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Research Article

Influence of Feeding Low-protein Diets Enriched with Lysine Plus Methionine, Citric Acid or their Combination on the Performance of Sinai Laying Hens

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Abstract

A 16-week study were conducted to investigate the effect of feeding low-protein diets enriched with lysine plus methionine (LM), citric acid (CA) or both on productive and reproductive performance of Sinai laying hens. Seven test diets were formulated. Diet 1 contained 15% CP and served as a control, diets 2 and 3 contained 14 and 13 % CP plus LM, diets 4 and 5 contained 14 and 13% CP but enriched with 0.2% CA while diets 6 and 7 had the same CP contents as diets 1 and 2 but fortified with CA plus LM. The birds were randomly assigned into their test diets; each treatment had three equal replications (ten ♀: one ♂). All birds were kept in naturally ventilated floor pens and had free access to fresh water and feed from 30 to 46 weeks of age and exposed to a daily photoperiod of 16 hr. Feeding the diet of 13% CP enriched with LM adversely affected egg production rate, feed conversion ratio and total egg mass but dietary supplementation with CA plus LM enhanced these traits to comparable levels as those of the control hens. Fertility and hatchability of eggs, blood parameters and most egg quality traits as well as economic efficiency of feeding were not negatively affected by dietary treatments. There was an important role for single or combined addition of LM and CA to low-protein diets for achieving optimal performance of Sinai laying hens, with no adverse effects on egg quality, reproductive performance or blood parameters.

Keywords: *Dietary Protein Inclusions; Reproductive Performance; Sinai Laying Hens*

Introduction

Proper nutrition is an important management strategy to optimize the productive performance of poultry. In fact, nutritionists are interested in using lower protein levels in feeds for laying hens as compared to the feeds normally used in the poultry industry, in order to minimize the cost of feeding [1]. Reducing dietary crude protein (CP) contents can lead to a deficiency in critical amino acids (AA) which may be met by formulating diets with normal CP levels [2]. In corn-soybean meal-layer diets methionine, lysine and threonine are generally considered the first, second and third limiting AA, respectively [3]. Thus, in practical feed formulation for poultry, there is an urgent need to use some of the synthetic commercial amino acids in order to meet the poultry requirements of limiting amino acids when they are fed on low-CP diets.

Numerous studies using low-crude protein diets supplemented with AA have been conducted on various classes of poultry. Various essential AA such as methionine, lysine, threonine and tryptophan have become economically available in recent years due to technological advances [4]. According to [5], usage of synthetic AA could reduce dietary crude protein content by10% for each phase of developing poultry. Scientific publications elucidated that promising results in terms of maintaining laying performance and maximizing profitability can be obtained by the use of low protein diets with supplemental AA [6,7]. In poultry industry, chickens can only utilize approximately 40% of the dietary protein; thus, inclusion of synthetic AA in their diets has become a common practice [8], and decreasing dietary crude protein level has many advantages such as minimizing the nitrogen excretion and reducing the production cost [9]. However, commercial synthetic AA must be included in poultry diets in order to meet their requirements of the limiting AA as dietary protein level is decreased [3].

In respect of organic acids [10] pointed out that adding organic acids to feed can lower the gastric pH. More recently, organic acids (acidifiers) have been reviewed to improve the digestibility of nutrients and increase the absorption of minerals [11]. They also stated that inclusion of organic acids into diets of pigs and poultry also leads to thinning of the intestinal lining which facilitates better absorption of nutrients and its efficient utilization. In this context [12], reported that citric acid (CA) as an organic acid can reduce the pH of the digesta which can result in increased dissociation between phytic acid and minerals. In addition, low gastric pH accelerates the conversion of pepsinogen to pepsin, which can improve the digestibility of proteins and the absorbability of AA and minerals [13]. Citric acid has been reported as a growth promoter, an acidifier, a bacterial inhibitor, an antioxidant and an antitoxin [14,15]. However, some studies have been indicated that dietary CA supplementation had no a beneficial effect of the productive performance of laying hens [16-18].

Therefore, the current study was carried out to determine the effect of feeding low-protein diets enriched with lysine plus methionine (LM), citric acid (CA) or their combination on laying performance, reproductive performance, egg quality, serum biochemical parameters and economic efficiency of Sinai laying hens from 30 to 46 weeks of age.

Materials and Methods

Housing and management of sinai laying hens

This study was conducted at El-Serw Poultry Research Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. The experiment lasted from October 2018 to January, 2019. Sinai chickens is a native breed in Egypt which is developed for meat and egg production. A total of 210 Sinai pullets and 21 cockerels of the same breed at 30 weeks of age were housed on deep litter in floor pens (ten ♀: one ♂ per pen) and kept in an open-sided building. The dimensions of each floor pen were 200 cm length and 150 cm width. The birds were randomly assigned into seven dietary treatments, each with three equal replications. At the onset of the experiment, birds were weighed and assigned to treatments based on approximately similar live body weights in all treatments. The laying house used herein was naturally ventilated and all birds were exposed toa daily photoperiod of 16 hr. Sinai chickens were provided their test diets and fresh water *ad libitum* throughout the duration of this study, from 30 to 46 weeks of age.

Test diets for Sinai laying hens

In an early study [19], estimated that the metabolizable energy (ME) and crude protein (CP) requirements of Sinai chickens were 2800 kcal ME per kg diet and 15% CP during the laying period in order to attain optimal performance. Such estimated requirements of ME and CP were used as a guide when calculating the nutrient contents of the test diets. Seven iso-energetic test diets were prepared and used. These diets were as follows: A basal diet was formulated to contain 15% CP to serve as a control diet while diets 2 and 3 contained 14 and 13% CP, respectively, but fortified with lysine and methionine (LM) to have the same LM contents as those of the control diet. Diets

4 and 5 were compounded to contain 14 and 13% CP, respectively, but enriched with 0.2% CA while diets 6 and 7 contained 14 and 13% CP, respectively, but fortified with CA and lysine plus methionine (LM). Ingredient composition and calculated analyses of the test diets are given in table 1 [20].

Ingredients (%)	Control	2	3	4	5	6	7
Yellow corn, ground	65.00	67.00	68.50	66.60	68.45	66.70	68.30
Soybean meal (44% CP)	18.80	15.20	13.00	16.70	13.50	15.10	13.00
Corn gluten meal (60% CP)	1.00	1.08	0.50	0.50	0.50	1.18	0.50
Soybean oil	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Wheat bran	3.44	4.80	5.99	4.24	5.59	4.90	5.99
Limestone, ground	8.16	8.16	8.16	8.16	8.16	8.16	8.16
Dicalcium phosphate	1.40	1.40	1.40	1.40	1.40	1.40	1.40
Vit. & Min. Premix[1]	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Common salt (NaCl)	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DL- Methionine (99%)	0.10	0.12	0.13	0.10	0.10	0.12	0.13
L-Lysine.HCl	...	0.14	0.22	....	....	0.14	0.22
Citric acid	...	...	...	0.2	0.2	0.2	0.2
Total	100	100	100	100	100	100	100
Calculated Analysis (As Fed Basis; NRC, 1994)							
Crude protein (%)	15.02	14.01	13.07	14.04	13.00	14.02	13.06
ME (kcal/kg)	2827	2840	2839	2826	2834	2833	2832
Crude fiber (%)	3.14	3.08	3.08	3.11	3.07	3.08	3.08
Ether extract (%)	4.25	4.34	4.40	4.30	4.39	4.33	4.39
Calcium (%)	3.47	3.46	3.46	3.46	3.46	3.46	3.46
Non-phytate P (%)	0.37	0.37	0.36	0.37	0.36	0.37	0.36
Methionine (%)	0.35	0.36	0.35	0.34	0.33	0.36	0.35
Lysine (%)	0.71	0.73	0.74	0.65	0.58	0.73	0.74
Meth. + Cyst. (%)	0.62	0.61	0.59	0.59	0.56	0.61	0.59
Feed cost/kg (EGP)[2]	4.63	4.51	4.40	4.65	4.50	4.69	4.58

Table 1: *Composition and nutrient contents of the experimental diets fed to Sinai laying hens from 30 to 46 weeks of age.*

[1]Each 3 kg of the premix contains: 100 million IU Vit A; 2 million IU Vit.D_3; 10g Vit. E; 1.0g Vit. K_3; 1.0g Vit B_1; 5g Vit B_2; 10 mg Vit.B_{12}; 1.5g Vit B_6; 30 g Niacin; 10g Pantothenic acid; 1g Folic acid; 50 mg Biotin; 300g Choline chloride; 50g Zinc; 4.0g Copper; 0.3g Iodine; 30g Iron; 0.1g Selenium; 60g Manganese; 0.1g Cobalt and a carrier ($CaCO_3$) to 3.0 kg. [2]EGP: Egyptian pounds.

Laying performance and egg quality of sinai laying hens

Body weights of hens in each replicate group were recorded at the beginning and at the end of the study, to assess body weight change during the experimental period. Egg production rate (Hen-day EPR; %), eggs produced/hen, average egg weight (AEW), total egg mass (TEM), daily feed intake (DFI) and feed conversion ratio (FCR; g feed: g egg) were recorded during whole experimental period. At 38 weeks of age, 18 eggs per treatment (6/replicate) were freshly collected at random to measure egg quality parameters.

Reproductive performance of sinai laying hens

The reproductive performance of Sinai laying hens were estimated as egg fertility and fertile hatchability. At 40 and 41 weeks of age, all eggs produced by hens of different dietary treatments were collected for four successive days. The eggs were set in forced air incubator. Egg fertility was estimated on the 18^{th} day of incubation by dividing the number of fertile eggs by the total eggs set into the incubator. Hatchability was calculated as total number of hatched chicks as a percentage of number of fertile eggs. It was calculated also a percentage of total eggs set into the incubator. All hatched chicks were immediately weighed to the nearest 0.1 gram.

Biochemical variables of blood serum

At 46 weeks of age, 3 blood samples were collected in non-heparinized test tubes from the wing veins of hens in each treatment. The blood samples were immediately centrifuged at 3500 rpm for 15 minutes, to separate the sera for biochemical analysis. The serum concentrations of total protein, albumin, creatinine, Ca and P and activity of transaminases (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) were determined by the available commercial kits.

Economic efficiency of feeding (EEF)

At the end of the study, economical efficiency for egg production during the whole experimental period (30 - 46 weeks of age) was calculated using the following equation: EEF (%) = 100 (Net return/Total feed cost). Total feed cost (TFC) was computed as total feed intake (TFI) multiplied by cost per kg feed. Net return was estimated as total return (sale price of total eggs produced) minus TFC.

Statistical analysis

Data were statistically analyzed using General Linear Model Procedures of the [21]. Differences between treatments were subjected to Duncan´s Multiple Range test [22]. The following model was used to study the effect of treatments on the estimated parameters as follows: $Y_{ij} = \mu + T_i + e_{ij}$

where: Y_{ij} = An observation, μ = Overall mean, T_i = Effect of treatment (i = 1 to 7) and e_{ij} = Random error.

Results and Discussion

Body weights of sinai laying hens

As shown in table 2, final body weight (FBW) at the end of study (46 weeks of age) of hens fed the control diet (15% CP) was significantly higher ($P \leq 0.05$) than those of hens received the diet containing 13% CP supplemented with 0.2% CA or 14% CP supplemented with lysine plus methionine (LM) and CA. However, FBW of hens fed the test diets containing 14 or 13% CP plus LM, 14% CP enriched with CA or 13% CP fortified with CA and LM were not significantly ($P > 0.05$) different from that of the control group. On the other hand, hens fed the diet containing 13% CP supplemented with CA had significantly lower ($P \leq 0.05$) mean of change in body weight (CBW) than that of the control ones while other dietary treatments did not differ from that of the control group.

The present results are in consistency with the findings obtained by [4], who reported that hens fed the diets contained high CP levels attained the best value of BWC. In addition, Hy-line layers fed 14% CP-diet achieved the highest value of body weight compared with those fed 13% CP-diet during egg production [23]. On the other hand, the current results seem to slightly contradict with those obtained by [24], who reported that FBW and CBW of commercial laying hens were not significantly affected by different dietary levels of CP (14, 16, 18 and 20%). Similarly [25], found no significant differences in live body weight gain of hens in response to feeding different levels of dietary protein.

Laying performance of sinai laying hens

The response of productive performance of Sinai laying hens to feeding low-protein diets enriched with lysine plus methionine (LM), citric acid (CA) or both during the whole experimental period (30 - 46 weeks of age) is shown in table 2. The results obtained revealed that hens fed the diets having 14 and 13% CP with supplemental LM or CA consumed significantly more feed ($P \leq 0.05$) than did the control group, but means of daily feed intake (DFI) of hens received 14 and 13% CP-diets enriched with LM plus CA were not significantly ($P > 0.05$) different from that of the control birds. It was observed that egg production rate (EPR, %) of hens fed the diet containing 13% CP plus LM was significantly lower ($P \leq 0.05$) than that of the control hens. While hens fed the diets containing 14% CP supplemented with LM or CA or fed 13% CP-diets enriched with CA alone or combined with LM achieved comparable means of EPR to that of the control ones. In addition, EPR of hens fed the diet containing 14% CP fortified with a combination of CA and LM was comparable to that of the control group but slightly higher than those of hens fed the diets having 13% CP enriched with CA alone or combined with LM. As for total egg mass (TEM) the hens fed the diets having 13% CP fortified with LM or CA exhibited significantly lower ($P \leq 0.05$) means of TEM than did the control ones but were not significantly ($P > 0.05$) different from those of hens fed the diets having 14% CP enriched with LM or CA, or the diet having 13% CP fortified with a combination of CA and LM. In addition, hens fed the diet having 14% CP enriched with both CA and LM achieved slightly higher TEM than did the control ones and those 14% CP enriched with CA alone. However, dietary treatments had no significant effect ($P > 0.05$) on average egg weight (AEW) during the whole experimental period. On the other hand, hens fed the diets containing 13, 14% CP plus LM or CA, or 13% CP enriched with a combination of CA and LM exhibited significantly inferior ($P \leq 0.05$) means of feed conversion ratio (FCR) to that of the control hens while FCR of hens fed the 14%-CP diet fortified with a combination of CA and LM was identical to that of the control group.

The present results showed that decreasing dietary protein level from 15 to 13% CP with maintaining the same levels of lysine and methionine could not sustain optimal productive performance of Sinai laying hens. By the same manner, reducing dietary crude protein content to 13% with supplementation of 0.2% CA resulted in an adverse effect on total egg mass. This result is in agreement with that of [26], who elucidated that reducing dietary CP level would reduce egg production. The reduction in productive performance due to reducing dietary protein concentration may be explained as follows: [27] pointed out that even with supplementation of essential amino acids (AA) too much reduction of protein may lead to a situation where part of the essential AA is diverted to the synthesis of non-essential AA due to a lack of non-specific nitrogen for this process, thus reducing body protein deposition and animal performance. Also, although both lysine and methionine were included into the diet containing 13% CP at the same level as in control diet, in this study, but such AA could not be fully metabolized for maximum protein utilization by the laying hens because other AA might become critical [28]. Also, the observed insignificant increase in the activity of serum AST of hens fed the 13% CP-diet without any supplement (Table 5) may be an indicator to an adverse effect on the liver function. On the other hand, feeding Sinai hens maintained on the diet containing 14% CP fortified with a combination of CA and LM could compensate for this slight reduction in their protein requirements and achieved productive performance comparable to that of the control hens. In the present study, inclusion of 0.2% CA into low-protein diets with LM was somewhat effective for restoring an optimal performance of Sinai laying hens without the need for further supplementation. In this regard, CA can stimulate feed intake by maintaining feed freshness and thus the palatability of the feed [29]. However, some studies illustrated that there is no positive effect of CA on the productive performance of laying hens [16-18].

Logically, feed intake (FI) of Sinai laying hens, determined herein, increased with reducing dietary protein level but average egg weight (AEW) was not affected by dietary treatments. These results harmonize with those of [25], who reported that laying hens fed low-protein diets enriched with AA recorded an increase in their FI than did the control ones, and they attributed this increase in FI to an amino acid "appetite" that occurred when their test diets contained marginal levels of essential AA. Similarly [4], detected no differences in egg weight of White Leghorn layers when they were fed low- protein diets supplemented with amino acids. In the same context [30], reported that CA adding to laying hen diets produced no positive influence on laying performance and FCR. In addition, dietary organic acid addition to hen's diet had no impact on egg performance, FI and FCR [31]. With the exception of feeding the diet having 14% CP enriched with both CA and LM, other dietary treatments, applied herein, caused a significant depression in FCR of hens compared with the control group. The reason for such a depression might be due to the fact that the increase in DFI was not parallel to the reduction in EPR of Sinai laying hens during the whole experimental period (Table 2).

Criteria	T1 15% CP Control	T2 14% (LM)	T3 13% (LM)	T4 14% (CA)	T5 13% (CA)	T6 14% LM+CA))	T7 13% (LM+CA)	SEM	Sig.
IBW, g	1392	1405	1387	1392	1395	1387	1395	3.06	NS
FBW, g	1708^{a}	1672^{ab}	1653^{ab}	1670^{ab}	1610^{b}	1628^{b}	1637^{ab}	9.83	*
CBW, g	316.7^{a}	266.7^{ab}	266.7^{ab}	278.3^{ab}	215.0^{b}	241.7^{ab}	241.7^{ab}	6.01	*
TEP/hen	76.07^{ab}	70.1^{bc}	66.17^{c}	69.53^{bc}	70.43^{abc}	77.47^{a}	70.47^{abc}	4.9	*
EPR, %	67.9^{ab}	62.6^{bc}	59.1^{c}	62.1^{bc}	62.9^{abc}	69.2^{a}	62.9^{abc}	0.96	*
AEW, g	48.74	49.21	49.78	49.94	48.56	48.33	48.82	0.24	NS
TEM, g	3707^{ab}	3443^{bc}	3293^{c}	3475^{abc}	3415^{c}	3745^{a}	3431^{bc}	43.4	*
DFI, g	106.2^{b}	108.3^{a}	107.8^{a}	108.5^{a}	108.1^{a}	107.6^{ab}	107.2^{ab}	0.21	*
FCR, g:g	3.21^{b}	3.52^{ab}	3.67^{a}	3.50^{ab}	3.55^{ab}	3.22^{b}	3.50^{ab}	0.06	*

Table 2: *Effect of feeding low-protein diets supplemented with lysine plus methionine (LM), CA or both on productive performance of Sinai laying hens from 30 to 46 weeks of age.*

$^{a-c}$: Means in the same row bearing different superscripts are significantly different (P ≤ 0.05).

IBW: Initial Body Weight; FBW: Final Body Weight; CBW: Change in Body Weight; TEP: Total Eggs Produced; EPR: Egg Production Rate; AEW: Average Egg Weight; TEM: Total Egg Mass; DFI: Daily Feed Intake; FCR: Feed Conversion Ratio and SEM: Standard Error of the Means, respectively.

On the other hand [6], elucidated that FI and FCR of Baheijlaying hens were not affected by dietary CP levels.

Egg quality of sinai laying hens

Data presented in table 3 showed that egg yolk index (EYI), Haugh units (HU), per cent yolk weight (YW) and per cent albumen weight (AW) were not significantly affected (P > 0.05) by dietary treatments. It was noted that egg weight (EW) of hens fed the diet containing 13% CP enriched with CA was significantly lower (P ≤ 0.05) than that of their control counterparts while egg weights of other experimen-

Traits	T1 15% CP Control	T2 14% (LM)	T3 13% (LM)	T4 14% (CA)	T5 13% (CA)	T6 14% LM+CA))	T7 13% (LM+CA)	SEM	Sig.
EW, g	53.63[a]	52.83[a]	49.80[ab]	50.70[ab]	46.33[b]	50.08[ab]	48.88[ab]	0.69	*
ESI, %	80.9[ab]	79.6[ab]	77.4[b]	77.9[ab]	80.5[ab]	81.0[ab]	82.5[a]	1.0	*
EST, mm	0.32[ab]	0.31[b]	0.33[ab]	0.31[b]	0.34[ab]	0.35[a]	0.34[ab]	0.01	*
EYI, %	20	22	21	21	21	22	21	0.02	NS
ESW, %	11.47[ab]	11.52[ab]	11.86[ab]	11.44[ab]	10.95[b]	11.50[ab]	12.22[a]	0.14	*
YW, %	31.08	30.77	31.94	33.49	35.79	33.46	34.61	0.67	NS
AW, %	57.45	57.72	56.20	55.07	53.26	55.04	53.17	0.70	NS
HU	74.08	73.63	77.81	75.96	71.61	73.33	71.67	1.07	NS
ESCa, %	33.5[c]	34.0[c]	38.0[ab]	38.0[ab]	36.0[bc]	40.0[a]	33.5[c]	0.67	*
ESP, %	1.05[b]	1.17[b]	1.25[b]	1.15[b]	1.20[b]	1.50[a]	1.05[b]	0.04	*

Table 3: *Effect of feeding low-protein diets supplemented with lysine plus methionine (LM), CA or both on egg quality traits of Sinai laying hens.*

[a-c]: Means in the same row bearing different superscripts are significantly different (P ≤ 0.05).

EW: Egg Weight; ESI: Egg Shape Index; EST: Egg Shell Thickness; EYI: Egg Yolk Index; ESW: Egg Shell Weight; YW: Yolk Weight; AW: Albumen Weight; HU: Haught Units; ESCa: Egg Shell Calcium; ESP: Egg Shell Phosphorus and SEM: Standard Error of the Means; respectively.

tal groups were insignificantly different from that of the control group (Table 3). Hens fed the diet containing 13% CP supplemented with LM plus CA achieved significantly higher (P ≤ 0.05) egg shape index (ESI) than did those fed the 13% CP-diet enriched with LM but other experimental groups did not differ from the control group in their ESI (Table 3). Egg shell thickness (EST) of hens fed 14% CP-diet fortified with LM plus CA was significantly higher (P ≤ 0.05) than those of hens 14% CP-diets fortified with LM or CA but EST of other dietary treatments did not differ from that of the control hens (Table 3). The per cent egg shell weight (ESW) of hens fed 13% CP-diet enriched with LM plus CA was significantly higher (P ≤ 0.05) than that of hens given 13% CP-diet plus CA alone but ESW of other dietary treatments were comparable to that of the control hens (Table 3). On the other hand, means of per cent egg shell calcium (ESCa) of hens received 13% CP-diets enriched with LM or CA, and 14% CP-diet fortified with LM plus CA were significantly higher (P ≤ 0.05) than that of the control group, but those of other experimental groups were comparable to that of the control hens. Similarly, per cent egg shell phosphorus (ESP) of hens received 14% CP-diet fortified with LM plus CA was significantly higher (P ≤ 0.05) than those of other dietary treatments.

In the present study, insignificant impact of added dietary lysine and methionine (LM) on Haugh unit and the percentages of yolk and albumen concurs with the findings reported by [32,33]. It is interesting to note that the common feature of egg quality traits, measured herein, is the lack of significant differences among the different experimental groups of hens. The absence of significant differences in EST and ESW among hens fed the low-protein diets enriched with LM and CA singly or in combination could be explained by the fact that eggshell synthesis requires only a small amount of protein or amino acids. In this respect [25], observed no significant differences in egg quality parameters of hens fed diets containing 16 - 17.5% CP-diets supplemented with lysine, methionine and threonine. In the same context [29], detected no significant effect on HU due to adding 0.2% CA in laying hens' diet, this result agrees with the present results. Organic acids have been reported to beneficially affect soft-shell + broken egg production of laying hens but had no influence on laying rate and eggshell strength or shell thickness [34].

Reproductive performance of sinai laying hens

As shown in table 4, dietary treatments did not significantly (P > 0.05) affect egg fertility (EF, %), fertile hatchability (FH, %) or total hatchability (TH, %) of eggs. It was interesting to note that means of TH% of eggs produced by hens fed the diets containing 13 or 14% CP supplemented with CA or LM plus CA were numerically higher (P > 0.05) than that of the control group. Similarly, eggs produced by hens fed the diets containing 13 or 14% CP enriched with LM, CA or LM plus CA exhibited slightly higher (P > 0.05) means of FH% than did their control counterparts. Concerning chick weight at hatch (CWH), hens fed the 14% CP diet enriched with CA produced significantly heavier chicks (P ≤ 0.05) than those of hens fed the 13% CP diet fortified with LM plus CA.

Criteria	T1 15% CP Control	T2 14% (LM)	T3 13% (LM)	T4 14% (CA)	T5 13% (CA)	T6 14% LM+CA))	T7 13% (LM+CA)	SEM	Sig.
EF, %	96.41	95.38	93.85	96.92	97.44	95.38	96.92	0.52	NS
TH, %	86.15	87.18	86.67	90.26	91.79	88.72	89.74	0.93	NS
FH, %	89.31	91.41	92.27	93.11	94.19	92.94	92.58	2.82	NS
CWH, g	35.73^{ab}	35.85^{ab}	35.28^{ab}	36.49^{a}	35.41^{ab}	35.61^{ab}	34.63^{b}	0.19	*

Table 4: *Effect of feeding low-protein diets supplemented with lysine plus methionine (LM), CA or both on reproductive performance of Sinai laying hens.*

$^{a-b}$: Means in the same row bearing different superscripts are significantly different (P ≤ 0.05).

EF: Egg Fertility; TH: Total Hatchability; FH: Fertile Hatchability; CWH: Chick Weight at Hatch and SEM: Standard Error of the Means, respectively.

Such beneficial effect on the reproductive performance of Sinai laying hens, observed herein, might be related to more balanced amino acid-supplemented diets or to CA supplementation. According to [35], dietary protein plays a major role in growth and reproductive performance of poultry. In addition [30], has been proposed that supplemental CA in laying hen diets could improve Ca availability by chelating Ca and reducing the formation of insoluble Ca-phytate-complexes. On the other hand, dietary supplementation with CA to laying and breeder hens can help the maintenance of the antioxidant system of the developing embryo. It is generally accepted that the hatching process is an oxidative stress and improvement in antioxidant defenses of the embryo can increase hatchability. In this regard, CA has been reported as a growth promoter, an acidifier, a bacterial inhibitor and an antioxidant [14,36].

Serum biochemical parameters of sinai laying hens

Results obtained on serum biochemical parameters of Sinai laying hens in response to feeding low-protein diets supplemented with LM, CA or their mixture are shown in table 5. Statistical analysis of the data obtained did not reveal any significant (P > 0.05) alternations among dietary treatments as for serum biochemical parameters. The previous parameters are vital indices of nutritional and physiological status of the bird. The lack of significant effect of dietary treatments on serum biochemical parameters of Sinai laying hens, detected herein, is in the line with the findings of [6], who found that blood plasma albumin concentration was not influenced by feeding diets of different CP levels. Also [37], observed no significant effects of feeding different levels of dietary protein on blood plasma concentrations of globulin and creatinine but levels of total protein and albumin of hens fed 20 and 18% CP-diets were greater than those fed on 16% CP-diet. On the contrary [24], reported that serum fractions of protein tended to increase but albumin to globulin ratio tented to decrease with increasing CP level from 14 to 18% for laying hens. Also [38], found that serum Ca and P levels were not affected due to organic acid

Parameters	T1 15% CP Control	T2 14% (LM)	T3 13% (LM)	T4 14% (CA)	T5 13% (CA)	T6 14% LM+CA))	T7 13% (LM+CA)	SEM	Sig.
TPR (mg/dl)	6.25	6.70	6.85	6.15	4.63	6.70	5.75	0.18	NS
ALB (mg/dl)	3.04	2.96	3.09	3.28	2.91	3.49	2.85	0.08	NS
GLO (mg/dl)	3.21	3.74	3.76	2.87	1.73	3.21	2.90	0.23	NS
A/G	0.95	0.82	0.83	1.17	1.84	1.11	0.99	0.10	NS
CRE (mg/dl)	8.33	7.00	6.00	5.67	9.67	7.70	11.37	0.54	NS
ALT (U/L)	2.65	3.00	4.05	3.00	3.15	4.00	4.80	0.26	NS
AST (U/L)	37.00	31.80	41.57	35.87	31.70	32.90	35.47	0.89	NS
Ca (mg/dl)	15.10	13.57	11.47	12.60	10.17	12.47	9.40	0.43	NS
P (mg/dl)	6.77	7.17	7.00	7.17	5.80	7.03	5.20	0.17	NS

Table 5: *Effect of feeding low-protein diets supplemented with lysine plus methionine (LM), CA or both on serum biochemical parameters of Sinai laying hens.*

NS: Non-Significant; TPR: Total Protein; ALB: Albumin; GLO: Globulin; A/G: Albumin Globulin Ratio; CRE: Creatinine; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase and SEM: Standard Error of the Means.

supplementation to layer diets. In agreement with the present results [39], reported that concentrations of total protein, albumin and globulin in blood plasma of broiler chicks were not affected by supplemental CA. But [40], found a decrease in blood plasma protein of broiler chickens due to feeding diets supplemented with different CA concentrations (0.0%, 0.2%, 0.4%, 0.6%, and 0.8%). This contradiction in response to citric acid supplementation could be attributed to the level of added CA, chemical composition of the test diet and hygienic conditions [15]. The observed slight decrease in the activity of AST in our study due to dietary supplements singly or in combination for Sinai laying hens may refer to an improvement in their liver function. Also, outcomes in the present study are in agreement with those reported by [41], who reported that CA did not negatively affect the activity ALT in broiler chickens. On the other hand [42], reported that chicks fed a diet supplemented with organic acids displayed significantly higher blood serum concentrations of Ca and P. They attributed this response to lowering the intestinal pH and the increase in the absorption of these macro elements by the utilization of these organic acids.

Economic efficiency of feeding (EEF)

Results concerning the economic efficiency of egg production as a result of feeding the Sinai laying hens on low-protein diets enriched with LM, CA or LM+CA are shown in table 6. It could be noted that all dietary treatments did not significantly ($P > 0.05$) differ from the control diet in respect of EEF of Sinai laying hens except for hens fed the diet containing 14% CP supplemented with 0.2% CA which achieved significantly inferior EEF to that of the control group.

Conclusion

These results imply an important role for single or combined addition of LM and CA to restore optimal performance of Sinai laying hens fed low-protein diets, with no adverse effects on egg quality, egg fertility, hatchability or blood parameters.

Items	T1 15% CP Control	T2 14% (LM)	T3 13% (LM)	T4 14% (CA)	T5 13% (CA)	T6 14% (LM+CA)	T7 13% (LM+CA)	SEM	Sig.
TFI/hen (kg)	12.95	12.72	12.88	12.75	12.63	12.63	12.72	0.08	NS
Cost/kg feed (EGP)	4.63^{b}	4.51^{b}	4.40^{c}	4.65^{a}	4.50^{b}	4.69^{a}	4.58^{b}	0.26	*
TFC, EGP	55.11^{b}	54.80^{b}	53.06^{c}	56.50^{a}	54.46^{b}	56.63^{a}	54.96^{b}	0.26	*
TEP/hen	76.07^{ab}	70.1^{bc}	66.17^{c}	69.53^{bc}	70.43^{abc}	77.47^{a}	70.47^{abc}	4.9	*
SPE, EGP	1.10	1.10	1.10	1.10	1.10	1.10	1.10	.....	.....
TR, EGP	83.67^{ab}	77.1^{bc}	72.78^{c}	76.49^{bc}	77.48^{abc}	85.21^{a}	77.51^{abc}	1.18	*
NR, EGP	28.56^{a}	22.31^{abc}	19.72^{c}	19.99^{c}	23.01^{abc}	28.58^{a}	22.55^{abc}	1.11	*
EEF, %	51.58^{a}	40.73^{ab}	37.17^{ab}	35.44^{b}	42.30^{ab}	50.47^{ab}	41.03^{ab}	1.96	*

Table 6: *Effect of feeding low-protein diets supplemented with lysine plus methionine (LM), CA or both on economic efficiency of feeding (EEF) of Sinai laying hens.*

$^{a-b}$: Means in the same row bearing different superscripts are significantly different ($P \leq 0.05$).

TFI: Total Feed Intake; TFC: Total Feed Cost; TEP: Total Eggs Produced; SPE: Sale Price of Egg; TR: Total Return; NR= Net Return; EE.

Ethics Approval

This study was undertaken with the approval of Mansoura University Ethics Committee.

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Competing Interests

None.

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