total serum protein and serum triglycerides of laying hens diet while plasma cholesterol was significantly (P≤0.01) increased when 12 or 18% DDGS diets were used. Jiang *et al*[14] found that HDL and LDL concentrations in serum were not significantly (P>0.05) influenced in hens fed diet up to 20% level of corn DDGS with vitamin E during the laying period (40 to 63 wk of age). Dietary inclusion of rice DDGS up to 25% did not affect the plasma content of total protein, glucose, cholesterol and triglyceride in juvenile red seabream (*Pagrus major*)[5]. Moreover, Abd El-Hack[1] indicated that increasing corn DDGS level significantly (P≤0.01) increased serum triglycerides, cholesterol and LDL for hens fed diet contained 22% DDGS in the diet.

Hematological profile

Statistical analysis revealed significant (P<0.01) changes due to dietary treatment of different levels of DDGS were observed in PCV and Hb. Inclusion of 5, 7.5 and 10% level of DDGS significantly (P<0.01) enhance the PCV and Hb values than 0% inclusion level but did not differ with each other in PCV. Whereas significantly (P<0.01) higher value of Hb was observed at 7.5 and 10% inclusion level of DDGS. Dietary addition of enzyme have significant (P<0.05) impact on Hb where, all value were improved, however, PCV did not show any significant (P>0.05) effect of enzyme. The data revealed that different levels of DDGS and enzyme either sole or in combination with each other (interaction effect) did not have any significant effect on PCV however, significant (P<0.01) interaction effect was found on Hb value. Contrary to present findings, Ghazalah *et al*[11] reported that DDGS level at 75% substitution for SBM significantly increased Hb % with enzyme 40 week-old bovans brown layers, while, in broiler, DDGS up to 15% insignificantly affected the hematological parameter[29].

Table 1: Impact of feeding rice DDGS with and without enzyme supplementation on sensory evaluation of 45th 54th week old layer's egg

Diet	DDGS (%)	Enz.	Appearance & Colour	Flavour	Texture	Juiciness	Acceptability
D1	0	-	6.63	6.67	6.50	6.67	6.83
D2	0	+	7.42	6.92	6.71	6.71	7.04
D3	5	-	6.63	6.63	6.55	6.50	6.88
D4	5	+	7.42	6.88	6.71	6.63	7.04
D5	7.5	-	6.88	6.83	6.58	6.38	6.88
D6	7.5	+	7.38	6.88	6.63	6.50	7.04
D7	10	-	6.88	7.00	6.54	6.54	7.04
D8	10	+	7.71	7.00	6.79	6.71	7.08
DDGS (%)							
	0		7.02	6.79	6.60	6.69	6.94
	5		7.02	6.75	6.63	6.56	6.96
	7.5		7.13	6.85	6.60	6.44	6.96
	10		7.29	7.00	6.67	6.63	7.06
Enzyme							
	-		6.75[a]	6.78	6.54	6.52	6.91
	+		7.48[b]	6.92	6.71	6.64	7.05
	Pooled SEM		0.069	0.055	0.052	0.053	0.057
Probability							
	DDGS		NS	NS	NS	NS	NS
	Enzyme		P<0.01	NS	NS	NS	NS
	Interaction		NS	NS	NS	NS	NS

NS- Non-significant (P>0.01)

Table 2: Impact of feeding rice DDGS with and without enzyme supplementation on egg cholesterol (mg/g yolk) of 45th to 54th week old layers

Diet	DDGS (%)	Enz.	Egg Cholesterol	
			4th week of trial	8th week of trial
D1	0	-	17.18	16.80
D2	0	+	17.96	16.66
D3	5	-	16.43	16.50
D4	5	+	16.22	16.44
D5	7.5	-	16.38	16.58
D6	7.5	+	16.12	16.56
D7	10	-	16.25	16.39
D8	10	+	16.15	16.39
DDGS (%)				
	0		17.57	16.73
	5		16.32	16.47
	7.5		16.25	16.57
	10		16.20	16.39
Enzyme				
	-		16.56	16.57
	+		16.61	16.51
	Pooled SEM		0.380	0.205
Probability				
	DDGS		NS	NS
	Enzyme		NS	NS
	Interaction		NS	NS

Table 3: Impact of feeding rice DDGS with and without enzyme supplementation on hemato-biochemical profile of 45th to 54th week old layers

Diet	DDGS (%)	Enz.	Albumin (g/dl)	Total Protein (g/dl)	Globulin (g/dl)	AG Ratio	Glucose (mg/dl)	Serum Cholesterol(mg/dl)	Serum Triglyceride(mg/dl)	Serum VLDL (mg/dl)	Serum HDL (mg/dl)	Serum LDL (mg/dl)	PCV	Hb
D1	0	-	1.97	6.05	4.08	0.49	214.67	187.88	543.77	108.75	40.00	36.28	22.10	8.73^{a}
D2	0	+	2.08	6.06	3.98	0.55	203.49	184.67	548.64	109.73	44.82	30.18	22.60	9.05^{a}
D3	5	-	2.21	6.32	4.11	0.54	189.91	187.45	551.88	110.38	49.30	27.89	23.60	9.61^{b}
D4	5	+	2.51	6.34	3.84	0.67	186.60	182.09	543.31	108.66	49.67	24.38	23.10	10.69^{c}
D5	7.5	-	2.23	6.52	4.29	0.54	185.84	176.94	541.45	108.29	49.88	22.03	24.10	10.87^{c}
D6	7.5	+	2.54	6.53	3.98	0.65	186.29	179.95	533.34	106.67	52.89	20.12	24.00	10.68^{c}
D7	10	-	2.68	6.69	4.01	0.69	185.97	178.45	534.03	106.80	51.92	22.28	25.10	10.90^{c}
D8	10	+	2.71	6.69	3.99	0.69	184.51	172.44	498.55	99.71	53.85	20.43	23.30	10.84^{c}
DDGS														
	0		2.02^{a}	6.05^{a}	4.03	0.52^{a}	209.08^{b}	186.27	546.20^{b}	109.24^{b}	42.41^{a}	33.23^{c}	22.35^{a}	8.89^{a}
	5		2.36^{b}	6.33^{ab}	3.98	0.60^{b}	188.25^{a}	184.77	547.59^{b}	109.52^{b}	49.48^{b}	26.13^{b}	23.35^{b}	10.15^{b}
	7.5		2.39^{b}	6.53^{b}	4.14	0.59^{b}	186.06^{a}	178.45	537.39^{b}	107.48^{b}	51.39^{b}	21.07^{a}	24.05^{b}	10.78^{c}
	10		2.69^{c}	6.69^{b}	4.00	0.69^{c}	185.24^{a}	175.44	516.29^{a}	103.26^{a}	52.88^{b}	21.35^{a}	24.20^{b}	10.87^{c}
Enzyme														
	-		2.27^{a}	6.40	4.12	0.57^{a}	194.10	182.68	542.78	108.56	47.77	27.12^{b}	23.73	10.03^{a}
	+		2.46^{b}	6.41	3.95	0.64^{b}	190.22	179.79	530.96	106.19	50.31	23.77^{a}	23.25	10.32^{b}
	Pooled SEM		0.037	0.072	0.069	0.015	2.094	1.829	3.478	0.696	1.074	0.731	0.179	0.108
Probability														
	DDGS		P<0.01	P<0.01	NS	P<0.01	P<0.01	NS	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01
	Enzyme		P<0.01	NS	NS	P<0.01	NS	NS	NS	NS	NS	P<0.01	NS	P<0.05
	Interaction		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	P<0.01

NS- Non-significant (P>0.01)

CONCLUSION

Findings of this experiment revealed that consistent decreased values of egg cholesterol were observed at higher inclusion levels of DDGS in combination with enzyme, however significantly (P<0.01) increased the hematological, serum protein profile and have lowering effect on serum lipid profile, which was found better effect after dietary addition of enzyme. There was no detrimental effect on sensory attributes of egg.

REFERENCES

1. Abd El-Hack, M.E., Enzymes drying to be used in low-cost animal fodder production for existing biotechnology company. Ph.D. thesis, submitted to Agriculture Zagazig University, Egypt (2015).
2. AOAC. Official Methods of Analysis. Association of Official Analytical Chemists. 17th Ed., Gaithersburg, Maryland, USA (2000).
3. Bor-Ling, S., Hsu, A.L. and Chen, Y.K., Effects of corn distiller's dried grains with soluble on the productive performance and egg quality of laying hens. Dept. of Ani. Sci. National Chia-Yi University and AGAPE Nutrition Consultant, Taiwan (2011).
4. Bruckert, E. and Rosenbaum, D., Lowering LDL-cholesterol through diet: Potential role in the stain ear. *Curr. Opin. in Lipid*. **22:** 43–48 (2011).
5. Choi, J., Rahman, M. M. and Lee, S.M., Rice Distillers Dried Grain is a Promising Ingredient as a Partial Replacement of

Plant Origin Sources in the Diet for Juvenile Red Seabream (Pagrus major), *Asian Australian Journal of Animal Science.* **27(12):** 1736–1743 (2014).

6. Doumas, B.T., Bayse, D.D., Borner, K., Carter, R.J., Elevitch, F., Garber, C.C., Graby, R.A., Mather, A., Peters, T. R. and Reeder, D.J., Russell, S.M., Schaffer, R., Westgard, J.O. and Reus, L.L.A., Method for the determination of total serum protein. II. Test for transferability. *C/tn. Chem*. **27**: 1651-1654 (1981).
7. Fenton, M. and Sim, J.S., Determination of egg yolk cholesterol content by on-column capillary gas chromatography. *J. Chrom.* **540:** 323–329 (1991).
8. Fossati, P. and Lorenzo, P., Serum triglycerides determined calorimetrically with an enzyme that produces hydrogen peroxide**.** *Chin. chemistry.* **28(10):** 2077-80 (1982).
9. Friedewald, W.T., Levy, R.L. and Fredickson, D.S., Estimation of concentration of low-density lipopration cholesterol in plasma without use of the Ultracentrifuge, *Clin. Chem*. **18(6):** 499-502 (1972).
10. Fudge, A.M., Avian complete blood count, Laboratory Medicine: Avian and Exotic pets, 1st Ed., Saunders Publishing Company, Philadelphia, Pennsylvania (2000).
11. Ghazalah, A.A., Abd-Elsamee, M.O. and AL-Arami, A.A., Use of distillers dried grains with solubles (DDGS) as replacement for yellow corn in laying hen diets. *Poult. Sci.* **31:** 191-202 (2011).
12. Glassman, M., Spark, A., Berezin, S., Schwarz, S., Medow, M. and Newman, L.J., Treatment of type IIa hyperlipidemia in childhood by a simplified American Heart Association diet and fiber supplementation. *Amer. J. Dis. Child*. **144:** 973–976 (1990).
13. Harris, P.C. and Wilcox, F.H., Studies on egg yolk cholesterol: effect of dietary cholesterol. *Poult. Sci.* **42:** 186–189 (1963).
14. Jiang, W., L. Zhang and A. Shan, The effect of vitamin E on laying performance and egg quality in laying hens fed corn dried distillers grains with solubles. Poult. Sci., **92:** 2956-2964 (2013).
15. Johnson, A.M., Rohlfs, E.M. and Silverman, L.M., Proteins. *In* : Burtis C.A. and Ashwood E.R. (Eds). Tietz Textbook of Clinical Chemistry. 3rd ed., WB Saunders Company, Philadelphia. 477-540 (1999).
16. Kaplan, L.A. and Pesce, A.J., Carbohydrate and metabolite–clinical chemistry: theory, analysis and co-relation. Ed. CV Mosby, Toronto, 1032-1327 (1984).
17. Kirby, R.W., Anderson, J.W., Sieling, B., Rees, E., Chen, W., Miller, R. and Kay, R., Oat-bran intake selectively lowers serum low-density lipoprotein cholesterol concentrations of hypercholesterolemic men. *Ameri. J. Clin. Nutri*. **34:** 824–829 (1981).
18. Larmond and Elizabeth Laboratory methods for sensory evaluation of food. Research branch, Canada, Dept. of Agri. 16-37 (1977).
19. Saunders, J.A. & Rosentrater, K.A. Survey of US fuel ethanol plants. *Bioresource Technology*, **100:** 3277-3284 (2009).
20. Sharma, I.J. and Singh, H.S., Student's Laboratory Manual of Veterinary Physiology, Kalyani Publishers, New Delhi, (2000).
21. Summers, J.D., Slinger, S.J. and Anderson, W.J., The effect of feeding various fats and fat by-products on the fatty acid and cholesterol composition of eggs. *Brit. Poult. Sci.* **7:** 127–134 (1966).
22. Sun, H., Lee, E.J., Samaraweera, H., Persia, M. and Dong, U.A., Effects of increasing concentrations of corn distillers dried grains with solubles on chemical composition and nutrient content of egg. *Poult. Sci.* **92:** 233–242 (2013).
23. Swiatkiewicz, S. and Koreleski, J., Effect of maize distillers dried grains with

soluble and dietary enzyme supplementation on the performance of laying hens. *J. Anim. Feed Sci.* **5:** 252-260 (2006).

24. Talpatra, S.K., Roy, S.C. and Sen, K.C. Estimation of Phosphorus, Chlorine, Calcium, Magnesium, Sodium and Potassium in feed stuffs. *Indian J. Vet. Sci.* **10:** 243-258. (1940).
25. Weiss, J.F., Naber, E.C. and Johnson, R.M., Effect of dietary fat and other factors on egg yolk cholesterol. 1. The 'cholesterol' content of egg yolk as influenced by dietary unsaturated fat and the method of determination. *Archives Biochem. Biophy*. **105:** 521–526 (1964).
26. Weiss, J.F., Johnson, R.M. and Naber, E.C., Effect of some dietary factors and drugs on cholesterol concentration in the egg and plasma of hen. *J. Nutri*. **91:** 119–128 (1967).
27. Williams, C.L., Bollella, M., Spark, A. and Puder, D., Effectiveness of a psyllium enriched step I diet in hypercholesterolemic children. *J. American Coll. Nutri.* **14:** 251–257 (1991).
28. Wybenga, D.R. and Pileggi, V.J., Estimation of Cholesterol. *Clinic. Chem.* **16:** 980 (1970).
29. Youssef, A.W., El-Azeem, N.A.A., El-Daly, E.F. and Monairy, M.M., The Impact of Feeding Graded Levels of Distillers Dried Grains with Solubles (DDGS) on Broiler Performance, Hematological and Histological Parameters, *Asian J. Poult. Sci*. **7(2):** 41-54 (2013).