

## Postprint: Optimal Dietary Metabolizable Energy and Protein Levels for Dawu Fen 1 Commercial Layer Pullets during the Late Rearing Period (10-17 Weeks of Age)

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### Abstract

This experiment was conducted to investigate the effects of dietary metabolizable energy and protein levels during the late rearing period (10-17 weeks of age) on growth performance, organ indices, small intestinal development, and peak laying period production performance and egg quality in Dawufen No. 1 commercial layer hens; and to determine the appropriate dietary metabolizable energy and protein levels for Dawufen No. 1 commercial layer hens during the late rearing period by establishing regression models between dietary metabolizable energy and protein levels and the measured indicators. This study comprised two experiments. Experiment 1: A total of 810 64-day-old layer hens were randomly selected and allocated to 9 groups, with 6 replicates per group and 15 birds per replicate. A 3×3 factorial design was employed, with three metabolizable energy levels [11.77 (high), 11.27 (medium), and 10.77 MJ/kg (low)] and three protein levels [16.50% (high), 15.50% (medium), and 14.50% (low)], resulting in 9 experimental diets that were fed to the respective groups for an 8-week period (10-17 weeks of age). Experiment 2: The grouping of experimental hens remained unchanged, and all hens were fed a uniform diet (metabolizable energy level: 10.91 MJ/kg; protein level: 15.98%) for a 14-week period (18-31 weeks of age). The results of Experiment 1 showed that: 1) Dietary metabolizable energy level had significant effects on average daily feed intake (ADFI), feed to gain ratio (F/G), and tibia length of layer hens ( $P < 0.05$ ); dietary protein level had significant effects on ADFI and tibia length ( $P < 0.05$ ); the interaction between dietary metabolizable energy and protein levels had significant effects on ADFI, average daily gain (ADG), and F/G ( $P < 0.05$ ). 2) Dietary metabolizable energy and protein levels and their interaction had no significant effects on organ indices of layer hens ( $P > 0.05$ ). 3) Dietary metabolizable energy level had

significant effects on jejunum, duodenum, and small intestine length ( $P < 0.05$ ); the interaction between dietary metabolizable energy and protein levels had significant effects on jejunum and small intestine length ( $P < 0.05$ ). The results of Experiment 2 demonstrated that: 1) Dietary metabolizable energy and protein levels during the late rearing period had no significant effects on production performance during the peak laying period ( $P > 0.05$ ), although ADFI during the peak laying period exhibited a decreasing trend with increasing dietary metabolizable energy level during the late rearing period; the interaction between dietary metabolizable energy and protein levels during the late rearing period had significant effects on ADFI, average daily egg production, and feed to egg ratio (F/E) during the peak laying period ( $P < 0.05$ ). 2) Dietary metabolizable energy level during the late rearing period had significant effects on yolk color and egg shape index during the peak laying period ( $P < 0.05$ ); dietary protein level during the late rearing period had significant effects on yolk color during the peak laying period ( $P < 0.05$ ); the interaction between dietary metabolizable energy and protein levels during the late rearing period had significant effects on shell thickness and egg shape index during the peak laying period ( $P < 0.05$ ). Through quadratic curve fitting of ADFI, F/G, jejunum length, duodenum length, small intestine length, and yolk color during the late rearing period, the appropriate dietary metabolizable energy levels for Dawufen No. 1 commercial layer hens during the late rearing period were determined to be 10.902, 10.720, 11.404, 11.446, 11.374, and 11.760 MJ/kg, respectively; through quadratic curve fitting of tibia length during the late rearing period, the appropriate dietary protein level was determined to be 15.300%. Based on these indicators, it is recommended that the dietary metabolizable energy level for Dawufen No. 1 commercial layer hens during the late rearing period (10-17 weeks of age) be 10.720-11.760 MJ/kg, and the protein level be 15.300%.

## Full Text

### Study on Optimal Dietary Metabolizable Energy and Protein Levels for Dawufen No.1 Commercial-Layer Hens in the Later Growing Period (10-17 Weeks of Age)

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## Abstract

This experiment aimed to investigate the effects of dietary metabolizable energy (ME) and protein levels during the later growing period (10–17 weeks of age) on growth performance, organ indices, and intestinal development of Dawufen No.1 commercial-layer hens, as well as their production performance and egg quality during the laying peak period. Regression models were established between dietary ME and protein levels and the measured parameters to determine the optimal dietary ME and protein levels for Dawufen No.1 commercial-layer hens during this critical growth phase. The study comprised two trials. In Trial 1, 810 sixty-four-day-old hens were randomly allocated to nine groups with six replicates of 15 birds each. A 3×3 factorial design was employed with three ME levels [11.77 (high), 11.27 (medium), and 10.77 MJ/kg (low)] and three protein levels [16.50% (high), 15.50% (medium), and 14.50% (low)], resulting in nine experimental diets that were fed to the respective groups for eight weeks (10–17 weeks of age). In Trial 2, the same grouping was maintained, but all hens were fed a uniform diet containing 10.91 MJ/kg ME and 15.98% protein for 14 weeks (18–31 weeks of age).

The results of Trial 1 demonstrated that dietary ME level significantly affected average daily feed intake (ADFI), feed-to-gain ratio (F/G), and tibia length ( $P < 0.05$ ). Dietary protein level significantly influenced ADFI and tibia length ( $P < 0.05$ ). The interaction between ME and protein levels significantly affected ADFI, average daily gain (ADG), and F/G ( $P < 0.05$ ). Neither dietary ME nor protein levels, nor their interaction, significantly affected any organ indices ( $P > 0.05$ ). Dietary ME level significantly impacted the length of the jejunum, duodenum, and total small intestine ( $P < 0.05$ ), while the interaction between ME and protein levels significantly affected jejunum and small intestine length ( $P < 0.05$ ).

Trial 2 results revealed that dietary ME and protein levels during the later growing period did not significantly affect production performance during the laying peak ( $P > 0.05$ ), though ADFI during the peak period tended to decrease as dietary ME level in the growing period increased. The interaction between ME and protein levels during the growing period significantly affected ADFI, average daily egg production, and feed-to-egg ratio (F/E) during the laying peak ( $P < 0.05$ ). Dietary ME level during the growing period significantly influenced yolk color and egg shape index during the peak period ( $P < 0.05$ ), with yolk color increasing significantly as dietary ME level rose. Dietary protein level during the growing period significantly affected yolk color ( $P < 0.05$ ). The interaction between ME and protein levels significantly impacted eggshell thickness and egg shape index during the laying peak ( $P < 0.05$ ).

Quadratic curve fitting between dietary ME level and ADFI, F/G, jejunum length, duodenum length, small intestine length, and yolk color yielded optimal ME levels of 10.902, 10.720, 11.404, 11.446, 11.374, and 11.760 MJ/kg, respectively. Quadratic fitting between dietary protein level and tibia length indicated

an optimal protein level of 15.300%. Based on these comprehensive results, the recommended dietary ME level for Dawufen No.1 commercial-layer hens during the later growing period (10-17 weeks) is 10.720-11.760 MJ/kg, with a protein level of 15.300%.

**Keywords:** Dawufen No.1 commercial-layer; metabolizable energy; protein; requirement; growing period; laying peak period

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## Introduction

The quality of pullets during the later growing period is closely correlated with their subsequent production performance in the laying phase. The later growing period represents a critical developmental stage when digestive capacity becomes increasingly robust and skeletal growth is most vigorous, making dietary nutrient levels directly determinant of replacement pullet quality. Previous research has demonstrated that dietary energy level significantly affects nutrient intake in laying hens, with excessive energy predisposing birds to fatty liver hemorrhagic syndrome, while inadequate energy reduces feather-pecking behavior. Furthermore, energy level during the growing period significantly influences follicle-stimulating hormone receptor (FSHR) mRNA expression in the ovary during the laying period. When dietary protein levels are excessive, pecking and feeding behaviors decrease while nesting and preening behaviors increase significantly. Reduced protein levels decrease phosphorus digestibility in the pre-cecal section and significantly lower nitrogen excretion. Additionally, the interaction between dietary ME and protein levels significantly affects digestive organ morphology and intestinal development in laying hens.

In poultry production, feed costs account for approximately 75% of total production expenses, making precise formulation of dietary nutrient levels a primary strategy for cost reduction. Dawufen No.1 commercial-layer hens, characterized by high egg production rates and strong stress resistance, represent a domestically developed breed introduced in 2013. Determining optimal dietary ME and protein levels for this breed during the growing period is therefore of significant practical importance. Although numerous studies have investigated optimal energy and protein levels for growing pullets, requirements vary considerably among breeds. Research on different breeds has reported optimal energy levels ranging from 11.60 to 13.38 MJ/kg and protein levels from 16.03% to 21%. One study demonstrated that providing ME and protein levels at 60% of NRC (1994) recommendations reduced growth rate but increased bone hardness. The present study utilized Dawufen No.1 commercial-layer hens to investigate the effects of dietary ME and protein levels and their interaction on growth performance, organ indices, intestinal development, and subsequent laying performance and egg quality, with the objective of establishing regression equations to determine optimal dietary ME and protein levels for this breed during the later growing period.

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## Materials and Methods

**1.1 Experimental Design and Grouping** Eight hundred ten 64-day-old Dawufen No.1 commercial-layer hens with similar body weight, good health status, and uniform genetic background were randomly divided into nine groups, each consisting of six replicates of 15 birds. The study comprised two trials. Trial 1 employed a 2-factor (ME and protein levels)  $\times$  3-level factorial design: ME levels of 11.77 (high), 11.27 (medium), and 10.77 MJ/kg (low); and protein levels of 16.50% (high), 15.50% (medium), and 14.50% (low). Nine experimental diets were formulated: high-ME high-protein (Group I), high-ME medium-protein (Group II), high-ME low-protein (Group III), medium-ME high-protein (Group IV), medium-ME medium-protein (Group V), medium-ME low-protein (Group VI), low-ME high-protein (Group VII), low-ME medium-protein (Group VIII), and low-ME low-protein (Group IX). All diets were mash form, with consistent nutrient levels other than ME and protein. The experimental period lasted eight weeks (10–17 weeks of age). Diet composition and nutrient levels are presented in Table 1. Trial 2 maintained the original grouping, with all hens fed a uniform diet containing 10.91 MJ/kg ME and 15.98% protein for 14 weeks (18–31 weeks of age). Diet composition and nutrient levels for this phase are shown in Table 2.

**1.2 Housing and Management** The feeding trial was conducted at the National Layer Breeding and Extension Base of Hebei Dawu Farming and Animal Husbandry Group. Hens were housed in a closed, longitudinally ventilated building in A-frame three-tier cages (45 cm  $\times$  40 cm  $\times$  45 cm) at three birds per cage. House temperature was maintained at 10–25°C with relative humidity of 55–70% and light intensity of 10 lx. Photoperiod was 10 hours daily. Manure was removed at 10:00 and 16:00 each day. Manual feeding was practiced, with birds provided ad libitum access to feed and water throughout the experimental period.

### 1.3 Measurements

**1.3.1 Growth Performance in Growing Period and Production Performance and Egg Quality in Laying Peak** Mortality was recorded daily. In Trial 1, feed troughs were cleaned at 20:00 on the last day of each week after feed withdrawal (water was provided). Feed consumption was recorded by replicate to calculate ADFI, ADG, and F/G for weeks 10–17. At 08:00 on the first day of each week, five pullets per replicate were selected for measurement of body weight, tibia length, chest breadth, and keel length. In Trial 2, eggs were collected from 16:00 to 16:30 daily during weeks 27–31. Egg number, feed consumption, and egg weight were recorded by replicate to calculate ADFI, average daily egg production, laying rate, and feed-to-egg ratio (F/E). On days 189, 196,

203, and 210, two eggs per replicate were randomly collected for egg quality analysis. Egg weight, albumen height, yolk color, and Haugh unit were measured using a SONOVA egg quality analyzer. Eggshell strength and thickness were measured using respective analyzers. Egg shape index, yolk percentage, and albumen percentage were calculated using the following formulas:

Egg shape index = longitudinal diameter / transverse diameter

Yolk percentage = (yolk weight / egg weight)  $\times$  100

Eggshell percentage = (eggshell weight / egg weight)  $\times$  100

**1.3.2 Organ Indices** At 119 days of age, one hen per replicate was randomly selected for slaughter. The heart, liver, spleen, thymus, pancreas, and bursa of Fabricius were dissected, trimmed of adherent fat, blotted dry, and weighed to calculate organ indices. The duodenum, jejunum, and ileum were separated and measured with a 0.01 m precision ruler to calculate relative lengths.

Organ index (%) = (organ weight / carcass weight)  $\times$  100

Relative length of intestinal segment (%) = (segment length / total small intestine length)  $\times$  100

**1.3.3 Dietary Sample Analysis** Dietary crude protein content was determined using the Kjeldahl method according to GB/T 6432–94.

**1.4 Statistical Analysis** Experimental data were processed using Excel 2016 and analyzed using SPSS 22.0. One-way ANOVA was used for data processing, with main effects and interactions analyzed using the general linear model (GLM) multivariate procedure. Differences among groups were assessed using LSD multiple comparison tests, with  $P < 0.05$  considered statistically significant. When interactions were significant, multiple linear equations were established using curve estimation in the regression procedure. When interactions were not significant but main effects were significant, quadratic equations for main effects were fitted. When quadratic effects were significant, the dietary ME and protein levels corresponding to maximum response values were derived from the quadratic functions and considered optimal.

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## Results

**2.1 Effects of Dietary ME and Protein Levels on Growth Performance in Later Growing Period** As shown in Table 3, chest breadth in Group I was significantly greater than in Groups IX and VIII ( $P < 0.05$ ). Tibia length in Group V was significantly shorter than in groups fed ME levels of 11.77 and 10.77 MJ/kg ( $P < 0.05$ ). ADFI and F/G in Group II were significantly lower than in Groups VIII and VII ( $P < 0.05$ ). Dietary ME level did not significantly affect chest breadth, keel length, body weight, or ADG ( $P > 0.05$ ), but significantly influenced ADFI, F/G, and tibia length ( $P < 0.05$ ). ADFI and F/G decreased as

dietary ME level increased, with medium- and low-ME groups being significantly higher than the high-ME group ( $P < 0.05$ ). Dietary protein level significantly affected tibia length and ADFI ( $P < 0.05$ ), with the low-protein group showing significantly higher ADFI than medium- and high-protein groups ( $P < 0.05$ ), but did not significantly affect other growth performance parameters ( $P > 0.05$ ). The interaction between dietary ME and protein levels significantly affected ADFI, ADG, and F/G during the later growing period ( $P < 0.05$ ).

**2.2 Effects of Dietary ME and Protein Levels in Later Growing Period on Production Performance During Laying Peak** Table 4 shows that ADFI during the laying peak was significantly lower in Groups I and II compared with Group IX ( $P < 0.05$ ). Feed-to-egg ratio (F/E) in Group II was significantly higher than in Groups I, III, V, and IX ( $P < 0.05$ ). Laying rate in Group II was significantly higher than in Group I ( $P < 0.05$ ) but did not differ significantly from other groups ( $P > 0.05$ ). Neither dietary ME nor protein levels during the growing period significantly affected production performance during the laying peak ( $P > 0.05$ ), although ADFI during the peak period tended to decrease as dietary ME level in the growing period increased. The interaction between dietary ME and protein levels during the growing period significantly affected ADFI, average daily egg production, and F/E during the laying peak ( $P < 0.05$ ).

**2.3 Effects of Dietary ME and Protein Levels in Later Growing Period on Egg Quality During Laying Peak** As presented in Table 5, eggshell strength in Group I was significantly higher than in Group VII ( $P < 0.05$ ). Egg weight in Group IV was significantly greater than in Group V ( $P < 0.05$ ). Albumen height in Group V was significantly lower than in Groups I and V ( $P < 0.05$ ). Yolk color in Groups I, II, III, and IV was significantly higher than in Groups V, VI, VII, VIII, and IX ( $P < 0.05$ ). Dietary ME level during the growing period significantly affected yolk color and egg shape index during the laying peak ( $P < 0.05$ ), with yolk color increasing significantly as dietary ME level increased, but did not significantly affect other egg quality parameters ( $P > 0.05$ ). Dietary protein level during the growing period significantly influenced yolk color ( $P < 0.05$ ) but did not significantly affect other egg quality traits ( $P > 0.05$ ). The interaction between dietary ME and protein levels during the growing period significantly affected eggshell thickness and egg shape index during the laying peak ( $P < 0.05$ ) but did not significantly influence other measured egg quality parameters ( $P > 0.05$ ).

**2.4 Effects of Dietary ME and Protein Levels on Organ Weight and Organ Indices in Later Growing Period** Table 6 shows that no significant differences were detected in any organ indices among groups during the later growing period ( $P > 0.05$ ). Dietary ME level, protein level, and their interaction did not significantly affect any of the measured organ indices ( $P > 0.05$ ).

**2.5 Effects of Dietary ME and Protein Levels on Intestinal Development in Later Growing Period** As shown in Table 7, ileum length, jejunum length, total small intestine length, and relative jejunum length in Group V were significantly greater than in Group VIII ( $P < 0.05$ ). Dietary ME level significantly affected jejunum, duodenum, and total small intestine length ( $P < 0.05$ ) but did not significantly influence other intestinal development parameters ( $P > 0.05$ ). Duodenum length in the low-ME group was significantly shorter than in the medium-ME group ( $P < 0.05$ ). Dietary protein level did not significantly affect any measured intestinal development indices ( $P > 0.05$ ). The interaction between dietary ME and protein levels significantly affected jejunum and small intestine length ( $P < 0.05$ ) but did not significantly influence other measured parameters ( $P > 0.05$ ).

## 2.6 Estimation of Optimal Dietary ME and Protein Levels

**2.6.1 Binary Regression Model Estimation** Using measured values of parameters showing significant interaction effects as dependent variables (Y), with dietary ME level as independent variable 1 (X) and protein level as independent variable 2 (X), binary regression analysis was conducted using the model  $Y = AX + BX + C$ . As shown in Table 8, dietary protein level was not significant in the linear equations for yolk color and F/G during the growing period, rendering the binary regression equations non-meaningful.

**2.6.2 Optimal Dietary ME Level for Dawufen No.1 Hens in Later Growing Period** Using dietary ME level as the independent variable (X) and measured values of parameters significantly affected by ME level as dependent variables (Y), monadic quadratic regression equations were established using the model  $Y = AX^2 + BX + C$ . Table 9 presents the significant quadratic regression equations for dietary ME level. Based on yolk color, F/G, ADFI during the growing period, and jejunum, duodenum, and small intestine length, the optimal dietary ME level for Dawufen No.1 commercial-layer hens in the later growing period is 10.720–11.760 MJ/kg.

**2.6.3 Optimal Dietary Protein Level for Dawufen No.1 Hens in Later Growing Period** Using dietary protein level as the independent variable (X) and parameters significantly affected by protein level as dependent variables (Y), monadic quadratic regression equations were established. The quadratic regression equation for ADFI during the growing period showed poor fit and was not adopted. Table 10 presents the significant quadratic regression equations for dietary protein level. Based on tibia length during the growing period, the optimal dietary protein level for Dawufen No.1 commercial-layer hens is 15.300%.

## Discussion

**3.1.1 Effects of Dietary ME Level on Growth Performance in Later Growing Period** Dietary ME level is associated with gastrointestinal tract tension, which can alter chyme particle size and consequently affect nutrient digestion and absorption. The present results showed that as dietary ME level increased, F/G and ADFI decreased while ADG increased, consistent with findings from several previous studies. Poultry possess an innate ability to regulate feed intake based on energy content, ceasing consumption when energy requirements are met. Therefore, feed intake decreases as dietary energy level increases. Quadratic regression fitting of the relationship between ADFI (Y) and dietary ME level (X) during the growing period yielded the equation  $Y = -33.224X^2 + 724.405X + 3827.66$  ( $R^2 = 0.526$ ), indicating that ADFI decreased as ME level increased within the range tested, reaching maximum value at 10.769 MJ/kg.

**3.1.2 Effects of Dietary Protein Level on Growth Performance in Later Growing Period** Previous research has identified dietary protein level as an important factor affecting feed intake in growing pullets. The current results showed that ADFI, ADG, and F/G decreased as dietary protein level increased, which differs from some studies but aligns with others. Discrepancies among studies may be attributed to differences in environmental conditions, dietary protein level ranges, and breed characteristics. Tibia length represents an important indicator for evaluating growth performance in laying hens. The significant effect of dietary protein level on tibia length suggests that increased protein may enhance calcium deposition and promote skeletal development. Quadratic regression fitting of tibia length (Y) against dietary protein level (X) produced the equation  $Y = -0.858X^2 + 26.254X - 95.502$  ( $R^2 = 0.861$ ), revealing that tibia length initially increased then decreased as protein level rose, reaching maximum at 15.300% dietary protein.

**3.1.3 Interaction Effects of Dietary ME and Protein Levels on Growth Performance** Previous research demonstrated that the interaction between dietary ME and protein levels in breeder diets significantly affected progeny growth performance. The current study found that this interaction significantly affected ADFI, ADG, and F/G in Dawufen No.1 pullets during the growing period, which differs from some previous findings. These discrepancies may reflect breed-specific differences in energy and protein requirements. Binary regression analysis revealed no significant correlation between measured parameters and dietary protein level.

**3.2 Effects of Dietary ME and Protein Levels During Growing Period on Production Performance During Laying Peak** Pullet quality during the growing period is closely related to subsequent laying performance. The present results showed that feeding different ME and protein levels during the growing period significantly affected ADFI, average daily egg production, and F/E during the laying peak, consistent with some studies but differing from

others. These variations may be breed-specific. One study found that dietary ME and protein levels significantly affected adiponectin mRNA expression in abdominal adipose tissue of broiler breeders. In the current study, Group I hens showed lower laying rates during peak production, possibly due to excessive abdominal fat accumulation resulting from high-ME, high-protein diets during the growing period, which subsequently reduced laying performance.

**3.3 Effects of Dietary ME and Protein Levels During Growing Period on Egg Quality During Laying Peak** Yolk color depends on the type and amount of carotenoids that poultry cannot synthesize and must obtain from the diet. Research indicates that excessive dietary fat can alter pigment structure and impair coloration, while adding 3-5% animal fat can enhance yolk color. The current results showed that feeding high-ME diets during the growing period improved yolk color during the laying peak. However, carotenoid metabolism is rapid, and yolk color is influenced not only by diet but also by physiological condition and environmental factors. Carotenoids are absorbed in the small intestine as diesters associated with low-density lipoproteins, enter circulation as free fatty acids, and are redeposited in yolk as diesters. The observed effects may be attributed to alterations in small intestinal physiology caused by different dietary ME levels during the growing period, which subsequently affected carotenoid absorption and utilization during the laying peak.

**3.4 Interaction Effects of Dietary ME and Protein Levels on Organ Indices in Later Growing Period** Healthy organs are essential for poultry growth, disease resistance, and environmental adaptation, with organ indices reflecting developmental status. The current results showed that the interaction between dietary ME and protein levels significantly affected pancreas weight and pancreas index, consistent with previous research. This indicates that pancreatic development is influenced by both dietary energy and protein levels. The interaction significantly affected ADFI, and since pancreatic secretions are required for protein, fat, and starch digestion, increased feed intake necessitates greater pancreatic secretion, which reflexively stimulates pancreatic development.

**3.5 Effects of Dietary ME and Protein Levels on Intestinal Development in Later Growing Period** The small intestine is the primary site for nutrient digestion and absorption and plays an important immune function. The current results showed that the interaction between dietary ME and protein levels significantly affected jejunum and small intestine length, though binary regression analysis indicated these effects were not significant for protein level alone. Dietary ME level significantly affected jejunum, duodenum, and small intestine length, consistent with previous research. This may occur because decreased dietary ME level increased ADFI, which promoted intestinal development. Quadratic regression equations for jejunum, duodenum, and small intestine length against dietary ME level were  $Y = -25.369X^2 + 578.602X + 3242.964$  ( $R^2 = 0.604$ ),  $Y = -5.411X^2 + 123.864X - 687.594$  ( $R^2 = 0.464$ ), and  $Y$

$= -49.263X^2 + 1120.601X - 6248.725$  ( $R^2 = 0.509$ ), respectively. The degree of digestive tract development in replacement pullets determines nutrient intake and absorption capacity during the laying period. Optimal ME levels for maximum jejunum, duodenum, and small intestine length were 11.376, 11.414, and 11.346 MJ/kg, respectively.

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## Conclusion

Based on comprehensive consideration of yolk color, ADFI and F/G during the growing period, jejunum, duodenum, and small intestine length, and tibia length, the recommended dietary ME level for Dawufen No.1 commercial-layer hens during the later growing period (10-17 weeks of age) is 10.720-11.760 MJ/kg, with a protein level of 15.300%.

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