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Effect of different energy and protein levels in Hubbard broiler diet on growth performance and some related blood metabolic parameters

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ABSTRACT

The present experiment was executed to detect the extent to which different energy and protein levels influence growth performance and some related blood metabolic parameters in Hubbard efficiency plus chicks. A total 234 one-day-old chicks were divided into 6 groups, 39 chicks in each. Group I, received a basal diet containing standard energy and protein. Group II, received a diet containing standard protein and 10% low energy (LE). Group III, received a diet containing standard protein and 20% low energy. Group IV, received a diet containing standard energy and 10% low protein (LP). Group V, received a diet containing standard energy and 20% low protein. Group VI, received a diet containing 20% low energy and 10% low protein. During the experiment that lasted 6 weeks, body weight, weight gain, feed intake, and FCR were recorded weekly. Serum glucose, insulin, and corticosterone levels were also measured. The results demonstrated that the body weight of all groups showed a marked decrease compared to control group except 10% LP group showed an unremarkable decrease in week 3 of age. In week 5 and 6, 20% LE-10% LP group and 20% LE group displayed the highest feed intake compared to control. Also, FCR, during whole experimental period, 20% LE-10% LP group and 20% LE group recorded a pronounced increase in FCR compared to control. Serum level of glucose and insulin revealed significant ($P < 0.05$) decrease in 20% LE and 20% LE-10% LP group. Whereas serum level of corticosterone displayed a significant ($P < 0.05$) increase in 20% LE and 20% LE-10% LP group compared to control group. In conclusion, low energy had a marked effect on growth performance and related metabolic blood hormones than low protein.

1. INTRODUCTION

Both genetic makeup and environmental issues, such as nutrition, impact an animal's growth rate and body weight. The neuroendocrine system is key to integrating genetic information with external factors like nutrition to manage the animal's growth (Zhao et al., 2004). Energy density in broiler diet regulates feed intake. The concentration of amino acids in the diet affects both the feed intake of broilers and the level of dietary energy (Zuidhof, 2019).

When designing a broiler's diet, it is crucial to include the appropriate amount of balanced dietary protein and amino acids (AA), as these are major cost factors in poultry diets, following energy, and have a significant effect on growth performance (Srilatha et al., 2018). The synergy between protein and energy underscores the relevance of maintaining an optimal calorie to protein (C:P) ratio for fulfilling peak performance and optimal carcass quality in broilers. Energy needs are essential in broiler production (El Sayed et al., 2017).

Indeed, feed consumption is a key aspect in improving the broilers' growth rate (Abdollahi et al., 2018). Moreover, dietary nutritional levels have a direct impact on feed intake (FI). Several studies have examined how feed intake (FI) is regulated based on dietary energy levels (Plumstead et al., 2007). Nevertheless, the outcomes are frequently inconsistent due to the complex physiological mechanisms that govern FI. Maintaining energy homeostasis involves a

multitude of external factors such as climate as well as internal stimuli including hormones and their receptors, all of which influence FI. Certainly, the concentration of dietary energy interacts with other nutrients, significantly impacting the intake of all nutrients in broilers (Classen, 2017).

For a considerable time, researchers and nutritionists held the belief that dietary energy density predominantly governed FI in broiler chickens (Ahiwe et al., 2018). However, Lemme et al. (2005) showed that aside from energy content of the diet, the concentration of amino acids also influences FI in broilers. The hypothalamus serves as a central hub for integrating signals from the brain, bloodstream, and digestive system to regulate feeding and maintain energy homeostasis (Lu et al., 2019). Within the hypothalamus, especially in the ARC (arcuate nucleus), two distinct groups of neurons are crucial for regulating feed intake, energy and glucose homeostasis. notably, AgRP/NPY-releasing neurons in the ARC are known to stimulate feeding behavior, acting as orexigenic factors in both avian species and mammals (Chen et al., 2018).

Chicken growth and development are predominantly regulated by the somatotrophic axis, as noted by Zhao et al. (2004). This axis, also known as the hypothalamus-pituitary growth axis, comprises essential components such as growth hormone (GH), insulin-like growth factors (IGF-1 and IGF-2), their associated carrier proteins and receptors, as well as other hormones like glucocorticoids and insulin (Nie et al., 2005). In poultry, corticosterone (CORT) serves as the

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principal glucocorticoid (GC) involved in regulating energy metabolism, feed intake, along immunological responses (Zulkifli and Azah, 2004). The goal of the present investigation was to assess the impact of energy level and protein concentration in the diet of broiler chickens on growth, feed intake, glucose, and some hormones related to growth such as insulin and corticosterone.

2. MATERIAL AND METHODS

This research was started on January 11, 2023, and lasted until February 21, 2023, covering a period of 42 days with an ethical approval number (BUFTM05-04-24) at the Center for Experimental Animal Research, Faculty of Veterinary Medicine, Benha University, Egypt.

2.1. Animals, experimental diets and scheme

Two hundred thirty-four one-day-old Hubbard efficiency plus chicks were split into 6 groups at random, each one containing 39 chicks distributed into 3 replicates, with 13 chicks per replicate.

Group I, chicks received a basal diet containing standard energy and protein (control group) (energy at the level of 3000.07, 3102.8, and 3200.82 Kcal/kg and protein at the level of 23.11, 21.10, 20.00 % for starter, grower, and finisher, respectively). Group II, chicks received a diet containing standard protein and 10% low energy (10% LE).

Group III, chicks received a diet containing standard protein and 20% low energy (20% LE).

Group IV, chicks received a diet containing standard energy and 10% low protein (10% LP).

Group V, chicks received a diet containing standard energy and 20% low protein (20% LP).

Group VI, chicks received a diet containing 20% low energy -10% low protein (20% LE-10% LP).

All six dietary treatments were designed using two levels of energy and protein across starter, grower, and finisher diets (Table 1).

All chicks were raised under equivalent environmental and hygiene standards. fresh and clean litter of wood shavings was used. Food and water were always available for the experiment. Water and food were always available during the experiment (42 days). During the experimental period, the feeding plan was divided into three stages: starter (day one: ten), grower (day eleven: twenty-two), and finisher (day twenty-three: forty-two).

2.2. Measurements of growth performance:

2.2.1. Body weight and body weight gain:

At the start of the experiment, the live body weight of the chicks was documented and subsequently measured on a weekly interval. Birds were weighed in the early hours of the day before feeding, using an electronic scale. The difference in body weight gain between two consecutive weeks was used to compute the live weight gain (g/broiler chick) at weekly intervals. While relative growth rate was calculated

according to Crampton and Lloyd (1959) as following:

$$\text{Average daily weight gain (ADG)} = \frac{\text{Total body weight gain}}{42}$$

$$\text{Relative growth rate (RGR)} = \frac{100 (w_2 - w_1)}{\frac{1}{2} (w_2 + w_1)}$$

2.2.2. Feed intake and feed efficiency

Regular experimental morning feeds were provided to the chicks. Daily feed consumption was determined by weighing the given and leftover feed, and then dividing this amount by the number of birds in each group each day (Kamel et al., 2020). The feed conversion ratio (FCR) was calculated by dividing the total feed consumed in grams by the weekly weight gain (g)

$$\text{FCR} = \frac{\text{Feed intake (g) /bird/week}}{\text{Body weight gain (g) /bird/week}}$$

2.3. Blood sampling for assessment of serum level of related-metabolic parameters

A blood sample was collected from each replicate of Hubbard chickens early in the morning (9 AM). The samples were taken using red (plain) vacutainer tubes and then centrifuged at 3000 rpm for 15-30 minutes. The serum was meticulously separated and stored at -20 °C until analysis of glucose, insulin and corticosterone levels.

2.4. Hormonal analysis

2.4.1. Measurement of glucose level

Glucose concentration was measured using the glucose oxidase method (Brake et al., 1981).

2.4.2. Measurement of insulin level

Serum insulin level was measured with IRI assay is a 2- site sandwich immunoassay using direct chemiluminescent technology with Atellica® IM Analyzer (Mianaris Medical Co., Ltd. For: Siemens Healthcare GmbH, HenkestraBe 127, 91052 Erlangen, Germany) as described by Argiles and Lopez-Soriano, (2001).

2.4.3. Measurement of corticosterone level

Corticosterone level was measured with Cor assay is a competitive immunoassay using direct chemiluminescent technology with Atellica® IM Analyzer (Siemens Healthcare GmbH, HenkestraBe 127, 91052 Erlangen, Germany) as described by Hawley et al., (2016).

2.5. Statistical analysis

The data were processed using SPSS (IBM Corp. 2019). IBM SPSS Statistics for Windows version 26.0, released by IBM Corp. in Armonk, NY (SPSS, 2019), was utilized. Mean differences were assessed using a one-way ANOVA, with the Duncan test applied for detailed comparison. The variance in the data was described using the mean and standard error, with significance set at P< 0.05.

3. RESULTS

3.1. Growth performance parameters

The impact of dietary energy and protein on broiler growth performance including body weight (BW), body weight gain (BWG), and relative growth rate (RGR) are shown in Table (2).

Table 1 Ingredients in kg/ton of starter, grower, and finisher broiler diets (as fed basis).

Ingredients	Starter						Grower						Finisher					
	G1	G2	G3	G4	G5	G6	G1	G2	G3	G4	G5	G6	G1	G2	G3	G4	G5	G6
Yellow corn	510.65	471	302	551.7	618.7	450.2	555.8	517	364.3	579.6	652.3	406.8	568.2	582.6	416.2	636.8	663	464.8
Soya bean meal 46	350	350	257.6	350	301.5	333.0	297	271	207	325.5	261.3	216	287	266	223	226	242.4	242
Wheat bran	-	56.8	287.6	-	-	160.3	-	99.4	284.5	-	-	285	-	55	264.5	-	-	245.5
Vegetable oil	29.5	5	5.2	32	23.7	5.3	38	5.3	5	46.5	34	5	53.5	5	5	41.9	48	5
Corn gluten meal	60	29	58.4	14.3	-	-	60	60	62	-	-	20	50	50	50	50	-	-
Mono calcium phosphate	17	16.4	14.1	17.7	18.1	16	16.5	15.4	13.3	16.7	17.2	13.6	14.4	13.8	11.6	14.8	15	12
Limestone	15.5	15.8	16.6	15.7	15.8	16.15	14.4	14	15.4	14.3	14.5	15.4	12.9	13	13.8	13	13	13.7
Sodium bicarbonate	2.28	2.2	2.7	2.5	3.1	2.4	2.8	2.9	3	2.7	3.3	3.3	2.3	2.5	2.5	3.1	3.1	2.5
DL-methionine	3	3.1	3	3.9	4.5	4	2.7	2.7	2.6	3.6	4	3.4	2.1	2.1	2.2	2.6	3.4	3
L lysine	3.4	3	4.7	3.8	5.3	3.8	3.4	3.8	4.8	3.1	4.9	5	2.3	2.6	3.2	4	4	3.1
Vitamin mineral premix1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Sodium chloride	2.6	2.4	2.1	2.3	1.8	2.3	2	2	1.8	2.1	1.6	1.7	2.4	2.3	2.3	1.9	1.9	2.3
L-threonine	1	1	1.5	1.7	2.5	1.9	0.1	1.2	1.5	1.5	2.3	2.1	0.6	0.7	1	1.4	1.9	1.5
Choline chloride	1	0.9	1.4	1	1.2	1.3	1.1	1.2	1.4	1	1.2	1.4	1	1.3	1.1	1	1	1.2
Sunflower meal 34% crude protein	-	40	399	-	-	-	-	-	30	-	-	17.9	-	-	-	-	-	-
L tryptophan	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-
Anti-clostridial	-	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Anti-toxin	0.1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

3.2.3. Corticosterone hormone level

Serum level of corticosterone displayed a significant ($P < 0.05$) increase in 20% LE and 20% LE-10% LP groups compared to control group. while 20% and 10% LP, and 10% LE did not differ appreciably from controls (Table 4).

Table 4 Effect of different dietary levels of energy and protein on glucose, insulin, and corticosterone serum level

Chicken groups	Glucose mg / dL	Insulin (μ U/ml)	Corticosterone (ng/ml)
Control	190.33 ^a ±0.88	34.11 ^a ±2.98	10.00 ^b ±2.52
10% low energy (10%LE)	169.67 ^a ±10.11	26.91 ^a ±0.42	10.00 ^b ±1.15
20% low energy (20%LE)	140.33 ^b ±6.64	18.83 ^b ±0.32	21.33 ^a ±0.88
10% low protein (10%LP)	179.67 ^a ±4.10	29.80 ^a ±4.19	7.00 ^b ±0.58
20% low protein (10%LP)	174.00 ^a ±1.53	34.26 ^a ±2.44	8.00 ^b ±0.58
20% low energy & 10% low protein (20%LE-10%LP)	127.00 ^b ±16.17	16.55 ^b ±1.15	22.33 ^a ±2.19

Values are expressed as mean \pm S.E.M. Values in the same column carrying different superscripts are significantly different at ($P < 0.05$).

4. DISCUSSION

It is widely acknowledged that the diet formulation and macronutrient ratios significantly impact both the performance and body composition of chickens. The levels of dietary protein and energy are critical for optimizing chick performance and economic returns (Abd El-Hady and Abd El-Ghany, 2003). The obtained results revealed that body weight of all groups showed a significant decrease except 10%LP group exhibited a non-substantial decrease in week 3 of age compared to control group. The lowest BW and BWG were observed in birds supplied with low-energy diets and low-protein diets. These results are supported by previous investigations, which have reported lowering dietary ME causing a linear reduction in growth performance and weight gain (Kamran et al., 2008). El-Faham et al. (2015) reported that chicks consuming a moderate dietary energy level showed the greatest decline in BW, with a decrease of 7.5%, compared to those fed diets with higher energy levels. Furthermore, Latham et al. (2016) found that a dietary energy level of around 3000 kcal/kg did not impact body weight in broilers, whereas lower caloric levels led to a reduction in BW. Research suggests that broiler chicks receiving low-protein or low-energy diets experienced considerably lower daily weight gain (Hassanien, 2006). Moreover, Reduction of dietary energy had a negative influence on BWG (Wang et al., 2020). Contrary to our results, regarding live BW and DWG, it is noteworthy that chicks fed a low-energy diet during the first period (0-3 weeks) showed the most negligible outcomes when compared to those fed diets with medium or high energy levels (El-Faham et al., 2015). Broilers fed the LE-LP diet exhibited considerably greater BWG and FI between days 22 and 35, as well as a higher final BW on day 35, compared to those on the NE diet (Miao et al., 2017).

The obtained findings displayed that the body weight of low protein groups showed a notable decrease compared to control group except 10%LP group showed a minor decrease in week 3 of age. These observations were corroborated by van Harn et al. (2019), who recorded that a notable drop in dietary protein levels exceeding 2 percentage points results in suboptimal growth performance in broilers. Similar results were found in this study, where lower dietary protein levels adversely affected BW and BWG of broilers throughout the experimental period. Ghazanfari et al. (2010) investigated rations with varying CP levels and found that lower protein levels adversely affected the FCR and BW of broilers at 0-32 days of age. However, Rabie et al. (1997) found no discernible variations in the growth performance of broilers provided with diets of varying protein content between 18 and 53 days of age. Therefore, it was noted from the results that the level of dietary crude protein might not

be as crucial as AA profile of the diet. When amino acids, especially the critical ones, were properly balanced, the protein content of the diet could be reduced without compromising growth performance. The outcomes of this investigation match those reported by Woyengo et al. (2023), who reported no discernible variations in WG following the reduction of dietary protein from twenty-three percent to twenty percent. The birds fed low levels of CP showed significantly ($P < 0.05$) higher BWG and better FCR as compared to the diets with high levels of CP (Srilatha et al., 2018). In the same aspect, Abbasi et al. (2014) reported that a 10% reduction in dietary CP level can be achieved without compromising growth performance during the finisher phase (25-42 days age). The observed decrease in growth rate could be attributed to lower-energy diets, which contain insufficient energy to support protein synthesis. This insufficiency forces the catabolism of amino acids to compensate, leading to reduced growth and poorer feed efficiency (Ghahremani et al., 2016).

In the present investigation, FI showed a non-significant difference between treated groups in the first 3 weeks. These findings align with Wang et al. (2020), who observed that the reduction of feed energy throughout the starter period did not influence FI, BW, and FCR of broiler chicks. However, in week 5 and week 6, chicks fed low-energy diets and low energy with low protein diet displayed the highest feed intake compared to control. These results are in line with Maharjan et al. (2021), who noted that broilers on lower ME diets consumed a greater amount of feed, likely to meet their physiological energy needs, which resulted in lower feed efficiency. A low-energy diet was also linked to enhanced agouti-related peptide (AgRP) mRNA expression in the hypothalamus, which stimulated FI (Xu, 2012). Therefore, low-energy (LE) diets led to increased feed intake (FI).

In this study, low protein groups showed nonsignificant difference changes in feed intake in comparison with the control group. These results are consistent with Malheiros et al. (2003), who found that broilers exhibited no differences in feed intake when fed iso-energy diets with reduced protein levels (15.8% CP). Similarly, according to Ferguson et al. (1998), decreasing dietary crude protein (CP) from 20.4% to 18.8% during the starter phase did not affect feed intake. On the other hand, Colnago et al. (1991) found that reducing crude protein (CP) levels led to a decrease in feed intake, even with essential amino acids provided. Feed intake was decreased in broilers on a low-protein diet relative to those on a control diet (Hada et al., 2013). Meanwhile, no changes in feed intake were observed when birds were supplied with 80% or 90% of their amino acid needs (Aviagen, 2007).

Regarding FCR, in our results during the whole experimental period, 20% LE-10% LP group and 20% LE group recorded a marked decrease in FCR compared to control. This result is supported by previous investigations, which have reported that feed conversion ratio showed an increase with lower energy content when diets were in mash form (Jafarnejad et al., 2010). Similarly, Latham et al. (2016) found that reducing dietary energy by 97 kcal/kg increased FCR between 0 and 42 days of age. Correspondingly, the feed conversion ratio (FCR) demonstrated a similar pattern, since birds on low-energy diets were less efficient in converting feed into body weight gain than those on medium or high-energy diets. The most favorable FCR has observed in chicks fed high-energy diets, while the least favorable FCR occurred in chicks fed low-energy diets. The conflicting findings regarding dietary energy and growth performance could be attributed to variations in nutrient levels, types of fat (vegan vs. animal), rearing environments, and the age of the birds.

It is explicit from the present results that serum levels of glucose and insulin revealed a significant decrease in chicks fed low energy diet and low energy with low protein diet compared to control group. Serum glucose (GLU) is a primary energy source essential for the growth of body tissues, including nervous system, renal medulla, red blood cells, and skeletal muscles (Hu et al., 2021). A failure to regulate this nutrient can lead to hypoglycemia (Nirmalan and Nirmalan, 2020). In the present study, low levels of energy lead to decrease glucose. Since glucose levels decreased, insulin also decreased as no stimulation of pancreas to secrete insulin. These results were aligned with Frayn (2009), who found that in the state of starvation or fasting, insulin secretion is reduced, and glucagon secretion is increased, which triggers catabolic processes and mobilizes glucose and free fatty acids (FFA). When glucose levels fall, glucagon secreted by α cells stimulates gluconeogenesis, increasing blood glucose by facilitating glycogen breakdown and releasing glucose from the liver. But it disagreed with those of other researchers like De Jong et al. (2002), who demonstrated that in birds, plasma glucose levels are usually well-maintained, even when fasting or starving.

GLU can be oxidized to supply energy and diverted into pathways for the synthesis of fatty acids (Uyeda and Repa, 2006). Therefore, glucose is crucial as an energy source and a primary factor for maintaining life in birds. It promotes the synthesis of new glucose from non-carbohydrate carbon sources through gluconeogenesis, a critical process for sustaining glucose levels needed for various metabolic functions (Dashty, 2013). The regulation of glucose homeostasis is achieved through the simultaneous, opposing actions of various hormones. Insulin lowers plasma glucose and aids in its conversion to glycogen, while glucagon functions as the primary opposing hormone, raising plasma glucose levels by promoting glycogen breakdown and glucose release from the liver (Nirmalan and Nirmalan, 2023). Due to the opposing roles of insulin and glucagon, an insulin deficiency results in elevated glucagon levels, which are essential for gluconeogenesis. Glucagon boosts gluconeogenesis in the liver by increasing both the quantity and activity of the liver enzymes involved. It also helps the liver absorb amino acids from the blood, converting them into glucose (Qaid et al., 2016).

In the present study, the serum level of corticosterone hormone displayed a marked increase in low energy and low energy with low protein group compared to control group. These results are supported by Ognik and Sembratowicz (2012), who demonstrated an increase in corticosterone levels, which is responsible for glucose production from carbohydrate, lipid, and protein reserves, leading to enhanced gluconeogenesis, causing decreases in skeletal muscle mass. When insulin is absent, corticosterone stimulates proteolysis and lipolysis, supplying substrates for gluconeogenesis and energy production. Khondowe et al. (2018) also observed that a low-energy diet significantly raised corticosterone levels. Avian metabolism is profoundly influenced by corticosterone, the primary glucocorticoid in birds, or by dexamethasone, which serves as a substitute. Glucocorticoid treatment in chickens results in reduced growth, particularly in skeletal muscle, and is associated with increased adiposity and liver weight (Yuan et al., 2008).

The observed lower growth rate in chicks fed low-energy diets in the current research is likely because of elevated corticosterone concentrations. Increased corticosterone stimulates proteolysis to provide amino acids for hepatic gluconeogenesis, raising blood glucose levels. Additionally,

lower insulin levels, which are crucial for carbohydrate and lipid metabolism regulation and for promoting growth through augmented protein synthesis and modifying the expression of growth-related genes contribute to the reduced growth rate. Birds primarily eat more to meet their energy needs, and they tend to consume greater quantities when their diet is lower in energy density. Despite this, when birds were given diets low in crude protein (CP) and metabolizable energy (ME), their feed intake increased. Moreover, this increased intake couldn't offset the negative impact on weight gain and feed conversion ratio (FCR) due to the inherent limitations on how much birds can consume. Moreover, it didn't lead to a full recovery of their final body weight. The hypothesis aligns with the results documented by Saxena et al. (2020).

5. CONCLUSIONS

In conclusion, concerning growth parameters energy deficiency caused a marked decrease in growth, and protein deficiency may probably be due to the upregulation of the level of corticosterone hormone and downregulation of glucose and insulin, so energy deficiency caused more marked effects on these hormones than protein deficiency.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

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