The Effect of Reducing Dietary Energy on Growth Performance, Intestinal Morphology and Intestinal Peptide and Amino Acid Transporters in Broiler Chicks

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Abstract

This study evaluated the effect of two levels of metabolizable energy (2950 and 2850 kcal/kg) on performance, intestinal morphology, and mRNA abundance of peptide (PepT1) and amino acid transporters ($b^{0,+}$ AT, B0AT, y^{+} LAT1 and CAT1) in broilers during the starter period (0-10 days of age). This is the first reported in this case in human or animals case. Two hundred and seventy day old male Ross 308 broilers were used according to a completely randomized design with two treatments, where each treatment applied to 9 replicate pens with 15 birds in each. The outcome variables were measured at the end of the starter phase. The mRNA abundance of PepT1, $b^{0,+}$ AT, B0AT, y^{+} LAT1 and CAT1 were assayed using real-time PCR. The results showed that the body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were not affected by the dietary treatments ($P>0.05$). Jejunal mRNA levels of the $b^{0,+}$ AT and B0AT decreased as metabolizable energy level decreased from 2950 to 2850 kcal/kg ($P\leq 0.05$). The transport systems PepT1, CAT1, and y^{+} LAT1 were not affected by the dietary treatments ($P>0.05$). There were no significant difference in villus height (VH), villus width (VW), villus surface area (VSA), crypt depth (CD), and villus height to crypt depth ratio (VCR) between treatments ($P>0.05$). These results demonstrated that dietary content of metabolizable energy affected the expression of amino acid transporters to modulate absorption of nutrients in broilers.

Introduction

The first days of life of today's broilers play a crucial role in their productivity because modern commercial broiler strains grow at a faster rate and need only a few weeks to attain slaughter weight. The development of digestive tract and muscle cell proliferation occur during the starter period, and therefore the post hatch nutrition of broiler chicks plays an important role in their productivity (Vieira and Moran 1999). The transition from embryogenic period to the early posthatch is characterized by major physiological and morphological changes in the gastrointestinal tract (GIT) allowing the chick to consume nutrients after hatching (Willemsen et al. 2010). The substantial changes also take place in the expression of many genes involved in growth control at the same time. There are many factors that can influence the GIT development such as breed, sex, feed form, disease, and dietary nutrient density.

The most expensive component of poultry diets is energy. The carbohydrate, fat, oil, and sometimes the dietary protein sources meet the bird's energy requirements. Fats and oils are the important sources of energy in poultry diet. In addition to supplying energy, fats and oils have wider physiological effects. These ingredients may decrease pulverulence, improve the absorption of fat-soluble vitamins, increase diet palatability, enhance the immune response and disease resistance, and change the synthesis of prostaglandin and thromboxane. It has been suggested that the fat has an extra metabolic effect and this effect could affect the feed intake and the retention time of feed in the GIT and consequently the nutrient digestion and absorption. (Mateos et al. 1981, 1982; Calder 2006; Cherian 2011; Classen 2017). In broiler chickens, early life energy requirement is provided by addition of fat to diet.

The mechanical and chemical activities of the GIT convert feed into its constituent components and then nutrient transporters move nutrients to the intestinal epithelial cells. Intestinal peptide and amino acid transporters are regulated by different factors, including genetic selection, stage of development, hormones, pathological states, pharmacological agents, intestinal development, form of diet, and nutrient density (Chen et al. 2005; Gilbert et al. 2008; He et al. 2013; Morales et al. 2017; Mahdavi et al. 2018; Osmanyanyan et al. 2018). The PePT1 transporter is capable of transporting all types of dipeptides and 8000 types of tripeptides from the epithelial layer into intestinal cells. Absorption through PePT1 is much more efficient and faster than absorption via amino acid transporters (Wang et al. 2017). The $b^{0,+}$ AT transporter is the main basic transporter in the epithelial layer that transports lysine into intestinal cells and simultaneously causes arginine to leave the cell and enter the intestinal lumen (Pineda et al. 2004). The CAT1 transporter carries lysine and arginine in the intestinal basolateral layer and factors such as nutrient type and density, hormones and growth factor modulate its gene expression. In the intestinal basolateral layer, neutral and basic amino acids are transported by y^+ LAT1. B^0 AT as the main transporter of neutral amino acids, especially methionine, leucine, isoleucine and valine, transport them from the intestinal epithelial layer into intestinal cells (Pineda et al. 2004; He et al. 2013; Wang et al. 2017).

Previous studies have evaluated the effect of various factors including sex, age, protein and amino acids levels, protein source and physical form of diet on the mRNA expression of intestinal peptides and amino acid transporters; however, there has been no attempt in human or animals to study the effect of dietary energy level on gene expression of intestinal peptide and amino acid transporters. Therefore, the aim of the present study is to find the effects of different dietary energy levels on the mRNA expression of intestinal peptide and amino acid transporters, growth performance, and intestinal morphology of broilers in the starter phase.

Materials And Methods

Experimental design and diets

A total of 270-day-old Ross 308 male chicks were housed in 18 pens in a completely randomized design with nine replicates of 15 birds each. They were arranged in two levels of ME (2950 and 2850 kcal/kg of diet) through 1 to 10 days of age. The nutrient contents of the feed ingredients were analyzed using NIRS™ DS2500 FOSS prior to dietary formulation. Dry matter, crude protein, total ash, and ether extract contents were determined according to (AOAC 2005). The formula and chemical composition of the diets are shown in Table 1.

Table 1
Ingredients and calculated nutrients of the diets.

	ME (Kcal/kg)	
Ingredients (g/kg)	2950	2850
Corn	518.00	518.00
Sunflower oil	29.00	17.20
Soybean meal	374.00	374.00
Corn gluten	30.00	30.00
DL-methionine	3.00	3.00
Lysine-HCl	2.50	2.50
L-threonine	0.70	0.70
Dicalcium phosphate	22.80	22.80
Limestone	9.70	9.70
NaCl	2.20	2.20
Sodium bicarbonate	2.10	2.10
Vitamin premix ^a	2.50	2.50
Mineral premix ^b	2.50	2.50
Choline chloride	1.00	1.00
Bentonite	0.00	10.80
Calculated Analysis		
Metabolizable energy, kcal/kg	2950	2850
Crude protein, %	23.20	23.20
Dig-Lysine, %	1.26	1.26
Dig-Met + Cys, %	0.91	0.91
Dig-Thr, %	0.80	0.80
Calcium, %	1.00	1.00
a. Each kg of vitamin premix contains 6 mg retinol, 0.1 mg cholecalciferol, 72 mg tocotrienol, 16 mg menodione, 4 mg thiamine, 20 mg riboflavin, 6 mg pyridoxine, 0.08 mg cobalamins, 120 mg niacin, 50 mg Ca-pantothenate, 2 mg folic acid, 0.08 mg biotin.		
b. Each kg of mineral premix contains 140 mg of Cu, 179 mg of Zn, 12.5 mg of Mn, 0.5 mg of I, 0.25 mg of Co, 0.4 mg of Se.		

	ME (Kcal/kg)	
Available P, %	0.48	0.48
Determined		
Dry Matter %	91.30	90.80
Crude Protein %	22.64	22.83
Ether Extract %	5.73	4.28
Ash %	6.32	6.45
a. Each kg of vitamin premix contains 6 mg retinol, 0.1 mg cholecalciferol, 72 mg tocotrienol, 16 mg menodione, 4 mg thiamine, 20 mg riboflavin, 6 mg pyridoxine, 0.08 mg cobalamins, 120 mg niacin, 50 mg Ca-pantothenate, 2 mg folic acid, 0.08 mg biotin.		
b. Each kg of mineral premix contains 140 mg of Cu, 179 mg of Zn, 12.5 mg of Mn, 0.5 mg of I, 0.25 mg of Co, 0.4 mg of Se.		

Performance and jejunal morphometry

The body weight was recorded on pen basis at 1 and 10 days of age. Feed intake and FCR was calculated at the end of the experiment. At d 10, one bird per replicate that had body weight close to the mean replicate was randomly sacrificed by neck dislocation, and their jejunum (10 centimeters to Meckel's diverticulum) was excised. Tissue samples were collected, and then fixed in 10% neutral buffered formalin solution. The jejunal tissue samples were processed by dehydration through a series of graded alcohols, cleared with xylene. Paraffin-embedded tissue sections (5 µm) were prepared using a CUT4055 manually microtome (microTec Laborgeräte GmbH, Germany). Slides were stained by Hematoxylin-Eosin method. The morphometric indices were determined using computer-aided light microscope image analyses (Olympus BX51TF, Japan). Morphometric parameters recorded included VH, from the tip of the villus to the crypt, mid-point VW, CD, from the base of the villi to the base of the crypt, and VCR ratio was calculated by dividing VH by CD. Villus surface area was also calculated as follows: $2\pi \times (VW/2) \times VH$; where $\pi = 3.14$, VW=villus width and VH=villus height (Sakamoto et al. 2000).

Real time PCR

On day 10, following post-mortem, samples from the region immediately prior to the Meckel's diverticulum (one bird from each pen) were aseptically dissected, rinsed with PBS and placed in a solution of RNAlater (Invitrogen, Carlsbad, CA, USA). Jejunum was selected because it is known as a main site of amino acids and peptide absorption in broilers (Chen et al. 2005). The tissue samples were transferred to -20°C until RNA could be extracted. The total RNA was isolated from 200 mg of the jejunum tissue using Trizol Reagent (Invitrogen Corp., Carlsbad, CA, USA) according to the manufacturer's recommended protocol and stored at -20°C. One microgram of total RNA was digested using DNase (Thermo Fisher Scientific) for 30 min at 37°C. The RNA quality was checked by 1.2% agarose gel electrophoresis, stained with 8 µg/ml ethidium bromide. Complementary DNA was synthesized from RNA

using the Mint-2 cDNA synthesis kit (DENA Zist Asia, Mashhad, Iran) according to the manufacturer’s recommended protocol. To ensure that there was no possible genomic DNA contamination, negative controls for all samples were also prepared by performing reverse transcription without enzyme mix. The following PCR conditions were used: 94°C for 2 min, and 40 cycles of 94°C for 15 s, 62°C for 15 s, 72°C for 20 s. All PCR was carried out using an Amplitronyx™ 6 Thermal Cycler (Nyx Technik, Inc. San Diego, CA).

Primers for the five genes chosen for verification by real-time PCR were described previously (Gilbert et al. 2008; Zhang et al. 2017) and are shown in Table 2. A housekeeping β-actin was used as an internal reference gene to normalize target gene transcript levels. Each of the cDNA samples was diluted 1:5 prior to real-time PCR and 1 μL diluted cDNA was used per real-time PCR reaction. Real-time PCR was performed in triplicate in 10 μL volume reactions that contained 2 μL SYBR Green Master Mix (DENA Zist Asia, Mashhad, Iran), 1 μL each of 2 μM forward and reverse primers, 5 μL H2O and 1 μL of diluted cDNA using a LightCycler®96 RT-PCR System (Roche, Swiss). The following protocol was used: (i) pre-incubation program (180 s at 95 °C); (ii) amplification, repeated 40 cycles (15 s at 95 °C, 15 s at 62 °C, 15 s at 72 °C); and (iii) a melting program (10 s at 95 °C, 1 min at 65 °C, 1 s at 97 °C). The relative expression ratio (R) of mRNA was calculated as $R = 2^{[C_{T(\beta\text{-actin})} - C_{T(\text{test})}]}$, where C_T = threshold cycle as described by Zeng et al. (2011).

Table 2
Sequences of real-time PCR primers used for selected genes.

SLC ^a name	Gene	GenBank ID	Primer Sequence Sense/Antisense
SLC15A1	PepT1	NM_204365.1	CCCCTGAGGAGGATCACTGTTGGCAGTT/ CAAAAGAGCAGCAGCAACGA
SLC7A9	b ⁰ +AT	NM_001199133.1	CAGTAGTGAATTCTCTGAGTGTGAAGCT/ GCAATGATTGCCACAACCTACCA
SLC7A1	CAT1	NM_001145490.1	CAAGAGGAAAACCTCCAGTAATTGCA/ AAGTCGAAGAGGAAGGCCATAA
SLC6A19	B ⁰ AT	XM_419056.4	GGGTTTTGTGTTGGCTTAGGAA/ TCCATGGCTCTGGCAGAGAT
SLC7A7	y ⁺ LAT1	XM_418326.4	CAGAAAACCTCAGAGCTCCCTTT/ TGAGTACAGAGCCAGCGCAAT
	β-actin	NM_205518.1	GTCCACCGCAAATGCTTCTAA/ TGCGCATTTATGGGTTTTGTT
^a SLC: solute carrier family.			

Statistical analysis

Data were analyzed using the General Linear Model procedure of SAS software Version 9.2 with a completely randomized design. If a significant effect was detected, the difference between treatments was separated using Duncan's multiple range test.

Results

Growth performance

The results of this study (Table 3) showed that reducing dietary energy by 100 kcal/kg did not impair the performance parameters of broilers in the starter phase ($P>0.05$). There was a slight trend towards an increased FI and FCR but not significantly when broilers fed diets with 2850 kcal ME/ kg compared with those fed 2950 kcal ME/kg.

Table 3
Effects of different levels of metabolizable energy of starter diet on the growth performance of broilers

ME (Kcal/kg)	Body weight gain (gr)	Feed intake (gr)	FCR
2950	191.08±19.11	217.42±11.82	1.15±0.20
2850	183.01±19.05	224.95±10.32	1.23±0.14
SEM	5.82	4.16	0.05
P-value	0.31	0.20	0.19

Intestinal peptide and amino acid transporters

Our results showed a significant effect of dietary energy level on the mRNA expression of amino acid transporters in the small intestine (Table 4). Reduction of dietary energy level caused a significant decrease in the mRNA expression of b0,+AT and B0AT transporters ($P\leq0.05$) but had no effect on the mRNA expression of PepT1, y+LAT1 and CAT1 ($P>0.05$).

Table 4
Effects of different levels of metabolizable energy of starter diet on mRNA abundance ($\times 10^{-3}$) of peptide and amino acid transporters of broilers

ME (Kcal/kg)	PepT1	b ⁰ +AT	CAT1	B ⁰ AT	y ⁺ LAT1
2950	0.78±0.20	140.99±48.92 ^a	1.16±0.32	124.67±25.28 ^a	2.08±0.86
2850	0.83±0.15	109.38±20.98 ^b	0.83±0.26	85.38±22.37 ^b	1.84±0.61
SEM	0.03	7.70	0.06	5.82	0.18
P-value	0.38	0.04	0.54	0.00	0.45
a,b Within a column, means with different superscripts differ significantly (P < 0.05).					

Intestinal morphology

The results also (Table 5) showed that there is no significant effect of feed energy on the intestinal morphology indices including VH, VW, CD, VCR and VSA (P>0.05).

Table 5
Effects of different levels of metabolizable energy of starter diet on the intestinal histomorphology of broilers

ME (Kcal/kg)	Villus height, μm	Villus width, μm	Crypt depth, μm	VCR	VSA ($\times 10^{-3} \mu\text{m}^2$)
2950	535.00±38.16	79.42±4.48	203.67±17.53	2.63±0.18	133.38±11.47
2850	494.89±35.44	85.08±7.89	187.00±14.36	2.65±0.17	132.54±17.47
SEM	11.99	1.99	5.13	0.05	4.14
P-value	0.18	0.46	0.24	0.68	0.43
VSA: Villus surface area; VCR: villus height to crypt depth ratio.					

Discussion

The results showed that decreasing dietary energy density had no significant effects on the FI, BWG, and FCR of broiler chickens. Similarly, it has been shown that the reduction of feed energy by 75 kcal/kg (from 3000 to 2925 kcal/kg) during the starter period did not affect BW, FI and FCR of broilers (Wan et al. 2020). However, Hu et al. (2018) found decreased body weight gain (BWG) when broilers fed diets with 3050 kcal ME/ kg compared with those fed 3200 kcal ME/kg. A previous study (Vieira et al. 2006) in broilers showed that the effect of dietary energy level ranged from 2,870 to 3,100 kcal/kg in starter period was not significant on the BWG of broilers at 7 days of age. Furthermore, Ghahremani et al. (2016)

showed that decreasing 100 kcal/kg ME did not affect the BWG, FI, and FCR of Cobb chicks, but a further reduction in dietary ME negatively affected the performance parameters.

Following the results, the mRNA expression of b0,+AT and B0AT transporters reduced with decreasing the dietary energy level; however, decreasing dietary energy level did not change the mRNA expression of PepT1, y+LAT1, and CAT1. This study is the first report on the effect of dietary energy on the mRNA expression of intestinal peptide and amino acid transporters in human or animals. In the present study, dietary energy level was changed by changing the amount of dietary oil. As mentioned previously, dietary fats and oils have the extra metabolic effect. Dietary fat has been shown to increase the feed retention time in the GIT, which in turn could improve digestion and absorption of nutrients (Mateos et al. 1981, 1982). One possible reason for the reduced expression of genes b0,+AT and B0AT could be the extra metabolic effect of fats and oils; the identification of possible metabolic pathways are unknown and requires further research. Our results did not support the adaptive regulation theory which states that sufficient or plenty substrate supply inhibits while any deficiency stimulates mRNA expression of amino acid transporters; this regulation takes place at the level of mRNA stability and synthesis (Hatzoglou et al. 2004; Majumder et al. 2009). Some factors regulate the expression of transporters genes and needs to be investigated further. For example, PPAR α (peroxisome proliferator-activated receptor α), which is a nuclear receptor, is activated by fatty acids (Shimakura et al. 2006).

The results also indicated that dietary energy density had no effect on the jejunal morphological indices, including VH, VW, CD, VCR and VSA. Intestinal development indicates the health of animals. New epithelial cells formed in the crypt, migrate and differentiate during their journey along the side of the villi. The efficiency of digestion and absorption of nutrients in the intestine depends on the development of the intestine. Reducing the energy level by 100 kcal/kg did not significantly impact the VH, CD, and VCR in duodenum of broilers while further reduction in energy level (180 kcal/kg) resulted in significant decreases in the intestinal morphometric parameters (Ghahremani et al. 2016). Kim et al. (2019) fed Pekin ducks with diets containing 2950, 3000, 3050, 3100 and 3150 kcal ME/kg from 21 to 42 days of age and did not observe any effect of dietary energy density on the VH and CD of the jejunum at 28, 35 and 42 days of age. By contrast, Zanganeh et al. (2015) found an increase in the VH and VCR in the jejunum and an increase in the CD of the ileum of broilers as the feed energy level increased by 100 kcal/kg, although the CD of the jejunum and the VH and VCR of the ileum were not affected by dietary treatments. The discrepancy between the results obtained in our study and previous studies can be attributed to differences in the length of experiment, energy level, age, sex, and strain of broilers, and other experimental conditions.

The results of the present study showed that decreasing the dietary energy concentration in the starter phase by 100 kcal/kg can induce intestinal mRNA expression of amino acid transporters changes without affecting the performance or intestinal structure during the first 10 days of age in broilers. It seems that unknown mechanisms involving gene expression of intestinal amino acid transporters may have affected the metabolism of nutrients. Decreasing the energy level of the starter diet by 100 kcal ME/kg reduces using energy sources, leading to a reduction in feed cost and increase profitability of

breeding. Further studies are needed to evaluate the effects of a wider range of energy, as well as the interaction between dietary energy and protein and amino acid on the expression of various nutrient transporters, intestinal morphology and nutrient digestibility.

Declarations

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Author Contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Reza Mahdavi, Shahab Ghazi Harsini and Ali Hossein Piray. The first draft of the manuscript was written by Reza Mahdavi and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability: The datasets generated during and/or analyzed during the current study are available from the Reza Mahdavi on reasonable request.

Ethics approval: This study was approved by the Animal Care and Use Committee of Razi University, with the approval number (IR.RAZI.REC.1400.066).

Consent to participate: Not applicable.

Consent for publication: Not applicable.

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