The effect of reducing dietary energy on performance, intestinal morphology and intestinal peptide and amino acid transporters in broiler chicks

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Abstract

This experiment evaluated the effects of two levels of metabolizable energy (ME; 2950 and 2850 kcal/kg) on performance, intestinal morphology, and mRNA abundance of amino acid (B^0AT , $b^{0,+}AT$, CAT1, and $y+LAT1$) and peptide transporters (PepT1) in broilers during the starter period (0-10 days of age). This is the first reported in this case in human or animal. Two hundred and seventy day-old male Ross 308 broilers were used according to a completely randomized design with two treatments with nine replicates and fifteen birds in each replicate. The outcome variables were measured at ten days of age. The mRNA abundance of PepT1, $b^{0,+}AT$, B^0AT , $y+LAT1$, and CAT1 were assayed using real-time PCR. The results showed that the dietary treatments did not affect feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) ($P>0.05$). mRNA abundance of the B^0AT and $b^{0,+}AT$ decreased as ME level decreased from 2950 to 2850 kcal/kg ($P\leq 0.05$). Dietary energy level did not have a significant effect on jejunal mRNA level of PepT1, CAT1, and $y+LAT1$ ($P>0.05$). The use of the different dietary energy levels did not affect the villus width (VW), villus height (VH), crypt depth (CD), villus surface area (VSA), and villus height to crypt depth ratio (VCR) ($P>0.05$). Results of the current experiment confirm that the dietary content of ME 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 occurs during the starter period, and therefore the post-hatch nutrition of broiler chicks plays an important role in their productivity. The transition from embryogenic to the post-hatch period is the most challenging step in broiler breeding and is characterized by dramatic changes (physiological and morphological) in the gastrointestinal tract (GIT), allowing the chick to efficiently digest and absorb nutrients (Vieira and Moran 1999; Willemsen et al. 2010). At the same time, the substantial changes also take place in the expression of genes involved in growth control. The main factors affecting the GIT development are sex, breed, dietary nutrient density, disease, and feed form.

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 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.

There are several factors that regulate the intestinal amino acid and peptide transporters including genetic improvement, age, intestinal development, medicinal agents, form of diet, pathological states, and nutrient density (Chen et al. 2005; Gilbert et al. 2008; He et al. 2013; Morales et al. 2017; Mahdavi et al. 2018; Osmanyan 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 energy level on mRNA abundance of intestinal amino acid and peptide transporters. The objective of this experiment was to identify the effects of two different dietary energy levels on the performance, intestinal morphology, and mRNA abundance of intestinal amino acid and peptide transporters of broiler chicks in the starter phase.

Materials And Methods

Experimental design

Two hundred and seventy one-day-old Ross 308 male broiler chicks divided into two treatments (two levels of ME including 2950 and 2850 kcal/kg of diet) in such a way that each treatment had nine replicate pens, and each pen had fifteen chicks through 1 to 10 days of age. The nutrient contents of the feedstuffs were analyzed prior to formulating the diets by NIRS™ DS2500 FOSS. Dry matter, crude protein, total ash, and ether extract contents were determined according to AOAC, 2005. The properties of the experimental diets are provided 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 one and ten days of age. FI and FCR was calculated at the end of the starter period. At d 10, one bird per replicate (closer to the mean body weight) was selected, sacrificed via cervical dislocation and 3 cm of its jejunum was excised at 10 centimeters anterior to the Meckel's diverticulum. Tissue samples were fixed in 10% neutral buffered formalin, dehydrated in graded alcohol series, cleared in xylene, and embedded in paraffin. Samples were cut using a CUT4055 manually microtome (microTec Laborgeräte GmbH, Germany). Tissue sections were stained with hematoxylin-eosin and digitalized with a computer-aided light microscope (Olympus BX51TF, Japan). The histological parameters recorded included VH, from the tip to the villus-crypt junction base, VW, at the half height point of villus, CD, from the base of the villus to the crypt base. The villus height to crypt depth ratio (VCR) was also calculated. VSA was calculated using the formula mentioned by Sakamoto et al. (2000)

$$VSA = 2\pi \times (VW/2) \times VH,$$

where π = 3.14, VW=villus width and VH=villus height (Sakamoto et al. 2000).

Real time PCR

Jejunal samples (5 cm centimeters anterior to Meckel's diverticulum) were removed from one bird per pen on day 10, rinsed with PBS and placed in a microtubes containing RNeasy lysis solution (Invitrogen, Carlsbad, CA, USA).

Chen et al. (2005) and Rodgers et al. (2012) proved that jejunum section as it is the primary site of peptide and amino acids absorption in broiler chicks. The intestinal samples were stored at -20°C until RNA extraction. Total RNA was isolated using Trizol Reagent (Invitrogen Corp., Carlsbad, CA, USA) from

200 mg jejunal sample using the manufacturer's recommended guidelines. One μg of RNA was treated and digested with DNase (Thermo Fisher Scientific) in 30 minutes at 37°C. The overall RNA quality was assessed by electrophoresis on agarose gel, and staining with ethidium bromide. Complimentary DNA (cDNA) was synthesized from isolated RNA using the Easy cDNA Synthesis Kit (DENA Zist Asia, Mashhad, Iran). A negative control prepared for all samples without enzyme mix, to ensure that there was no genomic contamination.

The following polymerase chain reaction conditions were as follows: initial denaturation at 94°C for 2 minutes, followed by 40 cycles of 94°C for 15 seconds, 62°C for 15 s, 72°C for 20 s. All polymerase chain reactions were carried out using an Amplitronyx Thermal Cycler (Nyx Technik, Inc. San Diego, CA).

All polymerase chain reactions were carried out by Amplitronyx Thermal Cycler (Nyx Technik, Inc. San Diego, CA) with the primers (shown in Table 2) designed by Gilbert et al. (2008) and Zhang et al. (2017). β -actin was used as an internal control for target gene expression normalization. All samples were subjected to Real time PCR in triplicate and each reaction contained 1 μL of diluted cDNA samples (1:5), 2 μL SYBR Green Master Mix (DENA Zist Asia, Mashhad, Iran), 5 μL H₂O, and 1 μL of each primer (forward and reverse primers) to reach a reaction volume of 10 μL . The following recommended protocol was used in LightCycler®96 RT-PCR System (Roche, Swiss): (1) One cycle at 95 °C for 180 s as pre-incubation program; (2) 40 repeated cycles (15 s at 95 °C, 15 s at 62 °C, 15 s at 72 °C) for amplification; and (3) one cycle at 95 °C for 10 s, at 65 °C 1 min, at 97 °C 1 s) as a melting program. The relative mRNA expression change in the target gene relative to β -actin was calculated with the following formula (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 ^o ,+AT	NM_001199133.1	CAGTAGTGAATTCTCTGAGTGTGAAGCT/ GCAATGATTGCCACAACCTACCA
SLC7A1	CAT1	NM_001145490.1	CAAGAGGAAAACCTCCAGTAATTGCA/ AAGTCGAAGAGGAAGGCCATAA
SLC6A19	B ^o 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.			

$$R = 2^{[C_{T(\beta\text{-actin})} - C_{T(\text{test})}]}$$

where R= relative expression ratio, and C_T = threshold cycle.

Statistical analysis

The effects of dietary treatments were analyzed in a completely randomized design with general linear model of SAS software Version 9.2 (SAS Institute, Cary NC 2017). When significant difference was detected, means were separated using Duncan's multiple range test, and statistical significance level was set at P< 0.05.

Results

Growth performance

The results of this experiment (Table 3) indicated 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 amino acid and peptide transporters

Our results showed a significant effect of dietary energy level on the mRNA abundance of intestinal amino acid transporters (Table 4). Reduction of dietary energy level significantly decreased the mRNA level of $b^{0,+}AT$ and B^0AT transporters ($P \leq 0.05$) but had no effect on the mRNA level 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^0AT	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

Table 5 shows 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 (×10 ⁻³ µm 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 FI, BW, and FCR of broiler chicks (Wan et al. 2020). However, Hu et al. (2018) found BWG when chicks fed diets with 3050 kcal ME/ kg compared with those fed 3200 kcal ME/kg. Vieira et al. (2006) reported that varying dietary energy level (2,870 to 3,100 kcal/kg) in starter period had no significant effect on the BWG of broiler chicks at 7 days of age. Furthermore, Ghahremani et al. (2016) showed that decreasing 100 kcal/kg ME had not significant effects on BWG, FI, and FCR of Cobb chicks, but a further reduction in dietary ME negatively affected the performance parameters.

The results also indicated that dietary energy density had no effect on the jejunal morphological indices, including VH, VW, CD, VCR and VSA ($P>0.05$). 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 intestinal digestion and absorption of nutrients 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 jejunal VH and CD at 28, 35 and 42 days of age. By contrast, Zanganeh et al. (2015) found an increase in the jejunal VH and VCR and an increase in the ileal CD of chicks 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.

Following the results, the mRNA expression of $b^{0,+}AT$ and B^0AT transporters reduced with decreasing the dietary energy level; however, decreasing dietary energy level had no effect on the mRNA level of $PepT1$, y^+LAT1 , and $CAT1$. This study is the first report on the effect of dietary energy on the mRNA abundance 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 $b^{0,+}AT$ and B^0AT 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 implies that substrate sufficiency inhibits (adaptive repression) while any substrate limitation stimulates the activity of the amino acid transporters (adaptive derepression) (Hatzoglou et al. 2004; Majumder et al. 2009). Some factors regulate the expression of transporters genes and needs to be investigated further. For example, $PPAR\alpha$ (peroxisome proliferator-activated receptor α), which is a nuclear receptor, is activated by fatty acids (Shimakura et al. 2006).

The results of the current study indicated 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 researches may be required to evaluate the effect of a wider range of energy, as well as the interaction between dietary energy 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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