

# Production Performance of Broilers Fed Rations Supplemented with Natural Clay and the Bioavailability of Clay Mineral

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## Abstract

The cost of commercial feed is very exorbitant and inorganic elements used in poultry feed production are not available and/or affordable to small scale farmers that own greater part of poultry business in Nigeria. This study evaluated production performance of broilers fed rations containing natural clay for a period of eight weeks and the bioavailability of clay mineral. A total of 80 unsexed *arbor acres* broiler chicks were equally and randomly allocated to four treatment groups (Treatments 1-4). Four treatment rations (isonitrogenous and isocaloric) were compounded and fed correspondingly to the treatment groups. Treatment 1 had no clay, 2 contained trace mineral elements from inorganic sources and both served as negative and positive controls respectively. Treatments 3 and 4 had 2% and 1% clay inclusion, respectively. Clay was found to increase trace mineral composition in feed, blood serum and liver tissue. Dose response to clay supplementation in favour of 2% inclusion confirms positive influence of treatment on production parameters and the bioavailability of clay minerals to the animal, and hence the possibility of clay being used as a replacement for trace minerals in broiler production.

**Keywords:** Broiler, Production, Clay, Trace minerals, bioavailability.

## 1. Introduction

There is an extensive global distribution of mineral element problems (FAO, 1993).

For instance, in 1980, there was about 80% reported nutritional disorders of moderate or high incidence, and mineral deficiencies or toxicities were involved in more than half of those whose causes were identified (FAO, 1993). The steady decline in trace mineral content of the soil results in a lowered nutritional value of plants and plant products (Loladze, 2014). Sequel to this, otherwise nutritious grass may fail to supply the needs of animals because certain trace elements are missing. Perhaps more important is the global concern over excess mineral output into the environment arising from intensive animal feeding operations (Gerber and Steinfeld, 2008). The most outstanding species of environmental concern are pig, poultry, and cattle, and the most important minerals are copper and zinc, which are widely used at high levels as growth promoters in the industry (Alonso, 2012). These minerals including iron are also reported to have homeostatic control at the site of intestine absorption; therefore, great emphasis has been placed on strategies for lowering the excretion of these most important minerals (NRC, 1998; Alonso *et al.*, 2004). According to Suttle (2010) this can be reduced by feeding minerals at levels that meet the best estimates of maximum individual requirements. Another approach is to use organic sources instead of inorganic ones that are supposed to have lower bioavailability (NRC, 1998; Alonso *et al.*, 2004).

Mineral elements are known to be essential dietary nutrients for all living organisms, including animals. Trace minerals however, are required in small amounts by animals, measured in parts per million (ppm) or part per billion (ppb); they are found in tissues as part of protein structure or as activators of enzyme systems (Thompson, 1970;

Alonso, 2012). Deficiencies of trace elements have been associated with retarded growth and some characteristic deficiency diseases (Thompson, 1970; Suttle, 2010).

Trace mineral needs of poultry have not received much attention over the last four and a half decades when compared to other aspects of nutrition such as, energy and protein (Leeson and Summers, 1997). A cursory review of information published in scientific journals over the last 15 years indicates only some 1.5% of research effort expended on trace minerals (Leeson and Summers., 1997; Alonso, 2012). The major reason for this lack of interest probably lies in their relative economic importance. When there are no overt deficiencies or excess of trace minerals in a diet, as is usually the case, trace minerals represent less than 0.5 percent of total diet cost (Leeson and Summers., 1997). This contribution is overwhelmed by the costs of energy and protein, and so naturally, the attention of researchers is focused on the needs for these macronutrients. However, in spite of the small cost contribution of trace minerals, their deficiencies when energy and protein are adequate will lead to high economic loss due to poor performance and unthriftiness (Leeson and Summers., 1997). The resurgence of interest in trace mineral nutrition has been brought about by worldwide concerns for the environment, particularly, the level of all nutrients in manure. Whereas in the Nigerian context, scarcity and un-affordability of trace mineral premix which is needed to boost feed quality for better productivity is of a greater concern.

Commercial trace mineral supplements are not readily available to small and middle-scale farmers which form the greater percentage of animal producers in Nigeria. Moreover, due to inefficient quality control of products sold in our markets and prolonged storage during importation and clearance at ports (for imported items) and at retail shops, the quality of feed and feed components sold to farmers may become sub-standard. Thus it becomes necessary for a search for cheaper and readily available functional alternatives to the conventional marketed supplement.

## 2. Materials and Methods

### 2.1 Experimental design

The study investigated the use of natural clay at two inclusion levels as trace mineral source in broiler diet using the completely randomized design. The experiment was performed at the poultry unit of the Teaching and Research Farm, Faculty of Veterinary Medicine, University of Nigeria Nsukka, Enugu State.

Four experimental units corresponding to treatment groups 1 to 4 (T1, T2, T3 and T4) were used. Each treatment group was further divided into four replicates (R1, R2, R3 and R4). Five broiler chicks were housed in each replicate unit and a total of 20 chicks per treatment group.

### 2.2 Experimental animal

A total of 80 day-old unsexed chicks of commercial *arbor acres* broiler strain were purchased from Farm Associates Enugu, Nigeria. The broilers were brooded together on a commercial broiler starter ration (Top feed®) for one week acclimatization period. Thereafter, they were placed on experimental starter and finisher diets for three and four weeks respectively in deep litter compartments.

### 2.3 Experimental diet

The composition of the treatment rations are as indicated on Table 1 (for the starter ration), and Table 2 (for the finisher ration).

Feed ingredients for compounding of experimental rations were purchased from Alpha farms feed mill Emene, Enugu. Three sets of clay samples were collected from Amuro in Onuimo Local Government Area of Imo-State, Nigeria. Commercial broiler vitamin - premix was purchased from Farm Associates Enugu; Trademark: M & B Broiler Premix. Inorganic Salts containing the various trace minerals were purchased from chemical stores in Nsukka and Enugu and were used to compound the Laboratory Trace mineral Premix (Lab. TMP) used in the positive control. River sand used in T1, T2 and T4 was collected from a river near Gariki in Enugu.

**Table 1: Percentage Composition of Experimental Diet (Starter Ration)**

Ingredients in Diets (%)	Experimental Diets			
	T1	T2	T3	T4
Maize	54.0	54.0	54.0	54.0
GNC	30.0	30.0	30.0	30.0
PKC	5.0	5.0	5.0	5.0
FM	6.0	6.0	6.0	6.0
BM	2.6	2.6	2.6	2.6
SALT	0.3	0.3	0.3	0.3
VP	0.1	0.1	0.1	0.1
Lab. TMP	-	0.1	-	-
Clay	-	-	2.0	1.0
Sand	2.0	1.9	-	1.0
TOTAL (%)	100	100	100	100

**Table 2: Percentage Composition of Experimental Diet (Finisher Ration)**

Ingredients in Diets (%)	Experimental Diets			
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
Maize	60.0	60.0	60.0	60.0
GNC	24.0	24.0	24.0	24.0
PKC	7.0	7.0	7.0	7.0
FM	4.0	4.0	4.0	4.0
BM	2.6	2.6	2.6	2.6
SALT	0.3	0.3	0.3	0.3
VP	0.1	0.1	0.1	0.1
Lab. TMP	-	0.1	-	-
Clay	-	-	2.0	1.0
Sand	2.0	1.9	-	1.0
TOTAL (%)	100	100	100	100

## 2.4 Method

Clay was dried and crushed. Mineral Analysis of the three clay samples was carried out using Spectrophotometer method (see Table 3 for result). River sand used was washed and dried to ensure that no nutrient enters the feed pool through it.

Feed ingredients were weighed, milled, and appropriately mixed at the feed mill unit of Alpha farms, Emene, Enugu. Laboratory compounded Trace Mineral Premix (Lab. TMP) was compounded from the inorganic salts in Animal Health and Production Laboratory, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Tables 3 and 4 show the inorganic salts from which Lab. TMP was compounded and the composition of the Lab. TMP, respectively.

**Table 3: Inorganic Salts from which Laboratory Trace Mineral Premix was compounded:**

S/No	Minerals	Chemical Formulae
1	Manganous chloride	MnCl <sub>2</sub> . 4H <sub>2</sub> O
2	Cupric sulphate	CuSO <sub>4</sub> .5H <sub>2</sub> O
3	Zinc sulphate	ZnSO <sub>4</sub> .7H <sub>2</sub> O
4	Cobalt chloride	CoCl <sub>2</sub> .6H <sub>2</sub> O
5	Ferrous sulphate	FeSO <sub>4</sub> .7H <sub>2</sub> O
6	Potassium iodide	KI
7	Selenium dioxide	SeO <sub>2</sub>

**Table 4: Composition of Laboratory Trace Mineral Premix:**

S/No	Minerals	Quantity (mg per Unit weight of Premix)
1	Copper (Cu)	8,000
2	Manganese (Mn)	64,000
3	Zinc (Zn)	40,000
4	Iron (Fe)	32,000
5	Selenium (Se)	160
6	Iodine (I)	800
7	Cobalt (Co)	400

## 2.5 Parameters investigated

Feed intake (measured daily), and growth performance (body weight and weight gain, measured on weekly basis), were recorded. Feed efficiency was calculated from these parameters.

Packed cell volume (PCV), was determined by the microhaematocrit method. The improved Neubauer haemocytometer method was used for the Red Blood cell (RBC) count, and Haemoglobin (Hb) concentration, was determined using the cyanomethaemoglobin method. Mortality rate within the different treatment groups was also recorded. At the end of the feeding trial, three birds per replicate of treatment groups were randomly picked, weighed, exanguinated and plucked. The eviscerated weights were determined before cutting the carcasses into parts. The dressing percentage was calculated as eviscerated weight divided by live weight and multiplied by 100. The weights of body parts were expressed as percentages of the eviscerated weight. These parameters were used to determine the carcass yield. Organs, namely, liver, heart, gizzard, kidneys, pancreas and spleen were excised, dried with blotting paper and the weight of each organ was determined and expressed as percent of body weight (Adejumo, *et al*, 2005). The feed cost benefit of broilers fed experimental diets was also ascertained (Adejumo, *et al*, 2005).

Feed, Liver and blood samples were analysed for zinc content using AOAC method (AOAC, 1975), (Table 6). According to Vinson and Bose (1981), the slope of a line graph of zinc concentration in blood serum, against zinc concentration in feed would represent the bioavailability of zinc.

## 3. Statistical Analyses:

Data collected on feed intake, live weight, weight gain and feed conversion efficiency were analyzed with computer software package (SPSS version 9.0), using analysis of variance (ANOVA) and treatment means compared by the Duncan's New Multiple Range Test (Daniel, 1995).

## 4. Results and Discussion

The three clay samples collected were designated clay<sub>(d)</sub>, clay<sub>(a)</sub>, and clay<sub>(r)</sub> for dark, ash, and red coloured clay respectively, and results of their trace mineral content are shown in Table 5.

Clay<sub>(a)</sub> has the highest trace mineral values for most of the trace minerals analyzed, namely, molybdenum, selenium, iodine, manganese, cobalt, calcium and also for iron. It was therefore chosen, for use in compounding of experimental diets, based on the result of the mineral analysis.

The essence of trace mineral supplementation is to augment the quantity of these minerals present in feeds especially in cases of deficiency.

**Table 5: Result of Mineral Analysis of Three Clay Samples (mg/100ml)**

Clay Samples	Clay (d)	Clay (a)	Clay (r)
Minerals (mg/100ml)			
Iron (Fe)	293.00	3.00	4.00
Zinc (Zn)	10.00	5.50	7.59
Copper (Cu)	12.00	2.40	16.00
Molybdenum (Mo)	0.05	1.70	1.40
Sulphur (S)	Trace	3.40	0.90
Iodine (I)	Trace	0.90	0.60
Manganese (Mn)	0.06	15.90	12.60
Cobalt (Co)	0.08	0.30	0.00
Calcium (Ca)	3,600	7,600	4,700
Phosphorus (P)	312	338	431

At the starter phase T1 (without additional trace mineral) recorded significantly higher values than T2 – T4 (whose diets were supplemented with either clay or Lab. TMP) for feed intake, live weight, weight gain, and feed conversion efficiency ( $p < 0.05$ ). At the finisher phase however, feed intake, live weight, and weight gain were significantly higher for T3 (clay inclusion at 2%) than the rest. These results suggest that the basal diet, without supplementation, contained enough trace minerals to support growth during the starter phase. Due to increased demand on biological function associated with the finisher phase of production, the requirements for the trace minerals probably increased, making the diet supply of minerals suboptimal at the finisher phase. In other words, marginal deficient trace mineral nutrition in T<sub>1</sub> during the growing phase possibly had a negative carryover effect into the finishing phase which resulted in decreased growth performance.

This result is similar to the finding by Chen and Moran (1995) who reported that growing birds and swine fed diets deficient in calcium and phosphorus had reduced finishing performance even when offered nutritionally adequate diets. The 2% sand added to the T1 group may have contributed to the better performance results in efficiency of feed utilization especially at the starter phase.

**Table 6: Summary of Performance Characteristics of Broiler Chicks (between week two and four – starter phase), with SEM in Brackets**

Parameters	Experimental Diets			
	T1 (No TMP)	T2 (Lab. TMP)	T3 (2% Clay)	T4 (1% Clay)
Initial weight (g)	160.00 (7.30)	162.86 (9.00)	159.78 (8.42)	161.10 (6.20)
Mean Feed Intake	2106.2 <sup>5a</sup> (39.56)	1986.7 <sup>5b</sup> (28.39)	1998.5 <sup>0b</sup> (35.68)	1902.0 <sup>0b</sup> (35.87)
Mean live weight (g)	991.00 <sup>a</sup> (27.96)	872.00 <sup>b</sup> (11.28)	949.25 <sup>a</sup> (22.07)	796.00 <sup>c</sup> (9.46)
Mean body weight gain (g)	831.00 <sup>a</sup> (27.96)	716.67 <sup>b</sup> (12.58)	789.25 <sup>a</sup> (22.07)	636.00 <sup>c</sup> (9.47)
Feed Conversion Efficiency (FCE)	0.41 <sup>a</sup> (0.02)	0.36 <sup>b</sup> (0.002)	0.39 <sup>c</sup> (0.004)	0.33 <sup>d</sup> (0.003)
Mortality (%)	0	0	0	0
Feed cost/kg (₹)	60.00	60.00	60.00	60.00
Cost of feeds consumed/chick (₹)	126.60	119.40	120.00	114.00
Feed cost/body weight gain (₹) (apparent cost per broiler)	51.91	42.98	46.80	37.62
Economic Efficiency (apparent cost/Net Revenue)	0.308	0.372	0.302	0.437
Economic Ranking	3	2	4	1

<sup>abc</sup> Different superscripts in a row indicate significant difference between the treatment means at the level of probability,  $P < 0.05$ .

Korean National Livestock Research Institute (KNLRI, 1999) reported that sand increased the 8<sup>th</sup> week body weight of broilers by 4% and Papaioannou *et al.*, (2004) fed pigs with diets containing 2% clinoptilolite type of clay and also reported significant improvement in FCE. It is however, known that sand functions as grit for chickens, in which case it aids the ventriculus (muscular stomach) of the chicken in feed grinding (Physical digestion) (KNLRI, 1999).

Clay inclusion at 2 % (T3) performed significantly better than same at 1 % (T4) in terms of live weight, weight gain, and to an extent feed intake and feed conversion efficiency ( $p < 0.05$ ). It suggests that trace minerals in clay are biologically available. The discrepancy observed in performance at starter phase between T1 and T2 which had 2 and

1.9 percent sand inclusions respectively raises a suspicion over the possibility of unavailability (biologically) and/or toxicity due to contamination from impure inorganic salts used in preparing the laboratory trace minerals used for T2. Mineral supplements are known to contain trace residues of toxic metals, especially cadmium and lead (EFSA, 2014<sup>1</sup>; EFSA, 2014<sup>2</sup>). Mineral supplements are therefore considered one of the feedstuffs with the highest toxic metal concentrations and the maximum admissible concentrations established by the European Union (EC Directive, 2002) are higher than that for most of the other feedstuffs (McBride, 1998).

Economic ranking favoured the less profitable dietary treatments. Leeson and Summers (1997) observed that experimental diets with lowest feed efficiency may not always be the most profitable because “economics, can detect the optimum use of lower rather than higher diet energy.” At the finisher phase the various parameters that determine feed cost benefit became more closely related resulting in similar economic efficiencies.

Mortality rate was not high except for T1. Post mortem of dead birds revealed no gross lesions specific to any disease. However, haemorrhage was noticed on the serosal and mucosal surfaces of organs suggesting that birds probably died from heat stress. This may have resulted from overheating of the brooding room in a bid to protect them from fluctuating temperatures between the day time and night. Treatment did not affect haematology parameters and carcass yield significantly ( $p > 0.05$ ).

For bioavailability study a trace element was picked for the study. Zinc was chosen based on the fact that methods of estimation vary and depend on the individual trace mineral (Finley, 2006), and availability of zinc is easily measured by an indirect absorption method which quantitates liver and serum level after feed ingestion.

**Table 7: Summary of Performance Characteristics of Broiler Chicks (between week two and eight - all through the experiment), with SEM in Bracket.**

Parameter	Experimental Diets			
	T1 (No TMP)	T2 (Lab. TMP)	T3 (2% Clay)	T4 (1% Clay)
Initial weight (g)	160.00 (7.30)	162.86 (9.00)	159.78 (8.42)	161.10 (6.20)
Mean Feed Intake	2106.2 <sup>a</sup> (39.56)	1986.7 <sup>b</sup> (28.39)	1998.5 <sup>b</sup> (35.68)	1902.0 <sup>b</sup> (35.87)
Mean live weight (g)	991.00 <sup>a</sup> (27.96)	872.00 <sup>b</sup> (11.28)	949.25 <sup>a</sup> (22.07)	796.00 <sup>c</sup> (9.46)
Mean body	831.00 <sup>a</sup>	716.67 <sup>b</sup>	789.25 <sup>a</sup>	636.00 <sup>c</sup>

weight gain (g)	(27.96)	(12.58)	(22.07)	(9.47)
Feed Conversion Efficiency (FCE)	0.41 <sup>a</sup> (0.02)	0.36 <sup>b</sup> (0.002)	0.39 <sup>c</sup> (0.004)	0.33 <sup>d</sup> (0.003)
Mortality (%)	0	0	0	0
Feed cost/kg (₹)	60.00	60.00	60.00	60.00
Cost of feeds consumed/chick (₹)	126.60	119.40	120.00	114.00
Feed cost/body weight gain (₹) (apparent cost per broiler)	51.91	42.98	46.80	37.62
Economic Efficiency (apparent cost/Net Revenue)	0.308	0.372	0.302	0.437
Economic Ranking	3	2	4	1

<sup>abc</sup> Different superscripts in a row indicate significant difference between the treatment means at the level of probability,  $P < 0.05$ .

Clay inclusion was found to increase trace mineral levels of feed as well as serum and liver concentration (Table 8), and result from dose – response assay confirms bioavailability of clay mineral (Figure 1).

A bioavailability score of 32% for zinc (Figure 1) may appear poor, however, inorganic sources of minerals are known for their poor biological availability (NRC, 1998; Alonso *et al.*, 2004). Report from other researchers (Boranic, 2000; Marthin-Kleiner *et al.*, 2001; Enemark *et al.*, 2003) indicate that trace mineral release from feed ingredients and bioavailability pattern is not systematic, which may be due to level of nutrient antagonists (binders) present in the feed, complexity of the diet and stress level for the animal, all of which affect nutrient release and bioavailability *in vivo*. However, Boranic (2000) reported that zeolites (a type of clay) when ingested with the diet led to a shift of pH and buffering capacity of gastrointestinal secretions, affected transport through the intestinal epithelium. Also changes in composition of intestinal flora and resumption of bacterial products, vitamins and microelements were also found. This observation informed the therapeutic use of encapsulated zeolite powders enriched with vitamins, oligoelements or other physiologically important ingredients for oral administration. Also, the humic shale type of clay which contains 60-70 minerals and trace elements, all in the fulvic colloidal hydrophilic form, is considered to be earths greatest store house of

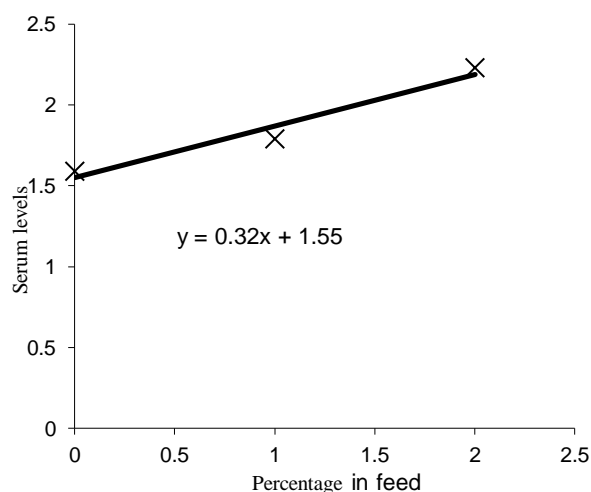
minerals and trace elements with some variations in mineral quantities. It is said to be an ideal supplement for human and livestock and are used for the remineralisation of farm lands and gardens (Mike, 2005).

## 5. Conclusion

Clay at 1% and 2% inclusion levels is well tolerated in broiler diets. However, 2% clay inclusion sustains better productivity than 1% and also improved performance characteristics such as feed intake, daily gain, feed efficiency and hence live weight. Use of clay in place of trace mineral premix in broiler diets attracted no significant effect on feed cost benefit of experimental diets. Results of this study therefore indicate the applicability and acceptability of clay supplemented diets for broilers as a safe, trace mineral supplement, at 2% level of inclusion. It is suggested that clay at 2% inclusion level may be used to replace commercial trace mineral premix in the formulation of broiler diets.

**Table 8: Laboratory Analysed Concentration of Zinc in Feed, Blood Serum and Liver; SD in Bracket.**

Samples	Experimental Diets			
	T <sub>1</sub> (No TMP)	T <sub>2</sub> (Lab. TMP)	T <sub>3</sub> (2% Clay)	T <sub>4</sub> (1% Clay)
Feed (µg/g)	17.82	44.55	62.37	59.40
Serum (µg/ml)	1.59 (0.24)	1.94 (0.02)	2.23 (0.06)	1.79 (0.18)
Liver (µg/g)	0.89 (0.35)	1.89 (0.83)	4.31 (0.83)	3.08 (0.14)



**Fig. 1:** A Graph of Zinc Concentration in Blood Serum, Against Zinc Concentration in Feed.

## References

- [1] Adejumo, D. O, Onifade, A. A., Olutunde, T. O. and Babatunde, G. M. The effect of concentration, age and duration of feeding supplementary yeast (Levucel SB®) in a high fibre diet on the performance of broiler chickens. In: Proc of 1st Nigeria Int. Poultry Summit (NIPS), Held at Ota, Nigeria: 69-76, (2005).
- [2] Alonso, M. L. Trace minerals and livestock: Not too much not too little. Bergh, Ø., Butaye, P., Kogut, M. H., and Whisnant, S. eds. ISRN. Veterinary Science: 1-18, (2012).
- [3] Alonso, M. L., Motaria, F. P., Miranda, M., Castillo, C., Hernandez, J., Benedito, J. L. Interactions between toxic (As, Cd, Hg, and Pb) and nutritional essential (Ca, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Zn) elements in the tissues of cattle from NW Spain. *Biometals*; 17(4): 389-397, (2004).
- [4] Association of Official Analytical Chemists, (AOAC), Official Method of Analysis, 12th ed., Washington D.C. (1975).
- [5] Boranic, M., What a Physician should know about Zeolites, L©: ec.vjesn. 122, 292-298, (2000).
- [6] Chen, X., and E. T. Moran (Jr.), Response of Broilers to omitting Dicalcium Phosphate from the Withdrawal Feed: Life Performance, Carcass Downgrading, and Further Processing Yields. *J. Appl. Poultry Residues* 3:74-79, (1994).
- [7] Daniel, W. W., *Biostatistics: A Foundation for Analysis in the Health Science* 6th ed. John Wiley and Sons Books, New York, (1995).
- [8] European Commission (E.C.) Directive 2002/32/EC of the European Parliament and of the Council, Undesirable substances in animal feed. *Journal of the European Commission, OJ, L140/10; e.12*, (2002).
- [9] European Food Safety Authority (EFSA), Opinion on the scientific panel on contaminants in the food chain on a request from the commission related to lead as undesirable substance in animal feed. Adopted on 2nd June, 2004. *The EFSA Journal*; 71: 1-20, (2004<sup>1</sup>).
- [10] European Food Safety Authority (EFSA) Opinion on the scientific panel on contaminants in the food chain on a request from the commission related to cadmium as undesirable substance in animal feed. Adopted on 2nd June, 2004. *The EFSA Journal*; 72: 1-24, (2004<sup>2</sup>).
- [11] Enemark, J. M., Frandsen, A. M., Thilsing-Hansen, T., Jorgensen, R. J.,

- Aspect of Physiological Effects of Sodium zeolite: A supplementation in Dry, Non-pregnant Dairy Cows fed Grass. *Silage Acta Vet, Scand. suppl*, 97, 97-117, (2003).
- [12] Food and Agricultural Organization (FAO), Animal Production and Health Paper 114 (Proc. of an FAO Expert Consultation Rome, 24-28th June, 1991: 104-120, (1993).
- [13] Finley, J.W., Bioavailability of Serum from foods. *Nutrition Reviews*, Vol. 64 (3): 146-151, (2006).
- [14] Gerber, P. J. and Steinfeld, H., Worldwide growth of animal production and environmental consequences. In: Trace elements in animal production systems, Schlegel, P., Durosoy, S., Jongbloed, A. eds. Wageningen Academic Publishers, The Netherlands.: 21-32, (2008).
- [15] Korean National Livestock Research Institute (KNLRI), Poultry Production: Low Cost Technology, KNLRI: 7, (1999).
- [16] Leeson, S, and Summers, J. D., Commercial Poultry Nutrition. 7th Rev. ed., (1997).
- [17] Loladze, I., Hidden shift of the ionone of plants exposed to elevated CO2 depletes minerals at the base of human nutrition. Baldwin, I.T., reviewing editor, eLife Sciences Publications Ltd.; 3: e02245, (2014).
- [18] Marthin-Kleiner, I, Z. Fleger-Mestric, R. Zadro, D Breljak, C. Stanoric, S. Jorda R. Stojkovic, M. Marusic, M. Radacic, and M. Boranic., The Effect of the Zeolite Clinoptilolite on Serum Chemistry and Haematopoiesis in Mice. In: *Veterinary Med. Czech*, 49, 2004 (10); Review Article 395 (Kaolin, Bentonites, and Zeolites): 389-399, (2001).
- [19] McBride, M. B., Growing food crops on sludge amended soils: Problems with the US environmental Protection Agency method of estimating toxic metal transfer. *Environmental Toxicology Chemistry*; 17: 2274-2281, (1998).
- [20] Mike, C. Online, Colloidal Minerals. <http://www.mikes.choice.com>.  
File://A:/colloidal.minerals.liquid.  
Supplement.concentrate.by.mike.choice.humik.shaleclay.htm. (2005).
- [21] National Research Council (NRC), Nutrient Requirement of Swine. National Academy of Sciences, National Academy Press, Washington DC, USA, (1998).
- [22] Papaioannou, D. S., Kyriakis, C.S., Alexopoulos, C., Tzika, E.D., Polizopoulou, Z.S., Kyriakis, S.C., A field study on the Effect of Dietary use of a Clinoptilolite – Rich Tuff, Alone or in combination with certain Anti-microbial on the Health status and Performance of weaned growing and finishing pigs. *Res. Vet. Science* 76: 19-29,. (2004)
- [23] Suttle, N. F., Mineral nutrition of livestock, Cabi Publishing Company (2010).
- [24] Thompson, D. J., Trace Elements in Animal Nutrition. International Minerals and Chemical Corporation (IMCC) Skokie, Illinois 60079: 1-28, (1970).
- [25] Vinson, J. A. and Bose, P., In: Comparison of the Bioavailability of Trace Elements... Procedure on Mineral Elements: 615-621, (1981).