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Wolde, S.; Negesse, T.; Melesse, A.

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**THE EFFECT OF DIETARY PROTEIN CONCENTRATION ON
NUTRIENT UTILIZATION OF RHODE ISLAND RED CHICKEN IN
WOLAITA (SOUTHERN ETHIOPIA)**

**[EFECTO DE LA CONCENTRACIÓN DE LA PROTEÍNA DIETÉTICA
SOBRE LA UTILIZACIÓN DE NUTRIENTES EN POLLOS RHODE
ISLAND RED EN WOLAITA (SURESTE DE ETIOPIA)]**

S. Wolde^a, T. Negesse^{b*} and A. Melesse^b

^a*Ministry of Agriculture and Rural Development, Southern Nations Nationalities
and Peoples Regional State, Wolaita Zone, Boditi, P.O.Box 54, Ethiopia, E-
mail: shewanwolde@yahoo.com*

^b*Animal and Range Science Department, College of Agriculture, Hawassa
University, E-mail: a_melesse@yahoo.com*

** P.O.Box 336, Ethiopia, E-mail: sgsdean@yahoo.com*

**Corresponding author*

SUMMARY

The effect of dietary crude protein (CP) concentration on feed intake, nutrient retention and efficiency of utilization of growing Rhode Island Red (RIR) chicks fed for a period of 12 weeks from 9 to 93 days of age was assessed and CP requirement determined in Wolaita, southern Ethiopia. Diets with 14% (T1), 16 % (T2), 18 % (T3), 20 % (T4) and 22 % (T5) CP levels were evaluated. At the beginning of trial, ten chicks whose average weight was closest to weight of chicks in experimental groups were slaughtered and at the end of the experiment, 3 selected male and 3 female (1 male and 1 female per replicate) were slaughtered for efficiency of utilization of protein (EUP) estimation. Dry matter intake (DMI) of chicks with different levels of CP was not different ($P>0.05$). T3 had the highest and T2 the lowest ($P<0.05$) intake values of most of the nutrients. Chicks in T1 retained significantly ($P<0.05$) lower protein than chicks fed on T3. Chicks were not different in energy, calcium and phosphorus retention ($P>0.05$). Chicks fed on T4 and T5 were lower in EUP than T2 ($P<0.05$) whereas chicks fed on T1 and T3 fall in between. Chicks fed different levels of dietary CP were not different in efficiency of energy and phosphorus utilization ($P>0.05$). Lower ($P<0.05$) efficiency of utilization of calcium was obtained on T1 than on T4, whereas chicks fed on T2, T3 and T5 fall in between. Optimum efficiency of nutrient utilization and retention were obtained at 16% dietary CP level and could be recommended as CP requirement of RIR chicks up to the age of 13 weeks under tropical climate. Intakes of energy (572 kJ/head), DM, Ca and P (40, 0.38 and 0.21 g/head, respectively) at 16% dietary CP level could also be recommended as daily allowances.

Key words: dietary crude protein level; efficiency of nutrient utilization; Rhode Island Red

RESUMEN

Se evaluó el efecto de la concentración de proteína (CP) dietética sobre el consumo de alimento, retención y eficiencia de utilización de nutrientes de pollos Rhode Island Red en crecimiento y se estimó el requerimiento de proteína. Las dietas se formularon para 14% (T1), 16 % (T2), 18 % (T3), 20 % (T4) y 22 % (T5) CP. Al inicio del experimento 10 aves con peso similar a los grupos experimentales fueron sacrificadas y al término del experimento 3 aves machos y 3 hembras por tratamiento fueron sacrificadas para estimar la eficiencia de utilización de la proteína (EUP). El consumo de alimento fue similar entre tratamientos ($P>0.05$). El consumo de nutrientes fue mayor en T3 y menor en T2 ($P<0.05$). Las aves en T1 tuvieron una menor retención de proteína en comparación con T3 ($P<0.05$). No se encontró diferencias en la retención de energía, Ca y P ($P>0.05$). Las aves del T4 y T5 tuvieron una menor EUP en relación a T2 ($P<0.05$) y T1 y T3 tuvieron valores intermedios. No se encontró diferencias en la eficiencia de utilización de energía y P ($P>0.05$). La menor eficiencia de utilización de Ca se encontró en T1 en comparación con T4 ($P<0.05$), T2, T3, y T5 y tuvieron valores intermedios. La eficiencia óptima de utilización y retención de nutrientes se obtuvo con 16% de CP, los cuales pudieran ser recomendados como requerimientos de estas aves hasta la edad de 13 semanas en condiciones tropicales. El consumo de energía (572 kJ/ave), materia seca, Ca y P (40, 0.38 y 0.21g/ave respectivamente) a un nivel de 16% de CP pudiera ser recomendado como dieta diaria.

Palabras clave: nivel proteína dietética; eficiencia de utilización de nutrientes; Rhode Island Red.

INTRODUCTION

Poultry production in Ethiopia has remained as a backyard venture. It is now emerging as an encouraging enterprise for rural smallholder farmers, landless laborers and also for big entrepreneurs who are maintaining large number of birds. Poultry rearing is not anymore considered as a non-prestigious occupation fit for only weaker sections of the society. It has rather become a fulltime job for many societies (Gopalakrishnan and Lal, 2004).

There are several constraints for poultry production; namely feeding, disease, predator, lack of skilled manpower, poor management and breed. According to Demeke (1996), availability, quality and cost of feed are the major constraints to poultry production in Ethiopia which is not self sufficient in cereal grains that form the bulk of concentrate feeds for poultry. There are shortages of protein supplements and micro-nutrients (vitamins and minerals) in the country which are needed for the preparation of balanced rations.

Quality and quantity of protein is important particularly for young, rapidly growing and mature high producing animals. Optimal use of protein is a must in any feeding system, because protein supplements are usually more expensive than energy feeds, and wasteful usage of the same increases the cost of production. Another consideration is that when excess protein is fed there will be increased elimination of nitrogen in feces and urine, which has implication on environmental pollution (Church and Kellems, 2002). In tropical areas, feeding high proteins to broilers is not recommended because protein has the highest heat increment value amongst all the nutrients (Furlan *et al.*, 2004) and impairs bird's performance.

There have not been consistent reports on the effects of dietary protein levels on the whole body composition of RIR chicken. In lower amount of protein in diets leads to poor carcass composition. It therefore becomes necessary to know the exact protein level for the optimum utilization of the nutrient thereby assisting in estimating protein requirement for the formulation of a well-balanced diet. In this context, comprehensive research is essential for a more detailed knowledge about whole body composition of chicks corresponding to the supply of proteins. The objective of this study was to evaluate the effects of dietary protein levels on nutrient retention and utilization of growing RIR chicken and determine nutrient requirements.

MATERIALS AND METHODS

Study site

The feeding trial with RIR chicks was conducted at Wolaita Soddo Poultry Husbandry Center located at 1884 meter above sea level, at 6.72-6.99°E and 37.61-37.88°N. The rainfall in the region is bimodal and ranges between 1201 and 1600 mm with a mean annual temperature ranging between 22 and 24°C.

Experimental diets

The major feed ingredients (maize, soybean, noug-Niger seed-cake and sunflower seed cake) were ground with a miller and mixed with limestone, methionine, lysine, rear premix and salt. Soybean meal, sunflower seed cake and noug seed cake were included at different proportions to obtain different protein concentrations. The level of protein in the diets was increased from 14.03 to 22.00 % by replacing maize and wheat bran with soybean meal, sunflower seed cake and noug seed cake.

The calculated energy, crude fiber, calcium and phosphorus contents of the diets were more or less similar. The proportion of feed ingredients and nutrient composition of experimental diets are presented in Table 1.

The contents of rear premix on kg diet basis: ash 655 mg, crude protein 135 mg, crude fat 2 mg, crude fiber 9 mg, lysine 90 mg, methionine 20 mg, threonine 5 mg, calcium 100 mg, sodium 135 mg, chloride 230 mg, copper 3,000 mg, iron 4,000 mg, manganese 6,000 mg, zinc 5,000 mg, cobalt 20 mg, iodine 80 mg, selenium 15 mg, vitamin A 1,000,000 IU, vitamin D3 200,000 IU, vitamin E 1,500 mg (Pre-Mervo, Utrecht. EXPVALK). The calcium content of limestone was assessed to be 35% (Boushy and Van der Poel, 2000). The nutrient composition in Table 1 was calculated based on the information presented in Table -2. The proximate analysis was carried out in the laboratory of National Veterinary Institute at Debrezeit, Ethiopia. The metabolizable energy values were calculated according to the method suggested by Wiseman (1987).

Management of experimental animals

Experimental rooms were disinfected with 37% formalin two weeks prior to the introduction of chicks. The chicks were maintained under deep litter management system and the waters and feeders were cleaned twice a week.

A total of 250 unsexed day-old Rhode Island Red (RIR) chicks were purchased from Bonga Poultry Husbandry Center and were kept under electric

brooder for nine days at Wolaita Soddo Poultry Husbandry Center. The under average chicks were segregated from the lot and a total of 200 chicks were randomly divided in to 20 replicates of 10 chicks each with initial average body weight of 51.8 ± 0.5 g. Four replicates were randomly assigned to each of the 5 treatment diets. The selected chicks were fed in group and provided with diets *ad libitum* for 84 days.

Chicks were vaccinated against New Castle and Gumboro diseases maintaining standard protocols. The amount of feed offered was weighed daily in the morning and fed to chicks in two halves. The daily increase in feed was 10% of what was offered on the previous day. Water was available *ad lib* through out the day. The feed leftover was collected and weighed prior to giving fresh feed for the day. The chicks were weighed individually at the beginning of the experiment and weekly till the end of the experiment.

Whole body analysis for nutrient retention

At the beginning of the experiment, 10 chicks, whose average body weight was about same as the average weight of the experimental chicks in the 20 groups, were selected and killed by dislocation of the neck. The slaughtered chicks were put in plastic bags and kept in a deep freezer at -20°C until they were processed for whole body chemical analysis. At the end of the experiment from randomly selected 3 replicates per treatment, 3 male and 3 female (1 male & 1 female per replicate) chicks were selected, the selected chicks weighed close to the average weight of their respective group and sex. And chicks were identified by leg banding. They were then fasted for 12 hours prior to slaughter with the aim to minimize the influence of gut content (digesta) on chemical composition of the whole body. They were then weighed and transferred to labeled plastic bags and put in a deep freezer.

The whole body of each chick was chopped while still frozen and retransferred to the freezer until it was minced using a commercial mixer, then put again back to the freezer. After thawing, 20 g of representative samples were taken from each of the homogenized samples for dry matter determination at 105°C and dried for 12 hours until constant weights were attained. The minced mass was dried in an oven at 65°C for 80 hours for nutrient analysis. After drying, it was ground using hand mortar.

The ground mass were sent to National Veterinary Institute (Ethiopia) for chemical analysis. The dry matter, mineral matter, lipids, nitrogen, calcium and phosphorous were analyzed. These values (on DM basis) multiplied by their respective total DM in the carcass gave the amount of nutrients deposited in the whole body. The amount of each nutrients retained

during the experimental period were calculated as a difference between initial and final concentration of nutrients in the body. The amount of each nutrient retained daily was also estimated by dividing the total amount of nutrient retained by the duration of the experiment.

Data analysis

Nutrient intake, nutrient retention and nutrient efficiency data were subjected to ANOVA using the software package SAS version 6.12. Duncan multiple range test was used for separating differences between treatment means, and differences were considered significant at $P \leq 0.05$ levels.

Nutrient retention data were also analyzed using the following polynomial regression model (SPSS Version 12.0 for Windows, 2003) and dietary crude protein level was a continuous variable: $y = c + bx_i + ax_i^2 + e_{ij}$, where c = estimated value of y when $x = 0$; b and a = linear and quadratic terms; x_i and x_i^2 = regression variables; e_{ij} = random error. Half the ratio of b/a or $1/2(b/a)$ is the point at which the equation levels off and represents the maximum possible value of the curve because b were positive and a negative.

RESULTS AND DISCUSSIONS

Chemical composition of feeds

Chemical composition of feed ingredients is presented in Table 2.

Nutrient intake

As shown in Table 3 the mean daily DM consumption of RIR chicks fed the different treatment diets were not significantly ($p > 0.05$) different which indicates that dietary CP level had no influence on DM intake of chicks. This could probably be related to the close proximate energy contents of the diets as energy level influences feed intake in birds (Melesse, 2007). It is possible that the palatability of feeds were perhaps not affected by crude protein (CP) levels as suggested by Pond *et al.* (2005). The result pertaining to feed intake and dietary CP level as obtained in the present study are in agreement with the observations of Kamran *et al.* (2000) and Oyediji *et al.* (2005). However, Pfeffer *et al.* (2000) reported that broiler chicks reared on diets containing various levels of CP prefer diets containing the lowest CP content. Contrary to the present findings, Urdaneta-Rincón and Leeson (2008) reported that chicks fed on low CP diet (17%) had a significantly ($P < 0.05$) reduced feed intake in comparison to the chicks reared on dietary CP ranging from 19% to 25%.

The CP intake of chicks was increased with CP level in the diets. Chicks fed on T2 had significantly ($p < 0.05$) lower energy intake than those on T3 whereas chicks fed T1, T4 and T5 fall in between the above. The calcium intake of chicks reared on T2 and T5 were significantly ($p < 0.05$) lower than T3 whereas chicks fed on T1 and T4 fall in between. Chicks raised on T2 and T4 consumed significantly ($p < 0.05$) lower phosphorus than those reared on T3 whereas chicks fed on T1 and T5 fall in between the above.

Nutrient retention

The chicks receiving lowest dietary crude protein (14%) resulted in significantly ($p < 0.05$) lower protein retention than those raised on 18% dietary CP (Table 3). The ANOVA indicated that chicks receiving 16%, 20% and 22% dietary CP fall in between and were not significantly different among each other. Protein retention increased linearly with increasing dietary CP level up to 18%, thereafter it remained constant. However, polynomial regression analysis revealed that CP retention was significantly influenced by dietary CP concentration and it (1.8 g CP/chick/d) leveled off at slightly higher (19.0%) dietary CP (Figure 1) than that obtained by ANOVA. The results indicate that the daily deposition of protein was independent of protein intake, or the deposition was impaired at higher crude protein levels perhaps due to higher heat increment that might have increased heat loss and maintenance requirement of chicks as suggested by Furlan *et al.* (2004). It could also be due to reduction in the utilization of nitrogen by the chicks because of high level of its excretion. The present findings are in consonance with the results obtained by Leeson & Summers (1997). However, Hai and Blaha (2000) and Kamran *et al.* (2000) observed that decrease in dietary CP level had no negative effects on carcass proteins.

The ANOVA and regression analysis ($y = -0.1887x^2 + 7.112x - 7.453$) showed that energy retention of chicks fed different levels of dietary CP were not significantly ($p > 0.05$) different, however, the numerical differences between T2 and diets with higher CP levels could be perhaps because the chicks on the rest of the diets might have used more energy to catabolize excess protein rather than depositing it in body tissues, which finds similarity with the result reported by Urdaneta-Rincón and Leeson (2008).

The ANOVA revealed that calcium and phosphorus utilization and retention values were not significantly different between groups, except for the chicks reared on T3 diet. The similarity of the same can be attributed to similarity in the ratio of Ca:P and lack of antagonism between the two minerals as suggested by Perry *et al.* (2004). However, the polynomial regression analysis showed that calcium retention, as opposed to P retention ($y = -0.00000583x^2 +$

$0.002102x - 0.004849$, $P \geq 0.05$), was significantly ($P \leq 0.05$) affected by the amount of dietary CP and maximum Ca retention (7.1 g Ca/head/d) was obtained at 19.3 % dietary CP (Figure 1).

Efficiency of utilization of nutrients

Efficiency of utilization of protein (EUP) was expressed as the ratio of CP retained per unit of CP intake (Table 3).

Polynomial regression analysis of efficiency of utilization of nutrients did not show significant differences among dietary CP levels. Efficiency of utilization of protein ($y = -0.0016x^2 + 0.0521x - 0.1653$) and calcium ($y = -0.0021x^2 + 0.0842x - 0.5755$) crossover each other at 17.6% dietary CP level. Differences in EUP among T1, T2 and T3 and among T3, T4 and T5 were not statistically significant. However, chicks fed on T2 had numerically the highest EUP which was significantly ($p < 0.05$) higher than that of T4 and T5. And chicks fed on T5 had numerically lowest EUP. The EUP linearly increased from T1 to T2 and then after it declined. The present findings find similarity with the results obtained by Pfeffer *et al.* (2000) who reported that protein retentions were more efficient in the lower protein diets when broiler chicken were fed diets with 225, 210, 190, 172, 153 g/kg CP from 3-6 weeks of age. However, CP intake of chicks was increased as the level of CP content in diets increased. This indicates that chicks fed above 18% CP might have excreted excess nitrogen rather than utilizing it or the crude protein was metabolized and increased heat increment.

Chicks fed on different levels of dietary CP were not significantly ($p > 0.05$) different in the ratio of energy deposited per unit of energy intake (efficiency of energy utilization, EUE), which is in agreement with the observations of Pfeffer *et al.* (2000). Chicks fed on T2 had the lowest energy intake but the highest energy retention resulting in the highest EUE. It could be assumed that the level of nutrients in T2 favored more deposition of energy at lower intake of energy.

Chicks fed on T1 had significantly ($p < 0.05$) lower ratio of calcium deposited per unit of calcium intake (efficiency of utilization of calcium, EUC) than those on T4, and chicks fed on T2, T3 and T5 fall in between. This indicates that pattern of consumption and deposition of calcium of chicks fed on T1 was less than the other diets and may also be attributed to higher level of calcium needed of chicks fed on low CP diet, because lower level of lysine (possibly also other amino acids) and/or protein intake have been reported to reduce Ca absorption (Pond *et al.*, 2005).

Table 1. The proportion of feed ingredients (% as fed) in the diets and nutrient composition of diets (%DM basis)

Ingredients, %	Treatment diets				
	T1	T2	T3	T4	T5
Maize (white)	38.70	36.20	30.80	29.70	25.00
Wheat bran	33.30	24.00	18.75	8.00	2.50
Soybean (roasted)	5.00	11.30	19.70	28.30	37.50
Noug seed cake	9.50	14.00	16.00	18.00	18.50
Sunflower cake (expeller)	10.50	11.50	11.75	13.00	13.85
Limestone	2.00	2.00	2.00	2.00	2.00
Salt	0.50	0.50	0.50	0.50	0.50
Rear premix	0.40	0.40	0.40	0.40	0.40
Lysine	0.05	0.05	0.05	0.05	0.05
Methionine	0.05	0.05	0.05	0.05	0.05
Total	100.00	100.00	100.00	100.00	100.00
Nutrient composition					
DM, %	92.15	90.85	92.00	92.40	92.60
CP, %	14.03	16.02	18.04	20.04	22.00
ME, MJ/kg DM	14.36	14.48	14.60	14.72	14.84
Crude fiber, %	11.53	11.55	11.90	11.87	11.96
Calcium, %	0.95	0.96	0.97	0.96	0.97
Total Phosphorus, %	0.52	0.52	0.53	0.53	0.54
Methionine, %	0.32	0.36	0.40	0.44	0.48
Lysine, %	0.69	0.84	1.03	1.23	1.43
Cystine, %	0.28	0.31	0.35	0.38	0.42
Threonine, %	0.55	0.63	0.72	0.81	0.91
Tryptophan, %	0.16	0.17	0.20	0.23	0.26
Isoleucine, (%)	0.60	0.69	0.78	0.89	0.99
Leucine, (%)	1.16	1.29	1.44	1.60	1.77
Phenylalanine, (%)	0.69	0.78	0.89	1.01	1.12
Tyrosine, (%)	0.32	0.38	0.48	0.56	0.67

Table 2. Chemical composition of feed ingredients

	Feed ingredients				
	Maize (white)	Wheat bran	Soybean (roasted)	Noug seed cake	Sunflower seed cake
Nutrients, % DM					
Dry matter	87.74	88.60	92.22	93.56	94.73
Mineral matter	1.60	4.35	5.75	10.80	5.86
Crude fiber	2.90	15.56	8.12	26.56	27.23
Crude protein	8.65	14.01	31.08	28.24	29.33
Nitrogen free extract	83.50	61.20	32.60	27.10	30.28
Fat	3.30	4.80	22.33	7.25	7.30
Calcium	0.06	0.45	0.28	0.60	0.31
Phosphorus	0.35	0.81	0.67	0.69	0.67
Amino acids ¹					
Methionine	0.18	0.23	0.62	0.31	0.80
Lysine	0.26	0.61	2.69	0.90	1.24
Cystine	0.18	0.32	0.66	0.34	0.64
Threonine	0.40	0.49	1.41	0.79	1.29
Tryptophan	0.10	0.20	0.51	-	0.41
Isoleucine	0.40	0.58	1.56	0.86	1.43
Leucine	1.10	1.07	2.75	1.34	2.22
Phenylalanine	0.50	0.64	1.78	0.87	1.66
Tyrosine	-	0.45	1.34	0.35	0.91

¹Amino acid content of feed ingredients (as fed basis) was estimated according to Tacon (1987), Lauridsen *et al.* (2004) and Perry *et al.* (2004).

Table 3. Nutrient (g/chick/d) and energy (kJ/chick/d) intakes, retentions (g/chick/d) and utilization efficiencies (g retained/g intake) of RIR chicks fed diets with varying dietary CP levels.

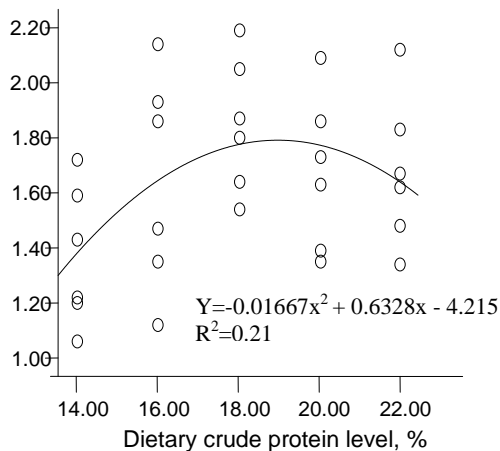
Parameters	Treatment diets					SEM
	T1	T2	T3	T4	T5	
DM intake	40.49	39.82	41.35	40.37	39.29	1.40
CP intake	5.81 ^a	6.31 ^b	7.62 ^c	8.01 ^d	8.70 ^e	0.13
Energy intake	598 ^{ab}	572 ^a	618 ^b	589 ^{ab}	586 ^{ab}	11.01
Calcium intake	0.39 ^{ab}	0.38 ^a	0.41 ^b	0.39 ^{ab}	0.38 ^a	0.01
Phosphorus intake	0.21 ^a	0.21 ^a	0.22 ^b	0.21 ^a	0.21 ^a	0.004
Protein retention	1.37 ^a	1.65 ^{ab}	1.85 ^b	1.68 ^{ab}	1.68 ^{ab}	0.12
Energy retention	53.68	61.52	57.92	57.79	58.68	4.17
Calcium retention	0.07	0.09	0.09	0.11	0.09	0.01
Phosphorus retention	0.02	0.02	0.02	0.03	0.02	0.002
EUP	0.24 ^{ab}	0.26 ^b	0.24 ^{ab}	0.21 ^a	0.19 ^a	0.02
EUE	0.09	0.11	0.093	0.10	0.10	0.01
EUC	0.18 ^a	0.25 ^{ab}	0.21 ^{ab}	0.27 ^b	0.24 ^{ab}	0.03
EUPH	0.10	0.12	0.10	0.11	0.11	0.01

Means within a row with different superscript letters are significantly different ($p < 0.05$).

EUP=Efficiency of utilization of protein EUC=Efficiency of utilization of calcium

EUE=Efficiency of utilization of energy EUPH=Efficiency of utilization of phosphorus

Crude protein retention, g/chick/d



Calcium retention, g/chick/d

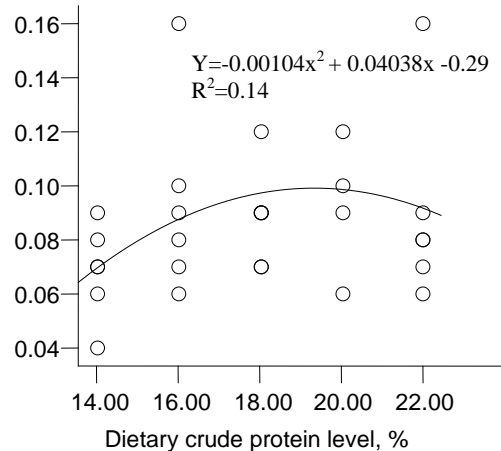


Figure1. The influence of dietary crude protein levels on crude protein and calcium retentions of Rhode Island Red chicks

Differences in the ratio of phosphorus deposited per unit of phosphorus intake (efficiency of utilization of phosphorus, EUPH) were not significantly different among chicks fed on different levels of dietary protein. Chicks fed T1 and T3 had similar EUPH but although insignificant had numerically lower than those fed T2, T4 and T5. Correlation between intake and deposition

of phosphorus for chicks fed on T1 and T3 were slightly less than those on other treatment diets.

Mortality

Mortality was encountered only in chicks fed on T1 (2.5%) and T5 (12.5%) with low and high dietary CP levels. Similar to result of no mortality obtained from

T4 (20% CP) was also reported by Tera (2007) of RIR chicks fed diets containing about same CP level. Tadesse (2007) reported that mortality of RIR chicks was 8.3% at 8 weeks of brooding period fed a diet containing 21.74% CP, which was slightly lower than the mortality (10%) observed at same age of the chicks fed T5 (22% CP) in this experiment. High mortality in chicks fed diets with the highest CP level could be partly explained by the fact that diets with high CP could raise heat stress causing mortality (Quentin *et al.*, 2005; and Furlan *et al.*, 2004). The recommendation of 16% dietary CP in this trial could be supported by the conclusion of these authors that a more concentrated starter diet reduced the resistance of broilers to stresses; and also by their recommendation to reduce dietary CP level in tropical areas for broiler chickens aiming to decrease the amount of heat produced and the harmful effects on birds' performance.

CONCLUSION

The present study indicates that the Rhode Island Red (RIR) chicks fed 16 % dietary crude protein (CP) were similar to chicks fed higher levels of CP in nutrient retention and efficiency of utilization of nutrients. Moreover, mortality was not observed amongst the chicks of the experimental group. It can be concluded that 16% dietary CP levels can be recommended as CP requirement of RIR chicks from 0 to 13 weeks of age raised under tropical climate for optimum nutrient utilization and retention, but if maximization of efficiency of utilization of both CP and Ca is sought, one could go as high as 17.6% CP. Intakes of energy (572 kJ/head), DM, Ca and P (40, 0.38 and 0.21 g/head, respectively) at 16% dietary CP level could also be recommended as daily allowances at 14.5 MJ ME/kg DM.

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