Pak. J. Agri. Sci., Vol. 57(6), 1653-1659; 2020
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/20.963
http://www.pakjas.com.pk

EFFECT OF DIFFERENT LEVELS OF ENERGY AND LYSINE ON THE PRODUCTION PERFORMANCE OF LAYING HENS

Muhammad Sharif[1,#], Awais Ali[1,2,#], Urooj Anwar[1], Farhan Ayaz Chisti[1], Saad Jameel[1], Muhammad Qamar Bilal[1], Mubasher Hussain[1], Fawwad Ahmad[1], Muhammad Yousaf[1], Abdul Naveed[3], ASM Golam Kibria[4], Hafiz Muhammad Ishaq[5] and Muhammad Aziz ur Rahman[1*]

[1]Institute of Animal and Dairy Sciences, University of Agriculture, Faisalabad, Punjab, Pakistan; [2]Livestock and Dairy Development Department Lahore, Punjab, 54000, Pakistan; [3]Office of Research Innovation and Commercialization, University of Agriculture, Faisalabad Punjab, Pakistan; [4]Dept. of Anatomy and Histology, Chittagong Veterinary and Animal Sciences University, Bangladesh. [5]Faculty of Veterinary and Animal Sciences, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan
#First and second author contributed equally to this manuscript
***Corresponding Author's e-mail: drazizurrahman@uaf.edu.pk**

The objective of the study was to evaluate the effect of different levels of energy and lysine on production performance of laying hens. For this purpose, 448 laying hens of body weight 1560±60 grams were used in 2×2 factorial arrangements with 14 replicates per treatment and 8 birds per replicate. The treatments were: standard energy (2800 kcal/kg) with standard lysine (0.67%), standard energy (2800 kcal/kg) with low lysine (0.65%), low energy (2750 kcal/kg) with standard lysine (0.67%) and low energy (2750 kcal/kg) with low lysine (0.65%). Feed intake, egg production, yolk height, albumin height, yolk diameter, egg specific gravity, egg breaking strength, eggshell thickness and blood metabolites were measured. Results showed that feed intake in first week was maximum ($P<0.05$) for diet with standard energy and low lysine while in last week feed intake was maximum ($P<0.05$) in low energy and low lysine diet. Results also showed that egg production, egg weight, egg quality parameters and blood metabolites were not affected ($P>0.05$) by dietary treatments. Nutrient digestibility was highest ($P<0.05$) for diet having low energy and standard lysine. It can be concluded that decreasing energy and lysine in late laying hens affects ($P<0.05$) feed intake and nutrient digestibility but had no effect ($P>0.05$) on egg production, egg weight, egg quality parameters and blood metabolites.
Keywords: Laying hens, energy, lysine, blood metabolites, egg quality parameters.

INTRODUCTION

The productivity of poultry industry has been enhanced in recent decades due to the consistent improvement in genetics (Richell, 1997; Younas *et al*., 2019). In correspondence to these improvements, nutritional requirements must be frequently redefined according to local economic, environmental and managemental conditions (Lima *et al.*, 2018; Abdullah *et al.*, 2019; Mehmood *et al*., 2019; Khater *et al*., 2020). However, developing countries are frequently following the recommendation of National Research Council (1995) irrespective to economic and climatic conditions (Kang *et al.*, 2018; Mehmood *et al*., 2020). The optimum level of energy and amino acid (AA) balance are two most valuable nutrients which should be defined in variable climatic conditions to optimise performance of laying hens (LH) (Onimisi *et al*., 2012; Elsayed *et al*., 2019).
Previous findings reported that changing metabolizable energy (ME) levels had variable effects on egg production (EP), feed efficiency and feed intake (FI) (Junqueira *et al.,* 2006). Kang *et al.* (2018) reported that EP was not effected by changing ME level in diet while FI was reduced. Similarly, Li *et al.* (2013) reported that increase in an ME levels (2700-2850 kcal/kg) had no effect on EP of the LH. Likewise, Perez-Bonilla*et al.* (2012) also reported that an increase in ME level from 2850 kcal/kg to 2950 kcal/kg did not change the EP. However, Perez-Bonilla *et al.* (2012) reported that increasing the ME level from 2650 kcal/kg to 2850 kcal/kg increased the EP in LH. Similarly, dePersio *et al.* (2015) also reported that increasing an ME from 2711 to 3017 kcal/kg increased the EP of LH. Variable results of energy on EP is encouraging researchers to redefine the ME requirements of LH.
Similar to ME levels in the diet of LH that influenced EP, lysine level also influence the EP and requirement of lysine varies due to managemental and nutritional changes (Novak *et al.,* 2004; Raheel *et al*., 2019). In a recent study, Spangler *et al*. (2019) reported that EP increased as the digestible lysine enhanced from 0.517 to 0.748 % in the diet. Cupertino*et al.* (2009) reported 0.724% digestible lysine requirement for Lohman Silver from 54 to 70 weeks. Rao *et al.* (2011) concluded that increasing the lysine level from 0.65 to 0.80% did not affect EP of LH. Onimisi *et al*. (2012) reported that

decreasing lysine level from 0.7% to 0.6% had no effect on the EP of LH. Burley *et al.* (2013) observed that reduced AA profile in diet of LH would be economical to a specific level without effecting laying performance of egg producing layers.

Based on previous studies, variable results of LH have been observed by different ME and lysine levels (Perez-Bonilla, *et al.*, 2012; Ding *et al.*, 2016; Kakhki *et al.*, 2016; Kang *et al.*, 2018; Spangler *et al.*, 2019; Sattar *et al*., 2019). However, none of researcher studied the effect of energy, lysine and their interactions collectively in LH. Therefore, a study wasplanned to check the effect of ME, lysine and their interactions on production performance of LH.

Table 1. Ingredient and nutrient composition (g/kg) of diets containing varying concentrations of metabolizable energy and lysine.

Ingredients %	[1]SESL	[2]LESL	[3]SELL	[4]LELL
Corn 14%	67.43	64.90	68.04	63.96
[5]SBM 46	16.38	16.58	14.86	15.19
Rapeseed Meal	3.00	3.00	3.00	3.00
Poultry by product Meal	1.41	1.00	2.64	2.96
Molasses	0.00	2.49	0.00	3.55
Poultry oil	0.28	0.00	0.00	
MCP	0.61	0.61	0.61	0.60
Calcium carbonate	9.03	8.96	9.03	8.94
Sodium Chloride	0.24	0.22	0.23	0.21
Sodium Bicarbonate	0.09	0.09	0.09	0.09
Lysine Sulphate	0.03	0.02	0.02	0.02
DL Methionine	0.14	0.14	0.11	0.11
[6]Vitamin+ Mineral Premix	0.87	0.87	0.87	0.87
Phytase Premix	0.50	0.50	0.50	0.50
Nutrient specifications of diets				
Metabolizable energy (Kcal/kg)	2800.00	2750.00	2800.00	2750.00
Crude Protein (%)	18.80	18.80	17.40	17.40
Calcium (%)	3.90	3.90	3.90	3.90
Phosphorus (%)	0.58	0.58	0.58	0.58
Avail. Phosphorous (%)	0.37	0.37	0.37	0.37
Sodium (%)	0.17	0.17	0.17	0.17
Chlorine (%)	0.17	0.17	0.17	0.17
Dig. Lysine (%)	0.67	0.67	0.65	0.65
Dig. Methionine (%)	0.33	0.33	0.32	0.32
Dig Methionine+Cysteine (%)	0.61	0.61	0.59	0.59
Dig. Arginine (%)	0.85	0.85	0.82	0.82
Dig. Valine (%)	0.58	0.58	0.56	0.56
Dig. Tryptophan (%)	0.14	0.14	0.14	0.14
Dig. Threonine (%)	0.46	0.46	0.45	0.45
Dig. Isoleucine (%)	0.53	0.53	0.51	0.51
Linoleic Acid (%)	2.00	2.00	2.00	2.00

[1]Standard energy standard lysine, [2]low energy standard lysine, [3]standard energy low lysine, [4]low energy low lysine, [5]soyabean meal, [6]Supplied per kg feed: 8,000 IU vitamin A, 1,800 IU vitamin D3, 12 mg vitamin E, 2 mg vitamin K3, 1 mg vitamin B1, 4 mg vitamin B2, 1 mg vitamin B6, 10 mcg vitamin B12, 0.40 mg folic acid, 0.04 mg biotin, 28 mg niacin, 11 mg calcium pantothenate, 6 mg Cu, 0.10 mg Co, 1 mg I, 50 mg Fe, 65 mg Mn, 45 mg Zn, 0.21 mg Se, 500 mg choline chloride 50%, 60 mg Coxistac® 12% , 12 mg antioxidant

MATERIALS AND METHODS

The experimental trial wasexecuted at Paroka Research Center, University of Agriculture, Faisalabad.

***Housing and management of birds*:** A total number of 448 white commercial Crystal NickLH at 64 weeks of age with average body weight of 1560± 60 grams were used in the current experiment. These birds were arranged in 2x2 factorial arrangement. A total of four treatments were used in such a way that each treatment had 14 replicates and each replicate had 8 birds. All experimental birds were reared in cages and brids welfare conditions were optimized as desribed in the study of Muhammad *et al.* (2019), the dimension of each cage for one replicate was 2 feet length, 2.5 width, 2 feet height (1.25 ft^3/bird). Four experimental diets were formulated; 1) standard energy with standard lysine (SESL) 2) standard energy with low lysine (SELL) 3) low energy with standard lysine (LESL) 4) low energy with low lysine (LELL). Experimental diets were formulated on the basis of digestible AA as described in recent studies (Hussain *et al.*, 2018; Hussain *et al.*, 2020).Birds were fed 105 grams of experimental feed per bird in 16 hours of light daily at 6 am. Relative humidity and temperatureof shedwere maintained at 55-65% and 20-25ºC, respectively. Water was offered ad libitum. Trial was lasted in 56 days.

***Data recording*:** Egg production was noted daily at 8:00 am. Hen's day egg production (HDEP) was calculated on weekly basis and was corrected for mortality. Weekly FI was recorded by subtracting the amount of ort from feed offer in whole week and corrected for mortality (Li *et al*., 2013). Hens mortality was recorded daily. Egg weight (EW) was noted per cage having two replicates of same treatment and average EW was recorded by dividing with total eggs of these replicates on daily basis using an electric weighing balance. Digestibility trial was conducted to estimate the digestibility of nutrients by indirect marker method using acid insoluble ash as defined by researchers (Muhammad *et al.*, 2016; Niu *et al.*, 2017; Xia *et al.*, 2018a). Nutrient composition of diet was dteremined by using standard methods as described in recent studies (Zhang *et al.*, 2015; Wang *et al.*, 2016; Xia *et al.*, 2018b; Xia *et al.*, 2018c; Demir *et al.*, 2019; Raza *et al.*, 2019; Rehman *et al.*, 2019). One egg per replicate was collected after one month and at end of the trial for measurement of egg shell thickness (EST), EW, specific gravity (SG), eggshell breaking strength (EBS), haugh units (HU), albumin height (AH), yolk height (YH), yolk diameter (YD) and yolk Index (YI). Eggshell thickness was measured by EST meter (model Osk13469, Peacock). Differential salts concentration in water was used to measure SG. Yolk and AH were measured by egg meter. Egg testing equipment (model Osk 13473, Ogawa Seiki Co., Ltd. Japan) was used to measure EBS and expressed as kg/cm^2.Haugh units were measured by recording AH and by incorporating AH and EW in the following formula (Eisen *et al.*, 1962);

$$H.U = 100 * \text{Log}H + 7.57 - 1.7\ W^{0.37}$$

H = AH (mm); W = EW in grams; Yolk index was measured by following formula; Yolk Index = YH / YD

***Serum biochemical parameters*:** Blood serology was conducted on day 30th and 56th of the trial. For that purpose, 3.5 ml approximate blood was collected from brachial vein. Samples were taken from one bird per replicate to avoid stress to the birds and were kept in Bolton gel & clot tube. After centrifugation, blood cells and serum were separated. Serum was collected in eppendorf tubes. Blood urea, blood glucose and blood cholesterol were calculated by BioMed-Urea, BioMed-Glucose L.S and BioMed-Cholesterol-LS kits respectively.

***Statistical investigation*:** Data were analysed by ANOVA using GLM procedure of minitab and mean values were compared using Tukey's test. Effects were declared significant at probability less than 0.05.

RESULTS

***Feed intake and nutrient digestibility*:** The effect of energy, lysine and their interaction on FI is shown in Table 2. Feed intake was improved (P<0.05) by energy and lysine interactions during the first week. Feed intake was more when lysine level was reduced keeping same energy level and there was no effect of changing lysine level at lower level of energy i.e. (2750 kcal/kg). Feed intake was not affected during week 1st, 2nd, 3rd, 4th, 5th and 6th, however, in week 7 FI was maximum in LELL diet (P<0.05).

Table 2. Effect of different levels of energy and lysine on weekly feed intake, crude protein and ether extract digestibility of laying hens.

	Energy			[8]P Value	Lysine			P Value	Energy*Lysine					P Value
	[1]SE	[2]LE	[3]SEM		[4]SL	[5]LL	SEM		SESL	SELL	LESL	LELL	SEM	
Weekly feed intake														
Week 1	93.0	94.0	1.24	0.59	91.03^{b}	96.04^{a}	1.24	0.02	89.32^{b}	96.79^{a}	92.74^{ab}	95.28^{ab}	1.75	0.03
Week 2	104.6	105	0.18	0.105	104.54	104.99	0.18	0.09	104.1	105	104.97	104.9	0.28	0.09
Week 3	104.3	104.67	0.249	0.28	104.40	104.55	0.25	0.68	104.5	104.09	104.33	105.00	0.352	0.14
Week 4	104.4	104.8	0.20	0.16	104.4	104.7	0.196	0.21	104.26	104.44	104.49	105.00	0.277	0.56
Week 5	104.5	104.71	0.194	0.42	104.61	104.58	0.194	0.91	104.81	104.17	104.42	105.00	0.275	0.03
Week 6	96.85	100.67	1.37	0.05	98.23	99.29	1.37	0.59	97.62	96.09	98.85	102.50	1.94	0.19
Week 7	97.88^{b}	101.75^{a}	0.973	0.006	99.54	100.13	0.973	0.67	98.82^{ab}	96.94^{b}	100.26^{ab}	103.32^{a}	1.38	0.08
Crude protein and ether extract digestibility														
[6]CP %	56.11	62.89	0.14	0.10	54.49	62.51	0.14	2.75	48.5^{b}	63.71^{ab}	64.48^{a}	61.3^{ab}	3.72	0.03
[7]EE %	90.03	90.33	0.11	0.85	88.91	91.45	0.11	1.04	89.09	90.97	88.72	91.93	1.83	0.66

[1]Standard energy, [2]Low energy, [3]Standard error of the mean of 14 replicates, [4]Standard lysine, [5] Low lysine, [6]Crude protein, [7]Ether extract
[8]a, b Means with different superscripts within a row differ significantly (P < 0.05)

Table 3. Effect of different level of energy and lysine level on egg production and egg weight of laying hen.

	Energy			[6]P Value	Lysine			P Value	Energy*Lysine					P Value
	[1]SE	[2]LE	[3]SEM		[4]SL	[5]LL	SEM		SESL	SELL	LESL	LELL	SEM	
Egg production														
Week 1	77.87	78.00	1.53	0.96	77.87	78.00	1.53	0.95	78.57	77.18	77.17	81.83	2.16	0.48
Week 2	81.20	82.65	2.10	0.67	82.26	81.59	2.10	0.82	81.48	80.91	83.04	82.26	2.97	0.97
Week 3	85.11	85.07	1.81	0.99	85.92	84.24	1.81	0.52	87.06	83.16	84.77	85.37	2.56	0.38
Week 4	84.69	88.12	1.76	0.17	86.76	86.05	1.76	0.77	86.55	82.82	86.97	89.27	2.49	0.23
Week 5	86.59	86.94	1.50	0.87	87.87	85.66	1.50	0.30	89.13	84.06	86.61	87.26	2.12	0.18
Week 6	83.83	82.88	1.75	0.70	84.35	82.35	1.75	0.42	86.53	81.12	82.17	83.58	2.47	0.17
Week 7	84.54	86.84	1.63	0.32	86.79	84.59	1.63	0.35	87.21	81.87	86.37	87.32	2.31	0.18
Egg weight														
Week 1	62.34	62.24	0.19	0.71	62.03	62.54	0.19	0.08	61.99	62.69	62.08	62.40	0.28	0.51
Week 2	61.57	61.09	0.19	0.09	61.40	61.27	0.19	0.65	61.41	61.74	61.39	60.80	0.27	0.10
Week 3	60.55	59.60	0.35	0.06	59.98	60.17	0.35	0.71	60.62	60.48	59.35	59.86	0.49	0.52
Week 4	61.05	60.74	0.22	0.34	61.12	60.67	0.22	0.16	61.28	60.82	60.96	60.52	0.31	0.99
Week 5	60.75	60.56	0.21	0.56	60.95	60.36	0.21	0.06	60.97	60.53	60.94	60.19	0.30	0.62
Week 6	61.02	61.06	0.16	0.87	61.35	60.73	0.16	0.01	60.97	61.07	61.72	60.39	0.23	0.00
Week 7	60.05	60.30	0.18	0.34	60.31	60.04	0.18	0.32	59.98	60.12	60.64	59.97	0.26	0.13

[1]Standard energy, [2]Low energy, [3]Standard error of the mean of 14 replicates, [4]Standard lysine, [5] Low lysine, [6]Means with no superscript within row are statistically non-significant (P>0.05)

Table 4. Effect of energy and lysine on egg quality parameters at day 30^{th} and 56^{th}

Parameters	Energy			[6]P	Lysine			P Value	Energy * Lysine					P
	[1]SE	[2]LE	[3]SEM	Value	[4]SL	[5]LL	SEM		SESL	LESL	LELL	SELL	SEM	Value
Day 30														
Specific gravity	1.088	1.090	0.001	0.20	1.090	1.088	0.001	0.35	1.090	1.090	1.097	1.085	0.002	0.20
Egg break strength	3.790	3.790	0.370	1.00	3.970	3.610	0.370	0.50	4.340	3.600	3.990	3.240	0.250	0.17
Eggshell thickness	0.386	0.393	0.008	0.52	0.395	0.384	0.008	0.39	0.391	0.399	0.387	0.380	0.011	1.00
Albumin height	6.390	6.380	0.290	0.98	6.310	6.460	0.290	0.69	6.340	6.290	6.500	6.430	0.380	0.87
Yolk height	17.17	17.22	0.240	0.89	17.21	17.18	0.240	0.92	17.17	17.25	17.18	17.17	0.340	0.91
Yolk diameter	42.56	42.53	0.380	0.95	42.02	43.07	0.380	0.06	41.86	42.19	42.87	43.27	0.530	0.50
Yolk index	0.400	0.410	0.000	0.85	0.410	0.390	0.000	0.13	0.410	0.410	0.400	0.400	0.010	0.72
Haugh Units	61.96	61.95	2.360	0.99	60.50	63.41	2.360	0.39	60.84	60.16	63.75	63.07	3.330	0.84
Day 56														
Specific gravity	1.090	1.088	0.74	0.51	1.090	1.089	0.74	0.002	1.091	1.088	1.089	1.089	0.002	0.510
Egg break strength	3.870	3.320	0.83	0.13	3.560	3.640	0.83	0.250	3.700	3.410	3.230	4.040	0.350	0.460
Eggshell thickness	0.400	0.390	0.18	0.18	0.400	0.390	0.18	0.010	0.410	0.390	0.380	0.390	0.010	1.000
Albumin height	6.540	6.530	0.27	0.98	6.690	6.390	0.27	0.430	6.570	6.816	6.260	6.520	0.380	0.515
Yolk height	17.27	16.96	0.37	0.40	17.29	16.95	0.37	0.250	17.38	17.19	16.74	17.19	0.360	0.730
Yolk diameter	41.26	42.09	0.32	0.24	42.03	41.42	0.32	0.430	41.18	42.88	41.30	41.54	0.610	0.120
Yolk index	0.420	0.400	0.86	0.17	0.410	0.410	0.86	0.010	0.420	0.400	0.410	0.410	0.010	0.590
Haugh Units	60.86	60.37	2.38	0.89	61.06	60.17	2.38	0.790	60.51	61.60	60.12	60.23	3.370	0.860

[1]Standard energy, [2]Low energy, [3]Standard error of the mean of 14 replicates, [4]Standard lysine, [5]Low lysine, [6]Means with no superscript within row are statistically non-significant (P>0.05).

Table 5. Effect of energy and lysine on blood metabolites at day 30^{th} and 56^{th}

Blood	Energy			[6]P	Lysine			P	Energy*Lysine					P
Metabolites	[1]SE	[2]LE	[3]SEM	Value	[4]SL	[5]LL	SEM	Value	SESL	SELL	LESL	LELL	SEM	Value
Blood metabolites at day 30^{th} of trial														
Glucose	138.0	146.6	12.3	0.63	135.8	148.8	12.3	0.472	131.9	144.2	139.7	153.4	17.35	0.970
Urea	3.928	5.594	0.91	0.22	4.748	4.775	0.91	0.980	4.200	3.660	5.300	5.890	1.280	0.667
Cholesterol	159.9	153.9	22.9	0.85	152.5	161.3	22.9	0.790	172.4	147.5	132.7	175.1	32.25	0.323
Blood metabolites at day 56^{th} of trial														
Glucose	135.8	129.1	4.94	0.360	127.9	136.9	4.94	0.227	127.6	144.0	128.3	129.9	6.97	0.314
Urea	4.150	4.304	0.53	0.845	4.129	4.329	0.53	0.795	4.225	4.083	4.033	4.575	0.748	0.658
Cholesterol	151.7	148.0	6.07	0.680	150.0	149.7	6.07	0.971	153.1	150.3	147.0	149.1	8.66	0.782

[1]Standard energy, [2]Low energy, [3]Standard error of the mean of 14 replicates, [4]Standard lysine, [5]Low lysine, [6]Means with no superscript within row are statistically non-significant (P>0.05).

Nutrient digestibility analysis is shown in Table 2. Results revealed that fat digestibility was not affected (P>0.05) by energy and lysine levels. Moreover, there was no interaction among energy and lysine for fat digestibility. Results showed that energy and lysine interaction effects protein digestibility (P<0.05). Protein digestibility was highest for LESL diet (P<0.05).

***Egg production and egg weight*:** The result of energy, lysine and their interaction on EP is shown in Table 3. Results revealed that EP was not affected by ME in week 1 and similarly no effect was seen in week 2, 3, 4, 5, 6 and 7. Egg production from the hens fed with diet containing different level of lysine was not affected throughout the experiment (P>0.05). There was no interaction among lysine and energy levels (P>0.05).

The result of energy, lysine and their interaction on EW is shown in Table 3. Results showed that changing ME level from 2800 to 2750 kcal/kg had no effect on EW (P>0.05). Similarly, varying lysine level from 0.67% to 0.65% had no effect (P>0.05) on EW. Interaction between energy and lysine levels was not significant (P>0.05) for EW throughout experiment.

***Egg quality parameters*:** Egg quality (EQ) parameters were not affected by varying ME levels (P>0.05). Similarly, lysine levels had no effect on EQ parameters (P>0.05). There was no effect of interaction among energy and lysine on EQ parameters on day 30^{th} and 60^{th} of the trial (P>0.05) as shown in Table 4.

***Serum biochemical parameters*:** Serum biochemicalanalysis is shown in Table 5. Results presented that blood glucose, cholesterol and urea were not affected by energy, lysine levels and their interaction on day 30^{th} and 56^{th} of trial (P>0.05).

DISCUSSION

The present research was performed to check the effect of energy and lysine on the production performance of the LH. In current study, HDEP, EW, EQ parameters and blood metabolites were not affected by ME, lysine levels and interaction among them. Protein digestibility was highest for LESL diet. Harms *et al.* (2000) reported that there was no statistical difference in EP when different energy density diets (2,519 (low), 2,798 (control) and 3,078 (high) kcal/kg) were used. Similar results were reported by Wu *et al.* (2007) that EP was not affected by decreasing ME level (3002 to 2447 kcal/kg) in eight commercial leghorn strains. Result of current study was in contrast to that of Mathlouthi *et al*. (2002) who reported that decreasing level of ME from 2753 to 2653 kcal/kg in brown LH resulted in decrease of EP. Similarly, Rao *et al.* (2013) reported that decreasing ME from 2800 to 2300 kcal/kg decreased the EP in white leghorn in tropical condition. The contrasting results of current study were may be due to low difference of ME (50 kcal/kg) in diet which made the effect non-significant. However, greater difference of ME (100 kcal/kg) in the experiment of Rao *et al.* (2013) and Mothlouthi *et al.* (2002) reflected that decreasing higher energy level in the diet of LH from recommended level decreased the energy available to the birds which led to lower EP (Enting *et al.*, 2007).

Lysine level did not affect the EP in present study. Novak *et al.* (2004) reported similar findings that EP was not affected by lysine in Dekalb Delta LH when lysine was fed from 860 to 959 mg. Prochaska *et al*. (1996) reported similar results that feeding lysine (677 to 1613 mg) in diet of Hy-Line W-36 had no effect on EP. Conflicting results were reported by Spangler *et al.* (2019) that decreasing lysine level from 0.979% to 0.565% of diet had decreasing effect on EP in 14, 18, 22 and 24 weeks in LSL breed of LH. The contrasting results of current study were due to low difference of lysine in dietary treatment as compared to results of Spangler *et al.* (2019), they used lysine levels from 0.565 to 0.979 with a lysine increase of 0.069 in 7 graded levels, which improved the EP. Feed intake results of current studies showed significant effect of energy and lysine interaction in first and last week of the trial. Feed intake was highest for diet when lysine level was below standard lysine at same level of energy. In the last week of current study, FI was highest in the LELL diet clearly indicating that birds eat more to fulfill their energy and lysine requirements. Gunawardana *et al.* (2008) reported similar findings, they concluded that FI decreased from 96.9 g to 94.9 g per day which was 2.1% as ME increased from 0-238 kcal/kg. Similarly, Kang *et al.* (2018) revealed that increased level of ME from 2850 to 3050 decreased the FI linearly. Bouyeh and Gevorgian (2011) reported similar results that decreasing lysine level increase FI of LH. Result of nutrient digestibility showed that digestibility of protein was highest in diet having standard lysine and low energy. High digestibility of protein in current study was probably due to low available energy to bird, so they utilized protein as energy source to maintain their production. The results of blood metabolites showed that lysine, energy levels and their interaction had no statistical effect on blood metabolites. Similar results have been described by Kakhki *et al.* (2016) that triglyceride and cholesterol were not affected by lysine. Very little research has been done on the relationship of nutrients with blood metabolites which can be explored further to have better understanding of nutrient requirement and their utilization in body of LH. Furthermore, digestive microflora data should be analyzed in future research as focused on recent studies (Qiu *et al.*, 2019a; Qiu *et al.*, 2019b; Qiu *et al.*, 2020).

***Conclusion*:** Based on findings of current study it is concluded that decreasing energy (2800 to 2750 kcal/kg) and lysine (0.67 to 0.65%) from standard requirement in LH had no effect on HDEP, EW, EQ parameters, BW, blood metabolites (urea, glucose, cholesterol) but significantly affect FI and nutrient digestibility. Therefore, it is suggested that low energy and lysine levels could be used in LH during late production stage. However, further research is required to explore with different levels of energy and lysine in different environmental conditions.

***Acknowledgment*:** Authors acknowledge the funding of Asia Poultry Feeds (Pvt.) Ltd. for the completion of research project. We also acknowledge the support of Office of Research Innovation and Commercialization, University of Agriculture, Faisalabad.

***Author's contribution*:** Muhammad Shareef and Aziz ur Rahman Planned the research project. Hafiz Muhammad Ishaq finalized the research plan. Awais Ali conducted the research trial. Muhammad Yousuf guided and helped in lab instrument running and data compiling. Urooj Anwar, Farhan Ayaz and Saad Jameel conducted lab analysis. Fawad Ahmed performed statistical analysis. Muhammad Qamar Bilal revised the manuscript. Mubashir Hussain finalized the manuscript. Abdul Naveed revised final draft.

REFERENCES

Abdullah, H.M., L.R. Bielke and Y.A. Helmy. 2019. Effect of arginine supplementation on growth performance and immunity of broilers: A Review. J. Glob. Inno. Agri. Soci. Sci. 7:141-144.

Burley, H.K., P.H. Patterson and M.A. Elliot. 2013. Effect of a reduced crude protein, amino acid-balanced diet on hen performance, production costs and ammonia emissions in a commercial laying hen flock. J. Appl. Poult. Res. 22:217-228.

Cupertino, E.S., P.C. Gomes, L.F.T. Albino, J.L. Donzele, H.H.D. Mello, M. Schmidt and A.A. Calderano. 2009. Digestible lysine requirements of laying hens from 54 to 70 weeks of age. Rev. Bras. Zootecn. 38:480-487.

Demir B, M. Sahin, A.G. Akcacik, S. Aydogan, S. Hamzaoglu and M.K. Demir. 2019.Effect of quinoa

(*Chenepodium Quinoa* Wild) flour addition on the nutritional and chemical properties of bread. J. Glob. Inno. Agri. Soci. Sci. 7:171-175.

Ding, Y., X.C. Bu, N.N. Zhang, L.L. Li and X.T. Zou. 2016. Effects of metabolizable energy and crude protein levels on laying performance, egg quality and serum biochemical indices of Fengda-1 layers. Anim. Nutr. 2:93-98.

Eisen, E.J., B. Bohren and H.E. Mckean. 1962. The Haugh Unit as a measure of egg albumen quality. Poult. Sci. 41:1361-1368.

Elsayed, M., F. El-Gohary, A. Zakaria and M. Gwida. 2019. Tracing of salmonella contaminations throughout an integrated broiler production chain in Dakahlia Governorate Egypt. Pak. Vet. J. 39:558-562.

Grobas, S., J. Mendez, C. De Blas and G.G. Mateos. 1999. Laying hen productivity as affected by energy, supplemental fat and linoleic acid concentration of the diet. Poult. Sci. 78:1542-1551.

Harms, R.H., G.B. Russell and D.R. Sloan. 2000. Performance of four strains of commercial layers with major changes in dietary energy. J. Appl. Poult. Res. 9:535-541.

Hussain, M., A. Mahmud, J. Hussain, S. Qaisrani, S. Mehmood and A. Rehman. 2018. Subsequent effect of dietary lysine regimens fed in the starter phase on the growth performance, carcass traits and meat chemical composition of aseel chicken in the grower phase. Braz. J. Poult. Sci. 20:455-462.

Hussain, M., A. Mahmud, J. Hussain, S.N. Qaisrani, S. Mehmood, S. Ahmad and A.U. Rehman. 2020. Effect of dietary amino acid regimens on growth performance and bodyconformation and immune responses in Aseel chicken. Indian J. Anim. Res. 54: 53-58.

Junqueira, O.M., A.C. de-Laurentiz, R. da-Silva-Filardi, E.A. Rodrigues and E.M. Casartelli. 2006. Effects of energy and protein levels on egg quality and performance of laying hens at early second production cycle. J. Appl. Poult. Res. 15:110-115.

Kakhki, R.A.M., A. Golian and H. Zarghi. 2016. Effect of dietary digestible lysine concentration on performance, egg quality and blood metabolites in laying hens. J. Appl. Poult. Res. 25:506-517.

Kang, H.K., S.B. Park, J.J. Jeon, H.S. Kim, K.T. Park, S.H. Kim, E.C. Hong and C.H. Kim. 2018. Effect of increasing levels of apparent metabolizable energy on laying hens in barn system. Asian-Australas J. Anim. Sci. 31:1766-1772.

Khater, H.F., H. Ziam, A. Abbas, R.Z. Abbas, M.A. Raza, K. Hussain, E.Z. Younis, T. Radwan and A. Selim. 2020. Avian coccidiosis: Recent advances in alternative control strategies and vaccine development. Agrobiol. Records 1:11-25.

Li, F., L.M. Zhang, X.H. Wu, C.Y. Li, X.J. Yang, Y. Dong, A. Lemme, J.C. Han and J.H. Yao. 2013. Effects of metabolizable energy and balanced protein on egg production, quality and components of Lohmann Brown laying hens. J. Appl. Poult. Res. 22:36-46.

Lima, M.B., N.K. Sakomura, E.P. Silva, J.C.P. Dorigam, N.T. Ferreira, E.B. Malheiros and J.B.K. Fernandes. 2018. The optimal digestible valine, isoleucine and tryptophan intakes of broiler breeder hens for rate of lay. Anim. Feed Sci. Tech. 238:29-38.

Mathlouthi, N., M. Larbier, M.A. Mohamed and M. Lessire. 2002. Performance of laying hens fed wheat, wheat-barley or wheat-barley-wheat bran based diets supplemented with xylanase. Can. J. Anim. Sci. 82:193-199.

Mehmood, A., M.H. Rasool, A.A. Anjum, M.A. Zahoor and M. Shafique. 2019. Pathotyping and genetic characterization of a highly virulent Newcastle disease virus strain isolated from recent outbreak in District Okara, Pakistan. Pak. Vet. J. 39:353-358.

Mehmood, K., R.M. Bilal and H. Zhang. 2020. Study on the genotypic and phenotypic resistance of tetracycline antibiotic in *Escherichia coli* strains isolated from free ranging chickens of Anhui Province, China. Agrobiol. Records 2:63-68.

Muhammad, A.U.R., C.Q. Xia and B.H.Cao. 2016. Dietary forage concentration and particle size affect sorting, feeding behaviour, intake and growth of Chinese Holstein male calves. J. Anim. Physiol. Anim. Nutr. 100:217-223.

Muhammad A.U.R., C. Xia, L. Ji, B. Cao and H. Su. 2019. Nutrient intake, feeding patterns, and abnormal behavior of growing bulls fed different concentrate levels and a single fiber source (corn stover silage). J. Vet. Behav. 33:46-53.

Niu, W., Y. He, C.Xia, M.A.U. Rahman,Q.Qiu, T.Shao,Y. Liang, L. Ji, H. Wang and B.H. Cao.2017. Effects of replacing Leymus chinensis with whole-crop wheat hay on Holstein bull apparent digestibility, plasma parameters, rumen fermentation and microbiota. Sci. Rep. 7:1-12.

Novak, C., H. Yakout and S. Scheideler. 2004. The combined effects of dietary lysine and total sulfur amino acid level on egg production parameters and egg components in Dekalb Delta laying hens. Poult. Sci. 83:977-984.

Onimisi, P.A., M. Orunmuyi, A.A. Musa, S. Bale and G.S. Bawa. 2012. Effects of feed lysine content on laying performance. Int. J. Appl. Res. Tech. 1:104-110.

Peguri, A. and C. Coon. 1991. Effect of temperature and dietary energy on layer performance. Poult. Sci. 70:126-138.

Perez-Bonilla, A., M. Frikha, S. Mirzaie, J. Garcia and G.G. Mateos. 2011. Effects of the main cereal and type of fat of the diet on productive performance and egg quality of brown-egg laying hens from 22 to 54 weeks of age. Poult. Sci. 90:2801-2810.

Perez-Bonilla, A., S. Novoa, J. Garcia, M. Mohiti-Asli, M. Frikha and G.G. Mateos. 2012. Effects of energy concentration of the diet on productive performance and egg quality of brown egg-laying hens differing in initial body weight. Poult. Sci. 91:3156-3166.

Prochaska, J.F., J.B. Carey and D.J. Shaffer. 1996. The effect of L-lysine intake on egg component yield and composition in laying hens. Poult. Sci. 75:1268-1277.

Qiu, Q., C. Gao, M.A.U Rahman, B. Cao and H. Su. 2020. Digestive ability, physiological characteristics, and rumen bacterial community of Holstein finishing steers in response to three nutrient density diets as fattening phases advanced. Microorganisms 8: 335.

Qiu, Q., C. Gao, Z. Gao, M.A. Rahman, Y. He, B. Cao and H. Su. 2019a. Temporal dynamics in rumen bacterial community composition of finishing steers during an adaptation period of three months. Microorganisms 7: 410.

Qiu, Q., Y. Zhu, X. Qiu, C. Gao, J. Wang, H. Wang, Y. He, M.A. Rahman, B. Cao and H. Su. 2019b. Dynamic variations in fecal bacterial community and fermentation profile of Holstein steers in response to three stepwise density diets. Animals 9: 560.

Raheel, I.A.R., A. Orabi and A. El Masry. 2019. Natural herbs CLEANACTIV®; immune-modulator, health activator and growth promoter in broiler chickens. Int. J. Vet. Sci. 8:267-270.

Rao, S.V.R., V. Ravindran, T. Srilatha, A.K. Panda and M.V.L.N. Raju. 2011. Effect of dietary concentrations of energy, crude protein, lysine and methionine on the performance of White Leghorn layers in the tropics. J. Appl. Poult. Res. 20:528-541.

Rehman, A., H. Hesheng, M. Rahman, X. Jingyi, L. Shuang, G. Yannan and S.H. Qamar. 2019. Evaluation of efficacy of compound chinese medicinal herbs against Mycoplasma synoviae using different Lab tests in mouse and chicken. Int. J. Agric. Biol. 22:647-654.

Richell, W.A. 1997. Breeding and genetics – a historical perspective. Poult. Sci. 76:1057-1061.

Rao, S.V.R., M.V.L.N. Raju, V. Ravindran and A.K. Panda. 2013. Influence of different concentrations of metabolisable energy at constant ratio to dietary protein, lysine, methionine, calcium and phosphorus on the performance of White Leghorn layers in the tropics. Anim. Prod. Sci. 53:523-530.

Raza H., F. Zaaboul, M. Shoaib, W. Ashraf, A. Hussain and L. Zhang. 2019. Physicochemical and structural characterization of microwave-roasted chickpea. J. Glob. Inno. Agri. Soci. Sci. 7:23-28.

Sattar, A.W.M., K.M. Sadek, A.R. Elbestawy, D.M. Mourad and H.S. El-Samahy. 2019. Immunological, histopathological and biochemical protective effect of date pits (*Phoenix dacrylifera* seeds) feed additive against aflatoxicated broiler chickens. Int. J. Vet. Sci. 8:198-205.

Spangler, H., P. Utterback, C.M. Parsons and P. Tillman. 2019. Determining the digestible lysine requirement of 22- to 47-week-old Lohmann laying hens using an increasing protein titration methodology. Poult. Sci. 98:1706-1715.

Wang, C., A. Muhammad, Z. Liu, B. Huang and B. Cao. 2016. Effects of ensiling time on banana pseudo-stem silage chemical composition, fermentation and in sacco rumen degradation. J. Anim. Plant Sci. 26: 339-346.

Wu, G., M.M. Bryant, P. Gunawardana and D.A. Roland. 2007. Effect of nutrient density on performance, egg components, egg solids, egg quality and profits in eight commercial Leghorn strains during phase one. Poult. Sci. 86:691-697.

Xia, C.Q., A.U.R. Muhammad, W. Niu, T. Shao, Q. Qiu, S. Huawei and B.H. Cao. 2018a. Effects of dietary forage to concentrate ratio and wildrye length on nutrient intake, digestibility, plasma metabolites, ruminal fermentation and fecal microflora of male Chinese Holstein calves. J. Integr. Agri. 17:415-427.

Xia, C., M.A.U. Rahman, H. Yang, T. Shao, Q. Qiu, H. Su and B. Cao. 2018b. Effect of increased dietary crude protein levels on production performance, nitrogen utilisation, blood metabolites and ruminal fermentation of Holstein bulls. Asian-Australas. J. Anim. Sci. 10: 1643-1653.

Xia, C., Y. Liang, S. Bai, Y. He, A.U.R. Muhammad, H. Su and B. Cao. 2018c. Effects of harvest time and added molasses on nutritional content, ensiling characteristics and in vitro degradation of whole crop wheat. Asian-Australas. J. Anim. Sci. 31: 354-362.

Younas, M., S. Rahman, S. Shams, M.M. Salman and I. Khan. 2019. Multidrug resistant carbapenemase-producing Escherichia coli from chicken meat reveals diversity and co-existence of carbapenemase encoding genes. Pak. Vet. J. 39:241-245.

Zhang, X., M.A.U. Rahman, Z. Xue, X. Wang, Y. He and B. Cao. 2015. Effect of post-pubertal castration of Wannan Cattle on daily weight gain, body condition scoring and level of blood hormone. Int. J. Agric. Biol. 17:334-338.

[Received 12 Aug. 2020; Accepted 22 Sept. 2020 Published (Online) 25 Oct. 2020]