

Blood profile of broiler chickens fed varying levels of crude fibre and energy in multi-fibre source-based diets without enzyme supplementation

¹Salami, R. I. and ²Odunsi, A. A.

¹Department of Agricultural Education (Animal Science Division), School of Vocational & Technical Education, Emmanuel Alayande College of Education, P.M.B 1010, Oyo, Oyo State, Nigeria.

²Department of Animal Nutrition and Biotechnology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.



¹Corresponding Author: risalami4@gmail.com,
risalami@eacoed.edu.ng, 08035666251

Abstract

This study sought to assess the effect of dietary crude fibre – energy relationships on the blood profile of unsexed Obamarshall strain of broiler chickens fed different levels of dietary crude fibre (CF) each at three levels of metabolisable energy (ME) in multi-fibre source-based diets from day-old to 56 days of age. Results indicated that neither CF nor ME and their interaction had significant effect on hematological indices measured at 50 days of age. The values of hematological indices obtained for the treatment groups fell within the standard values for normal chickens. Except for the blood cholesterol, there was no significant effect of varying levels of CF or ME on other serum metabolites and likewise their interaction effect. While increasing CF level tended to reduce significantly ($P < 0.05$) blood cholesterol, incremental levels of ME tended to increase ($P < 0.05$) blood cholesterol and also their interaction effects. The values of serum cholesterol in the birds ranged from 106 mg/dl for diet B to 181 mg/dl for diet D, which were within the range for normal chicken. Conclusively, the findings showed that blood profile was not adversely affected even at 12% CF and 3000 ME (Kcal/kg) diet.

Keywords: Broiler, crude fibre, metabolisable energy, calorie: protein ratio, blood

Introduction

Owing to its dynamic nature, blood is a major indicator or reflector of the physiological, pathological and nutritional statuses of the animal body. This is because the blood is susceptible to internal and external changes/stimuli provoked by nutrition, pests, diseases, metabolism, season and any other stimuli or stressors to which the animal may respond (Adejinmi *et al.*, 2000, Seth and Saxena, 2003 and Ladokun *et al.*, 2008). Evaluation of blood profile of farm animals is, therefore, pertinent in monitoring the metabolic status of farm animals, feed quality, disease diagnosis, prophylaxis and treatment as well as evaluation of adaptation traits to

various ecological/environmental factors. For fast-growing animals like broiler chickens, nutritional studies should therefore not be limited to the assessment of the biologic performance traits, carcass and organ evaluation but also to the evaluation of animal's blood profile. Several reports have been given on the effects of manipulation of nutritional factors on hematology and serum metabolites in simple-stomached animals (Akinola and Abiola, 1999; Adejinmi *et al.*, 2000 and Idowu *et al.*, 2003). For example, Hetland *et al.* (2004) indicated that lack of access to foraging material or deficiency of fibre in the diets for poultry may provoke stress as reflected in higher heterophils to

Blood profile of broilers fed multi-fibre source based- diets

lymphocytes ratio. According to these authors, the heterophils to lymphocytes ratio can be lowered by providing birds with adequate dietary fibre or access to foraging materials like litter material, which, will prevent or eliminate pecking behaviour among birds (Van Krimpen *et al.*, 2009; Esmail, 2012). Similarly, Bangbose *et al.* (2004) reported that values of serum total protein, packed cell volume, haemoglobin, albumin and red blood cells were depressed in 8 week-old cockerels due to increasing fibre levels ranging from 13 to 19% which might have impaired the utilization of nutrients, especially protein. Reports elsewhere also indicated that hematological and serum biochemical indices of poultry birds were altered by varying protein level in broiler finisher diets (Odunsi *et al.*, 2005), calorie: protein ratio in broiler diets (Idowu *et al.*, 2003) and by subjecting broilers to quantitative water restrictions (Iheukwumere and Hebert. 2003) below and beyond their tolerance limits.

On the basis of the impairment of digestion and absorption processes due to soluble and insoluble fibre in the monogastric animals (Hetland *et al.*, 2004 and Jozefiak, 2004) as well as the chelating action and binding capacity of fibre (Choct, 2006; Shang *et al.*, 2010), it is plausible to expect that varying levels of CF and ME in the diets of broiler chickens below and beyond their tolerance limits may cause changes in their blood profile. Against this backdrop, this study, therefore aimed at evaluating the

hematological and serum biochemical indices of broiler chickens fed varying levels of CF and ME in their diets (maintained at fixed calorie: protein ratio).

Materials and methods

The study was conducted at the Poultry unit of the Teaching and Research farm, Emmanuel Alayande College of Education, Oyo, Nigeria. Oyo is located approximately along latitude 7° 51' North of the equator and longitude 3° 57' East of the Greenwich meridian and 850m above sea level. The annual average rainfall is 1163mm while annual mean temperature is 27°C and annual mean relative humidity is 82% (Iwena, 2012).

Two hundred and sixteen (216) day-old unsexed Obamarshal broiler chicks were randomly allotted into nine treatment groups in a completely randomised design. Each group was replicated thrice with 8 birds per replicate. Nine treatment diets of the same formulation and nutritional specifications as those given in Salami and Odunsi (2017) were applied to the treatment groups under the same housing and management as previously described. The determined chemical compositions of the experimental diets appear in Table 1. The treatment diets were maintained at fixed calorie: protein ratios of 123:1 and 140:1 (based on calculated percent crude protein and ME values) for the starter and finisher phases respectively in accordance with the recommendation of Aduku (2004).

Table 1: Determined chemical composition of starter and finisher diets on dry matter basis

Chemical Component (%)	¹ Diets								
	2600 ME (kcal/kg)			2800 ME (kcal/kg)			3000 ME (kcal/kg)		
	(kcal/kg)ME(kcal/kg)			ME(kcal/kg)ME(kcal/kg)			(kcal/kg)ME(kcal/kg)		
	A (4)	B (8)	C(12)	D (4)	E (8)	F(12)	G (4)	H (8)	I (12)
StarterPhase:(0-4 weeks)									
Dry matter	90.1	90.0	89.7	90.29	89.70	89.91	89.85	89.31	89.05
Crude protein	20.45	20.55	21.00	22.74	22.35	22.80	23.65	24.55	23.79
Crude fat	5.35	7.14	10.27	6.30	10.23	12.18	8.47	12.54	16.04
Nitrogen – free extract	49.32	46.64	38.95	47.23	41.67	34.17	43.78	35.67	28.59
Crude fibre	4.09	7.85	11.75	4.20	7.80	11.85	3.97	7.65	12.0
Ash	12.06	12.20	13.16	11.84	12.44	12.53	13.22	13.04	13.01
Finisher Phase:(5-8 weeks)									
Dry matter	90.27	90.14	90.09	90.24	90.39	88.95	90.10	88.21	88.38
Crude protein	17.67	17.80	17.70	19.50	19.35	19.25	20.05	20.10	20.22
Crude fat	4.44	6.45	11.37	5.41	7.52	12.61	8.45	11.69	15.35
Nitrogen – free extract	52.06	49.13	42.80	49.85	45.73	37.40	48.30	39.52	32.83
Crude fibre	3.95	7.68	11.80	4.05	7.88	11.85	4.00	7.75	11.90
Ash	12.35	12.45	12.55	12.44	12.65	12.54	13.21	13.11	13.05

¹Means of triplicate determinations

Figures in parenthesis represent levels of crude fibre in the diets

At day 50 of the feeding trial, two birds per replicate were sampled for blood analysis. Blood was collected aseptically from the neck veins of the sampled birds for the measurement of hematological indices and serum metabolites. Heparinized bottles were used for collection of 2mls blood samples each for hematological indices while bottles without anti-coagulant were used for collecting 3mls blood samples for serum metabolites. The packed cell volume (PCV), and haemoglobin (Hb) were measured using the micro hematocrit and cyanomethemoglobin methods respectively as described by Mitruka and Rawnsley (1977). Erythrocyte count (RBC) and leukocyte count (WBC) were determined using the improved Neubauerhaemocytometer after the appropriate dilution (Schalm *et al.*, 1975). Differential leukocyte counts were determined by scanning Giemsa's stained slides in the classic manner (Schalm *et al.*, 1975).

Glucose and cholesterol were measured by spectrophotometric method according to Barham and Trinder (1972) and Flegg

(1973) respectively. Urea and creatinine were by urease and Folin-Wu filtrate methods respectively as described by Toro and Ackermann (1975). Total serum protein was determined using the biuret method as described by Reinhold (1953) while albumin was evaluated using the Bromocresol Green (BCG) method as described by Peters *et al.* (1982).

Data collected were subjected to analysis of variance in accordance with 3X3 factorial design made up of 3 levels of metabolisable energy (2600, 2800 and 3000ME (Kcal/kg)) and 3 levels of crude fibre (4, 8 and 12%) per energy level using SAS (2000) statistical package. Means were also separated using Duncan's Multiple Range Test (1955) of the same package at 5% probability level.

Results

There was no significant effect ($P>0.05$) of varying levels of dietary crude fibre and energy on the hematological indices of broiler finishers at 50 days of age (Table 2). Likewise, the values of hematological indices obtained for treatment groups

Blood profile of broilers fed multi-fibre source based- diets

revealed that they were not affected significantly ($P>0.05$) by the range of dietary crude fibre and metabolisable energy levels (Table 3), except the birds on 4% crude fibre diet containing 2800ME (Kcal/kg) (diet D). The birds placed on diet D had lower ($P<0.05$) value of eosinophils compared to birds on other diets. There were no significant differences

($P>0.05$) on serum biochemical indices with varying levels of crude fibre and metabolisable energy with the exception of serum cholesterol. While serum glucose and other metabolites were stabilized by the two variable factors, serum cholesterol tended to reduce ($P<0.05$) with increasing dietary crude fibre level and also increased ($P<0.05$) as dietary energy level increased (Table 4).

Table 2: Effect of varying levels of dietary crude fibre and energy on hematological indices of broiler finishers at 50 days of age

Hematological indices	Dietary crude fibre level (%)			± SEM
	4	8	12	
Packed cell volume (%)	29.1	29.2	29.8	0.81
Haemoglobin concentration (mg/dl)	9.71	9.57	9.06	0.40
Red blood cell ($\times 10^6$ /ml)	2.12	2.13	2.01	0.07
White blood cell ($\times 10^3$ /ml)	18.7	16.4	19.2	0.98
Heterophils (%)	28.7	31.8	29.2	0.41
Lymphocytes (%)	69.0	67.4	66.2	1.37
Monocytes (%)	2.22	2.11	2.67	0.36
Eosinophils (%)	2.44	2.89	3.00	0.62
Dietary ME level (kcal/kg)				
	2600	2800	3000	
Packed cell volume (%)	28.3	30.1	29.7	0.81
Haemoglobin concentration (mg/dl)	8.98	9.61	9.74	0.40
Red blood cell ($\times 10^6$ /ml)	2.20	2.10	1.97	0.07
White blood cell ($\times 10^3$ /ml)	16.6	18.7	19.1	0.98
Heterophils (%)	30.7	29.3	29.7	0.41
Lymphocytes (%)	66.6	68.7	67.4	1.37
Monocytes (%)	2.22	2.22	2.56	0.36
Eosinophils (%)	3.44	2.22	2.67	0.62

Means within the same rows without superscript are similar ($P>0.05$).

The interaction of both variable factors generally revealed no significant effect on the serum metabolites except the cholesterol. Serum cholesterol concentration was reduced significantly ($P<0.05$) as dietary crude fibre increased within the metabolisable energy levels (Table 5), while serum glucose was also

stabilized across diets except for diet B. The results indicated no adverse effects on haematology and blood chemistry in the broiler finishers fed diets containing 12%CF at 2800 and 3000ME (kcal/kg) (diets F and I and at the calorie: protein ratio of 140:1.)

Table 3: Interaction effects of varying dietary crude fibre and energy levels on hematological indices of broiler finishers at 50 days of age

Hematological indices	2600 ME (kcal/kg)			2800 ME (kcal/kg)			3000 ME (kcal/kg)			± SEM
	A 4	B 8	C 12%CF	D 4	E 8	F 12%CF	G 4	H 8	I 12%CF	
Packed cell volume (%)	29.0	28.0	28.0	30.0	28.3	32.0	28.3	31.3	29.3	1.3
Haemoglobin concentration (mg/dl)	9.7	9.4	7.8	9.9	9.5	9.4	9.5	9.8	9.9	1.1
Red blood cell (x 10 ⁶ /ml)	2.4	2.2	2.0	2.0	2.1	2.2	1.9	2.1	1.8	0.3
White blood cell (x 10 ³ /ml)	16.5	14.3	19.1	21.1	16.1	18.8	18.6	19.0	19.6	3.5
Heterophils (%)	28.0	34.3	29.7	29.0	30.0	29.0	29.0	31.0	29.0	3.1
Lymphocytes (%)	68.3	66.3	65.0	70.0	68.7	67.3	68.7	67.3	66.3	3.7
Monocytes (%)	2.3	2.3	2.0	2.0	1.7	3.0	2.3	2.3	3.0	0.9
Eosinophils (%)	3.0 ^a	3.3 ^a	4.0 ^a	1.7 ^b	2.7 ^{ab}	2.3 ^{ab}	2.7 ^{ab}	2.7 ^{ab}	2.7 ^{ab}	1.6

^{a,b}Means within the same row bearing different superscripts are significant (P<0.05).

Blood profile of broilers fed multi-fibre source based- diets

Table 4: Effect of varying levels of dietary crude fibre and energy on serum metabolites of broiler finishers

Serum metabolites (mg/dl)	Dietary crude fibre level (%)			± SEM
	4	8	12	
Total protein	8.5	8.6	8.5	0.2
Albumin	1.7	1.6	1.7	0.1
Creatinine	0.7	0.8	0.8	0.0
Urea	2.2	2.3	2.2	0.2
Glucose	204.5 ^a	188.1 ^b	201.9 ^a	2.3
Cholesterol	161.2 ^a	127.6 ^b	135.9 ^b	8.5
	Dietary ME level (kcal/kg)			
	2600	2800	3000	
Total protein	8.1	8.5	8.9	0.2
Albumin	1.7	1.7	1.7	0.1
Creatinine	0.7	0.8	0.8	0.0
Urea	2.1	2.2	2.3	0.2
Glucose	191.1 ^b	198.4 ^{ab}	205.1 ^a	2.3
Cholesterol	123.2 ^c	166.0 ^a	135.5 ^b	8.5

^{a,b,c}Means within the same row bearing different superscripts are significant (P<0.05).

Discussion

The similar values of hematological indices for the various treatment groups, suggest neither effect of CF nor ME levels. This is at variance with the depressed values of hematological indices in cockerel starters due to varying CF levels in their diets as reported by Bamgbose *et al.*(2004). The hematological indices and serum metabolites except cholesterol and glucose of the birds were not depressed by the 2600 ME (Kcal/kg) diet, indicating that maintenance functions were not jeopardized. This is probably an indication of nutritional adequacy of the diets fed as defined by similar calorie: protein ratio and the range of levels of CF and energy on the basis of health status of the birds. The values of hematological indices in this study, however, disagree with the decreasing values observed with increasing CF levels in rabbit diets fed rice bran (Alade *et al.*, 1998); decreasing crude protein level in the broiler finisher diets (Odunsi *et al.*, 2005); quantitative drinking water restriction to the broiler finishers (Iheukwumere and Herbert, 2003) and widening of calorie:protein ratio in broiler finisher diets (Idowu *et al.*, 2003). The

values of hematological indices for the treatment groups were generally within the values reported by Mitruka and Rawnsley (1977) and Ridwell (2011) for normal chickens.

Among the serum metabolites measured, only the serum cholesterol was attenuated or depressed as the dietary crude fibre increased within the energy levels. This is advantageous in the production of lean broiler carcass since fat is distasteful to the consumers of poultry meat because of incidence of cholesterol-related diseases (Shang *et al.*, 2010). However, serum cholesterol increased as dietary ME increased which, could be attributable to increased calorie consumption (Bartov *et al.*, 1974; Tion *et al.*, 2005). The cholesterol-lowering effect of increasing CF level was linked to the binding action of soluble fibre and lignin with the bile acids for excretion (Choct, 2001 and Jozefiak *et al.*, 2004). Impaired digestion and absorption of nutrients either via faster or slower rate of passage of digesta in the gut depending upon the proportions of soluble and insoluble fibres (Hetland *et al.*, 2004; Ibiyo and Atteh 2005) might also reduce serum cholesterol. The values of serum metabolites except

Table 5: Interaction effects of varying dietary crude fibre and energy levels on serum metabolites of broiler finishers

Serum metabolites(mg/dl)	2600 B		2800 E		3000 H	
	A 4	8	C 12%CF	D 4	F 12%CF	I 12%CF
Total protein	7.9	8.7	7.7	8.7	8.6	8.9
Albumin	1.7	1.5	1.8	1.7	1.8	1.8
Creatinine	0.7 ^{ab}	0.67 ^b	0.7 ^{ab}	0.8 ^{ab}	0.8 ^{ab}	0.8 ^{ab}
Urea	1.8	2.71	1.8	1.9	2.7	2.2
Glucose	206.2 ^a	168.4 ^b	198.7 ^{ab}	205.1 ^a	194.8 ^{ab}	200.5 ^a
Cholesterol	152.4 ^c	106.3 ^d	110.7 ^d	180.7 ^a	153.8 ^c	112.8 ^d

^{a,b,c,d}Means within the same row bearing different superscripts are significant (P<0.05)

glucose were within the normal range of values reported by Ridwell (2011). Except for diet B, the values of serum glucose for other diets were generally stabilized and the values exceeded the range of 152 to 182 mg/dl reported by Mitruka and Rawnsley (1997).

Findings from this study indicated no significant effect of varying CF and ME levels and their interaction on hematological and serum biochemical indices of the broiler finishers at 50 days of age except for blood cholesterol. Serum cholesterol tended to reduce with increasing dietary CF level within each energy level, while it increased with increasing dietary energy levels. Feeding broiler finisher chickens, a diet containing 12%CF and 3000 ME (kcal/kg) had no adverse effect on hematology and serum biochemical indices.

Acknowledgement

This report is part of the research work funded by TETFUND (Nigeria) for which the body is hereby thankfully acknowledged. The services rendered by Alhaja Titi Lawal of Animal Physiology Laboratory, Animal Science Department of University of Ibadan, Ibadan and the assistance received from Messrs, M. A. Haruna and O. Akindoye are worthy of note.

References

- Adejinmi, O. O., Adejinmi, J. O. and Adeleye, I. O.A. 2000.** The studies on the haematology and serum chemistry of broilers fed varying levels of soldier fly larvae meal diets. *Tropical Animal Production Investigation* 3: 169–179.
- Aduku, A. O. 2004.** Feeds and Feed composition table. Davcon computers and Business Bureau, Kaduna, Nigeria.
- Akinola, S. O. and Abiola, S. S. 1999.** Blood chemistry and carcass yield of cockerels fed melon husk diets. *Tropical Journal of Animal Science* 2(2): 39–44.
- Alade, N. K., Kwari, I. D. and Kabir, M. 1998.** Effects of dietary level of rice bran on mineral utilization and blood components of growing rabbits. *Nigeria Journal of Agricultural Education Vols 1 & 2*: 137–141.
- Bamgbose, A. M., Anjeniwa, A., Temitope, S., Ikhinnoya, I., Isah, O.A. and Omoikhoje, S. O. 2004.** Carcass and haematological parameters of cockerel starters fed rice milling waste-based diets. **Proc. of the 29th Ann. Conf. Nig. Soc. Anim. Prod.** pp 244-246.
- Barham, D. and Trinder, P. 1972.** *Analyst*. 97:142,
- Bartov, I., Bornstein, S. and Lipstein, B. 1974.** Effect of calorie to protein ratio on the degree of fatness in broilers fed on practical diets. *British Poultry Science*.15: 107 – 117.
- Choct, M. 2006.** Enzymes for the feed industry: past, present and future. *World Poultry Science Journal* Vol.62 (1): 5-15.
- Choct, M. and Kocher, A. 2001.** Non-starch carbohydrates: Digestion and its secondary effects in monogastrics (electronic copy) . <http://www-personal.une.edu.au/~mchoct/Nutsoc%20paper.pdf>
- Esmail, S.H. 2012.** Fibre plays a supporting role in poultry nutrition. *World Poultry*, Vol. 28 (1) <http://www.worldpoultry.net/chickens/nutritio>

- n/ayers/fibre-plays-a-supporting-role-in-poultry-nutrition-9965.html.
- Flegg, H. M. 1973.** *Ann Cain Biochem.*10:1350-1356.
- Hetland, H., Choct, M. and Svihus, B. 2004.** Role of insoluble non-starch polysaccharides in poultry nutrition. *World's Poultry Science Journal.* Vol. 60: 415–422.
- Ibiyo, L. M. O. and Atteh, J. O. 2005.** Response of starter broilers to diets containing graded levels of rice bran with or without Palm Oil, **Nigerian Journal of Animal Production**32(1): 39 - 45.
- Idowu, O. M. O., Erubetina, D., Oduguwa, O. O., Bamgbose, A. M. and Abiola, S. S. 2003.** Response of finishing broiler chickens fed three energy/protein combinations at fixed E:P ratio. *Nigerian Journal of Animal Production* 30 (2): 185–191.
- Iheukwumere, F. C. and Herbert, U. 2003.** Physiological responses of broiler chickens to quantitative water restrictions: haematology and serum biochemistry. *International Journal of Poultry Science*Vol 2(2): 117–119.
- Iwena, O.A. 2012.** Essential geography for senior secondary schools. 6th ed Lagos, Tonad Publishers Limited.
- Jozefiak, D., Rutkowski, A. and Martin, S. A. 2004.** Carbohydrate fermentation in the avian ceca: a review. *Animal Feed Science and Technology.*113: 1–15.
- Ladokun, A. O., Yakubu, A., Otite, J. R., Omeje, J. N., Sokunbi, O. A. and Onyeji, E. 2008.** Haematological and serum biochemical indices of naked neck and normally feathered Nigerian Indigenous Chickens in a sub humid tropical environment. *International Journal of Poultry Science* 7 (1): 55–58.
- Mitruka, B. M. and Rawnsley, H. M. 1977.** Clinical biochemical and haematological reference values in normal experimental animals. Masson Pub. U.S.A. Inc. N.Y. Pp. 88 & 140–143.
- Odunsi, A. A., Oladunjoye, I. O. and Emiola, I. A. 2005.** Response of finishing broilers fed varying dietary protein levels in a tropical Environment. *International Journal of Applied Agricultural and Apicultural Research* 1(1): 66 – 73.
- Peters, T., Biamonte, G. T. and Dumas, B. T. 1982.** Protein [total protein] in serum, urine and cerebrospinal fluid; albumin in serum. *Selected methods of clinical chemistry.* Vol. 9. W.R. Paulkner and S. Meites, Eds. Washington, DC: American Association for clinical chemistry.
- Reinhold, J. G. 1953.** Standard methods of clinical chemistry. In: Reiner, M. (Ed). New York 1: 88-89.
- Ridwell, C. 2011.** Comparative anatomy, histology and physiology of the chicken. Department of Pathology, Western College of veterinary Medicine, University of Saskatchewan, Saskatoon, Canada. [h t t p : / / c a l . vet.upenn.edu/projects/poultry/syllabus/pp37-44htm](http://cal.vet.upenn.edu/projects/poultry/syllabus/pp37-44htm).
- S.A.S Institute 2000.** Statistical Analysis System. User's Guide, Cary, N.C. USA.
- Salami, R. I. and Odunsi, A. A. 2017.** Carcass characteristics of finishing broiler chickens fed varying levels of crude fibre and energy in multi-fibre source-based diets. *Nigerian*

- Journal of Animal Production. In Press
- Schalm, O. W. Jane, N. C. and Carol, E. J. 1975.** Veterinary haematology. 3rd Edition. Lea and Febiger Philadelphia.
- Seth, N. and Saxena, K. K. 2003.** Haematological responses in a fresh water fish channapunctatus due to fenvalerate. *Bulletin of Environmental contamination and Toxicology*, 71(6): 1192 – 1199.
- Shang, H. M., Hu, T. M., Lu, Y. J. and Wu, H. X. 2010.** Effects of inulin on performance, egg quality, gut microflora and serum and yolk cholesterol in laying hens. *British Poultry Science.*, 51(6): 791 – 796.
- Sundu, B., Kumar, A and Dingle, J. 2006.** Palm kernel meal in broiler diet: effect on chicken performance and health. *World's Poultry Science Journal* 62 (2): 316 – 325
- Tion, M. A., Orga, M. T. and Adeka, I. A. 2005.** The effect of calorie to protein ratio of practical diets on performance and carcass quality of broiler chickens. *Nigerian Journal of Animal Production*, 32(2): 253 – 260
- Toro, G. and Ackermann, P. G. 1975.** Practical clinical chemistry. First Edition. Boston, Little Brown and Company: 237-238
- VanKrimpen, M. M., Kwakkel, R. P., Vander Peet – Schwering, C. M. C., den Hartog, L.A. and Verstegen, M. W. A. 2009.** Effects of nutrient dilution and non-starch polysaccharide concentration in rearing and laying diets on eating behaviour and feather damage of rearing and laying hens. *Poultry Science*. 88(4): 759 – 773.

Received: 25th August, 2016

Accepted: 12th March, 2017