Effect of dietary energy, protein, and a versatile enzyme on hen performance, egg solids, egg composition, and egg quality of Hy-Line W-36 hens during second cycle, phase two

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Primary Audience: Egg Producers, Nutritionists, Poultry Extension Personnel and Researchers

SUMMARY

This study was conducted to evaluate the effect of Rovabio, dietary energy, and protein on performance, egg composition, egg solids, and egg quality of commercial Leghorns in phase 2, second cycle. A 4 × 2 × 2 factorial arrangement of treatments comprising 4 dietary energy levels (2,791, 2,857, 2,923, and 2,989 kcal of ME/kg) and 2 protein levels (15.5 and 16.1%) with and without Rovabio was used. Hy-Line W-36 hens (n = 1,920, 87 wk old) were randomly divided into 16 dietary treatments (8 replicates of 15 hens per treatment). The trial lasted 12 wk. Dietary protein significantly increased feed consumption but decreased yolk color. As dietary energy increased from 2,791 to 2,989 kcal of ME/kg, feed consumption decreased from 98.0 to 94.9 g per hen daily, and yolk color increased from 5.27 to 5.56. There was a significant interaction among dietary protein, energy, and Rovabio on egg production, BW, egg mass, feed conversion, and yolk solids. Egg weight of hens fed the diets supplemented with Rovabio was significantly greater than that of hens fed the diets without Rovabio during wk 3 and 4. However, Rovabio did not significantly influence average egg weight (87 to 98 wk of age). Rovabio supplementation significantly increased BW of hens. These results suggest Rovabio had a small but significant influence on nutrient utilization of commercial Leghorns during phase 2 of the second cycle.

Key words: Rovabio, energy utilization, laying hen

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DESCRIPTION OF PROBLEM

In poultry operations, feed cost has always been a major issue. Enzyme supplementation as a feed additive has become common during the last 5 decades [1]. Enzymes are proteins, having unique abilities to catalyze biochemical reactions. Their usage is popular because of positive effects on hen performance and the lack of harmful effects on consumers. Utilization of most grains is influenced by the presence of indigestible complex carbohydrates, such as nonstarch polysaccharides (**NSP**). Enzymes are supplemented to the feed to improve nutritive value in those grains. Legume seeds also contain

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NSP-like hemicelluloses, mannan, and raffinose [2, 3]. Chickens do not produce some enzymes, such as galactosidases; thus, corn-soybean-based diets without supplemented enzymes such as xylanases and pectinases might result in gas accumulation in the gut and diarrhea [4, 5].

Rovabio is a natural mixture of enzymes produced by the organism *Penicillium funiculosum* [6]. Sims et al. [7] and Jakob et al. [8] reported that Rovabio significantly increased final BW and average daily BW gain of broilers and swine, respectively. However, no research has been conducted to evaluate the effect of Rovabio on laying hens.

Rovabio contains xylanases (endo-1,4-β-xylanase, α-arabinofuranosidase, β-xylosidase, feruloyl esterase, endo-1,5-α-arabinanase), β-glucanases [endo-1,3(4) β-glucanases, β-1,3-glucanase, endo-1,4-β-glucanase, cellobiohydrolase, β-glucosidase], mannanases (endo-1,4-β-mannanase, β-mannosidase), pectinases (pectinase, polygalacturonase, pectin esterase), and proteases (aspartic protease, metallo protease) [6]. The enzymes in this mixture work together to improve the utilization of feed ingredients. It is reported that a mixture of enzymes is more effective than a single enzyme [4].

Feed intake significantly decreased with increasing dietary energy or supplemental fat [9–11]. However, Summers and Leeson [12] and Jalal et al. [13] reported that there was no significant effect of dietary energy on feed intake. Decreased feed intake may have a great effect on cost of production. If feed intake cannot be linearly decreased by increased energy, increasing dietary energy by the addition of fat may not be economical. In addition, egg weight is also an important factor that can affect profits. Egg weight increased with increasing dietary energy [10, 11, 14, 15]. However, Jalal et al. [13] reported that there was no response of egg weight to increasing dietary energy by the addition of fat. The literature is very limited on the effect of dietary energy on hen performance, egg composition, egg solids, and egg quality in second cycle, phase 2. Therefore, it is necessary to have a better understanding of how to optimize the use of dietary energy to get optimal performance and profits of laying hens in the second cycle.

Numerous studies have been conducted to determine the effect of enzymes on availability of nutrients in broilers fed diets containing cereal grains that are rich in NSP. However, very few studies are available concerning the effect of enzymes in laying hens. Therefore, the goal of this study was to evaluate the effect of Rovabio, dietary energy, and protein on performance, egg composition, egg solids, and egg quality of commercial Leghorns in phase 2, second cycle (87 to 98 wk of age).

MATERIALS AND METHODS

A 4 × 2 × 2 factorial arrangement with 4 dietary energy levels (2,791, 2,857, 2,923, and 2,989 kcal/kg of ME) and 2 protein levels (15.5 and 16.1%) with and without Rovabio was used in this experiment. Those energy and protein levels of the experimental diets were calculated to meet the nutrient requirement specified by the Hy-Line W-36 management guide [16]. Ingredients and nutrient composition of the experimental diets are shown in Table 1. Hy-Line W-36 hens [17] (n = 1,920, molted at 66 wk) in their second cycle (87 wk old) were randomly divided among 16 treatments (8 replicates of 15 hens per treatment). Replicates were equally distributed into upper and lower cage levels to minimize the cage level effect. Three hens were housed in a 40.6 × 45.7 cm cage. All hens were housed in an environmentally controlled house with temperature maintained at approximately 26°C. The house had controlled ventilation and lighting (16L:8D). All hens were supplied with feed and water ad libitum. Animal housing and handling procedures during experimentation were in accordance with guidelines of the Institutional Animal Care and Use Committee of Auburn University. Feed consumption was recorded weekly for calculation of average daily feed consumption. Egg production was recorded daily, and egg weight and specific gravity were recorded once every 2 wk. Egg weight and egg specific gravity were measured using all eggs produced during 2 consecutive days. Egg specific gravity was determined using 9 gradient saline solutions varying in specific gravity from 1.060 to 1.100 in 0.005-unit increments [18]. Mortality was determined daily, and feed consumption was adjusted accordingly. Body weight was obtained by randomly weighing 3 hens (1 of 5 cages) per replicate. Egg mass (g of egg/hen per d) and feed conversion (g of feed/g of egg) were calculated from egg production, egg weight, and feed consumption. Feed samples were analyzed for enzyme activity [19].

Egg components were measured using 3 eggs from each treatment replicate at the middle (92 wk of age) and end (98 wk of age) of the experiment. Eggs were weighed and then broken. The yolks were separated from the albumen. Before yolk weight was determined, the chalaza was removed by forceps. Each yolk was rolled on a blotting paper towel to remove adhering albumen. The shells were cleaned of any adhering albumen and dried for 5 d. Albumen weight was calculated by subtracting the weight of yolk and shell from the whole egg weight.

Three eggs from each treatment replicate were randomly collected at the middle (92 wk of age) and at the end (98 wk of age) of the experiment for measuring whole solids. The yolk and albumen were mixed, and 5 to 6 g of homogenate was pipetted into an aluminum dish, with weight recorded to 0.001 g. The sample was dried in an oven for 24 h at 40.5°C [20] and then weighed. Three eggs per treatment replicate were used to analyze the yolk and albumen solids. After the yolk was separated from the albumen, 3 yolks and albumen per treatment replicate were mixed separately. The procedure for analyzing albumen and yolk solids was the same as the procedure for whole-egg solids content. Yolk color and Haugh units were measured (3 eggs from each replicate) at the middle (92 wk of age) and at the end (98 wk of age) of the experiment using an egg multitester, EMT-5200 [21]. Haugh units were calculated from the records of albumen height and egg weight using the following formula: $HU = 100 \log_{10} (H - 1.7 W^{0.37} + 7.56)$, where HU = Haugh units; H = height of the albumen (mm); and W = egg weight (g).

Data were analyzed by ANOVA using PROC MIXED of SAS Institute [22] for a randomized complete block with a factorial arrangement of treatments. Dietary energy, dietary protein, and Rovabio were fixed, whereas blocks were random. The factorial treatment arrangement consisted of 4 dietary energy levels and 2 protein levels with and without Rovabio, with 8 replicates per treatment. The following model was used to analyze the data:

$$Y_{ijk} = \mu + E_i + P_j + R_k + EP_{ij} + ER_{ik} + PR_{ik} + EPR_{ijk} + B_l + e_{ijk}$$

where Y_{ijk} = individual observation; μ = overall mean; E_i = dietary energy effect; P_j = protein effect; R_k = Rovabio effect; EP_{ij} = interaction between dietary energy and protein; ER_{ik} = interaction between dietary energy and Rovabio; PR_{ik} = interaction between protein and Rovabio; EPR_{ijk} = interaction among dietary energy, dietary protein, and Rovabio; B_l = effect of block; and e_{ijkl} = error component.

If differences in treatment means were detected by ANOVA, Duncan's multiple range test was applied to separate means. Contrast statements were used to test for linear or quadratic dietary energy effects. A significance level of $P \leq 0.05$ was used.

RESULTS AND DISCUSSION

There were no significant effects of dietary energy, protein, or Rovabio on average egg weight (Table 2). However, egg weight of hens fed the diets supplemented with Rovabio was significantly greater than that of hens fed the diets without Rovabio during wk 3 and 4 (hens at 89 and 90 wk of age). Egg weight of hens fed the high-protein diet was significantly greater than that of hens fed the low-protein diet during 97 and 98 wk of age (Table 2). The influence of dietary protein on egg weight in this study was consistent with that of Parsons et al. [23], Keshavarz [24], Leeson [25], Wu et al. [11], and Sohail et al. [15], who reported that egg weight of hens fed greater protein had a greater egg weight than the hens fed lower-protein diets.

The mechanism by which dietary protein and energy improves egg weight is well understood; Wu et al. [26] reported that increasing only dietary energy without the increase of other nutrient (protein and amino acid) levels did not improve egg weight, and both dietary energy and protein (amino acids) are important to optimize egg weight. However, conflicting results arise concerning the influence of supplemental energy on egg weight. Wu et al. [26] reported that egg weight linearly increased with increasing dietary energy in Bovans and Dekalb hens during the second cycle, whereas De Groote [27], Harms et al. [10], Jalal et al. [13], and Leeson

Table 1. Ingredients and nutrient content of the experimental diets

Item	P[1]: 16.1 R: −	P: 16.1 R: +	P: 16.1 R: −	P: 16.1 R: +	P: 16.1 R: −	P: 16.1 R: +	P: 16.1 R: −	P: 16.1 R: +	P: 15.5 R: −	P: 15.5 R: +	P: 15.5 R: −	P: 15.5 R: +	P: 15.5 R: −	P: 15.5 R: +	P: 15.5 R: −	P: 15.5 R: +
Ingredient																
Corn (8.5%)	66.07	66.07	64.51	64.48	62.98	62.94	61.44	61.42	67.60	67.60	66.08	66.05	64.54	64.49	63.01	63.00
Soybean meal (48.5%)	22.39	22.34	22.50	22.48	22.62	22.61	22.75	22.72	20.86	20.81	20.98	20.96	21.10	21.10	21.24	21.20
Limestone	5.12	5.12	5.12	5.12	5.12	5.12	5.12	5.12	5.12	5.12	5.12	5.12	5.12	5.12	5.12	5.12
Hard shell[2]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Dicalcium phosphate	1.50	1.50	1.51	1.51	1.52	1.52	1.52	1.52	1.51	1.51	1.52	1.52	1.52	1.52	1.52	1.52
Poultry oil	0.00	0.00	1.43	1.43	2.83	2.83	4.23	4.23	0.00	0.00	1.39	1.39	2.80	2.80	4.20	4.20
NaCl	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
Vitamin premix[3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[4]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.05	0.05	0.05	0.05	0.06	0.06	0.05	0.05
Rovabio[5]	—	0.05	—	0.05	—	0.05	—	0.05	—	0.05	—	0.05	—	0.05	—	0.05
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Calculated analysis																
CP	16.07	16.07	16.00	16.00	15.94	15.94	15.88	15.88	15.45	15.45	15.39	15.39	15.33	15.33	15.26	15.26
Crude fat	2.78	2.78	4.12	4.12	5.44	5.44	6.75	6.75	2.78	2.78	4.12	4.12	5.44	5.44	6.75	6.75
ME (kcal/kg)	2,791	2,791	2,857	2,857	2,923	2,923	2,989	2,989	2,791	2,791	2,857	2,857	2,923	2,923	2,989	2,989
Ca	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Available P	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Linoleic acid	1.31	1.31	1.61	1.61	1.90	1.90	2.20	2.20	1.32	1.32	1.62	1.62	1.92	1.92	2.21	2.21
Methionine	0.33	0.33	0.34	0.34	0.34	0.34	0.34	0.34	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Methionine + cystine	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.59
Tryptophan	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Isoleucine	0.79	0.79	0.78	0.78	0.78	0.78	0.78	0.78	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Lysine	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79

[1]P = dietary protein; R = Rovabio [6]. A plus (+) indicates diets supplemented with Rovabio; a minus (−) indicates diets without Rovabio supplementation.

[2]Hard shell = large-particle limestone (passing US mesh 4 and retained by US mesh 6) $CaCo_3$ [41].

[3]Provided per kilogram of diet: vitamin A (as retinyl acetate), 8,000 IU; cholecalciferol, 2,200 ICU; vitamin E (as DL-α-tocopheryl acetate), 8 IU; vitamin B_{12}, 0.02 mg; riboflavin, 5.5 mg; D-calcium pantothenic acid, 13 mg; niacin, 36 mg; choline, 500 mg; folic acid, 0.5 mg; vitamin B_1 (thiamine mononitrate), 1 mg; pyridoxine, 2.2 mg; biotin, 0.05 mg; vitamin K (menadione sodium bisulfate complex), 2 mg.

[4]Provided per kilogram of diet: manganese, 65 mg; iodine, 1 mg; iron, 55 mg; copper, 6 mg; zinc, 55 mg; selenium, 0.3 mg.

[5]Rovabio [6] was supplied at the rate of 0.05% (1.2 million xylanase and 10^5 β-glucanase units per 907 kg of feed).

Table 2. Influence of Rovabio [6], protein, and energy on egg weight (g) of Hy-Line W-36 hens of 87 to 98 wk of age

Item	Dietary energy (kcal/kg)	87–88 wk	89–90 wk	91–92 wk	93–94 wk	95–96 wk	97–98 wk	Mean egg weight (87–98 wk)
Protein (%)								
15.5		64.42	64.08	63.85	64.26	64.10	64.30	64.14
16.1		64.64	64.34	64.19	64.40	64.47	65.12	64.53
	2,791	64.36	63.74	63.97	64.15	64.00	64.37	64.10
	2,857	64.12	63.91	63.84	64.11	64.00	64.50	64.08
	2,923	64.74	64.68	64.63	64.76	64.61	64.98	64.73
	2,989	64.89	64.50	63.65	64.29	64.53	65.00	64.48
Rovabio[1]	−	64.33	63.86	63.89	64.13	64.05	64.65	64.15
	+	64.72	64.56	64.16	64.53	64.52	64.76	64.54
Pooled SEM		0.55	0.69	0.66	0.65	0.76	0.69	0.5
		Probability						
Protein		NS	NS	NS	NS	NS	0.02	NS
Energy		NS	NS	NS	NS	NS	NS	NS
Rovabio		NS	0.04	NS	NS	NS	NS	NS
Protein × energy		NS	NS	NS	NS	NS	NS	NS
Protein × Rovabio		NS	NS	NS	NS	NS	NS	NS
Rovabio × energy		NS	NS	NS	NS	NS	NS	NS
Protein × energy × Rovabio		NS	NS	0.03	NS	NS	NS	NS
Contrast								
Energy linear		NS	0.05	NS	NS	NS	NS	NS
Energy quadratic		NS	NS	NS	NS	NS	NS	NS

[1]A plus (+) indicates diets supplemented with Rovabio; a minus (−) indicates diets without Rovabio supplementation.

[25] reported that egg weight was not influenced by dietary energy. In this study, dietary energy had no influence on average egg weight. This result was in agreement with that of Jalal et al. [13], who reported that there was no response of egg weight to dietary energy in Hy-Line W-36 hens during the second cycle. Linoleic acid content of all experimental diets in this study was more than 1.3%. Grobas et al. [9] reported that linoleic acid content (more than 1.15%) in the diet had no effect on egg weight, and the NRC [28] recommended a linoleic acid requirement for laying hen of 1.0%. Therefore, it is believed that variations in linoleic acid content between diets had no influence on egg weight in this study.

Rovabio significantly increased egg weight during wk 89 to 90 of age, and there was a significant interaction among 3 factors on egg weight during wk 91 to 92 of age. The effect of Rovabio on egg weight was in agreement with Yoruk et al. [29], who reported that hens fed diets supplemented with a multi-enzyme similar to Rovabio had an increased egg weight in some weeks. Similarly, Wu et al. [4] reported that diets supplemented with β-mannanase, which is a constituent in Rovabio, significantly increased egg weight in some weeks, but not all. The effect of multi-enzymes on the performance of laying hens may not be explained by simply making NSP available as an energy source. Multi-enzymes help to improve the utilization of the nutrients found in the feed ingredients by decreasing the viscosity of digesta, which impairs the diffusion of nutrients and decreases the availability of nutrients for digestion and absorption [29].

There were no significant effects of Rovabio on feed intake (Table 3). However dietary energy and protein had significant effects on feed intake. As dietary energy increased, feed intake linearly decreased by 3.1%, from 98.0 to 94.9 g/hen per day. These results agree with those of Wu et al. [26], Sohail et al. [15], Grobas et al. [9], and Parsons et al. [23]. Increasing dietary protein increased feed intake from 95.9 to 97.5 g/hen per day, corresponding to a 1.65% increase in feed intake (Table 3). Parsons et al. [23] reported that increasing dietary protein from 18 to 20% increased feed intake from 104 to 107 g/

Table 3. Influence of Rovabio [6], protein, and energy on feed intake, mortality, and egg composition of Hy-Line W-36 hens of 87 to 98 wk of age

Item	Dietary energy (kcal/kg)	Feed intake (g/hen per d)	Mortality (%)	Egg composition (%) Yolk	Egg composition (%) Albumen	Egg composition (%) Shell	Egg composition (g) Yolk	Egg composition (g) Albumen	Egg composition (g) Shell
Protein (%)									
15.5		95.92	0.17	28.45	63.12	8.42	18.35	40.86	5.42
16.1		97.50	0.21	28.24	63.38	8.38	18.61	41.84	5.52
	2,791	97.96	0.09	28.12	63.38	8.50	18.29	41.40	5.53
	2,857	97.34	0.28	28.46	63.26	8.29	18.60	41.44	5.42
	2,923	96.63	0.18	28.31	63.38	8.31	18.42	41.34	5.40
	2,989	94.90	0.23	28.50	62.99	8.51	18.60	41.23	5.55
Rovabio[1]	−	96.35	0.19	28.52	63.14	8.35	18.61	41.32	5.44
	+	97.07	0.19	28.17	63.37	8.46	18.35	41.38	5.50
Pooled SEM		0.98	0.13	0.54	0.62	0.19	0.42	1.08	0.12
		Probability							
Protein		0.03	NS	NS	NS	NS	NS	NS	NS
Energy		0.02	NS	NS	NS	NS	NS	NS	NS
Rovabio		NS	NS	NS	NS	NS	NS	NS	NS
Protein × energy		NS	NS	NS	NS	NS	NS	NS	NS
Protein × Rovabio		NS	NS	NS	NS	NS	NS	NS	NS
Rovabio × energy		NS	NS	NS	NS	NS	NS	NS	NS
Protein × energy × Rovabio		NS	NS	NS	NS	NS	NS	NS	NS
Contrast									
Energy linear		0.01	NS	NS	NS	NS	NS	NS	NS
Energy quadratic		NS	NS	NS	NS	0.03	NS	NS	0.03

[1]A plus (+) indicates diets supplemented with Rovabio; a minus (−) indicates diets without Rovabio supplementation.

bird per day, and Wu et al. [11] also reported that increasing dietary protein from 14 to 16% increased feed intake from 102.9 to 105.6 g/hen per day.

Increasing dietary energy linearly decreased protein, TSAA, and lysine intake and increased dietary energy intake (Table 4). Although nutrient intake, such as protein and TSAA, decreased as dietary energy increased, egg production, egg weight, and egg mass did not decrease. Increasing fat content has an effect of slowing passage rate, which leads to increased digestibility of other nutrients, such as protein and amino acids [30]. This effect is called the extracaloric effect of fat. Reginatto et al. [31] reported that increasing dietary energy improved protein utilization in broilers. In this study also, increasing dietary energy by addition of poultry oil appeared to improve nutrient (protein, lysine, or TSAA) utilization.

Hens fed diets supplemented with Rovabio with low dietary energy levels (2,791 and 2,857 kcal of ME/kg) had a significantly greater BW than the hens fed diets without Rovabio at the high protein level (Table 5). These results suggest that Rovabio helps increase ileal digestibility of feed ingredients, increasing amino acid or energy availability, or both. Yoruk et al. [29] also reported a significant effect of a multi-enzyme that was similar to Rovabio on BW. Marsman et al. [32] found that improvement in the nutritional value of soybeans could be achieved with protease and glucanase enzyme supplementation, which are also constituents of Rovabio. Results of this study suggest that Rovabio has the ability to increase availability of energy, amino acids, or both from feed ingredients. Similarly, Sims et al. [7] and Jakob et al. [8] reported that broilers and swine fed diets supplemented with Rovabio had significantly increased BW.

There was a significant interaction among dietary protein, energy, and Rovabio on egg production (Table 5). Hens fed Rovabio-supplemented diets with high dietary protein had greater egg production than hens fed diets without Rovabio at high energy levels (2,923 and

Table 4. Influence of Rovabio [6], protein, and energy on energy, protein, TSAA, and lysine intake and egg quality of Hy-Line W-36 hens of 87 to 98 wk of age

Item	Dietary energy (kcal/kg)	Nutrient intake per hen daily: Energy (kcal)	Protein (g)	TSAA (mg)	Lysine (mg)	Egg quality: Haugh unit	Yolk color
Protein (%)							
15.5		278	14.9	565.9	757.7	73.57	5.50
16.1		281	15.7	604.5	809.3	72.96	5.34
	2,791	274	15.5	592.7	793.6	74.74	5.27
	2,857	278	15.4	588.9	788.5	73.73	5.33
	2,923	283	15.3	584.8	782.9	70.96	5.52
	2,989	284	15.0	574.4	768.9	73.64	5.56
Rovabio[1]	−	279	15.2	583.0	780.5	74.07	5.41
	+	281	15.3	587.4	786.5	72.46	5.43
Pooled SEM		2.83	0.15	5.95	7.97	2.13	0.14
		Probability					
Protein		NS	0.01	0.01	0.01	NS	0.02
Energy		0.03	0.02	0.02	0.02	NS	0.01
Rovabio		NS	NS	NS	NS	NS	NS
Protein × energy		NS	NS	NS	NS	NS	NS
Protein × Rovabio		NS	NS	NS	NS	NS	NS
Rovabio × energy		NS	NS	NS	NS	NS	NS
Protein × energy × Rovabio		NS	NS	NS	NS	NS	NS
Contrast							
Energy linear		0.03	0.02	0.02	0.02	NS	0.01
Energy quadratic		NS	NS	NS	NS	NS	NS

[1]A plus (+) indicates diets supplemented with Rovabio; a minus (−) indicates diets without Rovabio supplementation.

2,989 kcal of ME/kg). However, hens fed Rovabio-supplemented diets with low dietary protein had a lower egg production than hens fed diets without Rovabio at high energy levels. Results of dietary protein and energy on egg production in this study were consistent with that of Harms et al. [33] and Parsons et al. [23], who reported that egg production was not affected by dietary energy. Similarly, Parsons et al. [23], Sell et al. [34], Wu et al. [26], and Sohail et al. [15] reported that there was no consistent effect of dietary energy or dietary protein on egg production.

There was a significant interaction between dietary energy and Rovabio on egg albumen solids (Table 6). Rovabio significantly increased albumen solids at the lower energy level. There was a significant interaction among dietary protein, energy, and Rovabio on egg yolk solids (Table 6). However, a significant interaction was observed only with low dietary protein level (15.5%). Hens fed Rovabio with low dietary protein had greater egg yolk solids than hens fed diets without Rovabio at high energy levels (2,923 and 2,989 kcal of ME/kg). These results are important for the egg-breaker industry, because Rovabio could be used in some situations to increase solids with diets containing lower dietary protein. However, more research is needed to further explore the influence of Rovabio on hen performance, particularly in regard to the potential influence of Rovabio on egg weight in young hens and percentage of yolk solids, which would be useful to the breaker egg industry.

Dietary protein had a significant effect and dietary energy had a significant linear effect on egg yolk color (Table 4); as dietary energy increased by increasing poultry oil content in the diets from 0 to 4.2%, yolk color index increased from 5.27 to 5.56, resulting in a net increase of 0.29 units. These results suggest a relationship between dietary fat and egg yolk color. Xanthophyll is the major colorant responsible for the egg yolk color and it is highly fat soluble [35–37]. Because xanthophylls are fat soluble, with an increase of dietary fat, more xanthophylls may be absorbed and deposited in the egg yolk. These results agree with those reported by Morihiro et al. [38], Masahiro et al. [39], and Abu Serewa et al. [37]. Increasing dietary protein decreased egg yolk color because of a reduction in corn use, as the level of protein increased. This result was in agreement with that reported by Karunajeewa [40].

There was a significant interaction between dietary protein and energy on egg specific gravity (Table 5). As dietary energy increased, egg specific gravity decreased at a greater protein level. This may be due to decreased feed (Ca) intake with the increased supplemented fat. Harms et al. [10] also reported that as dietary energy increased, egg specific gravity decreased. There was a significant interaction among dietary protein, energy, and Rovabio on egg mass and feed conversion (Table 5). Hens fed diets supplemented with Rovabio at the high protein level

Table 5. Influence of Rovabio [6], protein, and energy on egg production, egg specific gravity, BW, egg mass, and feed conversion of Hy-Line W-36 hens of 87 to 98 wk of age

Item	Dietary energy (kcal/kg)	Egg production (%)	Specific gravity (unit)	BW (kg)	Egg mass (g/hen per d)	Feed conversion (g of feed/g of egg)
Protein (%)						
15.5		77.84	1.0784	1.74	49.96	1.92
16.1		78.65	1.0781	1.80	50.75	1.92
	2,791	79.11	1.0786	1.71	50.70	1.93
	2,857	78.20	1.0781	1.79	50.12	1.94
	2,923	78.34	1.0782	1.73	50.70	1.91
	2,989	77.34	1.0781	1.89	49.89	1.91
Rovabio[1]						
	−	78.22	1.0783	1.71	50.19	1.92
	+	78.27	1.0782	1.82	50.52	1.92
Interaction (protein × energy × Rovabio)						
15.5 × 2,791 × −		79.16	1.0783	1.75	50.58	1.90
15.5 × 2,791 × +		79.31	1.0789	1.74	50.72	1.95
15.5 × 2,857 × −		76.05	1.0777	1.62	48.29	1.99
15.5 × 2,857 × +		79.19	1.0777	1.86	51.06	1.93
15.5 × 2,923 × −		79.40	1.0791	1.52	51.28	1.90
15.5 × 2,923 × +		76.67	1.0785	1.76	49.83	1.89
15.5 × 2,989 × −		77.74	1.0779	1.85	50.03	1.87
15.5 × 2,989 × +		75.21	1.0788	1.81	47.86	1.95
16.1 × 2,791 × −		80.60	1.0783	1.62	51.68	1.91
16.1 × 2,791 × +		77.35	1.0788	1.72	49.81	1.97
16.1 × 2,857 × −		79.10	1.0790	1.67	50.42	1.92
16.1 × 2,857 × +		78.45	1.0780	2.00	50.72	1.94
16.1 × 2,923 × −		77.97	1.0779	1.84	50.32	1.93
16.1 × 2,923 × +		79.31	1.0773	1.81	51.37	1.91
16.1 × 2,989 × −		75.77	1.0781	1.85	48.91	1.96
16.1 × 2,989 × +		80.63	1.0771	1.90	52.77	1.85
Pooled SEM		1.19	0.0004	0.03	0.89	0.02
		Probability				
Protein		NS	NS	0.03	NS	NS
Energy		NS	NS	0.01	NS	NS
Rovabio		NS	NS	0.01	NS	NS
Protein × energy		NS	0.01	0.02	NS	NS
Protein × Rovabio		NS	NS	NS	NS	NS
Rovabio × energy		NS	NS	0.01	NS	NS
Protein × energy × Rovabio		0.02	NS	0.02	0.02	0.03
Contrast						
Energy linear		NS	NS	0.03	NS	NS
Energy quadratic		NS	NS	NS	NS	NS

[1]A plus (+) indicates diets supplemented with Rovabio; a minus (−) indicates diets without Rovabio supplementation.

Table 6. Influence of Rovabio [6], protein, and energy on egg solids of Hy-Line W-36 hens of 87 to 98 wk of age

Item	Dietary energy (kcal/kg)	Solids (%) Whole egg	Albumen	Yolk
Protein (%)				
15.5		24.93	12.12	53.26
16.1		25.61	12.30	52.69
	2,791	25.10	12.66	52.51
	2,857	24.95	12.03	52.97
	2,923	24.99	12.14	53.80
	2,989	26.05	12.01	52.63
Rovabio[1]				
	−	24.88	12.02	53.07
	+	25.66	12.40	52.89
Interaction (protein × energy × Rovabio)				
15.5 × 2,791 × −		25.31	11.85	52.20
15.5 × 2,791 × +		25.29	12.63	52.76
15.5 × 2,857 × −		24.91	11.95	54.35
15.5 × 2,857 × +		24.85	11.99	52.36
15.5 × 2,923 × −		24.15	12.33	53.69
15.5 × 2,923 × +		24.69	12.18	54.93
15.5 × 2,989 × −		24.99	12.29	52.74
15.5 × 2,989 × +		25.29	11.76	53.08
16.1 × 2,791 × −		25.26	11.84	52.83
16.1 × 2,791 × +		24.53	14.32	52.25
16.1 × 2,857 × −		24.89	12.13	52.81
16.1 × 2,857 × +		25.16	12.07	52.37
16.1 × 2,923 × −		24.92	11.87	53.22
16.1 × 2,923 × +		26.19	12.17	53.36
16.1 × 2,989 × −		24.62	11.92	52.71
16.1 × 2,989 × +		29.29	12.08	51.98
Pooled SEM		1.15	0.42	0.36
		Probability		
Protein		NS	NS	0.01
Energy		NS	NS	0.02
Rovabio		NS	NS	NS
Protein × energy		NS	NS	NS
Protein × Rovabio		NS	NS	NS
Rovabio × energy		NS	0.01	0.03
Protein × energy × Rovabio		NS	NS	0.01
Contrast				
Energy linear		NS	NS	NS
Energy quadratic		NS	NS	0.01

[1]A plus (+) indicates diets supplemented with Rovabio; a minus (−) indicates diets without Rovabio supplementation.

had a greater egg mass and improved feed conversion at a high energy level.

The influence of Rovabio on hen performance is complex, as indicated by interactions with protein and energy. Because increasing dietary energy significantly decreased feed intake, one would expect to see a decrease in feed intake with the addition of Rovabio if it increased dietary energy 66 kcal/lb, as suggested by the manufacturer [6]. However, because the difference (66 kcal/lb) in dietary energy levels between diets with and without Rovabio is much smaller than the difference that can be created with fat, it is difficult to statistically show an influence of Rovabio on feed intake even if it is there. Hens fed Rovabio had increased BW, apparently because they did not adjust dietary energy intake.

CONCLUSIONS AND APPLICATIONS

1. Increasing dietary energy by addition of poultry oil significantly decreased feed intake and increased yolk color.
2. Increasing dietary protein significantly increased feed intake, increased egg weight during last the 2 wk (wk 97 to 98 of age), and decreased yolk color.
3. The significant influence of Rovabio on hen performance was complex, as indicated by the significant Rovabio, dietary energy, and protein interactions on egg weight (91 to 92 wk of age), egg production, BW, egg mass, feed conversion, and albumen and yolk solids. These interactions suggest that Rovabio has at least some influence on utilization of energy, amino acids, or both.

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