EFFECT OF FEEDING LOW ENERGY AND PROTEIN DIETS ON EXPRESSION OF SOME GROWTH-RELATED GENES AND INTESTINAL MORPHOLOGY IN HUBBARD BROILERS

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ABSTRACT

This study aimed to assess the effects of varying dietary energy and protein levels on growth-related gene expression and intestinal morphology in Hubbard broilers. A total of 234 one-day-old chicks were used and divided into six groups, with 39 chicks per group. **Group I:** Received a basal diet containing standard energy and protein. **Group II:** Received a diet with standard protein and 10% reduced energy. **Group III:** Received a diet with standard protein and 20% reduced energy. **Group IV:** Received a diet with standard energy and 10% reduced protein. **Group V:** Received a diet with standard energy and 20% reduced protein. **Group VI:** Received a diet with 20% reduced energy and 10% reduced protein. Gene expression of IGF1, Myostatin, and Ghrelin was measured. Histological and morphometric analyses of the intestines were also conducted. The results revealed that IGF1 gene expression was downregulated in all groups, except for group IV, which showed no significant change compared to the control group. Group III and group VI exhibited the lowest IGF1 expression. For Myostatin, the highest expression levels were observed in group III and group VI, while group IV showed no significant difference ($p<0.05$) compared to the control group. Ghrelin gene expression was significantly upregulated in all groups, with the highest upregulation seen in group III and group VI. Regarding intestinal histomorphology, all groups showed a significant decrease in villi length compared to the control group. The jejunal crypt depth was significantly reduced ($p<0.05$) in the group III and group VI. In conclusion, low energy had a more pronounced effect on the expression of growth-related genes and intestinal indices than low protein.

Keywords: Low protein, low energy, intestinal morphology, gene expression, Hubbard chicks.

INTRODUCTION

In recent decades, the growth rate of broiler chickens has significantly increased, affecting their dietary needs and body composition (Fancher, 2014; Fouad & El-Senousey, 2014). Previously, nutritional experts and researchers believed that the energy density of broiler chicken diets was the primary factor governing feed intake (FI) (Ahiwe *et al.*, 2018; Fouad & El-Senousey, 2014). However, Ceylan *et al.* (2023) have shown that feed intake in broiler chickens is influenced by both the energy and amino acid

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content of the diet. Energy is allocated to various functions, including maintaining body weight and developing body proteins (Zuidhof, 2019). Different peptides and hormones have varying effects on food intake in broilers (Honda *et al*., 2012). Additionally, nutritional availability plays a crucial role in controlling body and muscle growth, either directly or indirectly affecting regulatory parameters (Carbone *et al*., 2012).

Insulin-like growth factor 1 (IGF-1) is a peptide growth factor essential for various aspects of growth, development, and metabolism (Werner, 2023). Plasma concentration of IGF-1 is influenced by nutrition (Lu *et al*., 2009). Under fasting conditions, the overexpression of IGF-binding protein 2 (IGFBP-2) reduces the availability of IGF-1 in the body, which negatively impacts chicken growth (Kita *et al*., 2005; Lu *et al*., 2009). Broiler chicks exhibit an increase in IGF-1 mRNA levels following refeeding (Fujita *et al*., 2017), highlighting the hormone's crucial role in growth and development, particularly when chickens are fed (Fujita *et al*., 2019). IGF-1 is a key regulator of chicken metabolism and muscle growth. For instance, research has shown that IGF-1 enhances protein content in chicken myotubes (Nakashima *et al*., 2017) and promotes the growth of chicken myoblasts (Yu *et al*., 2015).

Ghrelin functions as a hunger signal and plays a crucial physiological role in initiating meals (Cummings *et al*., 2001). Additionally, ghrelin affects various processes, including feeding patterns, gastrointestinal processes, and energy metabolism (Kaiya *et al*., 2002). It is important to note that complex interactions between genetic, environmental, and nutritional factors, which in turn influence endocrine secretions, ultimately determine growth outcomes.

Myostatin is a protein known for its role in inhibiting muscle growth. Its significance is primarily due to two key factors. Firstly, myostatin has a profound impact on muscle growth, highlighting its potential for influencing muscle development. It acts as a negative regulator of muscle growth, where reduced levels or inactivity of myostatin can lead to increased muscle growth through enhanced cell division and/or hypertrophy (Shoyombo *et al*., 2022).

Ultimately, starvation depletes skeletal muscle mass by inducing skeletal muscle proteolysis, which supplies amino acids for hepatic gluconeogenesis or energy synthesis (Mitch and Goldberg, 1996). Broilers exhibit rapid growth due to the exceptional ability of their intestinal epithelia to absorb nutrients efficiently and convert them into muscle. The gastrointestinal tract (GIT) is a complex and dynamic organ essential for nutrient absorption. It breaks down feed into its basic components through mechanical and chemical processes, and nutrient transporters then move these nutrients to the intestinal epithelial cells (Mahdavi *et al*., 2024).

The regulation of intestinal amino acid and peptide transporters is influenced by factors such as nutritional density, food composition, and intestinal development (Mahdavi *et al*., 2018; Osmanyan *et al.,* 2018). The intestinal mucosa, where nutrient absorption occurs, comprises various cell types and tissues, including epithelial and connective tissues (Mishra and Jha, 2019). The crypts and villi of the absorptive epithelium are crucial for the final stages of nutrient digestion and absorption in the small intestine (Wang and Peng, 2008).

Assessing intestinal development involves measuring the crypts, where new intestinal cells emerge, as well as the height and surface area of the villi. These measurements indicate the available space for digestion and absorption processes (Franco *et al*., 2006). Changes in intestinal morphology can be influenced by various factors, including stress, nutrient levels, and feed additives (Chen *et al*., 2019). Additionally, dietary nutrient density and feed form play crucial roles in shaping gastrointestinal tract (GIT) development (Ravindran & Abdollahi, 2021).

The objective of the current study was to evaluate the impact of dietary energy levels and protein concentrations on growth-related hormone genes, such as ghrelin, IGF-1, and myostatin, as well as to assess changes in intestinal indices, including villi length, villi width (VW), crypt depth (CD), and goblet cell number.

MATERIALS AND METHODS

1. Animals, experimental diets and design
This study was conducted under ethical approval number BUFTM05-04-24 at the Center for Experimental Animal Research, Faculty of Veterinary Medicine, Benha University, Egypt.

A total of 234 one-day-old Hubbard Efficiency Plus broiler chicks of both sexes were used in this study. The chicks were obtained from Pyramid Poultry Company, Cairo, Egypt. The chicks were randomly allocated into six groups, with 39 chicks per group. Each group was represented in three replicates, with 13 birds per replicate.

Group I (control group): Chicks received a basal diet with standard energy and protein.
Group II (10% LE): Chicks received a diet with standard protein and 10% reduced energy.
Group III (20% LE): Chicks received a diet with standard protein and 20% reduced energy.
Group IV (10% LP): Chicks received a diet with standard energy and 10% reduced protein.
Group V (20% LP): Chicks received a diet with standard energy and 20% reduced protein.
Group VI (20% LE - 10% LP): Chicks received a diet with 20% reduced energy and 10% reduced protein.
All six dietary treatments were designed with two levels of energy and protein for the starter (days 1-10), grower (days 11-24), and finisher (days 25-42) phases, according to Hubbard's requirements (2022). Nutrient specifications are detailed in Table 1.

The chicks were raised under identical conditions for 42 days, with unrestricted access to food (provided via an 8 kg plastic manual feeder) and water (from a 6 L plastic drinker). Fresh, clean bedding of 7 cm depth made from wood shavings was used and turned weekly. During the first two days, continuous light was provided using compressed filament lamps. Subsequently, a lighting regimen of 23 hours light and 1 hour dark (23L/1D) was maintained throughout the study to minimize the chicks' activity.

Heaters were used to regulate the environmental temperature according to the chicks' age, starting at 32°C and decreasing by 2°C per week until reaching 24°C by the end of the experiment. Adequate ventilation was ensured through the use of windows and negative-pressure fans, to remove moisture, dry the litter, and expel carbon dioxide and the produced ammonia from the birds' feces

2. Tissue samples
The expression analysis of ghrelin, IGF-1, and myostatin in the proventriculus, liver, and skeletal muscle, respectively, was performed using β-actin as the housekeeping gene for normalization. After slaughter, samples of skeletal muscle, proventriculus, and liver tissue, each weighing approximately 1 g, were aseptically collected from each bird and preserved in RNALATER. The samples were then stored at -80°C until RNA extraction.

3. mRNA expression of growth genes
Total RNA was isolated from proventriculus and skeletal muscle cells according to the manufacturer's protocol. The isolated RNA was then reverse transcribed into complementary DNA (cDNA) using the RevertAid Reverse Transcriptase kit (200 U/µL) (Thermo Fisher Scientific, USA, cat. no. EP0441). Primer sequences are detailed in Table 2. Real-time PCR was conducted using a Stratagene MX3005 P real-time PCR system and the Quantitect SYBR Green PCR kit (Metabion, Germany, cat. no. 204141).

Table 1: Ingredients and chemical composition 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	51.07	47.1	30.15	55.17	61.87	45.02	55.58	51.7	36.43	57.96	65.23	40.68	56.82	58.26	41.62	63.68	66.3	46.48
Soya Bean Meal 46% CP	35	35	25.76	35	30.15	33.30	29.7	27.1	20.7	32.55	26.13	21.6	28.7	26.6	22.3	22.6	24.24	24.2
Wheat bran	_	5.68	28.76	_	_	16.03	_	9.94	28.45	_	_	28.5	_	5.5	26.45	_	_	24.55
vegetable oil	2.95	0.5	0.52	3.2	2.37	0.53	3.8	0.53	0.5	4.65	3.4	0.5	5.35	0.5	0.5	4.19	4.8	0.5
Corn gluten meal	6	2.9	5.84	1.43	_	_	6	6	6.2	_	_	2	5	5	5	5	_	_
mono calcium phosphate	1.7	1.64	1.41	1.77	1.81	1.6	1.65	1.54	1.33	1.67	1.72	1.36	1.44	1.38	1.16	1.48	1.5	1.2
lime stone	1.55	1.58	1.66	1.57	1.58	1.62	1.44	1.48	1.54	1.43	1.45	1.54	1.29	1.3	1.38	1.3	1.3	1.37
Sodium Bicabonate		0.22	0.27	0.25	0.31	0.24	0.28	0.29	0.3	0.27	0.33	0.33	0.23	0.25	0.25	0.31	0.31	0.25
DL-Methionine	0.3	0.31	0.3	0.39	0.45	0.4	0.27	0.27	0.26	0.36	0.4	0.34	0.21	0.21	0.22	0.26	0.34	0.3
l lysine	0.34	0.3	0.47	0.38	0.53	0.38	0.34	0.38	0.48	0.31	0.49	0.5	0.23	0.26	0.32	0.4	0.4	0.31
Vit and minl premix1	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sodium chloride	0.26	0.24	0.21	0.23	0.18	0.23	0.2	0.2	0.18	0.21	0.16	0.17	0.24	0.23	0.23	0.19	0.19	0.23
L_Threonine	0.1	0.1	0.15	0.17	0.25	0.19	0.01	0.12	0.15	0.15	0.23	0.21	0.06	0.07	0.1	0.14	0.19	0.15
choline chloride	0.1	0.09	0.14	0.1	0.12	0.13	0.11	0.12	0.14	0.1	0.12	0.14	0.1	0.1	0.13	0.11	0.1	0.12
sunflower meals 34% CP	_	4	3.99	_	_	_	_	_	3	_	_	1.79	_	_	_	_	_	—
l tryptophan	_	_	_	_	0.01	_	_	_	_	_	_	_	_	_	_	_	_	—
Anti-clostridial	_	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Anti-mycotoxin	0.01	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

Chemical composition

Ingredients	Starter						Grower						Finisher					
	G1	G2	G3	G4	G5	G6	G1	G2	G3	G4	G5	G6	G1	G2	G3	G4	G5	G6
ME	3000.1	2700.1	2400.7	3000.1	3000.5	2400.2	3102.8	2790.4	2480.9	3101.5	3100.1	2480.0	3200.8	2880.5	2560.8	3200.4	3201	2561
Crude protein	23.1	23.1	23.1	20.8	18.5	20.8	21.1	21.1	21.1	18.99	16.9	19.0	20	20.0	20.0	18.0	16.0	18.0
Digestible protein	20.5	20.4	19.8	1826	15.9	17.9	18.7	18.5	18.0	16.7	14.6	16.0	17.8	17.7	17.3	15.8	13.9	15.4
Digestible fat	5.44	3	3.22	5.64	4.94	3.08	6.39	3.30	.35	7.08	6.03	3.31	7.9	3.31	3.38	6.93	7.43	3.29
Digestible fat poultry	2.15	2.04	1.99	2.10	2.23	2.00	2.27	2.31	2.17	2.11	2.32	2.13	2.26	2.40	2.23	2.45	2.34	2.16
Crude Fiber	2.20	3.33	4.82	2.24	2.20	3.42	2.13	2.86	4.57	2.20	2.14	4.43	2.1	2.56	3.99	2.05	2.10	3.93
Lysine	1.4	1.41	1.42	1.39	1.38	1.41	1.26	1.27	1.28	1.26	1.24	1.28	1.14	1.14	1.16	1.12	1.12	1.16
Lysine dig	1.27	1.27	1.27	1.27	1.27	1.27	1.15	1.15	1.15	1.15	1.15	1.15	1.03	1.03	1.03	1.03	1.03	1.03
Methionine	0.67	0.67	0.67	0.69	0.72	0.70	0.61	0.61	0.61	0.63	0.65	0.63	0.54	0.54	0.54	0.55	0.58	0.56
Methionine dig	0.64	0.64	0.63	0.67	0.70	0.67	0.58	0.58	0.57	0.61	0.63	0.60	0.51	0.51	0.51	0.53	0.56	0.53
Methionine +cystine	1.05	1.06	1.07	1.04	1.03	1.05	0.97	0.97	0.98	0.95	0.94	0.97	0.88	0.89	0.90	0.87	0.85	0.88
Methionine +cystine dig	0.95	0.95	0.95	0.95	0.95	0.95	0.87	0.87	0.87	0.87	0.87	0.87	0.79	0.79	0.79	0.79	0.79	0.79
Threonine	0.96	0.96	0097	0.95	0.93	0.96	0.79	0.89	0.89	0.87	0.85	0.88	0.8	0.81	0.82	0.79	0.78	0.80
Threonine dig	0.83	0.83	083	0.83	0.83	0.83	0.67	0.79	0.76	0.76	0.76	0.77	0.69	0.69	0.70	0.69	0.69	0.69
Tryptophan	0.26	0.28	0.27	0.25	0.23	0.26	0.23	0.23	0.24	0.23	0.20	0.23	0.22	0.22	0.23	0.19	0.19	0.23
Tryptophan dig	0.23	0.24	0.24	0.22	0.20	0.23	0.2	0.21	0.21	0.20	0.17	0.20	0.2	0.19	0.20	0.17	0.16	0.20

Ct values and amplification curves were analyzed using Stratagene MX3005P software. Gene expression levels were quantified using the "ΔΔCt" method (Yuan *et al.,* 2006). The Ct values of each sample were compared to the control group, and gene expression was estimated using the ratio (2−ΔΔCt) to assess the relative variance in gene expression among different samples.

Whereas ΔΔCt = ΔCt gene of interest – ΔCt Control
ΔCt gene of interest = Ct target – Ct β-actin
and ΔCt Control = Ct target – Ct β-actin

Table 2: Primer sequences of ß-actin, ghrelin, myostatin, and IGF1 genes for Real-time PCR.

Gene	Primer sequence (5'-3')	Reference
Ghrelin	CCT TGG GAC AGA AAC TGC TC	**Ghazanfari *et al.,* 2010**
	CAC CAA TTT CAA AAG GAA CG	
MSTN	GCT TTT GAT GAG ACT GGA CGAG	**El-Saway *et al.,* 2022**
	AGC GGG TAG CGA CAA CAT C	
IGF1	CAG AGC AGA TAG AGC CTG CG	**Amills *et al.,* 2003**
	TCT GCA GAT GGC ACA TTC AT	
ß. Actin	CCACCGCAAATGCTTCTAAAC	**Yuan *et al.,* 2007**
	AAGACTGCTGCTGACACCTTC	

4. Histological and Morphometrical Studies on intestinal villi.

At the end of the experiment (day 42), three birds from each group were slaughtered, and intestinal specimens were collected from several sections, specifically 2.5 cm segments of the ileum, mid jejunum, and duodenum. The tissues were fixed in 10% neutral-buffered formalin for three days. After fixation, the specimens were dehydrated by rinsing multiple times in absolute alcohol, and then embedded in paraffin wax. Serial 5-μm longitudinal sections were cut using a Leica Rotary Microtome (RM 2145, Leica Microsystems, Wetzlar, Germany) and placed on glass slides. The slides were stained with hematoxylin and eosin (H&E), following the protocol by Prakatur *et al.* (2019). Histomorphometric analysis was performed using ImageJ software (National Institutes of Health, MD, USA). Villus height was measured from the villus tip to the villus-crypt junction, villus width was measured at the midpoint of the villus, and crypt depth was the distance from the crypt-villus junction to the base of the crypt (Choe *et al*., 2012; Tenesa *et al*., 2016). Goblet cell density was calculated as the number of goblet cells per unit area (mm²).

5. Statistical Analysis

Statistical analyses were performed using SPSS Statistics (Version 26, IBM, USA). Group differences were evaluated using one-way ANOVA, followed by Duncan's multiple range test for post hoc analysis. Results are presented as means ± S.E.M., with statistical significance defined as $P < 0.05$. Figures were created using GraphPad Prism software (Version 10, USA).

RESULTS

1. Effects of different levels of energy and protein in the diets on Ghrelin, IGF1 and Myostatin relative gene expression

mRNA expression of ghrelin was significantly ($p < 0.05$) upregulated in all groups compared to the control group. The highest expression levels were observed in

the 20% LE-10% LP group and the 20% LE group (Table 3, Fig. 1A).

Regarding IGF-1 (Table 3, Fig. 1B), mRNA expression exhibited substantial (p < 0.05) downregulation in all groups compared to the control group. However, the 10% LP group did not show a significant difference from the controls. The lowest expression levels were observed in the 20% LE-10% LP group and the 20% LE group.

mRNA expression of Myostatin in skeletal muscle was upregulated in all groups compared to the control group, except for the 10% LP group, which showed non-significant (p > 0.05) upregulation (Table 3, Fig. 1C). The highest expression levels were observed in the 20% LE-10% LP group and the 20% LE group.

Table 3: Effects of different dietary levels of energy and protein on Ghrelin, IGF1 and Myostatin relative gene expression

parameters / Groups	Ghrelin	IGF1	Myostatin
Control	1.00 ^{e} ± 0.00	1.00 ^{a} ±0.00	1.00 ^{e} ± 0.00
10% LE	3.3 ^{cd} ± 0.26	0.66 ^{b} ± 0.03	6.15 ^{c} ± 0.27
20% LE	7.44 ^{b} ± 0.32	0.45 ^{c} ± 0.05	9.57 ^{b} ± 0.32
10% LP	2.53 ^{d} ± 0.24	0.95 ^{a} ± 0.02	1.99 ^{e} ± 0.11
20% LP	4.08 ^{c} ± 0.12	0.71 ^{b} ± 0.02	4.66 ^{d} ± 0.24
20% LE-10% LP	9.42 ^{a} ± 0.36	0.21 ^{d} ± 0.02	12.15 ^{a} ± 0.55

Values are expressed as mean ± S.E.M. Values in the same column carrying different superscripts are significantly different at (p < 0.05).

2. Effects of different dietary levels of energy and protein on the intestinal histomorphology of broilers

The effects of varying energy and protein levels in the diet on intestinal histomorphology are detailed in Table 4 and illustrated in Figures 2, 3, and 4.

Villus length in the duodenum significantly decreased (p < 0.05) in all groups compared to the control group. However, there was no appreciable variation in villus depth or crypt depth across the groups. The number of goblet cells significantly decreased (p < 0.05) in the 10% LE, 20% LE, and 20% LE-10% LP groups compared to the control group, whereas the 10% LP and 20% LP groups showed non-significant (p > 0.05) differences.

Jejunal villi length showed a significant (p < 0.05) decrease in all groups compared to the control group. Crypt depth in the jejunum was significantly reduced (p < 0.05) in the 10% LE, 20% LE, and 20% LE-10% LP groups compared to the control, while the 10% LP and 20% LP groups did not differ significantly (p > 0.05) from the control. Villi width in the jejunum significantly decreased in all groups compared to the control, except for the 10% LP group, which showed a non-significant decrease. Additionally, the number of goblet cells in the jejunum was significantly reduced (p < 0.05) in all groups compared to the control.

In the ileum, villi length significantly decreased (p < 0.05) in all groups compared to the control, except for the 10% LP group, which showed a non-significant decrease. There were no significant differences in crypt depth, villi width, or goblet cell number between the groups.

Table 4: Effects of different dietary levels of energy and protein on the intestinal histomorphology of broilers

Groups / Parameters	Control	10% LE	20% LE	10% LP	20% LP	20%LE-10%LP
Duodenum						
Villi length (μm)	$896.3^a \pm 6.6$	$616.2^{bc} \pm 6.7$	$578.2^c \pm 26.2$	$716.3^b \pm 31.6$	$633.3^{bc} \pm 47.2$	$429.9^d \pm 7.8$
Crypt depth (μm)	$116.9^a \pm 25.5$	$89.1^a \pm 2.8$	$64.4^a \pm 7.3$	$102.4^a \pm 7.8$	$98.3^a \pm 10.9$	$74.5^a \pm 10.2$
Villi width (μm)	$79.1^a \pm 6.6$	$92.9^a \pm 7.7$	$89.7^a \pm 10.9$	$104.5^a \pm 5.3$	$103.5^a \pm 7.5$	$82.6^a \pm 3.8$
Goblet cell number/mm2	$340.0^a \pm 30.5$	$262.4^{bc} \pm 9.7$	$197.5^c \pm 12.6$	$348.7^a \pm 10.8$	$319.2^{ab} \pm 10.1$	$201.4^c \pm 9.5$
Jejunum						
Villi length (μm)	$1212.2^a \pm 38.9$	$814.6^{bc} \pm 14.9$	$707.4^{cd} \pm 11.9$	$908.8^b \pm 27.4$	$838.4^b \pm 20.5$	$660.7^d \pm 22.2$
Crypt depth (μm)	$228.8^a \pm 9.7$	$172.7^b \pm 5.1$	$102.3^b \pm 5.1$	$190.2^{ab} \pm 5.9$	$177.2^{ab} \pm 10.9$	$105.8^b \pm 10.7$
Villi width (μm)	$143.5^a \pm 27.2$	$67.0^b \pm 16.1$	$67.9^c \pm 2.5$	$107.2^{ab} \pm 7.5$	$134.4^b \pm 12.9$	$64.7^c \pm 10.9$
Goblet cell number/mm2	$599.8^a \pm 16.8$	$345.8^{cd} \pm 18.3$	$292.6^d \pm 27.5$	$484.8^b \pm 11.8$	$397.5^c \pm 3.9$	$275.6^d \pm 9.7$
Ileum						
Villi length (μm)	$576.8^a \pm 14.0$	$404.5^{cd} \pm 25.6$	$402.3^{cd} \pm 5.9$	$507.1^{ab} \pm 25.2$	$428.4^{bc} \pm 31.1$	$310.2^d \pm 10.1$
Crypt depth (μm)	$79.3^a \pm 9.3$	$70.2^a \pm 9.04$	$82.8^a \pm 2.8$	$73.8^a \pm 7.04$	$64.4^a \pm 7.3$	$58.7^a \pm 7.7$
Villi width (μm)	$50.5^a \pm 13.2$	$69.8^a \pm 11.7$	$66.3^a \pm 8.0$	$66.1^a \pm 9.7$	$82.6^a \pm 9.2$	$99.8^a \pm 17.6$
Goblet cell number/mm2	$253.9^a \pm 27.4$	$271.8^a \pm 20.8$	$217.8^a \pm 6.5$	$231.2^a \pm 9.8$	$213.5^a \pm 5.5$	$123.1^a \pm 13.2$

Values are expressed as mean ± S.E.M. Values in the same row carrying different superscripts are significantly different at ($P < 0.05$).
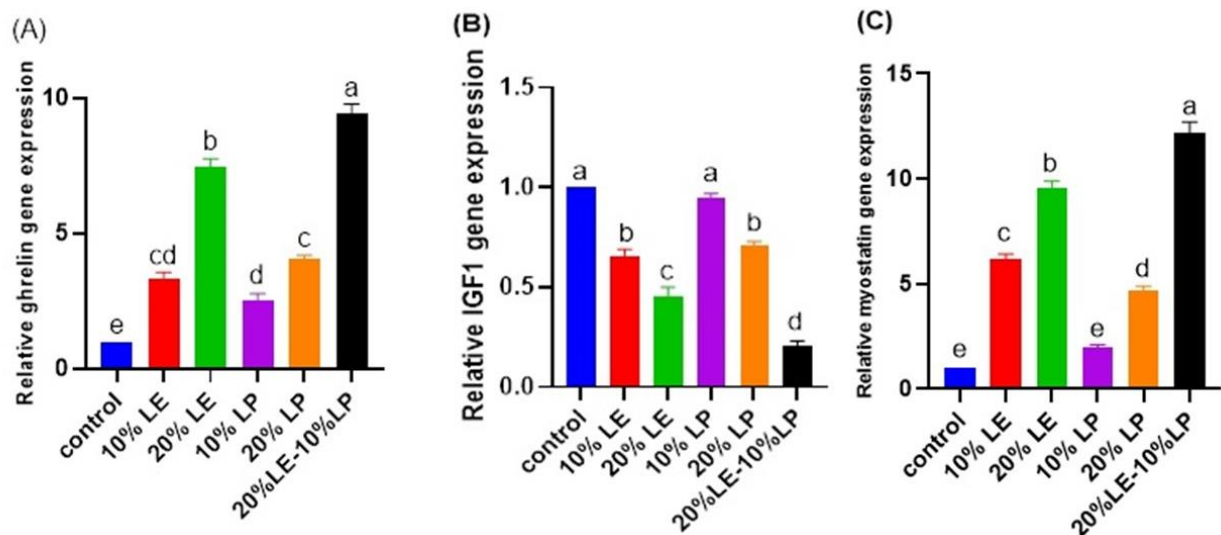
**Fig. 1:** Effects of different levels of dietary energy and protein on Ghrelin **(A),** IGF1**(B)** and Myostatin **(C)** relative gene expression. 10% LE :10% low energy group. 20%LE: 20% low energy group. 10%LP: 10% low protein group. 20%LP: 20% low protein group. 20%LE-10%LP: 20% low energy- 10% low protein group. Values are expressed as mean ± S.E.M. Values in the same column carrying different superscripts are significantly different at (p < 0.05).
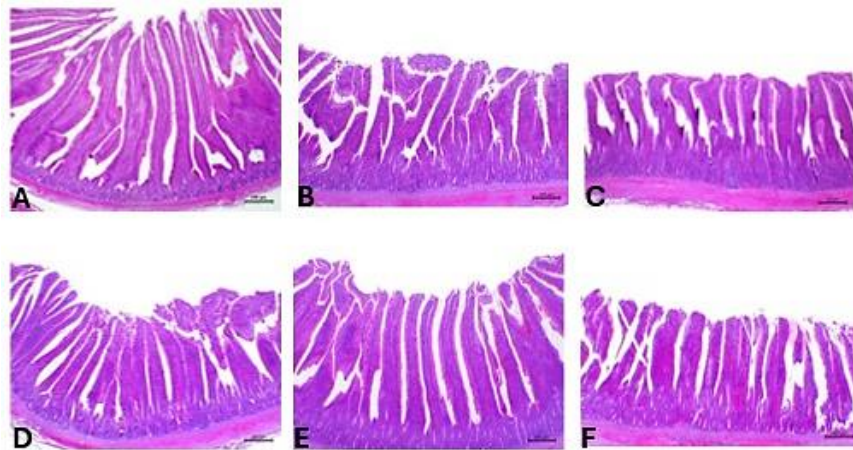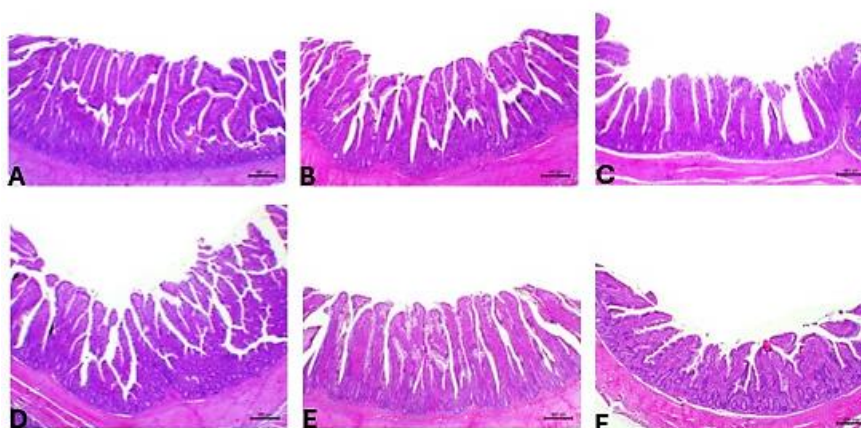
Fig. 2: Duodenal sections of broilers fed different levels of energy and protein in diets (H&E, 40x). **A:** control group. **B**: 10% low energy group (10% LE). **C:** 20% low energy group (20% LE). **D:** 10% low protein group (10% LP). **E:** 20% low protein group (20% LP). **F:** 20% low energy-10% low protein group (20%LE-10%LP).
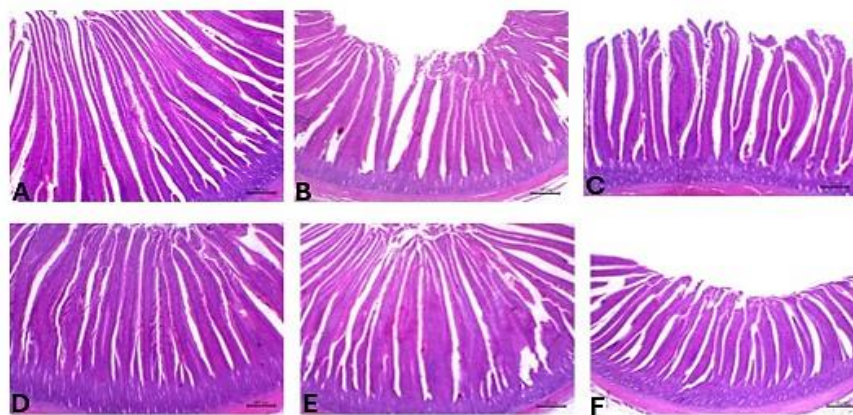
Fig. 3: Jejunal sections of broilers fed different levels of energy and protein in diets (H&E, 40x). **A:** control group. **B**: 10% low energy group (10% LE). **C:** 20% low energy group (20% LE). **D:** 10% low protein group (10% LP). **E:** 20% low protein group (20% LP). **F:** 20% low energy-10% low protein group (20%LE-10%LP).

Fig. 4: Ilium sections of broilers fed different levels of energy and protein in diets (H&E, 40x). **A:** control group. **B**: 10% low energy group (10% LE). **C:** 20% low energy group (20% LE). **D:** 10% low protein group (10% LP). **E:** 20% low protein group (20% LP). **F:** 20% low energy-10% low protein group (20%LE-10%LP).

DISCUSSION

Nutrition and genotype play critical roles in determining growth rate, body size, and weight in animals. The neuroendocrine system regulates growth by integrating genetic factors with external influences, such as nutrition (Zhao *et al*., 2004). In broiler chickens, the energy density of the diet is a key factor in regulating feed intake. Additionally, the concentration of amino acids in the diet impacts also feed intake.

Conflicting results have been reported regarding the influence of dietary energy levels and protein concentrations on feed intake and related hormones, such as ghrelin. Therefore, the current study aims to evaluate how different energy densities and protein concentrations in broiler chicken diets affect the expression of growth-related hormones. Additionally, the study seeks to explore the mechanisms through which these hormones regulate feed intake, and how this in turn, impacts growth rate and body size in broiler chickens.

The results of the current study revealed that deficiencies in dietary energy and/or protein in broiler chickens led to notable compensatory changes in feed intake, energy metabolism, and the gene expression of ghrelin, IGF-1, and myostatin. Specifically, the study demonstrated a significant upregulation of ghrelin gene expression in the proventriculus of broiler chickens under energy-deficient and protein-deficient conditions. Notably, the highest levels of ghrelin expression were observed in the energy-deficient groups, indicating that ghrelin gene expression is strongly influenced by dietary energy levels than by protein concentrations.

These findings are consistent with previous research by Ghazanfari *et al*. (2010), which reported that reduced dietary energy levels led to increased ghrelin gene expression. Additionally, ghrelin expression was found to be higher in birds under dietary restriction compared to those fed a basal diet. Elevated ghrelin levels were also observed in the proventriculus during fasting (a state of negative energy balance). Similar studies on quail and chickens have shown that calorie restriction increases plasma ghrelin concentrations, which subsequently decrease upon refeeding (Shousha *et al*., 2005; Kaiya *et al*., 2007). Ghrelin's primary role is thought to stimulate appetite and, consequently, increase energy intake, which is crucial for maintaining glucose and energy homeostasis (Sovetkina *et al*., 2020).

The results of the current study revealed an intriguing increase in feed intake in birds fed low energy (LE) and low energy-low protein (LE/LP) diets. This increase is likely attributed to the substantial upregulation of ghrelin gene expression in the proventriculus tissue of these diets. Sovetkina *et al*. (2020) similarly noted that during fasting, conditions of negative energy balance enhance the release of ghrelin, which then decreases upon refeeding in positive energy balance conditions, highlighting ghrelin's compensatory role in energy imbalance. Kaiya *et al*. (2013) also demonstrated that endogenous or central ghrelin significantly impacts the regulation of feed intake and appetite in birds.

Interestingly, while ghrelin gene expression was substantially elevated in chicks receiving a low protein diet, feed intake did not significantly differ compared to control chicks. This finding contrasts with Wang *et al*. (2020), who reported a significant downregulation of ghrelin gene expression in the proventriculus of laying hens fed a low protein diet. Wang *et al*. (2020) suggested that this downregulation in expression in turn reduced feed intake.

The conflicting results regarding the impact of dietary proteins and amino acid concentrations on ghrelin gene expression and feed intake might be attributed to differences between the types of birds

studied. Wang *et al.* (2020) focused on laying hens, while the current study was conducted on broiler chickens. This indicates that the effects of dietary protein concentration on feed intake and ghrelin expression may vary between broiler chickens and laying hens. Additionally, both appetite and feed intake are also regulated by various anorexigenic neuropeptides, which might play a role in the decreased appetite observed in low-weight-selected chicks (Yi *et al.*, 2015).

Finally, ghrelin functions not only as a hunger signal, but also as an indicator of energy consumption, playing a critical role in maintaining energy balance in broilers. The energy level in the diet is closely related to ghrelin secretion, as higher energy deficiencies lead to increased ghrelin expression, reflecting its role in compensating for negative energy balance and regulating feed intake (Sovetkina *et al.*, 2020).

Numerous studies on growth factors have revealed that nutritional conditions significantly influence the levels of circulating insulin-like growth factors (IGF-1 and IGF-2) and their gene expression (Tóth *et al.*, 2022). In this research, the mRNA expression of IGF-1 was downregulated in all groups fed low energy and low protein diets, except for the 10% LP diet group, which showed non-significant changes, compared to the control group. These findings are consistent with previous studies demonstrating reduced IGF-1 gene expression in broiler chickens subjected to restricted diets or fasting, with restored IGF-1 levels upon refeeding (Giachetto *et al.*, 2004; Richards *et al.*, 2005; Ayson *et al.*, 2007).

Protein depletion and energy deficiency have been shown to inhibit IGF-1 synthesis (Leaman *et al.,* 1990), as low nutrient or energy availability reduces IGF-1 production and secretion. This reduction in IGF-1 can increase autophagy, apoptosis,

and cell turnover, ultimately impairing growth (Adler and Bonduriansky, 2014). The expression of IGF-1 is closely correlated with plasma IGF-1 levels in broilers (Burnside and Cogburn, 1992). Food restriction typically lowers IGF-1 levels, leading to decreased IGF-1 gene expression and an increase in plasma growth hormone (GH) (Duncan *et al.*, 2015). This effect is attributed to the downregulation of growth hormone receptors during caloric restriction, which leads to reduced serum IGF-1 levels. Research on rats showed that hepatic IGF-1 mRNA levels are lower in fasted rats, compared to well-fed ones (Clemmons and Underwood, 1991). Conversely, some studies have reported increased IGF-1 levels in food-restricted broiler chickens compared to controls (Hocking *et al.*, 1994).

The observed decrease in IGF-1 gene expression in broilers fed low-energy diets in the current study could be attributed to elevated serum corticosterone levels. This is consistent with findings from Song *et al.* (2011), who reported that glucocorticoid hormones, such as corticosterone, can inhibit growth by reducing IGF-1 levels. Elevated corticosterone is known to negatively impact growth by affecting various metabolic processes, including the suppression of IGF-1 production. This suppression of IGF-1 could contribute to the observed stunted growth and reduced muscle development in broilers subjected to low-energy diets.

In chicken skeletal muscle, nutrient availability is a critical regulator of MSTN (myostatin) gene expression (Guernec *et al.,* 2004), which negatively impacts muscle growth and development by restricting both the quantity and size of muscle Fibers (Chen et al., 2021). The current study revealed a marked upregulation of myostatin gene expression in broilers fed low-energy and low-protein diets. This finding is consistent with previous research.

For example, Yang et al. (2009) observed that reduced protein and energy levels in the diet led to increased MSTN gene expression and decreased muscle production. Similarly, Ye et al. (2007) reported that birds fed diets low in both calories and lysine exhibited the highest MSTN expression, which was associated with the lowest breast muscle yield.

The study by Varadharajan (2022) also supports the finding that myostatin (MSTN) gene expression is increased in broilers fed low-protein diets. This observation aligns with the current research, which demonstrated higher MSTN expression in broilers receiving low-energy and low-protein diets. Varadharajan's study highlights that low-protein diets can elevate MSTN expression, indicating a potential inhibitory effect on muscle growth.

Conversely, the research by Guernec et al. (2003) reported a positive relationship between MSTN expression and breast muscle yield in broilers, particularly at days 28 and 42.

The findings from the current study regarding the impact of low energy and low protein diets on intestinal morphology in broiler chickens show a significant reduction in villus height and a shallower crypt depth across the jejunum, duodenum, and ileum. These observations are consistent with earlier research, such as the study by Ghahremani et al. (2016), which found that a reduction in dietary energy, particularly beyond 100 kcal/kg, led to a statistically significant decline in intestinal indices, including villus height and crypt depth.

Similarly, Ale Saheb Fosoul et al. (2016, 2018) and Abbasi et al. (2014) reported that decreased dietary energy intake was associated with a reduction in jejunal villus length, highlighting the impact of energy deficiency on gut morphology. These findings suggest that energy levels in the diet play a crucial role in maintaining intestinal health and development, as indicated by the significant changes observed in the intestinal indices under low energy conditions.

On the other hand, XU et al. (2003) reported a contrasting effect, where low-energy diets resulted in a marked reduction in duodenal villus height and an increased crypt depth.

The observations from the present study highlight that low energy and low protein diets in broiler chickens lead to a significant reduction in villus height and a shallower crypt depth, which can impair nutrient absorption and the overall performance. These findings align with some studies that have reported how decreased villus height and altered crypt depth negatively affect gut function and performance.

However, the results from other studies present a more nuanced view of the relationship between dietary energy levels and intestinal morphology. For instance, Ceylan et al. (2021) observed that reducing the metabolizable energy (ME) level in the diet led to an increase in villus height and surface area, without affecting crypt depth. This contrasts with the current study's findings and suggests that the impact of dietary energy on intestinal morphology might vary depending on specific conditions or methodologies.

Similarly, Mahdavi et al. (2024) and Kim et al. (2019) found that changes in dietary energy density had no significant effect on jejunal villus height, width, or crypt depth. These studies indicate that the effects of dietary energy on gut morphology might not always be straightforward, and can vary between different species or experimental setups.

Furthermore, Majdolhosseini et al. (2019) observed that while low ME diets did not affect gut morphology significantly, they did negatively impact growth performance. This suggests that the implications of

dietary energy levels might extend beyond gut morphology to affect overall health and performance.

The findings from the present study indicate that a reduction in dietary crude protein (CP) leads to a slight decrease in villus length in the duodenum, jejunum, and ileum compared to the control group, though the decrease is less pronounced than observed with reduced metabolizable energy (ME) diets. This observation aligns with some existing research, but also contrasts with other studies.

Laudadio *et al*. (2012) support these findings by noting that high and medium-CP diets lead to an increase villus height and a decrease crypt depth compared to lower-CP diets. This suggests that higher protein levels can enhance intestinal morphology, potentially improving nutrient absorption and gut health.

Conversely, Buwjoom *et al*. (2010) found that varying CP levels did not significantly affect villus height, crypt depth, or villus surface area.

The current study indicates that variations in energy and protein levels have differential effects on villus morphology across different sections of the intestine. Specifically, the width of the duodenal and ileal villi was largely unaffected by changes in dietary energy or protein levels. This observation aligns with Ceylan *et al*. (2021), who also reported no significant changes in villus width with decreasing metabolizable energy (ME) levels. The width of jejunal villi was notably affected by dietary energy levels, with severe energy deficiency leading to a significant reduction in villus width. This finding is consistent with Ceylan *et al*. (2023), who observed that diets with the lowest energy concentrations resulted in the smallest villus width in the jejunum.

CONCLUSION

Concerning to hormonal changes, low energy groups caused upregulation of ghrelin and myostatin gene expression and downregulation of IGF1 gene expression. So, energy deficiency caused a more marked effect on the expression of these growth-related genes than protein deficiency. Concerning intestinal morphology, energy deficiency caused marked changes in intestinal indices than protein deficiency.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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131

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تأثير تغذية علائق منخفضة من الطاقة اوالبروتين علي التعبير الجيني لبعض الجينات الخاصة بالنمو والتركيب النسيجي الخلوي لأمعاء الدجاج الهابرد

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اجريت هذه الدراسة في مركز بحوث التجارب بكلية الطب البيطري جامعة بنها لتقييم تأثير مستويات مختلفة من الطاقة الغذائية اوالبروتين في عليقة دجاج الهابرد علي التعبير الجيني لبعض الجينات الخاصة بالنمو وعلي تركيب الانسجة الخلوي تم استخدام ٢٣٤ كتكوت (الهابرد) بعمر يوم ، وتم تقسيم الطيور الي ست مجموعات متساوية العدد (٣٩) طائر وكل مجموعة قسمت الي ثلاث مكررات يحتوي كل مكرر علي (١٣) كتكوت وتم تقسيم المجموعات علي النحو الاتي:

(١) المجموعة الاولي : تتغذي علي عليقة تحتوي علي النسبة المثلي للطاقة والبروتين وسميت بالمجموعة الضابطة
(٢) المجموعة الثانية: تتغذي علي عليقة تحتوي علي البروتين القياسي ونقص مصدر الطاقة بنسبة ١٠%
(٣) المجموعة الثالثة: تتغذي علي عليقة تحتوي علي البروتين القياسي ونقص مصدر الطاقة بنسبة ٢٠%
(٤) المجموعة الرابعة: تتغذي علي عليقة تحتوي علي مستوى الطاقة القياسي ونقص مصدر البروتين بنسبة ١٠%
(٥) المجموعة الخامسة: تتغذي علي عليقة تحتوي علي مستوى الطاقة القياسي ونقص مصدر البروتين بنسبة ٢٠%
(٦) المجموعة السادسة: تتغذي علي عليقة تحتوي علي نقص مستوى الطاقة بنسبة ٢٠% والبروتين بنسبة ١٠%

ويمكن تلخيص النتائج التي تم الحصول عليها كالاتي:
اولا: التعبير الجيني للجريلين ، معامل شبيه الانسولين والميوستاتين
بالنسبة لجين الجريلين : كل المجموعات اظهرت زيادة في هذا الجين مقارنة بالمجموعة الضابطة واعلي زيادة في هذا الجين كان في مجموعة نقص الطاقة ٢٠% ومجموعة نقص كلا من الطاقة ٢٠%مع نقص البروتين ١٠%.
بالنسبة لجين معامل شبيه الانسولين ١: كل المجموعات اظهرت نقص فيه مقارنة بالمجموعة الضابطة ما عدا مجموعة نقص البروتين ١٠% لم تختلف عن المجموعة الضابطة واقل نقص في هذا الجين كان في مجموعة نقص الطاقة ٢٠% ومجموعة نقص كلا من الطاقة ٢٠% مع نقص البروتين ١٠%
بالنسبة لجين الميوستاتين : كل المجموعات اظهرت زيادة في هذا الجين مقارنة بالمجموعة الضابطة ما عدا مجموعة نقص البروتين ١٠% لم تختلف عن المجموعة الضابطة واعلي زيادة في هذا الجين كان في مجموعة نقص الطاقة ٢٠% ومجموعة نقص كلا من الطاقة ٢٠%مع نقص البروتين ١٠%.
ثانيا: تأثير نقص الطاقة والبروتين علي طول الزغبات المعوية وعمق الخبايا: اظهر فحص مورفولجيا القناة الهضمية ان كل المجموعات اظهرت نقص في طول الزغبات المعوية مقارنة بالمجموعة الضابطة. واقل طول للزغبات ظهر في معي الصائم في مجموعة نقص الطاقة ٢٠% ومجموعة نقص كلا من الطاقة ٢٠%مع نقص البروتين.
ونستنتج من ذلك انه كان لانخفاض الطاقة تأثير علي هذه الجينات الخاصة بالنمو والمؤشرات المعوية مقارنة بانخفاض البروتين. وتوصي الدراسة باستخدام علائق متزنة في الطاقة والبروتين لضمان الانتاجية المثلى.