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Effect of Dietary Energy Levels on the Reproductive Performance in Breeding Pigeons, and Growth Performance and Intestinal Health in Squabs

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The present study evaluated the effect of different dietary energy levels on reproductive performance in breeding pigeons, as well as growth performance and intestinal health in squabs. In total, 180 pairs of 12-month-old White King breeding pigeons were randomly assigned to five dietary treatments, each with six replicates of six pairs of birds, and fed diets containing 11.60, 11.80, 12.00, 12.20, and 12.40 MJ/kg for 46 days, respectively. Energy content beyond 12.00 MJ/kg shortened the laying interval (linear and quadratic, $P<0.05$), while boosting 38-day, 42-day, and 46-day laying rates (linear, $P<0.05$) in breeding pigeons. Except for the early stage of lactation, feed intake showed a linear and/or quadratic negative relationship with dietary energy content ($P<0.05$). Body weight at 1 week of age, average daily gain during the early growth stages, and serum total protein of squabs increased with increasing dietary energy content (linear, $P<0.05$); whereas alanine aminotransferase activity decreased (quadratic, $P<0.05$). Jejunal villus height and villus height to crypt depth ratio in squabs increased with increasing dietary energy levels (linear and quadratic, $P<0.05$), particularly in the 12.40 MJ/kg group. Higher dietary energy content increased jejunal malondialdehyde content (linear, $P<0.05$), total superoxide dismutase (T-SOD), and glutathione peroxidase activities (linear, $P<0.05$), as well as ileal T-SOD (linear and quadratic, $P<0.05$) and catalase (quadratic, $P<0.05$) activities in squabs. Hence, intakes greater than 12.00 MJ/kg altered the jejunal redox status. Finally, higher dietary energy content improved reproduction in breeding pigeons and intestinal morphology in squabs. Overall, 12.00 MJ/kg strikes the right balance as it promotes reproductive performance in breeding pigeons and intestinal health in squabs.

Key words: dietary energy levels, reproductive performance, breeding pigeons, growth performance, intestinal health, squabs

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Introduction

Pigeon meat is an excellent quality protein food source for humans because of its high protein and low fat content[1]. With improvements in living standards and nutritional awareness, market demand for pigeon meat is increasing, especially in China[2]. Squab pigeons are altricial birds that rely on crop milk from their parents. Parent pigeons live in pairs with a two-egg clutch[3]. They are characterized by a long breeding clutch interval encompassing 18 days of incubation and 28 days of lactation[4]. This special feeding pattern adversely affects the laying performance of breeding pigeons and the yield of squabs. Multiple strategies for improving parent pigeon breeding performance and squab growth have been attempted including dietary manipulation.

Energy is a key nutritional component in pigeon's diet as it is closely associated with growth, development, reproduction, and output[5,6]. Carbohydrates, lipids, and occasionally proteins[7] are the predominant source of dietary energy and a major contributor to feed costs[5]. Therefore, accurate provision of energy is vital for cost-effective and sustainable pigeon rearing. Many studies have been conducted to determine suitable energy levels for pigeons. Bu et al.[5] reported that as dietary metabolizable energy (ME) increased from 11.0 to 12.3 MJ/kg, body weight (BW), egg production, apparent nutrient digestibility, and feed

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conversion efficiency in laying pigeons were increased. Wang et al.[8] evaluated the growth performance of squabs fed by breeding pigeons received diets containing different energy densities. Their results showed higher final BW at 21 days of age, greater average daily weight gain, and better feed conversion efficiency from 1 to 21 days in the low-energy diet group (12.39 MJ/kg) than in the high-energy diet group (12.81 MJ/kg). Peng et al.[9] showed that administration of 12.2, 12.4 or 12.6 MJ/kg ME had no effect on reproduction performance of breeding pigeons, or on growth performance, slaughter performance, and meat quality of squabs. However, the same group[6] showed that 12.6, 12.8 or 13.0 MJ/kg exerted little effect on breeding pigeons but significantly affected growth performance, slaughter performance, and meat quality of squabs. Hence, the response of pigeons to dietary energy density is inconsistent, and optimal energy levels vary for different breeds, environments, and breeding patterns. Therefore, the objective of this study was to investigate the effect of dietary energy levels on the reproductive performance of breeding pigeons and the growth performance, intestinal morphology, and redox status of squabs. Our results provide a theoretical basis for improving the nutritional standards used in breeding pigeon performance and squab growth.

Materials and methods

Animal care

The experimental design and procedures of this study were reviewed and approved by the Institutional Animal Care and Use Committee of Henan University of Technology (Zhengzhou, People's Republic of China) before commencement of the trial (HAUT20231010).

Animals and experimental design

A total of 180 pairs (180 male and 180 female) of 12-month-old White King breeding pigeons with similar BW and reproductive performance were provided by Henan Tiancheng Pigeon Industry Co., Ltd. (Wugang, People's Republic of China). They were randomly assigned to five dietary groups and fed diets containing 11.60, 11.80, 12.00, 12.20, and 12.40 MJ/kg ME for 46 days. This time frame included 18 days for incubation and 28 days for lactation. Diets were formulated to meet the nutritional recommendations for pigeons (Table 1). Each treatment was replicated six times, with six pairs of breeding pigeons per replicate. All groups were housed in an air-conditioned room under a 16-h light/8-h dark cycle, and reared in cages, with one pair of breeding pigeons and three squabs per cage. Each cage was equipped

Table 1. **Ingredients and nutrient levels of the experimental diets.**

Dietary components	Metabolizable energy levels (MJ/kg)				
	11.60	11.80	12.00	12.20	12.40
Ingredients (%)					
Corn	53.75	54.00	53.20	52.00	51.35
Sorghum	3.75	3.75	3.75	3.75	3.75
Peas	18.75	18.75	18.75	18.75	18.75
Soybean meal	17.375	17.325	17.475	17.75	17.875
Soybean oil	0.00	0.50	1.325	2.30	3.10
Dicalcium phosphate	1.50	1.50	1.50	1.55	1.55
Limestone	2.15	2.15	2.15	2.10	2.10
L-Lysine	0.09	0.09	0.09	0.08	0.08
DL-Methionine	0.11	0.11	0.11	0.11	0.11
Premix[1]	1.00	1.00	1.00	1.00	1.00
Sodium chloride	0.30	0.30	0.30	0.30	0.30
Zeolite powder	1.225	0.525	0.35	0.31	0.035
Total	100.00	100.00	100.00	100.00	100.00
Calculated nutrient levels[2]					
Gross energy (MJ/kg)	14.94	15.33	15.72	15.88	16.07
Crude protein (%)	17.67	17.62	17.51	17.55	17.43
Calcium (%)	1.20	1.20	1.20	1.20	1.20
Total phosphorous (%)	0.60	0.60	0.60	0.60	0.60
Lysine (%)	0.96	0.96	0.96	0.96	0.96
Methionine (%)	0.35	0.35	0.35	0.35	0.35
Methionine + Cysteine (%)	0.64	0.64	0.64	0.63	0.63

[1]Premix provided per kilogram of feed: vitamin A, 4000.00 IU; vitamin D3, 1725.00 IU; vitamin E, 24.00 mg; vitamin K_3, 1.0 mg; vitamin B_{12}, 25.00 μg; vitamin B_1, 3.0 mg; riboflavin, 13.0 mg; niacin, 15.00 mg; choline chloride, 200.0 mg; pantothenic acid, 7.50 mg; vitamin B_6, 2.00 mg; biotin, 0.12 mg; folic acid, 0.55 mg; Fe, 35.0 mg; Cu, 10.0 mg; Mn, 55.0 mg; Zn, 35.0 mg; I, 0.20 mg; Se, 0.25 mg.
[2]Gross energy and crude protein were analyzed.

Table 2. **Effect of dietary energy levels on the reproductive performance in breeding pigeons.**

Reproductive performance	Metabolizable energy levels (MJ/kg)					SEM	*P*-value		
	11.60	11.80	12.00	12.20	12.40		ANOVA	Linear	Quadratic
Laying interval (days)	38.04^{a}	37.51^{a}	34.89^{b}	35.14^{b}	35.36^{b}	0.32	<0.001	<0.001	0.024
Fertility rate (%)	90.56	79.63	84.32	87.50	95.83	2.87	0.475	0.375	0.138
Hatchability (%)	82.88	94.44	90.34	88.01	92.68	2.02	0.429	0.366	0.484
38-day laying rate (%)	50.00^{b}	55.56^{b}	88.89^{a}	86.11^{a}	88.89^{a}	4.05	<0.001	<0.001	0.071
42-day laying rate (%)	91.67	86.11	94.44	97.22	100.00	1.69	0.080	0.018	0.400
46-day laying rate (%)	91.67	88.89	97.22	97.22	100.00	1.42	0.068	0.011	0.799

Different lowercase letters indicate statistical significance ($P<0.05$). SEM, standard error of the mean (n=6).

with a nest, perch, nipple drinker, and feeding trough. All birds had free access to water and health sand throughout the experimental period. Breeding pigeons were fed manually at regular intervals from 7:00 am, with *ad libitum* access to feed, and fasted every evening at 19:00.

Laying performance of breeding pigeons

The 38-day, 42-day, and 46-day laying rates (%) were calculated as: number of pigeons laying eggs on the 38th, 42nd, and 46th day / pigeon number × 100%. The fertilization rate (%) was calculated as: number of fertile eggs / total number of laying eggs × 100%. Between the 3rd and 5th day of incubation, an egg stained with blood indicated fertilization when it was illuminated by a flashlight; otherwise, it was not fertilized. Hatchability (%) was calculated as: number of hatched eggs / total number of fertile eggs × 100. The laying interval was always calculated from the day after the second egg was laid in the first clutch to the day before the first egg was laid in the subsequent clutch.

Feed intake and BW change in breeding pigeons

Daily feed consumption was recorded to determine feed intake (FI) in each group. The feed was usually weighed at feeding time, and the remaining feed in the feeder was weighed every evening at 19:00. All breeding pigeons were individually weighed before the start of the experiment (day 0) and at the end (day 46). The final BW was subtracted from the initial BW to calculate the weight loss per pair of birds in each group.

BW change and sample collection of squabs

During the lactation period, all squabs were weighed at 0, 1, 2, 3, and 4 weeks to determine average daily gain (ADG). ADG per bird was calculated over a 1-week interval (0–1, 1–2, 2–3, and 3–4) by subtracting the BW at the end of the first week from the initial BW and then dividing by the number of days. The final BW was subtracted from the initial BW and then divided by the number of days to calculate total daily gain per bird in each group. At the end of the experiment, 12 squabs (28 days of age) from each group were randomly selected and weighed after 12 h of fasting. Blood samples were collected and centrifuged to harvest the serum. Then, the collected serum was stored at -80°C until further analysis. The squabs were euthanized by decapitation, and approximately 2-cm-long segments of the mid-jejunum and ileum were collected, flushed with physiological saline, and fixed in 4% polyformaldehyde to assess intestinal morphology. The jejunum and ileum were opened longitudinally along the mesenteric border, and their contents were removed by squeezing and washing with ice-cold physiological saline. The mucosa was subsequently scraped from the washed and everted intestine using a sterile glass slide, pooled, placed into cryogenic vials, and immediately snap-frozen and stored at -80°C until further analysis.

Serum biochemical parameters of squabs

Glucose, total cholesterol, triglycerides, high- and low-density lipoprotein cholesterol levels, as well as alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase activities in the serum were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, People's Republic of China).

Intestinal morphology of squabs

After fixation, jejunal and ileal samples were dehydrated in a graded alcohol series, cleared in xylene, embedded in paraffin, sectioned to a thickness of 5 μm, stained with hematoxylin-eosin, and photographed under a light microscope (RVL-100-G; ECHO Laboratories, San Diego, CA, USA). In each section, villus height (VH) and crypt depth (CD) were measured using Image-Pro Plus 6.0 software (Media Cybernetics, San Diego, CA, USA). The ratio of VH to CD was calculated.

Intestinal redox status of squabs

Jejunum and ileum samples were homogenized with precooled physiological saline at a ratio of 1:9 (w/v) and then centrifuged to collect the supernatant, which was employed to assay the activities of catalase (CAT), total superoxide dismutase (T-SOD), glutathione peroxidase (GPX), and malondialdehyde (MDA) using commercial kits (Nanjing Jiancheng Bioengineering Institute).

Statistical analysis

Data were analyzed using one-way ANOVA with SPSS software (version 27.0, SPSS Inc., Chicago, IL, USA). Results are expressed as means and pooled standard errors. An orthogonal polynomial contrast test was performed to determine the linear and quadratic effects of increasing dietary ME concentrations. Differences between groups were tested using Tukey's post-hoc test. $P<0.05$ was considered statistically significant.

Table 3. **Effect of dietary energy levels on the body weight of breeding pigeons (g/pair).**

Weight parameters	Metabolizable energy levels (MJ/kg)					SEM	*P*-value		
	11.60	11.80	12.00	12.20	12.40		ANOVA	Linear	Quadratic
Initial weight	1156.66	1144.59	1160.25	1137.64	1131.29	6.80	0.624	0.234	0.638
Final weight	1072.22	1087.57	1066.47	1070.37	1042.39	6.28	0.248	0.084	0.239
Weight loss	84.44^{ab}	57.02^{b}	93.78^{a}	67.27^{ab}	88.90^{a}	3.51	0.003	0.426	0.220

Different lowercase letters indicate statistical significance (P<0.05). SEM, standard error of the mean (n=35 or n=36).

Table 4. **Effect of dietary energy levels on feed intake of breeding pigeons.**

Feed intake	Metabolizable energy levels (MJ/kg)					SEM	*P*-value		
	11.60	11.80	12.00	12.20	12.40		ANOVA	Linear	Quadratic
Incubation (g/pair/day)									
1–18 days	90.12^{a}	88.08^{ab}	87.98^{ab}	85.33^{b}	85.38^{b}	0.50	0.004	<0.001	0.634
Lactation (g/pair/day)									
1–7 days	101.53	99.35	103.77	100.92	105.52	0.79	0.093	0.075	0.311
7–14 days	233.24^{a}	212.74^{b}	214.66^{b}	216.11^{b}	216.49^{b}	2.10	0.006	0.020	0.008
14–21 days	270.57	251.80	253.53	245.64	251.14	3.05	0.093	0.033	0.111
21–28 days	253.59^{a}	238.19^{ab}	239.13^{ab}	230.88^{b}	233.93^{ab}	2.47	0.026	0.005	0.135
1–28 days	214.73^{a}	200.52^{ab}	202.77^{ab}	198.39^{b}	201.77^{ab}	1.85	0.033	0.022	0.046
Whole experimental period (g/pair/day)									
1–46 days	165.97^{a}	158.71^{b}	157.85^{b}	156.18^{b}	156.23^{b}	0.99	0.003	<0.001	0.044

Different lowercase letters indicate statistical significance (P<0.05). SEM, standard error of the mean (n=6).

Results

Production performance of breeding pigeons

As shown in Table 2, increasing the amount of energy in the diet from 11.60 and 11.80 MJ.kg to 12.00, 12.20, and 12.40 MJ/kg shortened the laying interval (linear and quadratic, P<0.05), while increasing the 38-day laying rate of breeding pigeons (linear, P<0.05). Higher energy levels promoted, albeit not significantly (P>0.05), the 42-day and 46-day laying rate of breeding pigeons, but not the fertility rate or hatchability (P>0.05).

As presented in Table 3, weight loss of a pair of breeding pigeons was higher in the 12.00 and 12.40 MJ/kg groups compared to the 11.80 MJ/kg group (P<0.05). Instead, there was no difference across groups with respect to initial and final weights of breeding pigeon pairs (P>0.05).

As shown in Table 4, during incubation, lactation (7–14, 21–28, and 1–28 days), and the entire experimental periods (1–46 days), FI displayed linear and/or quadratic inhibition at increasing dietary energy content (P<0.05). During the incubation period, breeding pigeons in the 12.20 and 12.40 MJ/kg groups had lower FI compared to the 11.60 MJ/kg group (P<0.05). During lactation, FI of breeding pigeons was higher in the 11.60 MJ/kg group (P<0.05) than in the other four groups from 7 to 14 days or the 12.20 MJ/kg group in the 21–28-day and 1–28-day intervals. Additionally, FI of breeding pigeons was higher in the 11.60 MJ/kg group (P<0.05) than in the other four groups throughout the entire experimental period.

BW change of squabs

A linear increase in parental dietary energy content caused an increase in BW at 1 week of age and ADG in the early growth stages (0–1 weeks) (Table 5, P<0.05). BW of squabs at 1 week of age was higher in the 12.40 MJ/kg group than in the 11.60 MJ/kg group (P<0.05); whereas ADG was higher for the 12.00 and 12.40 MJ/kg groups at this growth stage than the 11.60 MJ/kg group (P<0.05). Instead, BW at 2, 3, and 4 weeks of age and ADG at 1–2 weeks, 3–4 weeks, and 0–4 weeks were not altered by dietary treatments (P>0.05).

Serum biochemical parameters of squabs

Although no significant difference was observed among treatments (Table 6, P>0.05), total protein in the serum of squabs increased linearly (P<0.05) with increasing levels of dietary energy, and alanine aminotransferase activity decreased quadratically (P<0.05). No significant changes were observed for the concentrations of glucose, urea nitrogen, creatinine, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, total cholesterol, triglycerides, or the activities of aspartate aminotransferase and alkaline phosphatase in the serum of squabs (P>0.05).

Intestinal morphology of squabs

As shown in Table 7 and Figure 1, jejunal VH and VH:CD ratio increased (linear and quadratic, P<0.05) with increasing energy levels in breeding pigeon diets. The jejunal VH of squabs

Table 5. **Effect of dietary energy levels on the growth performance of squabs.**

Growth performance	Metabolizable energy levels (MJ/kg)					SEM	*P*-value		
	11.60	11.80	12.00	12.20	12.40		ANOVA	Linear	Quadratic
Body weight (g/bird)									
0 week	15.78	15.73	15.94	15.96	16.04	0.04	0.107	0.012	0.839
1 week	139.81[b]	144.00[ab]	150.68[ab]	150.17[ab]	151.32[a]	1.30	0.015	0.001	0.214
2 week	341.40	346.71	347.92	349.58	352.63	2.15	0.562	0.097	0.821
3 week	443.50	464.51	455.81	462.40	460.06	2.61	0.082	0.089	0.145
4 week	474.91	491.75	488.73	495.91	491.68	2.86	0.170	0.061	0.179
Average daily gain (g/bird/day)									
0–1 week	17.72[b]	17.75[ab]	19.25[a]	19.17[ab]	19.33[a]	0.22	0.019	0.002	0.455
1–2 week	28.80	28.15	28.18	28.48	28.76	0.27	0.903	0.897	0.348
2–3 week	14.58	16.36	15.41	16.12	15.35	0.31	0.389	0.556	0.183
3–4 week	4.49	3.78	4.70	4.79	4.52	0.24	0.701	0.530	0.987
0–4 week	16.40	17.00	16.89	17.14	16.99	0.10	0.175	0.066	0.177

Different lowercase letters indicate statistical significance ($P<0.05$). SEM, standard error of the mean (n=35 or n=36).

Table 6. **Effect of dietary energy levels on the serum biochemical parameters of squabs.**

Serum parameter	Metabolizable energy levels (MJ/kg)					SEM	*P*-value		
	11.60	11.80	12.00	12.20	12.40		ANOVA	Linear	Quadratic
Glucose (mmol/L)	9.82	8.38	10.58	8.90	9.45	0.33	0.278	0.926	0.965
Total protein (g/L)	34.39	30.87	36.71	38.30	39.00	1.08	0.110	0.029	0.637
Urea nitrogen (mmol/L)	1.79	2.33	1.87	1.71	1.71	0.10	0.291	0.289	0.366
Creatinine (μmol/L)	44.41	42.11	49.00	42.02	38.82	2.02	0.607	0.438	0.363
Aspartate aminotransferase (U/L)	7.80	5.69	6.17	7.23	5.73	0.30	0.087	0.207	0.461
Alanine aminotransferase (U/L)	7.32	4.53	4.91	5.83	6.81	0.36	0.055	0.906	0.006
Low-density lipoprotein cholesterol (mmol/L)	5.81	6.16	6.91	6.10	6.85	0.22	0.409	0.195	0.677
High-density lipoprotein cholesterol (mmol/L)	6.97	7.00	6.63	6.92	6.49	0.17	0.847	0.401	0.864
Total cholesterol (mmol/L)	8.55	7.04	8.36	7.62	7.63	0.21	0.139	0.388	0.559
Triglyceride (mmol/L)	1.04	1.10	1.25	1.07	1.35	0.06	0.389	0.156	0.819
Alkaline phosphatase (U/L)	1985.10	1965.18	1926.06	1906.84	1923.37	18.99	0.686	0.188	0.567

SEM, standard error of the mean (n=12).

Table 7. **Effect of dietary energy levels on the intestinal morphology of squabs.**

Intestinal parameter	Metabolizable energy levels (MJ/kg)					SEM	*P*-value		
	11.60	11.80	12.00	12.20	12.40		ANOVA	Linear	Quadratic
Jejunum									
Villus height (μm)	344.68[b]	330.21[b]	307.12[b]	329.96[b]	398.74[a]	6.95	<0.001	0.012	<0.001
Crypt depth (μm)	43.78	47.24	47.75	37.95	41.44	1.93	0.471	0.311	0.528
Villus height to crypt depth	8.28[ab]	7.76[ab]	7.32[b]	8.89[ab]	10.16[a]	0.31	0.029	0.020	0.025
Ileum									
Villus height (μm)	274.15	277.94	288.82	267.77	300.15	3.95	0.072	0.124	0.429
Crypt depth (μm)	42.06	44.61	39.73	48.04	43.10	1.18	0.241	0.505	0.854
Villus height to crypt depth	6.77[ab]	6.39[ab]	7.67[a]	5.72[b]	7.24[ab]	0.21	0.029	0.853	0.731

Different lowercase letters indicate statistical significance ($P<0.05$). SEM, standard error of the mean (n=12).

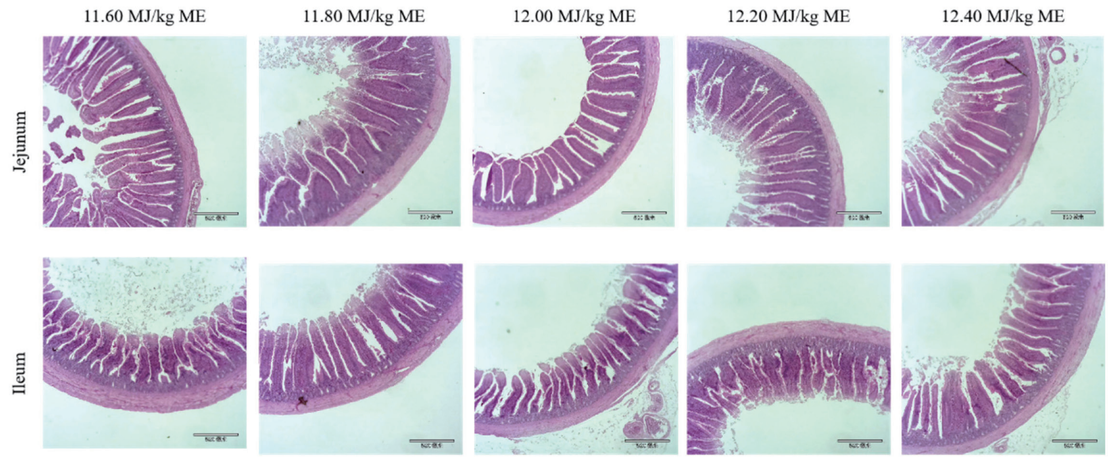

Figure 1. **Representative images of the intestine stained by hematoxylin and eosin; scale bar, 520 µm.**

Table 8. **Effect of dietary energy levels on the intestinal redox status of squabs.**

Redox parameter	Metabolizable energy levels (MJ/kg)					SEM	*P*-value		
	11.60	11.80	12.00	12.20	12.40		ANOVA	Linear	Quadratic
Jejunum									
MDA (nmol/mg protein)	2.68^{b}	2.74^{ab}	4.84^{ab}	4.74^{a}	4.90^{a}	0.29	0.010	0.001	0.379
T-SOD (U/mg protein)	73.80	66.26	72.06	81.27	92.61	3.40	0.125	0.028	0.141
GPX (U/mg protein)	143.82^{b}	130.31^{b}	173.69^{ab}	159.57^{b}	241.46^{a}	10.14	0.003	0.001	0.086
CAT (U/mg protein)	145.32	127.09	142.47	148.17	171.20	6.04	0.240	0.089	0.150
Ileum									
MDA (nmol/mg protein)	0.74	1.08	1.02	1.03	0.67	0.08	0.361	0.729	0.053
T-SOD (U/mg protein)	103.42^{b}	120.65^{ab}	138.06^{ab}	150.06^{a}	124.80^{ab}	4.61	0.014	0.020	0.014
GPX (U/mg protein)	58.20	60.86	67.75	50.83	58.60	3.00	0.524	0.667	0.592
CAT (U/mg protein)	140.00	156.80	182.49	171.98	150.55	5.06	0.056	0.293	0.007

Different lowercase letters indicate statistical significance (P<0.05). MDA, malondialdehyde; T-SOD, total superoxide dismutase; GPX, Glutathione peroxidase; CAT, catalase; SEM, standard error of the mean (n=12).

was higher in the 12.40 MJ/kg group than in all other groups (P<0.05). The VH:CD ratio in the jejunum of squabs was higher in the 12.40 MJ/kg group than in the 12.00 MJ/kg group (P<0.05). The latter had a higher ileal VH:CD ratio than the 12.20 MJ/kg group (P<0.05). There was no significant difference in intestinal CD and ileal VH among treatments (P>0.05).

Intestinal redox status of squabs

As shown in Table 8, a higher energy content in the diet increased jejunal MDA (linear, P<0.05), along with jejunal T-SOD and GPX activities (linear, P<0.05) and ileal T-SOD activity (linear and quadratic, P<0.05) in squabs. Jejunal MDA of squabs was higher in the 12.20 and 12.40 MJ/kg groups than in the 11.60 MJ/kg group (P<0.05). The 12.40 MJ/kg group attained higher jejunal GPX activity in squabs than the 11.60, 11.80, and 12.20 MJ/kg groups (P<0.05). Ileal T-SOD activity was higher in the 12.20 MJ/kg group than in the 11.60 MJ/kg group (P<0.05), but was similar to that in the 11.80, 12.00 and 12.40 MJ/kg groups (P>0.05). Ileal CAT activity in squabs increased quadratically with increasing levels of dietary energy (P<0.05), but the difference was not significant (P>0.05). Jejunal CAT activity, ileal MDA content, and GPX activity did not differ among dietary treatments (P>0.05).

Discussion

Dietary energy density plays an important role in reproductive performance of livestock[10,11] and birds[12]. Lotfi et al.[13] observed the increases in egg production, egg quality, and hatchability in Japanese quail when dietary energy was supplemented at 11.51, 12.41, and 12.77 MJ/kg. Van Emous et al.[12] reported that a high-energy diet (12.55 MJ/kg) during the second phase of laying increased hatchability and the number of chicks suitable for commercialization compared to a standard diet (11.72 MJ/kg). In laying pigeons, increased energy content in the diet effectively improves egg production[5]. In agreement with these studies, dietary incorporation of progressively higher levels of energy (i.e., exceeding 12.00 MJ/kg) shortened the laying interval and boosted the 38-day laying rate of breeding pigeons. Previous studies failed to find a reproductive response in European Mimas breeding pigeons subjected to the energy-dense "2+3" (12.20, 12.40, and 12.60 MJ/kg)[9] and "2+4" (12.60, 12.80, and 13.00 MJ/kg)[6] lactation patterns. The different effects of various levels of dietary energy on the reproductive performance of breeding pigeons may be related to lactation patterns and species specificity. Additionally, in the present study, increasing levels of dietary energy linearly and/or quadratically decreased FI in breeding pigeons during incubation, lactation, and the entire experimental period, reflecting results from broilers[14], hens[15], domestic pigeons[16], breeding pigeons[6], and laying pigeons[5]. This finding could be attributed to the fact that birds adjust FI in response to dietary energy levels[17].

Squabs represent a major commodity for the pigeon market. Their growth and development depend entirely on the crop milk produced by their parents, whose diet defines the milk[4]. The nutrients in breeding pigeon feed not only meet the nutritional requirements of the breeding pigeons, but also the production regimens of squabs. Assessing breeding pigeon reproduction, as well as growth, development, and health status of squabs is essential for establishing nutritional standards for pigeon feed. A previous study showed that differences in dietary energy levels of sows affected offspring[18]. Recently, Peng et al.[6] reported that dietary energy levels affected BW gain of squabs only in the early growth stages (1–7 days). Similarly, in the present study, we found that increasing dietary energy levels (from 12.00 to 12.40 MJ/kg) caused a linear increase in BW and ADG of squabs at an early age, but not in the middle and later growth stages. This initial positive effect may be explained by digestive tract development and muscle cell proliferation occurring during the first few days of a bird's life[19,20]. Interestingly, Peng et al.[9] reported that parental dietary energy levels (12.2, 12.4 and 12.6 MJ/kg) had no effect on growth performance of European Mimas squabs during winter. Discrepancies between studies may be due to distinct genotypes, rearing patterns, environmental conditions, and other factors.

The intestine is a major site for digestion and absorption of dietary nutrients, and its function is highly dependent on changes to its morphology[21], which can predict gut health status. New epithelial cells produced in the crypts migrate and differentiate toward the top of the villus. Increased VH augments the villus surface area, thereby boosting nutrient absorption and transport[22]. The VH:CD ratio can serve as an indicator of enterocyte turnover rate[23]. A higher VH:CD ratio indicates a lower turnover rate and maintenance requirement for the intestinal epithelium, but possibly improved growth. In Peking ducks from 21 to 42 days of age, dietary energy of up to 12.24–13.18 MJ/kg had no effect on jejunal VH and CD[24]. Likewise, Mahdavi et al.[7] found that an increase in dietary ME levels from 11.92 to 12.34 MJ/kg did not result in any significant changes in jejunal VH, CD, villus surface area, and VH:CD ratio in broiler chicks at 10 days of age. Consistently, Peng et al.[9] reported that the CD, VH, and VH:CD ratio in the jejunum of squabs at 21 days of age were not influenced by increasing levels of dietary energy (12.2 to 12.6 MJ/kg). In contrast, the findings of the current study show that higher dietary energy promoted jejunal VH and VH:CD ratio, particularly at 12.40 MJ/kg. Hence, increasing dietary energy supplementation may support a healthy intestinal morphology in squabs. Similarly, Liu et al.[25] reported that a maternal high-energy diet markedly increased intestinal VH in fetuses and piglets. Discrepancies between studies may have resulted from differences in energy levels, duration of experiments, and animal species.

Oxidative stress arises from an imbalance between oxidants and antioxidants[26]. Oxidative stress in the intestine is dangerous because it impairs cellular function or damages tissues due to reactive oxygen species and reactive nitrogen species. High-energy diet-induced oxidative stress has been observed in experimental animals, including livestock and poultry. Adebowale et al.[27] found that elevated dietary energy density induced oxidative stress in weaned piglets, as suggested by increased MDA content in the intestine. Lasker et al.[28] reported signs of oxidative stress in the serum and liver of rats fed a high-energy diet, as evidenced by enhanced lipid peroxidation, inhibition of antioxidant enzymes (e.g., SOD and CAT) activities, and decreased glutathione level. A recent study showed that increasing dietary energy up to 14.44 MJ/kg in pigeons increased MDA while decreasing total antioxidant capacity in the serum[16]. In agreement with these findings, we report that higher dietary energy levels such as 12.20 MJ/kg boosted jejunal MDA content, as well as T-SOD and GPX activities in squabs, suggesting a relationship between jejunal oxidative stress and dietary energy content. In the present study, increased MDA content and antioxidant enzyme activity at higher energy levels may have occurred in response to an abundance of free radicals. In addition, the different responses of antioxidant enzymes in different animals to high-energy diets may be related to discrepancies in animal tolerance and tissues. Although intestinal morphology of squabs in the high-energy diet group was good in the present study, oxidative stress could be influenced by gut microbiota[29]. In laying hens, increased dietary energy levels promote harmful bacteria such as *Sutterella* in the cecum[30]. However, whether increased dietary energy levels interfere with gut microbiota content and composition in pigeons or squabs, has rarely been reported. Interestingly, ileal T-SOD and CAT activities of squabs increased linearly and/or quadratically with increasing energy levels, but MDA content was not altered. The distinct responses of T-SOD, GPX, CAT, and MDA in the jejunum and ileum of squabs to different parental dietary energy levels point to tissue specificity. A recent study found that a high-energy diet (12.6 MJ/kg) significantly increased plasma total antioxidant capacity in squabs at 21 days of age compared to a less dense energy diet (12.2 MJ/kg), but had no effect on MDA and SOD activity[9]. As no studies regarding the effect of dietary energy content on the redox status in the intestine of squabs have been reported, we cannot compare our results to other findings.

Conclusion

In conclusion, increasing dietary energy supplementation improved the reproductive performance of breeding pigeons and intestinal morphology in squabs, but altered the intestinal redox status and had no effect on the final BW of squabs. Our results suggest that 12.00 MJ/kg could be used in nutritional management to enhance reproductive performance of breeding pigeons and intestinal health in squabs.

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Author Contributions

Jinrong Wang, Kang Cheng, and Yong Zhang designed the study. Jingyi Niu, Daizi Hu, and Jinxiu Yao conducted the experiments, collected the samples, and performed all analyses. Mingjun Yang provided experimental sites. Kang Cheng, Hongyue Zhao, and Jingyi Niu performed statistical analyses, interpreted the data, and prepared the tables. Kang Cheng drafted the manuscript.

Conflict of interest

The authors declare no conflicts of interest.

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