

Energy Type and Amount in the Diets of Broiler Chickens: Effects on Performance and Duodenal Morphology

Research Article

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ABSTRACT

The present study was conducted to investigate the effects of dietary energy sources and levels on performance and small intestinal morphology in broiler chickens. A total of 600 one-day-old broiler chicks were randomly divided into five treatments with four replicates each. Chicks were fed diet based on corn as main energy source and energy level based on Cobb 500 manual instruction considered control group (C), basal diet with 3% lesser energy than control (T1), basal diet with 6% lesser energy than control (T2), basal diet based on corn and soy oil level according to Cobb 500 manual instruction (T3), basal diet based on corn and soy oil with 3% upper energy (T4) for 42 days. Results showed that chicks in T3 group had higher body weight, body weight gain and duodenum villus height compared to control group (C) and improved feed conversion ratio (FCR) at day 42 of age ($P < 0.05$). Chicks in T2 group exhibited the lowest body weight (BW), body weight gain (BWG) and FCR but the highest feed intake ($P < 0.05$). Feeding of diet T4 improved daily weight gain and duodenal villus height while caused concurrently increased FCR. Energy levels greater than Cobb recommendation significantly increased the villus height of the duodenum and decreased crypt depth compared to the control group ($P < 0.05$). In order to achieve a higher weight more energy is needed than the recommended manual instruction for Cobb 500 but to have better feed conversion ratio the energy level recommended manual instruction is sufficient.

KEY WORDS broiler chickens, duodenum morphology, energy, performance, soy oil.

INTRODUCTION

The small intestine integrity in terms of both morphology and function is critical for animal development and growth (Ziegler *et al.* 2003). The intestinal epithelium of broiler chicken is responsible for the growth potential after hatching (Uni *et al.* 1998) and the development of intestinal morphology and function contributes to body weight increase of broiler chickens (Tarachai and Yamauchi, 2000). Small intestine of chickens develops rapidly during the first

5 days after birth (Noy and Sklan, 1998). Development of the intestinal villi in the chicken's early life could increase efficiency of nutrient utilization and enhance the growth performance. Furthermore, an increase in villi height may increase the intestinal surface area and nutrient absorption (Soltan, 2009). The small intestine is a metabolically active organ (Spratt *et al.* 1990) and many factors can affect its development (Thompson and Applegate, 2006). Energy is an important nutrient that constitutes the largest component of the diet and thus affects on potential growth perform-

ance, feed cost and profitability. The majority of the energy in poultry diets is obtained from carbohydrates while fat and protein can also yield energy (Leeson and Summers, 2005). Vegetable oils such as soy oil are frequently included in broiler diets to increase the energy density of the diet, improve efficiency and increase nutrient digestibility in broilers (Monfaredi *et al.* 2011). Soy oil also provide varying quantities of the essential fatty acid of linoleic acid (Leeson *et al.* 2001). Another important role of soy oil in diets is inhibition from de novo lipogenesis in broiler chickens (Wongsuthavas *et al.* 2011). Nutrient density is an important factor that may affect animal intestine development. High apparent metabolizable energy or high amino acid densities in the diets fed to broilers from 8 to 21 days of age improved their feed conversion ratio (Wang *et al.* 2014). Chickens fed a higher nutrient density diet grow faster throughout all growing phases (Nahashon *et al.* 2005; Zai *et al.* 2013). Intestinal structures may also be further modified to adapt to nutrition manipulation during growing phases and it seems to change energy levels can affect the small intestine structure. The appropriate structure of the small intestine can causes better nutrient absorption and ultimately improves growth performance in broiler chickens. In previous studies, experimental diets were provided according to broilers nutrients requirement suggested by NRC (1994). While nutritional needs of different strains of broiler chicken published by the companies of broiler breeder in recent years. In this experiment, energy and protein level in control diet were used according to Cobb 500 instruction manual, 2012. Genetic changes into improve performance that are provided by the companies producing strains of broiler chickens, because in this present study was decided, the effects of dietary energy level was used 3% lesser and upper based on Cobb 500 manual instruction on performance. Also, information on the effect of energy level in diets according to Cobb 500 manual instruction on duodenal morphology and performance of broiler chicken is lacking. Therefore, the aim of this study was to evaluate the effect of dietary energy sources and levels on growth performance and small intestinal morphology in broiler chickens.

MATERIALS AND METHODS

A total of 600 one-day-old Cobb 500 broiler chicks were obtained from a local hatchery randomly allocated to one of 5 dietary treatments (4 replicates per treatment and 30 chicks per replicate). Detailed descriptions of bird management (water, feed, light program and pen environment) and the arrangement of treatment groups in the broiler facility were presented in a companion study by Cobb 500 broiler chickens (Cobb 500, 2010). The lighting, tempera-

ture and air conditioning program used was consistent with the specifications in the Cobb lineage manual during the experimental period (Cobb 500, 2010). Feed intake and live body weight were recorded weekly and then feed conversion ratio (FCR) was calculated. Dead chicks were collected daily and weighed at the time of carcass removal; carcass weights were included in the FCR calculations.

In this experiment, chicks were fed by basal diet based on corn and energy level by Cobb 500 manual instruction as control group (C), basal diet with 3% lesser energy than control (T1), basal diet with 6% lesser energy than control (T2), basal diet based on corn and soy oil energy level according to Cobb 500 manual instruction (T3), basal diet based on corn and soy oil with 3% upper energy (T4). The experimental diets formulated by using (Cobb 500, 2012) (Table 1) and animal and poultry feed formulation (UF-FDA) software. Diets and fresh water were provided *ad libitum* during this experiment. Birds were fed the starter diet from day 1 to 10, the grower diet from day 11 to 28 and finisher diet from day 29 to 42. The experimental diets in mashed form were based on corn, soybean meal and in some treatments the gluten of corn and soy oil were used to adjust the levels of protein and energy contents.

The following growth performance variables were evaluated: body weight (BW), body weight gain (BW gain), feed intake (FI), daily feed intake (DFI) and feed conversion ratio (FCR). The birds were weighed on the first day of the experiment, then weighed weekly throughout the remaining experimental period (7 to 42 d of age). The feed was provided weekly and the leftover feed were weighed weekly to calculate the feed conversion ratio.

At 28 d, 4 birds per pen (20 chicks/dietary treatment) were dissected for determination of duodenum. Birds were weighed and killed by cervical dislocation. Duodenal samples (2 cm in length) were obtained from of the midpoint of each chick. The intestinal segments were flushed with cold PBS and fixed in 10% neutral buffered formalin phosphate for subsequent morphological examination.

The duodenum samples were processed by dehydration through a series of graded alcohol solutions (50, 70, 80, 90, 95 and 100%) cleared with xylene and embedded in paraffin. Paraffin sections (5- μ m thickness) were mounted on glass slides. The slides were stained using routine procedures for Mayer's hematoxylin and eosin (Yaghobfar *et al.* 2006).

Villi were photographed under alight microscope (Olympus CX31RBSF attached cameraman) using the method presented by (Wang *et al.* 2015). Morphometric parameters of duodenum villi were performed at a magnification of 40X and goblet cells were performed at measurement of 400X all measurements were analyzed using image j software.

Table 1 Ingredients and chemical composition of the experimental diets for broiler chicks

Ingredients (%)	Starter (0-10 days old)					Grower (11-28 days old)					Finisher (29-42 days old)				
	C	T1	T2	T3	T4	C	T1	T2	T3	T4	C	T1	T2	T3	T4
Corn grain	63.3	63.2	62.1	58.2	54.2	69.2	68.8	67.7	65.3	59.3	70.2	71.47	71.5	65.1	62.4
Soybean meal	22.6	28.9	31.5	31.9	32.2	18.0	23.4	26.2	24.0	28.4	19.3	20.0	24.2	25.8	25.0
Soybean oil	-	-	-	2.5	4.14	-	-	-	2.00	5.00	-	-	-	3.50	5.00
Corn gluten meal	9.12	3.01	-	2.67	4.68	8.22	3.27	-	4.21	2.96	6.21	4.2	-	1.47	3.50
Dicalcium phosphate	2.07	2.06	2.06	2.05	2.05	1.90	1.90	1.90	1.90	1.90	1.70	1.70	1.70	1.70	1.70
Calcium carbonate ¹	1.06	1.03	1.01	1.01	1.01	1.05	1.05	1.05	1.05	1.05	0.92	0.92	0.920	0.900	0.900
Salt	0.380	0.380	0.380	0.380	0.380	0.370	0.370	0.370	0.370	0.370	0.32	0.32	0.320	0.320	0.320
DL-methionine	0.340	0.410	0.450	0.400	0.390	0.220	0.270	0.300	0.250	0.250	0.180	0.220	0.270	0.220	0.200
L-lysine	0.530	0.400	0.350	0.310	0.360	0.440	0.340	0.280	0.320	0.220	0.360	0.360	0.270	0.210	0.220
L-threonine	0.100	0.110	0.130	0.080	0.090	0.100	0.100	0.120	0.100	0.050	0.090	0.090	0.100	0.060	0.040
Vitamin and mineral per-mix ²	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500
Choline chloride	-	-	-	-	-	-	-	-	-	-	0.220	0.220	0.220	0.220	0.220
Filler ³	-	-	1.52	-	-	-	-	1.58	-	-	-	-	-	-	-
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Calculated nutrient content															
ME (kcal/kg)	3035	2934	2853	3035	3120	3108	3014	2921	3108	3201	3185	3085	2990	3185	3275
CP (%)	21.0	20.3	19.7	21	21.5	19.0	18.4	17.8	19.0	19.5	18.0	17.4	16.8	18.0	18.5
Ca (%)	0.900	0.900	0.900	0.900	0.900	0.840	0.840	0.840	0.840	0.840	0.760	0.760	0.760	0.760	0.760
Available phosphorus (%)	0.450	0.450	0.450	0.450	0.450	0.420	0.420	0.420	0.420	0.420	0.380	0.380	0.380	0.380	0.380
Na (%)	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.160	0.160	0.160	0.160	0.160
Digestible methionine (%)	0.670	0.670	0.670	0.670	0.670	0.530	0.530	0.530	0.530	0.530	0.480	0.480	0.480	0.480	0.480
Digestible lysine (%)	1.18	1.18	1.18	1.18	1.18	1.05	1.05	1.05	1.05	1.05	0.950	0.950	0.950	0.950	0.950
Digestible threonine (%)	0.770	0.770	0.770	0.770	0.770	0.690	0.690	0.690	0.690	0.690	0.650	0.650	0.650	0.650	0.650

ME: metabolizable energy and CP: crude protein.

C: control; T1: basal diet with 3% lesser energy than control; T2: basal diet with 6% lesser energy than control; T3: basal diet based on corn and soy oil energy level according to Cobb 500 manual instruction and T4: basal diet based on corn and soy oil with 3% upper energy.

¹ Per kg contains: Ca: 23% and P: 18.5%.

² Supplied by Razak Co., Tehran, Iran, and provided per kilogram of diet: vitamin A: 11000 IU; vitamin D₃: 2000 IU; vitamin E: 18 IU; vitamin K: 4 mg; vitamin B₁₂: 0.015 mg; Thiamine: 1.8 mg; Riboflavin: 6.6 mg; Calcium pantothenic acid: 12.0 mg; Niacin: 30.0 mg; Pyridoxine: 2.9 mg; Folic acid: 1.0 mg; choline: 260.0 mg; Manganese: 64.5 mg; Zinc: 33.8 mg; Iron: 100.0 mg; Copper: 8.0 mg; Iodine: 1.9 mg and Selenium: 0.25 mg.

³ Inert filler used to complete diet formulations to 100%.

Morphometric parameters recorded included total villus height (VH) (from the tip to the bottom of each villus), mid-point villus width (VW), crypt depth (CD) (from the base to its opening), villus: crypt ratio (V:C; calculated by villus height by crypt depth) and goblet cell count (GC count) according to the procedure of (Wang *et al.* 2015).

A randomized completely design with 4 replications was used to test for effects of dietary treatment on growth performance (BW, BW gain, FI and FCR) and duodenal morphology parameters. All parameters were analyzed using SPSS (SPSS, 2010). Data derived from experiment were analyzed by a one way ANOVA. Significant differences among means were determined by Duncan's multiple range test ($P < 0.05$).

RESULTS AND DISCUSSION

The body weight, body weight gain, feed intake, and feed conversion ratio at the 28 and 42 days of age of the birds relative to the energy sources and levels in the diet are shown in Tables 2 and 3. The results showed that dietary treatments significantly affected on BW, BW gain, FI and FCR at 28 and 42 d ($P < 0.05$). In this study it was shown that using of the T3 and T4 increased body weight and daily weight gain.

Table 2 Effects of dietary energy sources and levels on growth performance in d 28 of age

Treatment	Feed intake (g/day/bird)	Live weight (g)	Daily weight gain (g/day/bird)	FCR
C	58.4 ^b	1279 ^b	40.2 ^{ab}	1.45 ^c
T1	61.2 ^{ab}	1199 ^d	39.6 ^b	1.54 ^b
T2	66.8 ^a	1086 ^e	38.6 ^b	1.72 ^a
T3	59.9 ^b	1243 ^c	41.1 ^{ab}	1.44 ^c
T4	62.2 ^{ab}	1337 ^a	41.3 ^a	1.50 ^{bc}
P-value	0.002	0.000	0.158	0.000
SEM	0.811	19.825	0.384	0.026

C: control; T1: basal diet with 3% lesser energy than control; T2: basal diet with 6% lesser energy than control; T3: basal diet based on corn and soy oil energy level according to Cobb 500 manual instruction and T4: basal diet based on corn and soy oil with 3% upper energy.

The means within the same row with at least one common letter, do not have significant difference ($P > 0.05$).

SEM: standard error of the means.

FCR: feed conversion ratio.

But feed conversion ratio was significantly higher for the birds that fed T4 diet compared to T3. Feed intake and feed conversion ratio were significantly increased, while body weight and daily weight gain were significantly reduced for the broilers fed diets with T1 and T2 compared to control diet. Birds fed high level of energy exhibited the best BW and daily weight gain (DWG). Using of the basal diet based on corn and soy oil energy level according to Cobb 500 manual instruction (T3) exhibited better growth parameters

compared with basal diet based on corn and energy level by Cobb 500 manual instruction as control group (C).

Table 3 Effects of dietary energy sources and levels on growth performance in d 42 of age

Treatment	Feed intake (g/day/bird)	Live weight (g)	Daily weight gain (g/day/bird)	FCR
C	86.2 ^{bc}	2160 ^b	47.6 ^b	1.81 ^c
T1	88.4 ^{ab}	2093 ^c	45.3 ^c	1.94 ^b
T2	89.3 ^a	2030 ^d	43.3 ^d	2.06 ^a
T3	84.3 ^c	2188 ^b	48.5 ^b	1.73 ^d
T4	87.4 ^b	2260 ^a	50.0 ^a	1.74 ^d
P-value	0.002	0.000	0.000	0.000
SEM	0.490	18.690	0.579	0.029

C: control; T1: basal diet with 3% lesser energy than control; T2: basal diet with 6% lesser energy than control; T3: basal diet based on corn and soy oil energy level according to Cobb 500 manual instruction and T4: basal diet based on corn and soy oil with 3% upper energy.

The means within the same row with at least one common letter, do not have significant difference ($P > 0.05$).

SEM: standard error of the means.

FCR: feed conversion ratio.

Morphological examination showed that dietary treatments significantly affected on duodenum villus height, crypt depth and villus height: crypt depth ($P < 0.05$) (Table 4).

In this experiment, significantly higher villus height and villus height: crypt depth also lower crypt depth were observed in the duodenum of the birds fed T3 and T4 diets as compared with the control diet. The results showed that birds fed diets with low levels of energy (T1 and T2 diets) significantly exhibited lower villus height and villus height: crypt depth also higher crypt depth compared with the control. Birds fed dietary treatment with basal diet based on corn and soy oil with 3% upper energy (T4) significantly exhibited the highest villus height and villus height: crypt depth also lowest crypt depth versus the control.

No dietary effect was apparent for villus wide and goblet cell count at the duodenum (Table 4). But birds fed dietary treatment with low levels of energy (T1 and T2 diets) exhibited the highest villus wide and the lowest goblet cell count. Birds fed dietary treatment with basal diet based on corn and soy oil energy level according to Cobb 500 manual instruction (T3) exhibited the lowest villus wide and highest goblet cell count.

The results of this study indicating that different levels of energy significantly affected on BW, BW gain, FI and FCR were consistent with previous reports (Yang *et al.* 2007; Min *et al.* 2012). Both ME and crude protein (CP) significantly affected on BW and BW gain at 42 days of age ($P < 0.05$), ME levels can significantly affected on FI ($P < 0.05$) while CP did not (Min *et al.* 2012). Feed intake was reduced with energy increase to 3100 kcal/kg and a concurrent improvement on FCR was found when energy

increased to 3000 kcal/kg (Vieira *et al.* 2006). Our findings indicated that feeding broilers with soy oil can lead to significant improvements in growth. The results showed that using of basal diet based on corn and soy oil energy level according to Cobb 500 manual instruction (T3) exhibited the best FCR results, that was also consistent with previous observations (Houshmand *et al.* 2011). Soy oil is frequently included in broiler diets to increase the energy density of the diet, improve efficiency and increase nutrient digestibility in broilers (Monfaredi *et al.* 2011). The results showed that birds fed dietary treatment basal diet based on corn and soy oil energy level according to Cobb 500 manual instruction (T3) exhibited better BW, BW gain and FCR compared to control at 42 days of age. It seems, to add soy oil as source of energy was caused to better growth performance in broiler chicks. In previous researches, the positive effect of soy oil on growth performance of broilers is well documented (Griffiths *et al.* 1977; Scaife *et al.* 1994). The final live weight (FLW) was significantly highest in broiler chickens fed dietary treatment with normal energy and normal protein (NENP) and was lowest in broiler chickens fed dietary treatment with low energy and high protein (LEHP) ($P < 0.05$). The BW gain also followed similar trend as FLW. Feed conversion ratio was significantly better in broiler chickens fed dietary treatment NENP, a trend also exhibited by the protein efficiency ratio (PER) ($P < 0.05$) (Dairo *et al.* 2010). The results showed that birds fed dietary treatment with low levels of energy exhibited the lowest BW, BW gain and FCR and the highest feed intake. Lower energy diets containing insufficient energy for the protein synthesis have a consequence catabolism of AA to make up this deficiency; this would in turn result in reduced growth and poorer feed efficiency.

Birds fed dietary treatment with high level of energy significantly exhibited the highest BW and BW gain ($P < 0.05$). Increased live weight was mostly due to higher ME consumption in same unit of diets by chickens, similarly supplementation of oil caused a positive trend in cumulative live weight gain (g/bird) of broilers at different ages (Das *et al.* 2014). In this study, observed limitation to expected FI increment was in part probably due to limitation in physical capacity of gastrointestinal tract. Our explanation is in agreement with that claimed by Griffiths *et al.* (1977) and Kamran *et al.* (2008).

In our study, birds fed basal diet based on corn and soy oil with 3% upper energy (T4) exhibited higher FI compared to control. This is in disagreement with Leeson *et al.* (1996). In this experiment, it seems that other reasons could affect on broiler chickens feed intake according to the same supply of nutrients in the diet and changing dietary energy levels. It seems, that energy density of rations had changed BW, BW gain, FI and FCR.

Table 4 Effects of energy sources and levels on duodenal morphology (μm) in 28 d

Treatment	Villus height (μm)	Villus width (μm)	Crypt depth (μm)	V/C ¹	Goblet cell (count/mm) ²
C	375 ^b	51.0	60.0 ^{ab}	6.25 ^c	140
T1	350 ^{bc}	54.0	66.0 ^{ab}	5.30 ^d	138
T2	301 ^c	62.0	74.0 ^a	4.06 ^e	137
T3	475 ^a	50.0	54.2 ^b	8.76 ^b	147
T4	527 ^a	52.0	50.5 ^b	10.43 ^a	146
P-value	0.000	0.626	0.027	0.000	0.606
SEM	20.338	2.784	2.852	8.132	2.201

¹ V/C: villus height to crypt depth ratio.

² Goblet cell densities were calculated as the number of goblet cells per unit of villus height.

C: control; T1: basal diet with 3% lesser energy than control; T2: basal diet with 6% lesser energy than control; T3: basal diet based on corn and soy oil energy level according to Cobb 500 manual instruction and T4: basal diet based on corn and soy oil with 3% upper energy.

The means within the same row with at least one common letter, do not have significant difference ($P>0.05$).

SEM: standard error of the means.

FCR: feed conversion ratio.

The results showed that birds fed dietary treatment with low levels of energy significantly exhibited the lowest duodenum villus height and VH:CD ratio also the highest crypt depth ($P<0.05$). A shortening of the villus and large crypt can lead to poor nutrient absorption, increased secretion in the gastrointestinal tract, and lower performance (XU *et al.* 2003).

Chicks exhibited a lower feed conversion ratio (FCR) and a higher BW gain when their crypts were shorter (Wang *et al.* 2015). In this study, birds fed dietary treatment with high level of energy significantly exhibited the highest duodenum villus height, villus height: crypt depth but the lowest crypt depth ($P<0.05$). Dietary treatment did not affect duodenum villus width and goblet cell count. But birds fed dietary treatment with low levels of energy exhibited the highest villus width and the lowest goblet cell count. The results showed that birds fed basal diet based on corn and soy oil energy level according to Cobb 500 manual instruction (T3) exhibited the lowest villus width and the highest goblet cell count. The results were in agreement with previous reports by Fan *et al.* (1997), Tarachai and Yamauchi (2000) and Xu *et al.* (2003).

Chickens fed a higher nutrient density diet grow faster throughout all growing phases (Nahashon *et al.* 2005; Zai *et al.* 2013). In contrast, increases in the villus height and villus height: crypt depth were directly correlated with increased epithelial cell turnover (Fan *et al.* 1997) and longer villi were associated with activated cell mitosis (Samanya and Yamauchi, 2002). The villi play a crucial role in the digestion and absorption processes of the small intestine, as is the first to make contact with nutrients in the lumen (Gartner and Hiatt, 2001). Dietary fat and probiotic supplementation significantly increased villus height of the duodenum. Villus height is known to correlate positively with nutrient absorption (Tarachai and Yamauchi, 2000) but improvement in broiler growth occurred among those fed soy oil diets only. Due to this reasons, using of the T3 and T4 diets exhibited the best duodenum villus height compared to control.

In this research, it seems that soy oil and nutrient density improved morphological parameters and growth performance.

Goblet cell secrete mucin in the digestive tract to protect the intestinal membrane from digestive enzyme degradation and pathogen invasion (Wang *et al.* 2015). The results showed that birds fed basal diet based on corn and soy oil energy level according to Cobb 500 manual instruction (T3) exhibited numerous goblet cell counts. Basal diet based on corn and soy oil energy level according to Cobb 500 manual instruction (T3) improved performance and small intestine structure compared to control.

In this study, there was positive relationship between soy oil consumed in diets and small intestine structure with growth performance in Cobb 500 broiler chickens. Birds fed basal diet based on corn and soy oil energy level according to Cobb 500 manual instruction (T3) exhibited the best growth performance, probably resulting from improved morphological parameters.

CONCLUSION

The results showed that the higher energy level rather than nutritional needs based on Cobb 500 broiler chicken requirements as specified in the manual was affective on performance and morphological parameters. These effects indicate that the current nutritional recommendations are not sufficient for achieving the full genetic potential of current broiler strains for body weight gain but in terms of feed conversion ratio the most appropriate level of energy obtained from Cobb 500 broiler chicken requirements in instruction manual.

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