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Impact of Enzyme-Supplemented Diets with Varying Metabolizable Energy Levels on Growth Performance, Egg Quality, and Blood Parameters in Laying Hens

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Abstract

This study examined the impact of reducing dietary metabolizable energy (ME) and supplementing with exogenous enzymes on performance, egg quality, and blood parameters of laying hens from 42 to 56 weeks of age. Four hundred and thirty-two laying hens were divided into twelve groups with six replicates (6 birds/replicate) and fed diets with varying levels of ME (control, 2.5% lower, or 5% lower) with or without a combination of two types of enzymes (Avizyme (1502) and Phyzyme (XP)). Hens fed diets with 2.5% lower ME exhibited reduced feed intake (FI), egg mass (EM), and a poorer feed conversion ratio (FCR) compared to the control group ($P < 0.05$). Reducing dietary ME by 5% did not significantly affect FI, egg production (EP), FCR and EM ($P > 0.05$). Enzyme supplementation generally improved FI and FCR at some points, but separate addition showed a greater benefit compared to combining them ($P < 0.05$). The use of enzymes resulted in a decrease in the Haugh unit compared to the control diet ($P < 0.05$). Additionally, the decrease in the energy content of the diet reduced the egg shape index ($P < 0.05$). Furthermore, the simultaneous addition of enzymes and decrease in energy increased the blood uric acid levels ($P < 0.05$). This study demonstrates that reducing metabolizable energy in corn-soybean meal diets for laying hens negatively impacts performance, with enzyme supplementation failing to fully compensate for these detrimental effects.

Introduction

The need to reduce feed costs in poultry production has been a longstanding concern amongst industry professionals. Given the rising trend of grain prices, the animal feed industry has been striving to lower nutritional expenses. Laying hens require a lot of energy for the demanding process of egg production (EP), making it important to find ways to reduce costs without compromising their health (Li *et al.* 2013). One approach that has been explored is reducing metabolizable energy (ME) in poultry diets (Novak *et al.* 2007; Scheideler *et al.* 2005). While this approach has the potential to yield cost savings, it also carries the risk of nutrient deficiencies and reduced performance. Over the past 15 years, the use of commercially available exogenous enzymes in the poultry industry has witnessed a significant increase, leading to better production efficiency by enhancing nutrient digestion and absorption while reducing nutrient loss through excreta (Ulo, 2022). So, by using enzymes to improve nutrient digestibility and utilization, we could achieve a slight reduction in ME, balancing cost savings with optimal performance (Ulo, 2022). According to Buchanan *et al.* (2007), exogenous enzymes hydrolyze non-starch polysaccharides (NSPs), which could potentially be utilized by the animal, thereby increasing the efficiency of feed energy utilization. The enzymes can release cell content, making it available for enzymatic digestion and increasing the digestibility of all nutrients (Slominski *et al.* 2006; Enenebeaku *et al.* 2018). This process, in turn, helps to reduce pollution associated with poultry manure and allows for the use of lower-cost ingredients (Bedford and Schulze, 1998; Cook *et al.*, 2000; Douglas *et al.*, 2000; Costa *et al.*, 2008). Numerous enzymes, such as carbohydrases, proteases, phytases, and lipases, are used to enhance the nutritional value of animal feed (McCleary, 2001). Commercial enzyme preparations usually contain a variety of enzymes rather than a single enzyme, which is beneficial when feed rations are made up of ingredients with different compositions. Although corn nutrients are generally highly available, enzyme supplementation has been

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reported to improve feed conversion ratio (FCR) by 2 to 3% (Cowan, 1993). Moreover, the study by Cowan (1993) highlights the presence of non-digestible carbohydrates in soybean meal, which can be broken down and utilized by hens through the addition of appropriate enzymes in their feed. Two primary methods exist for incorporating these external enzymes into formulated diets. The first, referred to as the "over the top" approach, offers an economical way to enhance performance. This approach entails adding enzymes to regular diets without modifying the current nutrient levels. The other way to address the issue is to modify the composition of the diet by decreasing the quantity of nutrients and incorporating external enzymes to regain the standard diet's nutritional value. Both techniques attain cost reductions, but the second technique yields the most substantial savings. Research on Avizyme®1502 (AVI), an enzyme preparation containing xylanase, amylase, and protease, demonstrates its effectiveness in improving nutrient availability, minimizing nutrient loss, enhancing live bird performance, and reducing feed costs (Zanella *et al.* 1999; Novak *et al.* 2007; Hahn-Didde and Purdum, 2014; Suharsono *et al.* 2019). Scheideler *et al.* (2005) observed improved EP in specific hen strains with reduced ME diets and AVI 1500 supplementation. Moreover, Phyzyme®XP (PHY) breaks down phytate, which is present in every vegetal ingredient, making phosphorus and other elements available for metabolism and animal use (Selle and Ravindran, 2007). According to Hahn-Didde and Purdum (2014), reducing the dietary ME and enzyme addition had no negative effect on productive performance and egg quality traits of laying hens. This study investigated the effects of dietary ME level and enzyme supplementation on productive performance, egg quality traits, and blood parameters in laying hens fed corn-soybean meal-based diets.

Materials and methods

The animal care procedures followed in the experiment were approved by the ethics committee of the Razi University in Kermanshah, Iran. A total of 432 Lohmann LSL-Classic laying hens were assigned to 12 treatments with six replicates per group and six hens per replicate. The hens were between 42 and 56 weeks of age throughout the study. They were housed in a wire cage (Three birds were housed per 45 × 45 × 45 cm wire cage) on a 16 h lighting schedule, temperature of 24 ± 2 °C, and relative humidity of 30-40%. About 110 grams of food per hen was considered daily, and access to water was ad libitum. The experiment employed a 3 × 4 factorial arrangement with twelve dietary treatments. The factors were ME level (2750, 2681, and 2612 kcal/kg of diet) and enzyme supplementation (without enzyme, 0.0375% of AVI (37.5 g/t), 0.006% of PHY (6 g/t), and AVI&PHY) (Table 1). Avizyme® (1502) (Danisco Animal Nutrition, Marlborough, UK) contained a minimum of 800 units/g of *α*-amylase from *Bacillus amyloliqufaciens*, 8,000 units/g of proteases from *Bacillus subtilis*, and 600 units/g of *β*-xylanase from *Trichoderma longibrachiatum*. Phyzyme®XP 5000G (Danisco Animal Nutrition, Marlborough, UK), which originates from the bacteria *Escherichia coli* and is produced by *Schizosacchromyces pombe*, was formulated to contain 0.30% available P and a Ca adjustment as recommended by Phycheck software tool (10% decrease). Recommendations for dietary nutrients were based on Lohmann LSL's classic commercial management guide (Table 1).

Productive performance

Following a one-week adaptation period to the experimental diets, data collection commenced. Daily egg number and weight were recorded, and these data were used to calculate EP and egg weight (EW). Moreover, FCR was calculated based on the above data. Additionally, egg mass (EM) was calculated by following formula: (Egg mass (g) = Egg production (%) x Average egg weight (g)). To calculate the amount of feed intake (FI), about 110 gr/hen/day of feed was weighed and given to the hens on a daily basis, and at the end of each week, the amount of feed residual was recorded. These data were expressed in four time intervals: 42 to 46 days, 47 to 51 days, 52 to 56 days, and for the entire experimental period (42 to 56 days). During the entire experimental period mortality was recorded and used for data correction.

Egg quality traits

In the final week of the experiment (56 weeks), two eggs were collected per replicate for three consecutive days for further analysis. These eggs were then evaluated for various quality traits, including Haugh unit, egg shape index, yolk index, shell weight, shell thickness, and yolk color. The egg shape index was calculated by measuring the egg's length and width with a compass and using the formula: (width/length) × 100. The Haugh unit was calculated using the established formula developed by Eisen *et al.* (1962). Shell thickness was measured at three locations on the egg (air cell, equator, and sharp end) using a dial and pipe gauge, with the final value representing the average of these measurements. Yolk color was assessed using the Roche fan color scale. Yolk height (H) was measured with a tripod micrometer (Mitutoyo, 0.01 mm, Japan), while yolk diameter (D) was measured with a compass (Swordfish, 0.02 mm, China). The formula used to calculate the yolk index was YI = (H/D) × 100.

Blood parameters

Two laying hens were picked at random from each replicate at the end of the experiment (56 weeks), and

0.3 ml of blood was collected from the bronchial wing vein in the test tubes. The blood samples were then centrifuged for 15 minutes at 1008 g, and the resulting sera were preserved at -20°C until the desired parameters were measured. Albumin, uric acid, glucose, triglyceride, and cholesterol levels were determined using Pars Azmun kits, following the manufacturer's instructions (Pars Azmoon, Tehran, Iran).

Table 1. Experimental diet composition and calculated nutritional composition

Ingredients (%)	ME (%)[a]											
	100	100	100	100	97.5	97.5	97.5	97.5	95	95	95	95
Corn	67.57	64.95	65.61	63.66	64.58	62.73	63.27	61.48	61.65	59.75	60.40	58.53
Soybean meal	21.23	19.49	19.96	19.24	20.76	20.03	20.34	19.70	20.11	19.67	19.80	19.33
Wheat Bran	0.00	4.47	3.88	4.48	3.65	4.00	4.00	4.00	7.00	7.00	7.00	7.00
Limestone	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Oyster Shells	5.44	5.47	5.54	5.54	5.46	5.46	5.53	5.53	5.48	5.48	5.55	5.55
DCP[b]	1.64	1.58	1.02	1.03	1.58	1.60	1.03	1.05	1.54	1.55	0.99	1.00
Common salt	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Vit. Premix[c]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Min. Premix[d]	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	0.22	0.17	0.16	0.17	0.16	0.17	0.16	0.17	0.16	0.17	0.16	0.17
Lysine-HCL	0.09	0.03	0.02	0.03	-	0.02	0.01	0.03	0.01	0.02	0.01	0.02
Sand (Inert filler)	-	-	-	2.00	-	2.15	1.85	4.19	0.24	2.52	2.28	4.55
Avizyme (1502)	-	0.0375	-	0.0375	-	0.0375	-	0.0375	-	0.0375	-	0.0375
Phyzyme (XP)	-	-	0.006	0.006	-	-	0.006	0.006	-	-	0.006	0.006
Nutrient composition (as fed basis)												
ME (Kcal/kg)	2750	2750	2750	2750	2681	2681	2681	2681	2612	2612	2612	2612
Crude protein (%)	14.69	14.69	14.69	14.69	14.69	14.69	14.69	14.69	14.69	14.69	14.69	14.69
Calcium (%)	3.64	3.64	3.64	3.64	3.64	3.64	3.64	3.64	3.64	3.64	3.64	3.64
Available Phosphorus (%)	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
Sodium (%)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Crude fiber (%)	2.31	2.68	2.64	2.64	2.63	2.60	2.72	2.56	2.91	2.85	2.87	2.81
Lysine (%)	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72
Methionine + Cystine (%)	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
Threonine (%)	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54

[a]ME= Metabolizable energy.
[b]DCP= Dicalcium phosphate.
[c]Vitamin mixture per kg of diet provides the following: vitamin A, 7,700,000 IU; vitamin D3, 3,300,000 IU; vitamin E, 6600 mg; vitaminK3, 550 mg; thiamine, 2200 mg; riboflavin, 4400 mg; vitamin B6, 4400 mg; Ca pantothenate, 550 mg; nicotinic acid, 200 mg; folic acid, 110 mg; choline chloride, 275,000 mg; biotin, 55 mg; vitamin B12, 8.8 mg.
[d]Mineral mixture per kg of diet provides the following: Mn, 66,000 mg; Zn, 66,000 mg; Fe, 33,000 mg; Cu, 8,800 mg; Se, 300 mg.

Statistical analysis

The data were analyzed using a completely randomized design with a 3 × 4 factorial treatment structure. This analysis was conducted using the General Linear Model (GLM) procedure of SAS software (SAS, 2015). Prior to analysis, the Kolmogorov-Smirnov test verified the normality of the data. Throughout the analysis, a significance level of $P < 0.05$ was employed. To compare treatment means, Duncan's multiple-range test (Duncan, 1955) was used. The following linear model was applied:

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + e_{ijk}$$

where: Y_{ijk} represents the measured characteristic, μ represents the overall mean, A_i represents the main effect of ME level, B_j represents the main effect of enzyme type, AB_{ij} represents the interaction between ME level and enzyme type, e_{ijk} represents the residual error. If the interaction term AB_{ij} was statistically significant, the main effects A_i and B_j were not considered for interpretation.

Results

The effect of experimental treatments on FI in the 3 phases, 42 to 46, 47 to 51, 52 to 56, and the entire experimental period (42 to 56) is shown in Table 2. In the first phase, the enzyme effect and in the third phase, the effect of ME on FI was significant ($P < 0.05$). Also, in the entire experimental period, a significant effect on the main effects of enzyme and ME on the FI was observed ($P < 0.05$). In the first phase and the entire experimental period, laying hens fed with enzymes separately (AVI or PHY) had more FI than the simultaneous use of two enzymes (AVI&PHY). Also, in the third phase and the entire period of the experiment, the layers fed by 100 and

95% ME consumed more feed than the level of 97.5% ($P < 0.05$).

In the first phase of the experiment, as well as the entire period of the experiment, feeding the birds with diets containing 97.5 and 95% ME increased the FCR compared to the treatment with 100% ME ($P < 0.05$) (Table 2). In the third phase, the main effect of the enzyme on the FCR was significant, so the highest FCR was recorded in the diet containing AVI&PHY and the lowest in the diet containing the AVI enzyme ($P < 0.05$).

Table 2. Effect of metabolizable energy and various enzyme supplements on the feed intake and feed conversion ratio of laying hens

		Feed intake (g/hen/day)				Feed conversion ratio (g feed: g/egg)			
		42-46wk	47-51wk	52-56wk	42-56wk	42-46wk	47-51wk	52-56wk	42-56wk
Main effects									
ME (%)									
	100	108.9	109.9	109.8^{a}	109.6^{a}	1.80^{b}	1.89	1.92	1.87^{b}
	97.5	108.1	109.9	108.8^{b}	108.9^{b}	2.08^{a}	2.10	2.01	2.06^{a}
	95	108.3	110.0	109.6^{a}	109.3^{ab}	2.01^{a}	1.91	1.94	1.95^{ab}
ENZ									
	N	108.4^{ab}	109.9	109.2	109.2^{ab}	1.98	2.03	1.90^{ab}	1.97
	AVI	109.0^{a}	109.9	109.7	109.6^{a}	1.93	1.81	1.88^{b}	1.87
	PHY	108.9^{a}	109.9	109.7	109.5^{a}	1.98	2.05	1.95^{ab}	1.99
	AVI+PHY	107.5^{b}	109.9	109.1	108.8^{b}	1.94	1.97	2.11^{a}	2.01
Interactions									
ME (%)	ENZ								
100	-	109.0	110.0	109.6	109.6	1.93	1.85	1.83	1.87
100	AVI	109.0	110.0	109.8	109.6	1.85	1.87	1.84	1.85
100	PHY	109.5	110.0	110.0	109.8	1.97	1.85	1.85	1.89
100	AVI+PHY	108.6	109.0	110.0	109.3	1.86	2.03	1.97	1.95
97.5	-	107.5	109.8	108.0	108.3	1.87	1.99	1.95	1.94
97.5	AVI	109.6	110.0	109.3	109.6	1.91	1.88	1.84	1.87
97.5	PHY	108.6	110.0	109.3	109.5	2.02	2.12	2.05	2.06
97.5	AVI+PHY	107.5	110.0	108.6	108.6	1.98	2.08	1.96	2.01
95	-	108.8	109.0	110.0	109.6	1.93	2.01	1.91	1.95
95	AVI	108.6	110.0	109.8	109.5	1.91	2.04	2.06	2.00
95	PHY	108.8	110.0	109.8	109.6	1.87	1.98	1.89	1.91
95	AVI+PHY	106.8	110.0	109.1	108.6	1.85	1.91	1.91	1.89
SEM		1.58	0.11	1.47	0.89	0.12	0.16	0.14	0.12
P-Value									
ME		0.16	0.22	0.02	0.03	0.01	0.07	0.57	0.01
ENZ		0.01	0.69	0.49	0.03	0.93	0.19	0.01	0.29
ME*ENZ		0.53	0.69	0.75	0.42	0.75	0.24	0.15	0.31

ME= Metabolizable energy, ENZ= Enzyme, N= No enzyme addition, AVI= Avizyme (1502), PHY= Phyzyme (XP).
a–c Means with no common superscript within each column are significantly ($P < 0.05$) different.

According to the results of Table 3, there was no significant difference in the EP ratio between different experimental treatments ($P > 0.05$).

In the second phase as well as the whole period of the experiment, the main effect of ME on EM was significant, so we had the highest EM in the diet with 100% ME ($P < 0.05$) (Table 3). Also, in the third phase, a significant interaction effect between ME and enzymes on EM was observed ($P < 0.05$). The highest amount of EM was observed in diets containing 100% ME + 0% enzyme (60.54), 100% ME + AVI (59.92), and 97.5% ME + AVI (59.86), which had a significant difference with diets containing 97.5% ME + PHY (54.24) and 95% ME + AVI (53.72).

The results related to the effect of the experimental treatments on the quality traits of eggs of laying hens are shown in Table 4. According to the results of Table 4, no significant difference was observed in EW, yolk index, yolk color, shell weight, and thickness ($P > 0.05$). In this study, the interaction effect of the experimental treatments on the egg shape index and the main effect of the enzymes on the Haugh unit were significant ($P < 0.05$). The lowest amount of egg shape index was observed in the diet containing 95% ME along with AVI, PHY, and AVI&PHY, which were significantly different from other treatments ($P < 0.05$). Moreover, the use of enzymes in the diet of laying hens decreased the Haugh unit compared to the control treatment ($P < 0.05$).

Table 3. Effect of metabolizable energy and various enzymes supplements on the egg production ratio and egg mass of laying hens

		Hen-day egg production (%)				Egg mass (g/hen/day)			
		42-46 wk	47-51wk	52-56wk	42-56wk	42-46 wk	47-51wk	52-56wk	42-56wk
Main effects									
ME (%)									
	100	90.8	90.4	91.1	90.8	57.4	58.2^{a}	58.9	58.2^{a}
	97.5	89.0	86.5	88.3	87.9	55.9	55.0^{b}	56.3	55.7^{b}
	95	91.9	87.7	89.6	89.7	57.4	55.7^{ab}	56.7	56.6^{ab}
ENZ									
	N	89.8	88.3	89.6	89.2	57.0	56.7	57.9	57.2
	AVI	91.9	89.5	89.8	90.4	57.8	57.3	57.8	57.6
	PHY	89.8	87.5	90.0	89.1	56.1	56.0	57.3	56.5
	AVI+PHY	90.7	87.5	89.2	89.1	56.8	55.1	56.2	56.0
Interactions									
ME (%)	ENZ								
100	-	88.9	91.4	91.6	90.7	57.0	59.8	60.5^{a}	59.1
100	AVI	93.3	91.2	92.5	92.4	58.8	59.0	59.9^{a}	59.2
100	PHY	88.3	91.8	91.3	90.5	55.5	59.4	59.4^{ab}	58.1
100	AVI+PHY	92.5	87.3	88.9	89.6	58.5	54.6	55.9^{abc}	56.3
97.5	-	91.2	87.8	88.2	89.1	57.5	55.6	55.8^{abc}	56.3
97.5	AVI	90.5	92.1	92.0	91.5	57.4	58.7	59.8^{a}	58.6
97.5	PHY	87.2	82.4	86.2	85.2	54.5	52.6	54.2^{bc}	53.8
97.5	AVI+PHY	87.1	83.6	86.6	85.8	54.3	53.1	55.4^{abc}	54.3
95	-	89.2	85.7	88.9	88.0	56.4	54.8	57.5^{abc}	56.2
95	AVI	91.8	85.2	84.9	87.3	57.1	54.4	53.7^{c}	55.1
95	PHY	94.0	88.4	92.5	91.7	58.2	56.0	58.3^{abc}	57.5
95	AVI+PHY	92.4	91.6	91.9	92.0	57.7	57.6	57.2^{abc}	57.5
SEM		4.76	6.60	5.49	4.78	3.33	4.39	4.01	3.36
P-Value									
ME		0.11	0.11	0.20	0.11	0.20	0.03	0.06	0.04
ENZ		0.53	0.78	0.97	0.82	0.50	0.44	0.54	0.47
ME*ENZ		0.16	0.06	0.06	0.08	0.29	0.07	0.04	0.13

ME= Metabolizable energy, ENZ= Enzyme, N= No enzyme addition, AVI= Avizyme (1502), PHY= Phyzyme (XP).
a–c Means with no common superscript within each column are significantly ($P < 0.05$) different.

According to the results obtained in Table 5, the experimental treatments did not cause any significant difference in blood parameters except blood uric acid concentration, so the interaction effect of ME and enzymes on uric acid content was significant ($P < 0.05$). The highest amount of uric acid in the blood serum of laying hens was observed in diets containing 97.5% ME + AVI&PHY, 95% ME + 0% enzyme, and 97.5% ME + AVI, and the lowest was in other treatments.

Discussion

The results showed that a 2.5% reduction in ME decreased FI and increased FCR of laying hens, however, a 5% reduction in ME did not affect FI or FCR compared to the control diet throughout the experiment. These findings are inconsistent with the prevailing theory that laying hens tend to increase FI when fed low-caloric density diets. Harms *et al*. (2000) found that hens which were given a low-energy diet (2,519 kcal/kg) ate 8.5% more feed than those hens which were provided with a control diet (2,798 kcal/kg). However, it should be noted that feed was not consumed freely in this study, which limits the precision of the results (Latshaw *et al*. 1990; Valkonen *et al*. 2008). Furthermore, the 2.5% reduction in ME level was found to decrease EM compared to the control diet, possibly due to the lower FI in these groups. When energy is less readily available relative to protein, hens might prioritize body maintenance over growth performance and this could lead to decreased FI and EM (Li *et al*., 2013). Previous research has suggested that modern strains of laying hens do not adjust their FI when fed lower ME diets with and without enzyme supplements, possibly due to the ME level not being low enough (Jalal *et al*. 2006). Another study found that the level of dietary ME did not significantly affect FI, EP and EM in either strain of layers. However, FCR increased in the lower ME (2805 kcal/kg) groups compared to the control groups (2890 kcal/kg) (Scheideler *et al*. 2005). Various factors can affect the results of different tests, including the age and breed of the laying hens, the composition of the diet, and its energy to protein content.

Table 4. Effect of metabolizable energy and various enzyme supplements on egg quality traits of laying hens at 56 weeks of age

		Egg Weight (g)	Egg index	Yolk color	Yolk index	Haugh unit	Shell weight (g)	Shell thickness ($mm10^{-2}$)
Main effects								
ME (%)								
	100	64.82	73.50[a]	6.59	38.14	79.09	5.97	38.88
	97.5	64.70	74.34[a]	6.66	39.30	79.43	6.04	38.47
	95	64.49	51.29[b]	6.79	39.31	81.5	6.00	37.95
ENZ								
	N	66.26	68.00	6.70	38.24	82.56[a]	6.08	38.61
	AVI	64.29	65.79	6.79	39.13	79.51[b]	6.00	38.20
	PHY	64.52	65.69	6.64	38.92	79.17[b]	5.99	38.44
	AVI+PHY	63.61	66.03	6.59	39.38	78.82[b]	5.94	38.50
Interactions								
ME (%)	ENZ							
100	-	66.75	72.92[a]	6.72	37.68	81.69	6.05	39.17
100	AVI	63.29	74.34[a]	6.67	37.58	77.48	5.84	38.72
100	PHY	64.91	72.98[a]	6.61	39.24	78.82	6.05	38.95
100	AVI+PHY	64.34	73.79[a]	6.39	38.08	78.40	5.97	38.72
97.5	-	65.86	72.84[a]	6.61	39.34	81.42	6.07	39.06
97.5	AVI	65.06	74.25[a]	6.78	39.73	79.59	6.05	38.11
97.5	PHY	63.85	75.28[a]	6.56	37.82	78.75	5.97	38.11
97.5	AVI+PHY	64.04	75.00[a]	6.72	40.33	78.00	6.07	38.61
95	-	66.17	58.27[b]	6.78	37.72	84.59	6.15	37.61
95	AVI	64.54	48.79[c]	6.95	40.10	81.47	6.12	37.78
95	PHY	64.82	48.82[c]	6.78	39.70	79.97	5.98	38.28
95	AVI+PHY	62.46	49.31[c]	6.67	39.76	80.07	5.78	38.17
SEM		3.25	3.91	0.35	2.11	4.13	0.36	1.58
P-Value								
ME		0.940	0.001	0.157	0.095	0.096	0.835	0.131
ENZ		0.101	0.250	0.352	0.412	0.032	0.685	0.885
ME*ENZ		0.854	0.002	0.779	0.208	0.970	0.698	0.925

ME= Metabolizable energy, ENZ= Enzyme, N= No enzyme addition, AVI= Avizyme (1502), PHY= Phyzyme (XP), mm= millimeters.

a–c Means with no common superscript within each column are significantly ($P < 0.05$) different.

In our study, we observed that using two enzymes simultaneously (AVI&PHY) resulted in a decrease in FI and an increase in the FCR, compared to using them separately. However, the difference with the control diet was not significant. A study by Juanpere *et al*. (2005) revealed an intriguing interaction between enzymes. When birds were fed corn-based diets, phytase and α-galactosidase exhibited an antagonism, leading to a poorer FCR. This phenomenon was not observed in wheat or barley-based diets, suggesting a potential link to the inherently lower phytase activity levels found in corn. Cowieson and Adeola (2005) offer another perspective on enzyme interactions. Their work suggests that limitations in certain nutrients can dampen the positive effects introduced by other enzymes. For example, even if phytase successfully liberates P from phytate, its overall impact on performance might be negligible if ME is restricted in the diet. In such a scenario, the bird's ability to utilize the liberated P would be hampered. In contrast to our findings, Tiwari *et al*. (2010) reported that the addition of a cocktail of xylanase, amylase, and protease (XAP) to corn-soybean meal-based diets did not improve broiler performance on its own. However, when XAP and phytase were combined, they observed an additive effect on growth performance. Similarly, Scheideler *et al*. (2005) did not find any significant difference in FI, EP, EM, and FCR between laying hens fed with a control diet and AVI 1500. Additionally, Bhanja *et al*. (2005) did not observe any beneficial effect on the performance of broiler breeders fed diets with 0.18% non-phytate phosphorus supplemented with phytase (500 FYT/kg) enzyme. In this study, the interaction effect of enzyme and ME on the measured production performance traits was not significant, except for EM at month 3, which was lower in 97.5% ME + PHY and 95% ME + AVI groups than in other treatments. Traditional thought would suggest that diets based on corn and soybeans, would not be improved by enzyme. While enzyme supplementation can improve nutrient utilization in poultry diets, its effectiveness depends on several factors. Diets low in NSPs, like a corn-soybean meal, may not benefit as much compared to those rich in NSP like rye, wheat, or

barley (Douglas *et al*., 2000; Persia *et al*., 2002). Studies by Douglas *et al*. (2000) and Jalal *et al*. (2006) support this, showing minimal performance improvements in birds fed low-energy corn-soybean meal diets with enzyme supplements. Similarly, Sohail *et al*. (2003) found no significant interaction between ME and enzyme supplementation on laying hen performance. However, some studies contradict these findings. Zanella *et al*. (1999) observed successful performance in broilers fed diets with reduced energy and AVI 1500 enzyme supplement, suggesting improved energy utilization. Cowieson and Ravindran (2008) also reported positive effects of enzyme supplementation on deficient diets. Additionally, Scheideler *et al*. (2005) observed improved EP in specific hen strains with reduced ME diets and AVI 1500 supplementation.

Table 5. Effect of metabolizable energy and various enzyme supplements on blood parameters of laying hens at 56 weeks of age

		Albumin (g/dL)	Uric acid (mg/dL)	Glucose (mg/dL)	Triglycerides (mg/dL)	Cholesterol (mg/dL)
Main effects						
ME (%)						
	100	7.30	8.25^{b}	194.55	722.49	1123.39
	97.5	6.89	9.55^{a}	218.72	721.51	1122.97
	95	7.17	9.77^{a}	200.79	715.06	1167.46
ENZ						
	N	7.04	8.91^{b}	198.01	699.65	1030.23
	AVI	6.93	8.97^{b}	206.95	728.89	1129.93
	PHY	7.30	8.61^{b}	195.28	738.56	1251.92
	AVI+PHY	7.22	10.26^{a}	218.51	711.64	1139.68
Interactions						
ME (%)	ENZ					
100	-	6.73	7.92^{d}	197.44	722.92	1026.05
100	AVI	6.44	8.19^{d}	217.13	739.42	1087.88
100	PHY	8.01	8.54^{d}	155.26	720.99	1346.90
100	AVI+PHY	8.03	8.37^{d}	208.38	706.65	1032.74
97.5	-	7.41	8.39^{d}	218.90	706.33	929.97
97.5	AVI	6.39	7.75^{d}	185.38	743.11	1075.35
97.5	PHY	7.07	8.88^{cd}	235.89	731.41	1275.88
97.5	AVI+PHY	6.73	13.21^{a}	234.73	705.21	1210.71
95	-	6.99	10.45^{bc}	177.70	669.71	1134.67
95	AVI	7.98	10.99^{b}	218.34	704.17	1226.59
95	PHY	6.84	8.44^{d}	194.69	763.30	1133.00
95	AVI+PHY	6.91	9.21^{bcd}	212.43	723.08	1175.62
SEM		1.33	1.51	75.11	66.67	388.23
P-Value						
ME		0.564	0.001	0.516	0.915	0.901
ENZ		0.836	0.009	0.789	0.308	0.407
ME*ENZ		0.101	0.001	0.652	0.592	0.825

ME= Metabolizable energy, ENZ= Enzyme, N= No enzyme addition, AVI= Avizyme (1502), PHY= Phyzyme (XP).
a–c Means with no common superscript within each column are significantly ($P < 0.05$) different.

This study highlights the potential trade-off between enzyme supplementation and egg shape index. While reducing ME and adding enzymes can be cost-effective, it may lead to a decrease in egg shape index, a crucial quality factor (Alkan and Türker, 2021). Lotfi *et al.* (2018) further emphasize the link between higher energy and protein levels in diets and improved egg shape index. This improvement is because higher energy levels can promote the development of a thicker and stronger shell, which can resist deformation during laying. It is also possible that the stress created as a result of reducing the energy of the diet caused a decrease in the egg shape index. Stress can cause the muscles in the oviduct to contract irregularly, which can deform the egg as it passes through. In this study, the Haugh unit of laying hens fed with a diet without enzymes was higher than using enzymes in the diet. According to Sohail *et al.* (2003) and Scheideler *et al.* (2005), there was no effect of AVI 1500 and ME on EW, dry shell percentage, Haugh units, EW, and yolk percentage between different treatments. Um *et al.* (1999) observed no effect on eggshell strength, egg-specific gravity, or eggshell thickness when phytase (250 FTU/kg) enzyme was added to the diet of laying hens.

Different dietary treatments had no significant effect on serum biochemical metabolites except for uric acid. According to the obtained results, the reduction of dietary ME and the simultaneous addition of enzymes increased the concentration of uric acid. Biochemical parameters in the blood may reflect the physiological state of the birds (Lin *et al.* 2000). Khondowe *et al.* (2021) reported that birds experiencing low dietary energy stress may have increased blood uric acid concentration due to protein catabolism for energy generation resulting from elevated corticosterone levels (Virden *et al.* 2007).

Conclusion

Reducing corn-soy ration energy did not affect egg production but decreased feed intake and egg mass and worsened feed conversion ratio compared to the optimized diet. Adding either, but not both, AVI and PHY enzymes improved performance. Energy reduction and enzyme addition also lowered egg shape index and increased blood uric acid. Our findings suggest that reducing corn-soy energy without precise enzyme tailoring negatively impacts laying hen performance.

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