ESTIMATION OF THE REQUIREMENT OF METABOLIZABLE ENERGY AND CRUDE PROTEIN FOR GROWING IN RHODE ISLAND RED CHICKENS, UNDER TROPICAL CONDITIONS OF SOUTHEASTERN MEXICO†

[ESTIMACIÓN DEL REQUERIMIENTO DE ENERGÍA METABOLIZABLE Y PROTEINA CRUDA PARA POLLOS RHODE ISLAND RED EN CRECIMIENTO BAJO CONDICIONES TROPICALES EN EL SURESTE DE MÉXICO]

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SUMMARY

The objective of the current study was to estimate the requirement of metabolizable energy (ME) and crude protein (CP) for growing Rhode Island Red male chickens from 17 to 119 days-old, under tropical conditions of Yucatan, Mexico. The experiment consisted in three feeding phases (17–56, 57–91, 92–119 days old), using a factorial 2x3 design for each one. Two constant ME (2900 and 3100 kcal/kg DM), and three CP concentrations (% DM) that varied with age: 22, 20 and 18% (17–56 days-old); 20, 18 and 16% (57–91 days-old); and 18, 16 and 14% (92–119 days-old) were used. Two batches of 72 Rhode Island Red male chickens each one was used; the first one for the first feeding phase and the second batch for the second and third feeding phases. Response surface procedures to obtain the best ME and CP concentrations were carried out. The body weight gain (BWG) and feed intake (FI) were greater in the first feeding phase with 2900 kcal ME/kg DM in the diet; however, in the second phase there was not significant effect of energy concentration. From 17–91 days-old, the FI increased with 2900 kcal ME/kg DM in the diet. Chickens had a higher BWG from 92–119 days-old when 16% CP was included in the diet. Protein efficiency was better from 17–119 days-old when the lower CP concentration was included in the diet. In conclusion, the greatest BWG were obtained, when 2900 kcal ME/kg DM, for the three feeding phases, was used. The best approximations of CP requirement in the diet for the first, second and third feeding phases were 21.3%, 19.2% and 17.1%, respectively.
Keywords: Requirements; slow growing; response surface; Rhode Island Red chickens.

RESUMEN

El objetivo del presente estudio fue estimar el requerimiento de energía metabolizable (ME) y proteína cruda (PC) de pollos machos de Rhode Island Red de 17 a 119 días de edad, en condiciones tropicales de Yucatán, México. El experimento consistió en tres fases de alimentación (17–56, 57–91, 92–119 días), utilizando un diseño factorial 2x3 para cada una. Dos concentraciones constantes de ME (2900 y 3100 kcal / kg MS) y tres concentraciones de PC (% DM) que variaron con la edad: 22, 20 y 18% (17–56 días); 20, 18 y 16% (57–91 días); y 18, 16 y 14% (92–119 días). Se utilizaron dos lotes de 72 pollos machos Rhode Island Red cada uno; el primero para la primera fase de alimentación y el segundo lote para la segunda y tercera fases de alimentación. Se realizaron procedimientos estadísticos de superficie de respuesta para obtener las mejores concentraciones de ME y CP. El aumento de peso corporal (BWG) y la ingesta de alimento (FI) fueron mayores en la primera fase de alimentación con 2900 kcal ME / kg DM en la dieta; sin embargo, en la segunda fase no hubo un efecto significativo de la concentración de energía. Entre los 17 y los 91 días de vida, el FI aumentó con 2900 kcal ME / kg de MS en la dieta. Los pollos tuvieron un BWG más alto de 92 a 119 días de edad cuando se incluyó un 16% de PC en la dieta. La eficacia de la proteína fue mejor desde los 17 a los 119 días de edad cuando se incluyó la menor concentración de PC en la dieta. En conclusión, los mayores BWG se obtuvieron, cuando se utilizaron 2900 kcal ME / kg DM, para las tres fases de alimentación. Las mejores aproximaciones del requerimiento de PC en la dieta para la primera, segunda y tercera fase de alimentación fueron 21.3%, 19.2% y 17.1%, respectivamente.
Palabras clave: Requerimientos; crecimiento lento; superficies de respuesta; pollos Rhode Island Red.

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INTRODUCTION

Slow growing poultry are commonly used in backyard poultry farming as they allow resource-limited people to produce their food (Centeno e*t al*., 2007). In addition, this poultry type is used in free range and semi-intensive grazing systems because they have some characteristics such as survive in adverse circumstances, low appetite and adaptable to low-nutrient density diets that make them easily adaptable to the climatic conditions of the tropics (Rizzi *et al*., 2013).

There are several reports showing that slow-growing chickens are less efficient in meat production (Almasi *et al*., 2015; Rizzi *et al*., 2013; Wang *et al*., 2013) but their meat may be healthier compared to commercial poultry meat because it contains less fat and more polyunsaturated fatty acids than medium- and rapid-growing broilers (Martínez-Pérez *et al*., 2017).

Price of crude protein (CP) and metabolizable energy (ME) sources for animal diets have been increasing, which affect the cost of meat animal production (Ren *et al*., 2013; Rosegrant *et al.*, 2013). Therefore, determining nutrient requirements and adequate feed formulation is a critical issue, because there is a direct relationship between the animal performance and the nutrient density in the feed (Horsted *et al*., 2010).

Furthermore, slow growing poultry does not seem to require a high concentration of CP and ME in the diet for growing and development (Sundrum *et al*., 2006). However, despite the National Research Council (NRC, 1994) proposes requirements of ME and CP for broilers, there are no consistent reports of requirements for slow-growing breeds, such as Rhode Island Red type of chickens (Wolde *et al*., 2011b).

Thus, it is important to know the energy and protein requirements of slow growing breeds birds with the purpose of implementing feeding strategies to take full advantage of the amount of nutrients offered in the diet. The objective of the current study was to estimate the requirement of ME and CP for growing Rhode Island Red male chickens from 17 to 119 day-old, under tropical conditions of the southeast of Mexico.

MATHERIALS AND METHODS

Study site

The experimental work was carried out from July to November 2016, at the animal nutrition area of the Faculty of Veterinary and Animal Science, University of Yucatan, Mexico (FMVZ-UADY). The climate in the region is subhumid with an average annual rainfall of 1100 mm; rainfall mainly occurring during summer (June to October). The average annual temperature is 26°C, with average maximum of 36 °C in May, and average minimum temperature 16 °C in January.

Animal management

Two batches of one-day-old Rhode Island Red male chickens were bought. The first batch was used for the first feeding phase (17–56 days-old) and the second batch for the second and third feeding phases (57–91 and 92–119 days-old, respectively). Both batches arrived in July 2016. At the arrival of the first batch, the chickens were weighted and assigned into six groups with similar body weight means. Chickens were kept in floor cages from 0–16 days old and each group was fed one of the six experimental diets used in the first feeding phase. When the chickens were 17-days-old, they were individually allocated in elevated wired cages (40 x 40 cm), using a completely randomised design.

At the arrival of the second batch, the birds were randomly assigned to floor cages. The chickens remained in floor cages during 7 weeks. At week 8, they were weighted, individually allocated in elevated wired cages and assigned to six groups with the same means to start the test at week 9 of age (57 days-old). The diet given to the chicken in this period contained 20% CP and 3100 kcal ME/kg DM. In the third phase, the same chickens of phase two were used. The chickens were weighted, and body weight gain was recorded weekly.The floor cages used in both batches were equipped with a push-fit drinker and tray feeders during the first week of age. Automatic bell drinkers and hanging feeders were used later on.

Characteristics of diets

Three phases were assigned in the test with a different feeding program for each one. The feeding program consisted of two levels of ME (2900 and 3100 kcal/kg DM), which were constant along the test and three amounts of CP that decreased by 2% from one phase to another. In the first phase, 18, 20 and 22% CP in diets were used. In the second and third phases, 16, 18 and 20% and 14, 16 and 18% of CP in the diets were used, respectively. Lysine and methionine percentage were balanced according to broiler requirements (NRC, (1994), maintaining an approximated ratio of 2:1 (100:46).

Soybean meal and maize were used as the main ingredients for diet formulation. The feed was prepared on site (experimental farm) and samples were taken for chemical analysis at the animal nutrition laboratory, FMVZ-UADY (Table 1).

Table 1. Analysed and calculated values of the ingredients of experimental diets.

Metabolizable energy (kcal/kg DM)	2900					3100				
Crude protein (% DM)	22	20	18	16	14	22	20	18	16	14
Ingredients										
Yellow maize	45.60	49.40	64.12	67.72	68.10	53.32	59.80	66.20	73.17	78.70
Soybean meal 45%	37.00	30.51	27.50	22.0	14.00	39.36	33.79	28.50	22.70	17.50
Wheat bran	11.00	14.80	5.10	7.70	15.30	-	-	-	-	-
Soybean oil	3.71	2.58	0.50	-	-	4.67	3.60	2.50	1.40	1.00
Calcium carbonate	2.08	2.10	2.15	2.20	2.20	2.10	2.20	2.20	2.20	2.30
Methionine 99%	0.19	0.21	0.23	0.25	2.70	0.19	0.21	0.20	0.13	0.10
Vitamins premix	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Minerals premix	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
NaCl	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.0	100.00	100.00	100.0
Feed analysis (%)										
Dry matter	89.77	90.21	89.87	90.13	89.93	89.46	90.38	90.26	90.43	90.13
Crude protein	23.02	20.55	18.81	16.32	14.72	22.88	20.72	18.61	16.66	14.31
Crude fibre	2.96	2.78	2.17	2.19	2.87	2.56	1.93	2.38	1.57	1.51
Calculate analysis										
Metabolizable energy (kcal/kg DM)	2902	2900	2923	2924	2900	3100	3099	3100	3108	3143
Total phosphorus %	0.42	0.43	0.36	0.36	0.39	0.35	0.33	0.32	0.31	0.29
Calcium %	0.90	0.90	0.90	0.91	0.90	0.90	0.92	0.91	0.90	0.93
Total lysine (%)	1.20	1.05	0.94	0.81	0.64	1.22	1.08	0.94	0.80	0.66
Methionine (%)	0.51	0.50	0.50	0.44	0.41	0.51	0.50	0.47	0.38	0.33
Tryptophan (%)	0.28	0.25	0.22	0.19	0.16	0.28	0.24	0.21	0.16	0.15
Met + Cyst (%)	0.68	0.63	0.59	0.55	0.49	0.68	0.63	0.58	0.53	0.48

Chickens were offered feed *ad libitum* every day and the leftovers were weighed weekly to calculate the cumulative intake. The ME intake, CP intake, protein efficiency ratio (PER, gain per gram of protein intake) and energy efficiency ratio (EER, gain per 1 kcal ME intake) were also determined. At the end of the third phase, animals were sacrificed at the slaughterhouse of the FMVZ-UADY, and carcass yield was expressed as the percentage of final live weight when abdominal fat and nonedible components were removed (head, feet, liver, gizzard, heart, blood, feathers and gastrointestinal tract).

Statistical analyses

The experiment consisted in a completely randomized design with a factorial arrangement of treatments (12 replicates per treatment). The body weight gain (BWG), feed intake (FI), feed conversion (FC), crude protein intake (CPi), metabolisable energy intake (MEi), protein efficiency ratio (PER), energy efficiency ratio (EER), carcass performance and abdominal fat, were analysed through analyses of variance with the statistics package SAS version 9.4.

The mathematical model was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + e_{ijk}$$

Where:

Y_{ijk} is the ijk-th observation in the i-th level of factor A (ME) and j-th level of factor B (CP); μ is the general mean; α_i is the effect of i-th level of factor A; β_j is the j-th level of factor B; $\alpha\beta_{ij}$ is the interaction of i-th level factor A with the j-th level of factor B; and e_{ijk} is the random error.

Since BWG was the unique variable for which requirements were obtained in each feeding phase, it was analysed to determine the best response level, using the RSM tool of the statistics package Minitab® 17. The mathematical model was:

$$Y_{ijk} = \beta_0 + \beta_i a_i + \beta_j b_j + \beta_{ii} a_i^2 + \beta_{jj} b_j^2 + \beta_{ij} ab_{ij} + e_{ijk}$$

Where:

Y is the response of interest (BWG); β_0 is the intercept and β_i linear effect of factor A on BWG; β_j is the linear effect of factor B on BWG; β_{ii} is the quadratic effect of factor A on BWG, β_{jj} is the quadratic effect of factor B on BWG; β_{ij} is the interaction of factor A with levels of factor B on BWG; a_i is the effect of level factor A; b_j is the level of factor B; ab_{ij} is the interaction of level factor A with level of factor B; and e_{ijk} is the random error.

RESULTS

Higher BWG, FI and FCR, were obtained with a diet containing 2900 kcal ME/kg DM compared to the 3100 kcal ME/kg DM diet from 17 to 56 days-old in chickens. The birds that consumed 2900 kcal ME had a higher CP intake and ME intake but less PER (Table 2). From the period 57–91 days-old, chickens that consumed 2900 kcal ME had higher FI, CP intake and EER but same FCR, compared with those consuming 3100 kcal ME in the diet (Table 3). From the time between 92 to 119-days-old, higher EER with 2900 kcal ME was observed (Table 4).

From 92–119 days of age, CP had a significant effect on BWG, where the highest response, was observed with 16% in the diet. Highest CP intake was observed with 16 and 18% CP in the diet, while 14% CP resulted in better PER. From the time the birds were 57–91 days-old, 18 and 20% CP had the lowest FCR and highest EER, whereas with 18 and 16% CP, birds had the best PER. CP intake was significantly higher with higher CP levels in the diet (22, 20 and 18%) for each feeding phase (17–56, 57–91 y 92–119 days of age, respectively). Nevertheless, the PER was lower with these CP levels in the diets. A significant interaction of CP intake and ME intake as factors was observed for FI, from 57–91 days old (Figure 1, 2 and 3). There was no effect of the main factors or interaction between them, neither for carcass yield nor for abdominal fat. Table 5 shows the response surface for BWG in the three feeding phases.

Table 2. Metabolizable energy and crude protein effect on body weight gain (g), feed intake (g), feed conversion (g/g), crude protein intake (g), protein efficiency (g/g), metabolizable energy intake (kcal) and energy efficiency (%) from 17–56 days.

Factors	BWG	FI	FC	CPi	MEi	PER	EER
				ME (kcal/kg)			
2900	852.9[a]	1832.2[a]	2.15[a]	366.3[a]	5313.4[a]	2.55[b]	0.175
3100	779.0[b]	1577.0[b]	2.02[b]	317.2[b]	4888.5[b]	2.70[a]	0.174
				CP (%)			
18	802.0	1699.8	2.12	306.0[b]	5065.1	2.87[a]	0.173
20	831.3	1752.7	2.11	350.6[a]	5243.1	2.60[b]	0.173
22	818.6	1679.3	2.05	369.4[a]	5020.2	2.41[c]	0.177
SEM	7.28	20.73	0.02	4.21	62.79	0.02	0.002
				P value			
ME	< 0.0001	< 0.0001	0.0008	< 0.0001	0.001	<0.0001	NS
CP	NS	NS	NS	< 0.0001	NS	0.0005	NS
ME x CP	NS	NS	NS	NS	NS	NS	NS

BWG= body weight gain; FI= feed intake; FC= feed conversion; CPi= crude protein intake; MEi= metabolizable energy intake; PER= protein efficiency ratio; EER= energy efficiency ratio; NS= not significant.

Table 3. Metabolizable energy and crude protein effect on BWG (g), feed intake (g), feed conversion (g/g), crude protein intake (g), protein efficiency (g/g), metabolizable energy intake (kcal) and energy efficiency (%) from 57–91 days.

Factors	BWG	FI	FC	CPi	MEi	PER	EER
ME (kcal/kg DM)							
2900	795.46	2035.76[a]	2.57	366.41[a]	5903.7	2.19	0.135[a]
3100	765.18	1919.32[b]	2.52	346.07[b]	5949.9	2.22	0.129[b]
CP (%)							
16	756.0	2009.73	2.66[a]	321.56[c]	6013.2	2.35[a]	0.126[b]
18	798.3	1958.69	2.46[b]	352.56[b]	5877.5	2.27[a]	0.136[a]
20	787.8	1966.39	2.50[b]	393.28[a]	5888.2	2.01[b]	0.134[a]
SEM	8.02	18.02	0.02	3.25	54.42	0.02	0.001
P value							
ME	NS	0.0029	NS	0.0017	NS	NS	0.0025
CP	NS	NS	0.0001	<0.0001	NS	<0.0001	0.0002
ME x CP	NS	0.0219	NS	0.0126	0.0238	NS	NS

BWG= body weight gain; FI= feed intake; FC= feed conversion; CPi= crude protein intake; MEi= metabolisable energy intake; PER= protein efficiency ratio; EER= energy efficiency ratio; NS= not significant.

Table 4. Metabolizable energy and crude protein effect on BWG (g), feed intake (g), feed conversion (g/g), carcass performance, abdominal fat (g/kg), crude protein intake (g), protein efficiency (g/g), metabolizable energy intake (kcal) and energy efficiency (%) from 92–119 days.

Factors	BWG	FI	FC	Carcass performance	Abdominal fat	CPi	MEi	PER	EER
ME (kcal/kg)									
2900	797.79	2828.21	3.55	67.31	33.01	453.1	8201.8	1.78	0.097[a]
3100	795.40	2777.83	3.52	67.63	31.00	436.6	8611.3	1.84	0.093[b]
CP (%)									
14	761.35[b]	2739.87	3.62	67.08	38.21	383.5[b]	8229.1	1.99[a]	0.093
16	851.57[a]	2925.84	3.44	67.43	32.95	467.9[a]	8762.2	1.83[b]	0.098
18	778.68[ab]	2732.88	3.55	67.85	25.45	495.0[a]	8195.9	1.58[c]	0.095
SEM	13.50	14.56	0.32	0.27	3.46	7.43	138.82	0.02	0.001
P value									
ME	NS	NS	NS	NS	NS	NS	NS	NS	0.0189
CP	0.0006	NS	NS	NS	NS	<0.0001	NS	<0.0001	NS
ME x CP	NS	NS	NS	NS	NS	NS	NS	NS	NS

BWG= body weight gain; FI= feed intake; FC= feed conversion; CPi= crude protein intake; MEi= metabolizable energy intake; PER= protein efficiency ratio; EER= energy efficiency ratio; NS= not significant.

Table 5. Orthogonal analysis of metabolizable energy (ME) and crude protein (CP) effect on body weight gain.

Term	17-56 days *P value*	57-91 days *P value*	92-114 days *P value*
Linear	0.000	0.061	0.107
CP	0.337	0.135	0.461
ME	0.000	0.064	0.864
Quadratic			0.0044
CP			0.0044
SEM	8.48	8.02	13.50
R^2	27.3	7.79	16.39

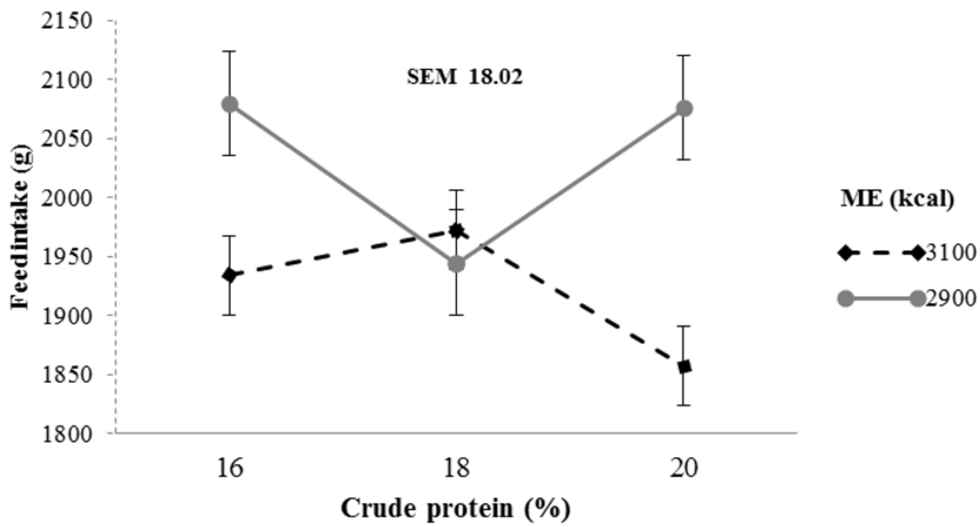

Figure 1. Interaction of crude protein and metabolizable energy for feed intake since 57-91 days old.

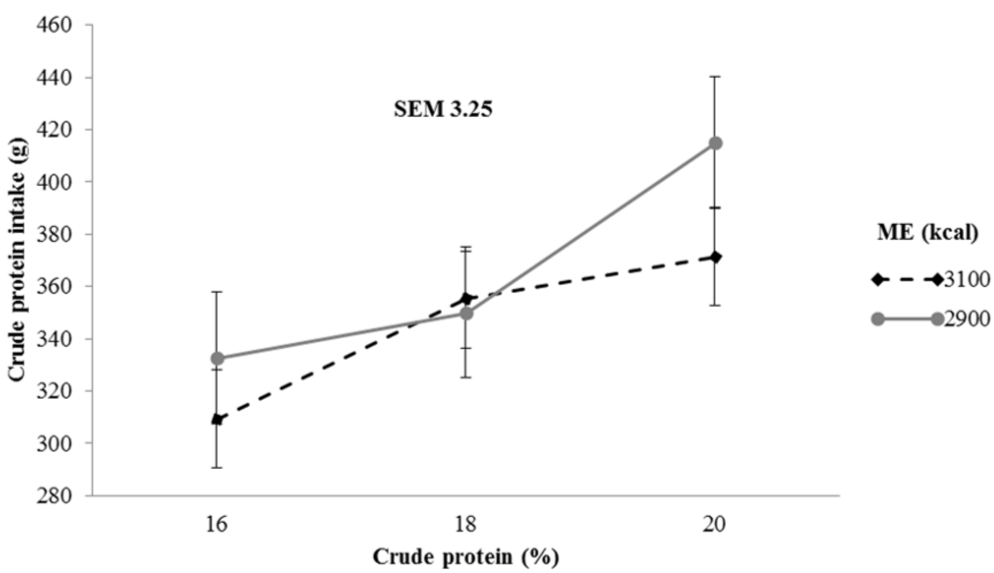

Figure 2. Interaction of crude protein and metabolizable energy for crude protein intake since 57-91 days old.

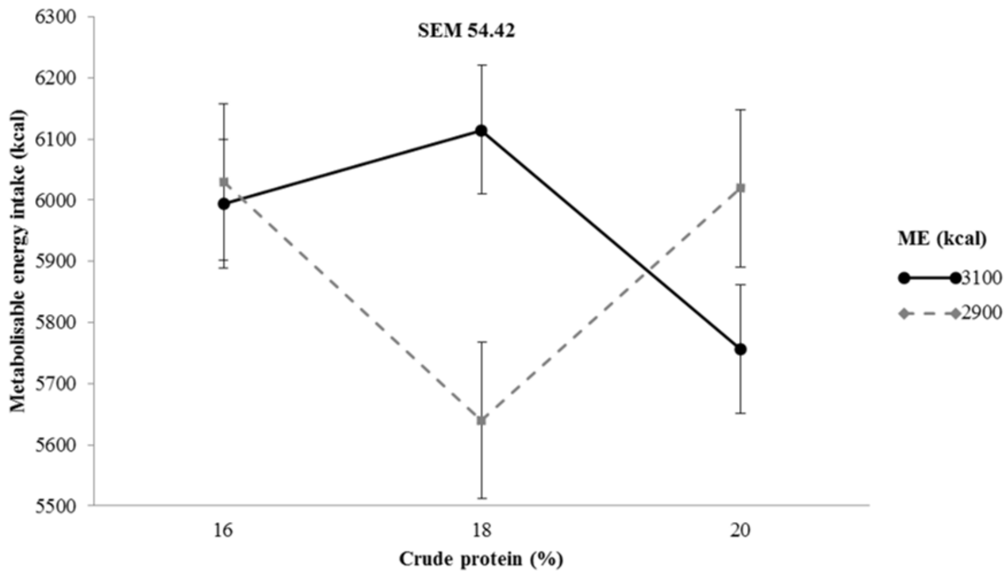

Figure 3. Interaction of crude protein and metabolizable energy for metabolizable energy intake since 57-91 days old.

For the BWG response surface of the first feeding phase CP had neither linear nor quadratic significant effect; however, ME had a linear effect. The response surface analysis indicates that the optimum level for CP in the diet was 23.0% and 2900 kcal/kg ME to reach 862 g BWG. The model explaining the BWG response was:

BWG= 1843 – 0.3708 (ME) + 4.11 (CP)

The BWG response surface for the second feeding phase showed that CP had neither linear nor quadratic effect and ME concentration had no effect on BWG. Response surface analysis indicates that the optimum level for both CP and ME were 20.7% and 2900 kcal/kg, respectively, to reach 811 g BW. The BWG response was explained by the following model:

BWG: 1116 – 0.1577 (ME) + 7.37 (CP)

In the response surface for the third feeding phase it was observed that the CP had a quadratic effect while ME had no effect. The response surface analysis indicates that the optimum amount for CP in the diet is 17.1% and 2900 kcal/kg ME to reach 852 g BW. The model explaining the BWG response was:

BWG: – 3621 + 0.024 (EM) + 525 (PC) – 15.66 $(PC)^2$

DISCUSSION

Effect of metabolizable energy

Some recommendations regarding dietary ME concentrations in slow-growing chickens and their effect on BWG are described in the literature. Nahashon *et al*. (2006) reported 3100 kcal ME/kg DM; Raju *et al*. (2004) and Li *et al*. (2013) mentioned 2900 kcal ME/kg DM and Haunshi *et al*. (2012) found that 2800 kcal ME/kg DM was sufficient to maximise the BWG. These reports indicate that the range of ME for slow-growing chickens could be between 2800 and 3100 kcal ME/kg DM. The differences reported may be attributed to the particular growth rate of the genotypes used in each study.

The results of the current study are within the range of previous reports, considering that in the first feeding phase (17–56 days old) the RIR chickens had highest BWG consuming 2900 kcal ME/kg DM. This is attributed to the higher feed intake and consequently higher CP and ME intakes in comparison to those chickens whose diet contained 3100 kcal ME/kg DM, which, in an attempted to regulate their ME intake, consumed less ME and therefore, had lower BWG. Zhao *et al*. (2009) and Wang *et al*. (2013) also described a positive relationship between high feed intake and high BWG in chickens.

In the last two feeding phases (57–91 and 92–119 days old), there was no effect of dietary ME concentration on the BWG, in agreement with Raju *et al*. (2004), Haunshi *et al*. (2012) and Abudabos (2014). However, in the second feeding phase (57–91 days old), the birds significant decreased feed intake when they consumed 3100 kcal ME/kg DM, causing both lower CP intake and EER, compared to those that consumed 2900 kcal ME/kg DM in the diet.

In the period of 92–119 days of age, EER was significantly better with 2900 kcal ME/kg DM in the diet, although the BWG and feed intake were similar. This could be due to the high ME: CP ratio in the diet with 3100 kcal ME/kg DM.

The PER result was lower when the diet contained 2900 kcal ME/kg DM compared to 3100 kcal, probably due to the low caloric content in the first diet, inducing the chickens to consume more feed; therefore, BWG: CP intake ratio was higher compared to those chickens consuming diets with 3100 kcal ME/kg DM. The differences in the feed intake from the current study indicate that it depends on the concentration of ME in the feed, because the chickens eat to meet their energy needs (Haunshi *et al*., 2012; Iqbal *et al*., 2014; Rama Rao *et al*., 2014).

Carmona *et al*. (2016) mentioned that abdominal fat increases as the age advances; in addition, Raju *et al*. (2004) and Akbari *et al*. (2016) reported that this increment is directly proportional to the ME in the diet. The carcass yield weight also increments when both age advances and ME increases in the diet. However, from the time chickens were 92–119 days-old, the phenomenon, neither for abdominal fat accumulation nor for carcass yield was observed in the current study; this may be due to the fact that there were no differences in the ME intake.

Effect of crude protein

In slow-growing chickens, Mosca *et al*. (2016) recommended 16% CP in the diet for birds from 0 to 180 days-old; while Li *et al*. (2013) mentioned that 17% CP should be used from 0 to 60 days-old, and Haunshi *et al*. (2012) reported 18% CP from 0 to 56-days-old. These recommendations indicate that the requirement of CP in slow-growing chickens could be between 16 and 18% when given a single concentration during the growth period.

However, the current study used three schemes where the CP in the diet ranged between 14 and 22%. Similarly, Wolde *et al*. (2011) used five diets (14, 16, 18, 20 and 22% CP) for 1–91 days-old RIR chickens and recommended 16% CP in the diet. This result differs with the current study, in which the chicken growth was divided in phases because the requirement of nutrients is higher in the early age; likewise, it is related to the rate of protein accretion in the body that is higher in the first two weeks of life (Kang *et al*., 1985; Wen *et al*., 2016).

The response surface analysis (RSM) yielded intermediate levels (21.3, 19.2 and 17.1% CP per phase) as ideal to improve the BWG response. In contrast, when using the ANOVA tool, the recommendations varied (18, 16 and 16% CP). The use of RSM was considered since it adjusted the behaviour of the dependent variable per the independent variables, when performing a multiple regression analysis (Khuri *et al*., 2006; Mehri *et al*., 2012).

Using the RMS (from 92–119 days-old), it was found that the CP level in the diet had a quadratic effect on the BWG. A difference of 27 g CP intake between both diets caused a significant decrease of 73 g BWG, even though, the CP intake had no significant effect on BWG when diets had 16 and 18%. This difference could be due to an excessive CP intake, since there is a direct relationship between CP intake and uric acid level in blood, derived from the amino acid catabolism of the diet (Hernández *et al*., 2012). According to Buyse *et al*. (1992) there is greater nitrogen excretion or fat deposition through lipogenesis which leads to a higher energy expenditure affecting the growth performance of chickens.

In the current study, the CP in the diet had no effect on the feed intake in agreement with Alagawany *et al*. (2011) and Zeweil *et al*. (2011), feeding laying hens, and Wolde *et al*. (2011) feeding RIR chickens. They found high CP intake when the diet had high CP concentration. From 57–91 days, FCR significantly worsened with the 16% CP level in the diet, associated with a low CP intake in the low dietary levels and *vice versa*. Cheng *et al*. (1997) and Bregendahl *et al*. (2002) agreed with those findings and reported high FCR with low CP dietary levels. From 17–56 and 92–119 days of age, the PER was better when chickens consumed low amounts of CP, in agreement with Cheng *et al*. (1997) and Haunshi *et al*. (2012).

From 57–91 days-old, the EER and PER were higher with 18% CP in the diet. It means that with 100 kcal ingested, there will be 13.6 g BWG and for every 100 g CP intake, there will be 227 g BWG. However, with a significant decrease of 16% in the EER, 20% of the PER decreased. This is due to the CP intake that directly affects these measurements (Cheng *et al*., 1997; Haunshi *et al*. 2012).

In the current study, no effect of dietary CP on abdominal fat was observed, in agreement with Awad *et al*. (2014) and Wolde *et al*. (2011). Although in the third phase (92–119 days old) there was a quadratic effect of CP on GDP, this suggests that the N from amino acids could be eliminated by the excreta as reported previously by Hernández *et al*., (2012). However, the number of observations in our study was not enough to show significance in the detected trend (13 g/kg abdominal fat difference).

Interaction of factors

The interaction of the main factors (ME and CP) was significant on FI, CP intake and ME intake during the second feeding phase (57–91 days old). The best response was obtained with 20% CP and 2900 kcal in the diet. On the other hand, the chickens that consumed the diet with 16% CP and 3100 kcal, had a high feed intake. However, the lower CP intake obtained was not enough to cover the requirement for 57–91 days and the ME intake was very high, which could contribute to the greater deposition of body fat (McLeod *et al*., 1991; Zeng *et al*., 2015), taking into account that fat synthesis requires higher ME than protein synthesis.

With the diets of 18% CP, the concentration of ME had no influence on the CP intake, consuming similar amounts of CP as the 20% diet with 3100 ME. This last diet had high nutrient concentration, low feed intake, high CP intake and low ME intake. The imbalance in nutrient intake could cause adverse effects on the growth rate (Kamran *et al*., 2008) an effect observed in the response surface analysis trend.

CONCLUSION

The best dietary level of ME requirement for the three feeding phases was 2900 kcal/kg to obtain greater BWG. On the other hand, 3100 kcal/kg ME decrease FI and FCR, but BWG was negatively affected. The carcass yield and abdominal fat content were not affected with the CP and ME levels tested. An approximation of CP requirement in the diet for the first, second and third feeding phase would be 21.3%, 19.2% and 17.1%, respectively.

REFERENCES

Abudabos, A.M. 2014. Effect of fat source, energy level and enzyme supplementation and their interactions on broiler performance. South African Journal of Animal Science. 44(3): 280-287. DOI: 10.4314/sajas.v44i3.10

Akbari S.M., Sadeghi, A.A., Aminafshar, M., Shawrang P., Chamani, M.. 2016. The effect of using different energy sources on growth and some carcass characteristics in Cobb 500 broiler chicks. Research Opinions in Animal & Veterinary Science. 6(3): 84-88. DOI: 10.20490/ROAVS/16-014

Alagawany, M., El-Hindawy, M.M., Ali, A.A., Soliman. M.M. 2011. Protein and total sulfur amino acids relationship effect on performance and some blood parameters of laying hens. Egyptian Journal of Nutrition and Feeds. 14(3): 477-487. https://www.researchgate.net/profile/Mahmoud_Alagawany/publication/258763293_Protein_and_Total_Sulfur_Amino_Acids_Relationship_Effect_on_Performance_and_Some_Blood_Parameters_of_Laying_Hens/links/00b7d528e824de5610000000.pdf

Almasi, A., Andrassyne B.G., Milisits, G., Kustosne, P.O., Z. Suto. 2015. Effects of different rearing systems on muscle and meat quality traits of slow- and medium-growing male chikens. British Poultry Science. 56(3): 320-324. DOI: 10.1080/00071668.2015.1016478

Awad E.A., Fadlullah, M., Zulkifli, I., Farjam, A.S., Chwen, L.T. 2014. Amino acids fortification of low-protein diet for broilers under tropical climate: Ideal essential amino acids profile. Italian Journal of Animal Science. 13(2):3166. DOI: 10.4081/ijas.2014.3166.

Bregendahl, K., Sell, J.L., Zimmerman, D.R. 2002. Effect of low protein diets on growth performance and body composition of broiler chicks. Poultry Science. 81(8): 1156-1167. https://doi.org/10.1093/ps/81.8.1156

Buyse, J., Decuypere, E., Berghman, L., Kuhn, E.R., Vandesande, F. 1992. The effect of dietary protein content on episodic growth hormone secretion and on heat production of male broilers. British Poultry Science. 33(5): 1101–1109. DOI: 10.1080/00071669208417552

Carmona, J.M., lopez-Bote, C.J., Daza, A., Rey, A.I. 2016. Fat accumulation, fatty acids and melting point changes in broiler chick abdominal fat as affected by time of dietary fat feeding and slaughter age. British Poultry Science. Advance online publication. DOI: 10.1080/00071668.2016.1187715

Centeno, S.B., López, C.A. Juárez, M.A. 2007. Producción avícola familiar en una comunidad del municipio de Ixtacamaxtitlán, Puebla. Técnica Pecuaria en México. 45(1): 41-60. https://cienciaspecuarias.inifap.gob.mx/index.php/Pecuarias/article/view/1788/1782

Cheng, T.K., Hamre, M.L., Coon C.N. 1997. Effect of environmental temperature, dietary protein, and energy levels on broiler performance. The Journal of Applied Poultry Research. 6(1):1-17. https://doi.org/10.1093/japr/6.1.1

Haunshi, S., Panda, A.K., Rajkumar, U., Padhi, M.K., Niranjan, M., Chatterjee, R.T. 2012. Effect of feeding different levels of energy and protein on performance of Aseel breed of chicken during juvenile phase. Tropical Animal Health and Production. 44(7): 1653–1658. DOI: 10.1007/s11250-012-0120-6.

Hernández, F., López, M., Martínez, S., Megías, M.D., Catalá, P., Madrid, J. 2012. Effect of low-protein diets and single sex on production performance, plasma metabolites, digestibility, and nitrogen excretion in 1- to 48-day-old broilers. Poultry Science. 91(3):683-692. DOI: https://doi.org/10.3382/ps.2011-01735.

Horsted, K., Allesen-Holm B.H., Hermansen, J.E. 2010. The effect of breed and feed-type on the sensory profile of breast meat in male broilers reared in an organic free-range system. British Poultry Science. 51(4): 515–524. DOI: 10.1080/00071668.2010.506907.

Iqbal, Z., Mughal, A., Kamran, Z., Ali, A., Ahsan, U. 2014. Effect of constant ME:CP at different levels of CP and ME on growth performance and meat characteristics of broilers from 1-28 days. Archiva Zootechnica. 17(2): 43-53. https://www.researchgate.net/profile/Zafar_Iqbal12/publication/282947746_Effect_of_constant_MECP_at_different_levels_of_CP_and_ME_on_growth_performance_and_meat_characteristics_of_broilers_from_1-28_days/links/56da214908aebe4638bba08f/Effect-of-constant-MECP-at-different-levels-of-CP-and-ME-on-growth-performance-and-meat-characteristics-of-broilers-from-1-28-days.pdf

Kamran, Z., Sarwar, M., Nisa, M., Nadeem, M.A., Ahmad, S., Mushtaq, T., Ahmad. T., Shahzad, M.A. 2008. Effect of lowering dietary protein with constant energy to protein ratio on growth, body composition and nutrient utilization of broiler chicks. Asian-Australian Journal of Animal Science. 21(11): 1629-1634. doi: https://doi.org/10.5713/ajas.2008.70586.

Kang, C.W., Sunde, M.L., Swick, R.W. 1985. Growth and protein turnover in the skeletal muscles of broiler chicks. Poultry Science. 64(2): 370-379. DOI: 10.3382/ps.0640370.

Khuri, A.I. 2006. Response Surface Methodology and Related Topics. World Scientific Publishing Company. Inc, Danvers, MA.

Li, Q., Xu, Z., Liu, L., Yu, H., Rong, H., Tao, L., Zhang, X. 2013. Effects of breeds and dietary protein levels on the growth performance, energy expenditure and expression of avUCP mRNA in chickens. Molecular Biology Reports. 40(4): 2769-2779. DOI: 10.1007/s11033-012-2030-0.

McLeod, M.G. 1991. Fat deposition and heat production as responses to surplus dietary energy in fowls given a wide range of metabolisable energy: Protein ratios. British Poultry Science. 32(5): 1097-1108. https://doi.org/10.1080/00071669108417432

Mehri, H., Davarpanah, A.A., Mirzaei, H.R. 2012. Estimation of ideal ratios of methionine and threonine to lysine in starting broiler chicks using response surface methodology. Poultry Science. 91(3): 771–777. DOI: http://dx.doi.org/10.3382/ps.2011-01818.

Mosca, F., Kuster, C.A., Stella, S., Farina, G., Madeddu, M., Zaniboni, L., Cerolini, S. 2016. Growth performance, carcass characteristics and meat composition of Milanino chickens fed on diets with different protein concentrations. British Poultry Science. 57(4): 531-537. DOI: 10.1080/00071668.2016.1174768.

Nahashon, S., Adefope, N., Amenyenu, A., Lema, M. 2006. Growth and carcass characteristics of French Guinea broilers fed diets with varying concentrations of metabolizable energy. Journal of Sustainable Agriculture. 27(4): 25-43. DOI: 10.1300/J064v27n04_04.

Raju, M.V.L.N., Shyam-Sunder, G., Chawak, M.M., Rama-Rao. S.V., Sadagopan V.R. 2004. Response of naked neck (Nana) and normal (nana) broiler chickens to dietary energy levels in a subtropical climate. British Poultry Science. 45(2): 186-193. DOI: 10.1080/00071660410001715786.

Rama-Rao, S.V., Ravindran, V., Raju, M.V.L.N., Srilatha, T., Panda, A.K. 2014. Effect of different concentrations of metabolisable energy and protein on performance of White Leghorn layers in a tropical climate. British Poultry Science. 55(4): 532-539. DOI: dx.doi.org/10.1080/00071668.2014.935997.

Rizzi C., Contiero, B., Cassandro, M. 2013. Growth patterns of Italian local chicken populations. Poultry Science, 92(8): 2226-2235. DOI: 10.3382/ps.2012-02825.

SirrI, F., Castellini, C., Bianchi, M., Petracci, M., Meluzzi, A., Franchini, A. 2011. Effect of fast-, medium- and slow-growing strains on meat quality of chickens reared under the organic farming method. Animal, 5(2):312-319. DOI: 10.1017/S175173111000176X.

Van Emous, R.A., Kwakkel, R.P., Van Krimpen, M.M., Van Den Brand, H., Hendriks, W.H. 2015. Effects of growth patterns and dietary protein levels during rearing of broiler breeders on fertility, hatchability, embryonic mortality and offspring performance. Poultry Science. 94(4): 681-691. DOI: 10.3382/ps/pev024.

Wang, X.Q., Chen, X., Tan, H.Z., Zhang, D.X., Zhang, H.J., Wei, S., Yan, H.C. 2013. Nutrient density and slaughter age have differential effects on carcase performance, muscle and meat quality in fast and slow growing broiler genotypes. British Poultry Science. 54(1): 50-61. DOI: 10.1080/00071668.2012.745927.

Wen, Z.G., Du, Y.K., Xie, M., Li, X.M., Wang, J.D., Yang, P.L. 2017. Effects of low-protein diets on growth performance and carcass yields of growing French meat quails (France coturnix coturnix). Poultry Science 96(5): 1364–1369. DOI: dx.doi.org/10.3382/ps/pew321.

Wolde, S., Negesse, T., Melesse, A. 2011. The effect of dietary protein concentration on nutrient utilization of Rhode Island Red chicken in Wolaita (southern Ethiopia). Tropical and Subtropical Agroecosystems, 14(1): 271-278. http://www.revista.ccba.uady.mx/ojs/index.php/TSA/article/view/585/524

Zeng, Q.F., Cherry, P., Doster, A., Murdoch, R., Adeola, O., Applegate, T.J. 2015. Effect of dietary energy and protein content on growth and carcass traits of Pekin ducks. Poultry Science. 94(3): 384-394. DOI: 10.3382/ps/peu06.

Zeweil, H.S., Abdalah, A.A., Ahmed, M.H., Marwa, R.S.A. 2011. Effect of different levels of protein and methionine on performance of baheij laying hens and environmental pollution. Egyptian Poultry Science. 31(2): 621-639. https://pdfs.semanticscholar.org/723f/2dff0413734eba0dbc5a326c6235ba06ff72.pdf