





# Influence of Low and High-density Diets with Yeast Supplementation on Feed Intake, Nutrient Digestibility, Egg Production and Egg Quality in Hy-line Brown Laying Hens

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## ■ Keywords

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## ABSTRACT

A total of 432 laying hens (40 weeks old) were used in a 10-weeks feeding trial. The birds were randomly assigned to 1 of 4 treatments with 18 replications (6 birds per replication) in a 2×2 factorial arrangement with low-density diet (LD), and high density (HD) diets supplemented with or without (0.1 %) of yeast. Laying hens feed intake during the 5<sup>th</sup>, 6<sup>th</sup> and 10<sup>th</sup> weeks has significantly increased ( $p < 0.05$ ) in LD diet and HD diet supplemented with (0.1 %) of yeast supplementation. However, egg production and broken rate was not affected with or without yeast and density diet. The quality of egg and shell color during the 5<sup>th</sup>, 6<sup>th</sup>, and 7<sup>th</sup> weeks has significantly reduced ( $p < 0.05$ ) by yeast supplementation with HD and LD diet. Eggshell strength was significantly improved at week 4, moreover albumin height was also significantly improved by yeast supplementation diets at week 5. During week 1 and 2 the yolk color was higher in LD diet and HD diet compared with yeast supplementation. However, eggshell strength was significantly improved on HD diets than LD diets during the 4<sup>th</sup> and 7<sup>th</sup> weeks. Eggshell color and albumen height were significantly decreased in laying hens fed HD or LD diets during week 5 and 6. The apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen (N), and Energy was not affected ( $p < 0.05$ ) by laying hens fed with or without yeast and HD than LD diets. In summary, 0.1% of yeast supplementation proved a positive impact on feed intake and egg quality of layer chicken.

## INTRODUCTION

Nutritional supplements are known as substances that have been applied in animal meals to enhance the quality of feed and quantity of animal products. Different scientific researches were performed to determine the efficacy and mechanism of yeast action. Several yeast products have been developed and used as growth promoters in livestock feed in the past decades. Yeasts with high levels of enzymes, vitamins, and other nutrients have been shown to improve egg production (Yalcin *et al.*, 2008; Tapingkae *et al.*, 2017). In poultry, yeast products have been found to be more effective than other probiotics in improving the characteristics or traits of birds Resinger *et al.*, 2012; Yasar & Desen 2014; Yasar & Yegen (2017). Moreover, yeast has a beneficial impact on the hematology of the blood, resulting in improved animal welfare (Agazzi *et al.*, 2011). Previous studies have favorable results on broiler's dietary supplementation with *S. cerevisiae* (Wallace, 1994; Newbold *et al.*, 1995; Gao, 2008). *S. cerevisiae* makes beneficial changes in the gut microbial population and maintains its natural microbial flora by stimulating the growth and proliferation of beneficial bacteria (Kumar *et al.*, 2019).



Earlier study Abdulrahman, (2013) stated that feeding broiler chicks with 0.1 % *S. Cerevisiae*, had reduced the strength of aflatoxins and increased the body weight. However, *S. Cerevisiae* has decreased the risk causing factors and increased the biochemical importance of nitrogen compounds in the animal digestive tract (Stanley *et al.*, 1993; Ozsoy *et al.*, 2018). Moreover, (Obeidat *et al.*, 2018) reported that the addition of yeast with high and low fiber diets in animals and birds could test the hypothesis that the level of the fiber in the diet can affect the efficacy of yeast performance. However, few studies have determined the effect of dietary supplementation of brewer's yeast on brown laying hens (Yousefi & Karkoodi, 2007). In contrast, some experiments indicate that there was no effect of yeast on dietary intake, feed efficiency, egg yield, and egg size in laying hens (Nursoy *et al.*, 2004; Sacakli *et al.*, 2013; Yalcin *et al.*, 2014). The findings of the above experiment showed a few controversies regarding the effect of adding yeast to laying hen's diets. Therefore, the current research has been conducted to determine the impact of LD and HD diets with or without yeast supplementation on feed intake, nutrient digestibility, egg production, and egg quality in Hy-line brown laying hens.

## MATERIALS AND METHODS

The experiment protocol used in this research was approved by the Animal Care and Use Committee of Dankook University (DK-1-1708).

### Source of brewer's yeast

The brewer's yeast (*S. cerevisiae*) was provided by platinum brewery company, Seoul, South Korea. As per Platinum Brewery company's information, the brewer's yeast contained 4,240 kcal/kg DE, 53.2 % crude protein, 1.8 % crude fat (ether extract) 5.2 % ash, source of brewer's yeast was previously described (Zhang *et al.*, 2019).

### Experimental Design, Birds and Housing

A total of 432 (Hy-line brown) laying hens (40 weeks) were used in a 10-weeks experiment. Laying Hens were randomly allotted to 1 of 4 treatments, 18 replications (6 birds per replication) in a 2x2 factorial arrangement with HD and LD diets supplemented with or without 0.1 % of brewer's yeast (Table 1). All nutrient diets were formulated to meet or exceed the recommendation of NRC, (2012) except for LD diets, which were below NRC recommendations for

**Table 1** - Composition of Basal diet (as-fed basis).

Items	Brewer's yeast
DE (kcal/kg)	4.240
Crude protein	53.2
Crude fat	1.8
Crude fiber	0.8
Ash	5.2
Moisture	6.8
<b>Amino acids</b>	
Arginine	2.3
Histamine	1.5
Ile	236
Leucine	296
Lysine	3.20
Methionine	0.88
Cysteine	0.53
TSAA	1.20
<b>Vitamin B complex</b>	
Thiamin	3.50
Riboflavin	4.50
Niacin	30.00
Vitamin B <sub>6</sub>	2.30
Folate	0.13
Vitamin B <sub>12</sub> (µg)	0.40
<b>Minerals</b>	
Calcium	0.15
Potassium	1.11
K	1.48
Calcium	10.0
Magnesium	0.33
Sodium (Na)	0.08
Se, ppm	0.91

brown laying hens and fed in mash form (Table 2). Each cage was provided with free access to water and feed by nipple drinkers and feeders. The laying hens were housed in an environmentally controlled and windowless room. Room temperature was maintained at 21±1°C and had a daily lighting schedule of 16 h light and eight hours dark.

### Experimental Procedures, Sampling, and Chemical Analyses

Daily records of egg production and egg broken rate, weekly records of feed intake were maintained. The egg production was expressed as an average hen-day production. Also, the quality of the egg was checked weekly from 1 to 10 wk. A total of 180 eggs (5-eggs per treatment) were randomly selected at 5 p.m. and used for quality analysis at 8 p.m. on the same day. The egg quality was determined at 8 p.m. on the day of collection. The weight of the egg was measured using an egg multi tester (Touhoku Rhythm Co. Ltd., Tokyo, Japan). Eggshell breaking strength was determined with the eggshell force gauge (model


**Table 2** – Dietary composition of low and high nutrient density diets and their analysis.

Raw Materials	Low %	High%
Corn (7mm)	52.7	46
Rice	-	5
Wheat bran	10.99	17.33
Soybean meal (CP 45)	157.0	161.9
Corn gluten	-	0.67
Sesame Meal	2.0	1.5
DDGS (Corn, USA)	20.0	18.4
Palm Kernel Meal	1.85	-
Tallow	0.7	0.7
Limestone	11.01	9.76
MDCP	0.06	-
Salt	0.05	0.11
Methionine (99%, DL-Form)	0.05	0.06
Lysine (50%)	0.27	0.06
Vitamin premix	0.1	0.1
Choline (50%)	0.1	0.1
Mineral premix	0.1	0.1
total	100	100
Calculation Composition, %		
Dry matter	89.28	89.28
moisture	0.72	0.72
Crude protein	5.70	5.70
Crude Fat	4.01	4.01
Crude Fiber	3.09	3.09
Crude ash	4.45	4.45
ME (Kcal/kg)	2770	2770
Calcium (%)	4.31	4.31
Tri-calcium phosphate	0.37	0.37
Lysine	0.76	0.76
Methionine	0.38	0.38
Cystamine	0.27	0.27
Threonine	0.58	0.58
Trypsin	0.16	0.16

<sup>1</sup>Tricalcium phosphate contains 32% calcium and 8% phosphorus according to the NRC (1994).

<sup>2</sup>Vitamin premix provided (mg/kg diet): 25,000 IU vitamin A; 2,500 IU vitamin D3; 0 mg vitamin E; 2 mg vitamin K3; mg vitamin B; 5 mg vitamin B2; mg vitamin B6; 5 mg vitamin B12; 500 mg folic acid; 35,000 mg niacin; 0,000 mg Ca-Pantothenate and 50 mg biotin.

<sup>3</sup>Mineral premix provided (mg/kg diet): 8 mg Mn; 60 mg Zn; 25 mg Cu; 40 mg Fe; 0.3 mg Co; .5 mg I and 0.5 mg Se. <sup>4</sup>calculation value

II, Robotmation Co., Ltd., Tokyo, Japan). A dial pipe gauge (Ozaki MFG. Co., Ltd., Tokyo, Japan) was used to measure eggshell thickness, which was determined based on the average thickness of the rounded end, pointed end, and the middle of the egg, excluding the inner membrane. Finally, egg weight, yolk color, and Haugh unit (HU) were determined using an egg multi-tester (Touhoku Rhythm Co. Lt., Tokyo, Japan). Chromium oxide (Cr<sub>2</sub>O<sub>3</sub>, 2 g/kg) was added to the laying hen's diets as an indigestible marker for days before excreta collection to determine the apparent nutrient digestibility of dry matter (DM), nitrogen (N),

and energy (E). Excreta samples from each pen were pooled and stored at -20°C until the analysis. Before chemical analysis, the excreta samples were thawed and dried at 70°C for 72 h; then, they were ground fine by 1 mm screen, later stored in the refrigerator at -20°C until analysis (Mountzouris *et al.*, 2010). DM, N, and energy were conducted under the methods established by the AOAC (2000) Chromium levels were determined via UV absorption spectrophotometry (Shimadzu, UV-1201, Japan) according to (Williams *et al.*, 1962). The digestibility was then calculated using the following formula:

$$\text{Digestibility (\%)} = \{1 - [(Nf \times Cd) / (Nd \times Cf)]\} \times 100$$

Where Nf: nutrient concentration in excreta (%DM), Cd: chromium concentration in the diet (%DM), Nd: nutrient concentration in the diet (%DM), and Cf: chromium concentration in excreta (%DM).

Feed samples were collected at the start of the experiment, and then ground to pass through a 1-mm screen, after nitrogen was determined (Kjeltec 2300 Nitrogen Analyzer; Foss Tecator AB, Hoeganaes, Sweden), and crude protein was calculated as N × 6.25. The gross energy was determined using a bomb calorimeter (Mode 1241; Parr Instrument Co., Molin, IL, USA).

### Statistical analyses

All the data were analyzed in a 2×2 factorial using the GLM procedure of the SAS program SAS Inst. Inc., Cary, NC). Software package (2000). A number of 18 replications was used as the experimental unit. Supplementation of yeast and HD and LD on feed intake, nutrient digestibility, egg production, and egg quality was unaffected. The data were expressed as the standard error of the mean (SEM), and *p* values <0.05 were considered to statistical significance.

## RESULT

### Feed intake and egg production

There was no significant effect of yeast supplementation or density diet on feed intake during weeks 1, 2, 3, and 4. (Table 3). However, during the 5<sup>th</sup>, 6<sup>th</sup>, and 10<sup>th</sup> week, a significant increase (*p*>0.05) in feed intake was seen in birds fed LD diet compared with the birds fed HD diet. The supplementation of yeast showed trends in improvement in feed intake during weeks 5 and 6 (*p*=0.08, 0.06, respectively). The egg production and egg broken rate were neither significantly affected by yeast supplementation nor the diet density.



**Table 3** – Effects of low and high –density diets and with or without brewer’s yeast supplementation on feed intake performance, egg quality and egg broken rate in laying hens

Items	LD Diet		HD Diet		SEM	<i>p</i> -value		
	-Ye	+Ye	-Ye	+Ye		Den	Ye	Den × Ye
<b>Feed intake (g)</b>								
Week 1	120	120	120	120	0.00	0.00	0.00	0.00
week 2	120	120	120	120	0.00	0.00	0.00	0.00
Week 3	120	120	120	120	0.00	0.00	0.00	0.00
Week 4	120	120	120	120	0.00	0.00	0.00	0.00
Week 5	123	125	131	132	0.95	<.0001	0.08	0.54
Week 6	121	124	130	131	0.76	<.0001	0.06	0.39
Week 7	127	127	128	129	1.25	0.22	0.74	0.34
Week 8	126	126	128	129	1.55	0.12	0.59	0.91
Week 9	128	129	130	130	1.41	0.27	0.86	0.95
Week 10	125	134	133	134	0.70	<.0001	0.00	0.91
<b>Egg production (%)</b>								
Week 1	85.91	84.33	86.11	88.29	1.67	0.27	0.22	0.85
week 2	86.51	84.33	85.71	87.70	1.30	0.12	0.33	0.93
Week 3	88.49	87.70	87.50	90.48	18.0	0.31	0.62	0.55
Week 4	86.51	84.52	86.31	86.51	1.26	0.48	0.48	0.39
Week 5	85.50	85.30	86.70	88.10	1.29	0.54	0.14	0.64
Week 6	84.33	86.51	84.92	88.89	1.49	0.56	0.33	0.05
Week 7	82.94	84.13	86.71	85.32	1.26	0.53	0.13	0.65
Week 8	83.93	84.13	86.71	85.32	1.26	0.53	0.13	0.65
Week 9	85.32	85.32	87.50	86.90	1.63	0.85	0.26	0.86
Week 10	85.52	85.12	87.90	86.90	2.14	0.89	0.34	0.75
<b>Egg broken rate (%)</b>								
Week 1	0.00	0.26	0.00	0.71	0.00	0.08	0.43	0.04
week 2	0.00	0.00	0.00	0.048	0.15	0.12	0.12	0.120
Week 3	0.00	0.24	0.24	0.24	0.15	0.95	0.17	0.95
Week 4	0.22	0.48	0.22	0.00	0.46	0.17	0.41	0.09
Week 5	0.43	0.74	0.00	0.23	0.44	0.55	0.94	0.30
Week 6	0.00	0.76	0.00	0.25	0.39	0.22	0.53	0.53
Week 7	0.00	0.98	0.24	0.48	0.45	0.20	0.42	0.78
Week 8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Week 9	0.00	0.25	0.23	0.25	0.21	0.53	0.58	0.58
Week 10	0.00	0.00	0.21	0.00	0.10	0.32	0.32	0.32

<sup>1</sup>Abbreviation: HD - High-density, LD – Low-density, With or without yeast (-yeast +yeast). <sup>2</sup>Number of replicates: 18 replications (6 birds per replication). <sup>3</sup>Standard error of means (SEM). <sup>4</sup>*p*-value (*p*<0.05).

### Egg quality

The results of the egg quality with or without yeast supplementation to HD and LD diets are present in table 4. The supplementation of 0.1% yeast into the diet of laying hens significantly reduced (*p*<0.05) shell color during weeks 5, 6, and 7. However, a significant improvement (*p*<0.05) was seen in the eggshell strength during week four and albumen height during week 5 in birds fed yeast supplemented diet. During weeks 1 and 2, the yolk color was higher in the birds fed the LD diets compared with the HD diets. A significant increase in the eggshell strength (week 4) and the egg weight (week 4 and 7) was seen in the birds fed the HD diets than the LD diets whereas a significant reduction in shell color (week 5), and albumen height (week 6) was observed in the birds fed the HD diets.

### Apparent total tract nutrient digestibility

The effects of yeast supplementation to LD and HD diets on the ATTD of DM, N, and energy are presented in Table 5. The nutrient digestibility remained unaffected (*p*<0.05) in the birds fed yeast supplemented versus non –supplemented diets and between the birds fed HD versus LD diets.

## DISCUSSION

The present research aimed to investigate the effect of HD and LD diets, diets with or without yeast, performance on the feed intake, egg quality, and nutrient digestibility of laying hens. Previous research indicated that yeast supplementation in laying hens’ diet had a beneficial impact on feed intake (Ozsoy



**Table 4** – Effects of low and high-density diets and with or without brewer's yeast supplementation on egg quality in laying hens.

Items	LD Diet		HD Diet		SEM	p-value		
	-Ye	+Ye	-Ye	+Ye		Den	Ye	Den x Ye
<b>Week 1</b>								
Egg weight, g	63.95	61.83	63.94	62.80	0.84	0.63	0.06	0.63
Yolk color	7.15	7.10	6.80	7.0	0.06	0.04	0.00	0.32
HU	88.62	91.11	90.76	85.82	1.25	0.8	0.28	0.00
Albumen height	8.17	8.50	8.53	7.6	0.19	0.26	0.12	0.00
Shell color	10.03	10.63	8.93	11.80	0.49	0.84	0.00	0.04
Egg shell strength kg/cm <sup>2</sup>	4.16	3.65	3.66	4.10	0.13	0.78	0.72	0.02
Eggshell thickness, mm <sup>2</sup>	46.01	45.18	44.48	46.90	0.55	0.97	0.12	0.00
<b>Week 2</b>								
Egg weight, g	64.70	63.72	64.98	63.55	0.74	0.94	0.12	0.76
Yolk color	7.11	7.12	6.81	7.03	0.07	0.01	0.16	0.16
HU	88.04	90.29	88.40	90.29	2.21	0.40	0.22	0.80
Albumen height	8.4	8.23	8.23	8.84	0.45	0.46	0.39	0.66
Shell color	11.47	11.70	11.70	11.90	0.43	0.82	0.50	0.82
Egg shell strength kg/cm <sup>2</sup>	4.30	4.35	4.17	4.30	0.13	0.53	0.47	0.85
Eggshell thickness, mm <sup>2</sup>	39.98	39.84	39.99	39.36	0.41	0.57	0.35	0.57
<b>Week 3</b>								
Egg weight, g	65.40	63.06	65.54	63.0	0.75	0.97	0.00	0.91
Yolk color	6.86	7.13	6.75	7.30	0.33	0.93	0.24	0.91
HU	94.08	96.44	90.41	93.76	8.38	0.48	0.16	0.31
Albumen height	9.92	9.17	9.17	9.14	0.52	0.46	0.48	0.52
Shell color	10.67	10.60	9.87	9.90	0.33	0.05	0.77	0.77
Egg shell strength kg/cm <sup>2</sup>	3.98	4.04	3.51	3.76	0.11	0.00	0.15	0.34
Eggshell thickness, mm <sup>2</sup>	37.66	37.20	37.46	36.47	0.39	0.23	0.08	0.51
<b>Week 4</b>								
Egg weight, g	62.81	62.90	64.00	64.80	0.66	0.03	0.5	0.59
Yolk color	6.56	6.77	6.44	6.81	0.08	0.89	0.00	0.37
HU	84.90	86.83	87.02	85.55	0.75	0.59	0.76	0.04
Albumen height	7.37	7.70	7.81	7.72	0.72	0.82	0.31	0.11
Shell color	10.07	9.43	9.63	11.00	0.24	0.38	0.16	0.00
Egg shell strength kg/cm <sup>2</sup>	3.69	4.23	4.18	4.25	0.12	0.03	0.02	0.03
Eggshell thickness, mm <sup>2</sup>	46.47	45.98	46.98	46.72	0.58	0.29	0.51	0.82
<b>Week 5</b>								
Egg weight, g	63.09	63.26	62.11	62.40	0.75	0.24	0.76	0.96
Yolk color	7.01	7.10	6.41	6.99	0.08	0.00	0.00	0.01
HU	82.97	86.5	80.01	83.41	1.86	0.12	0.07	0.94
Albumen height	7.27	7.78	7.01	7.42	0.20	0.17	0.04	0.66
Shell color	10.50	10.17	10.13	8.60	0.44	0.03	0.03	0.18
Egg shell strength kg/cm <sup>2</sup>	3.83	4.01	4.15	4.08	0.21	0.30	0.65	0.44
Eggshell thickness, mm <sup>2</sup>	38.92	38.18	39.21	37.81	0.61	0.94	0.09	0.61
<b>Week 6</b>								
Egg weight, g	63.83	64.12	64.44	62.43	0.74	0.46	0.23	0.13
Yolk color	6.71	6.97	6.54	7.04	4.92	0.32	0.36	0.32
HU	90.27	89.17	88.05	87.88	0.92	0.07	0.6	0.61
Albumen height	8.84	8.16	8.08	7.90	0.18	0.01	0.40	0.18
Shell color	12.50	11.53	11.60	10.77	0.45	0.07	0.03	0.07
Egg shell strength kg/cm <sup>2</sup>	3.69	4.02	4.22	3.98	0.16	0.22	0.92	0.08
Eggshell thickness, mm <sup>2</sup>	39.83	37.06	45.06	38.79	0.95	0.02	0.00	0.73
<b>Week 7</b>								
Egg weight, g	61.50	65.93	65.12	65.80	0.67	0.02	0.00	0.01
Yolk color	6.69	6.53	6.93	6.82	0.08	0.00	0.13	0.90
HU	79.57	80.59	83.84	78.11	0.69	0.62	0.19	0.86
Albumen height	6.67	6.95	7.43	6.56	0.25	0.59	0.29	0.05
Shell color	10.37	9.93	10.63	9.10	0.37	0.47	0.01	0.18
Egg shell strength kg/cm <sup>2</sup>	4.06	3.66	3.58	3.66	0.09	0.00	0.59	0.24
Eggshell thickness, mm <sup>2</sup>	39.30	38.44	39.0	38.73	0.59	0.81	0.48	0.57
<b>Week 8</b>								
Egg weight, g	6.65	64.34	66.12	62.56	3.90	0.48	.64	0.69
Yolk color	6.97	7.01	6.84	6.69	13.34	0.89	0.99	0.94
HU	87.79	89.47	87.65	84.77	2.26	0.13	0.90	0.48
Albumen height	8.35	8.59	8.24	7.44	13.16	0.86	0.90	0.92
Shell color	10.53	10.27	9.27	9.60	0.39	0.06	0.71	0.71
Egg shell strength kg/cm <sup>2</sup>	3.83	3.60	4.01	3.91	13.86	0.90	0.99	0.95
Eggshell thickness, mm <sup>2</sup>	37.67	35.13	38.12	38.1	8.42	0.86	0.90	0.92
<b>Week 9</b>								
Egg weight, g	65.3	64.29	65.91	62.95	3.86	0.60	0.82	0.72
Yolk color	6.71	6.78	6.85	6.79	13.36	0.89	0.99	0.95
HU	89.51	84.47	87.84	83.14	1.39	0.08	0.10	0.61
Albumen height	11.57	7.55	9.29	7.30	13.02	0.85	0.91	0.98
Shell color	10.10	10.37	9.30	9.97	12.84	0.87	0.97	0.95
Egg shell strength kg/cm <sup>2</sup>	3.65	3.63	3.64	3.69	13.89	0.90	0.99	0.95
Eggshell thickness, mm <sup>2</sup>	35.90	35.70	36.50	36.74	8.49	0.87	0.98	0.93
<b>Week 10</b>								
Egg weight, g	64.43	66.20	64.88	62.50	3.90	0.51	0.99	0.62
Yolk color	6.86	6.83	6.30	6.89	13.37	0.88	0.97	0.94
HU	83.30	86.20	85.42	87.22	1.62	0.54	0.42	0.47
Albumen height	6.84	7.71	7.83	7.91	3.24	0.91	0.97	0.94
Shell color	8.80	9.77	9.37	11.33	12.86	0.92	0.94	0.96
Egg shell strength kg/cm <sup>2</sup>	3.47	3.88	9.94	3.76	3.87	0.90	0.98	0.94
Eggshell thickness, mm <sup>2</sup>	36.01	35.32	36.78	36.93	8.49	0.88	0.99	0.94

<sup>1</sup>Abbreviation: HD - High-density, LD - Low-density, With or without yeast (-yeast +yeast) <sup>2</sup>Number of replicates: 18 replications (6 birds per replication) <sup>3</sup>Standard error of means (SEM). <sup>4</sup>p-value: (p<0.05).




**Table 5** – Effects of low and high-density diets and with or without brewer's yeast supplementation on digestibility in laying hens.

Items	Ld Diet		HD Diet		SEM	p-value		
	-Ye	+Ye	-Ye	+Ye		Den	Ye	Den × Ye
DM	74.89	74.60	75.38	74.96	0.58	0.48	0.56	0.90
Nitrogen	73.31	72.81	73.76	73.45	0.53	0.49	0.31	0.63
Energy	75.49	75.13	75.78	75.61	0.58	0.52	0.66	0.87

<sup>1</sup>Abbreviation: HD - High-density, LD – Low-density, With or without yeast (-yeast +yeast)

<sup>2</sup>Number of replicates: 18 replications (6 birds per replication)

<sup>3</sup>Standard error of means (SEM<sup>2</sup>).

<sup>4</sup>p-Value (p<0.05).

*et al.*, 2018), which agrees with our study. In the present study, yeast supplementation or diet density was significantly improved on feed intake of laying hens. On the other hand, Liu *et al.* (2002) and Sacakli *et al.* (2013) stated that the inclusion of yeast culture supplementation (0.2%) had a significant effect on feed intake during the overall experiment in laying hens. In contrast, Sehu *et al.* (1997) and Sacakli *et al.* (2013) demonstrated that inactivated yeast diets at levels of 5, 10, or 15% did not affect feed intake in quails. Maybe this contradictory result between various experiments was due to the animals, variation in the amount of yeast concentration, and differences in dietary compositions. The present study revealed that dietary inclusion of yeast and density diets in laying hens had no statistical difference in egg production and broken rate. These results are consistent with those of previous studies (Ayanwale *et al.*, 2006; Asli *et al.*, 2007; Yousefi & Karkoodi, 2007) and egg broken rate (Day *et al.*, 1987; Alabi *et al.*, 2011). (Hassanein & Soliman, 2010) demonstrated that hens egg production was positively affected in higher concentration 0.4, 0.8, 1.2, and 1.6% of yeast supplementation. However, Araujo *et al.* (2017) and Park *et al.* (2020) reported that the addition of the diet of the breeder hens with the hydrolyzed yeast resulted in a 2.14% improvement in egg production and broken rate. Therefore, it may be due to the bird's age, heat stress, inadequate nutrient problems in a feed (calcium and vitamin D3), and yeast concentration.

In the present study, yeast supplementation with LD and HD diets had a significant reduction during the 5<sup>th</sup> to the 7<sup>th</sup> wk in eggshell color in laying hens, which is in agreement with the previous study (Odabasis *et al.*, 2007). On the other hand, in longitudinal research on the effect of brown laying hen's eggshell color, no difference was observed between the eggshell color during weeks 35 to 75. Still, on the 25<sup>th</sup> wk, the eggshell color significantly increased compared to all other age groups (Samiullah *et al.*, 2014). However, Hutt (1949) and Wei *et al.* (1992) suggested that hens' ages and generations might cause less pigmentation on

individual eggs. Also, the eggshell strength and albumen height were significantly increased by the inclusion of yeast supplementation diet in this study. A similar result was observed by (EL-Kaiaty *et al.*, 2019). Also, (Alabi *et al.*, 2011) reported that a yeast supplementation diet had a positive impact on eggshell strength and albumen height. In contrast, (Hosseini *et al.*, 2006) stated that there were no positive effects of yeast supplementation on hen's eggshell strength. However, Park *et al.* (2020) indicated that yeast supplementation did not have a significant effect on eggshell breaking strength. The significant improvement in egg strength may be due to calcium absorption, age of birds, and yeast concentration in the feed. In this present study, the yolk color was higher on birds fed LD compared with HD diets. Similarly, the previous report showed that yolk color was significantly affected by a high concentration of yeast supplementation reported by (Parvu & Paraschivescu, 2014). Moreover, (Martinez *et al.*, 2010) showed that yolk color was not affected by yeast products (*S. cerevisiae*). That may be due to the different nutrients in feed such as yeast and corn and soybeans. In the present study, eggshell strength and egg weight was significantly higher on bird's fed HD than LD diet. These results are consistent with those of other researches (Swain *et al.*, 2011). Previously Wu *et al.* (2007) stated that the high nutrient density diet has significant improvement in egg weight. On the other hand, Jalal *et al.* (2006) and Hayam *et al.*, (2015) stated with contradictory statements that high nutritional density diet intake of laying hens had no significant effect on egg weight. Therefore, the results of the present study may be due to the age of the chickens and the concentration of yeast.

The significantly reduced eggshell color and albumen height was observed in birds fed the HD diets. Similar results were observed in the previous reports of Koiyama *et al.* (2017) and Shi *et al.* (2009). The albumen height was not affected by HD or LD diets (Lu *et al.*, 2019). (Menezes *et al.*, 2012) showed that the laying hens age and room temperature had an effect on albumen height; hens age at 35 weeks (5.836 mm)



compared with 50 weeks (4.487 mm). Our results may be due to the laying period (phase) of the chickens and the physiological changes in the egg composition.

In the current research, dietary yeast supplementation had no significant effect on nutrient digestibility of DM, Nitrogen, and Energy with HD or LD diets. The outcome of the study can moderately explain the lack of effect of yeast supplemented on production performance. Similarly, Park *et al.* (2020) showed that (*S. cerevisiae*) did not have a significant effect on nutrient digestibility of DM. The inclusion of yeast supplementation diets did not affect the nutrient digestibility of nitrogen in laying hens (Cai *et al.*, 2014). Also, the nutrient digestibility of DM or energy was not affected by yeast culture supplementation (from 500 to 75,000 mg/kg) on weaning pigs and chicken (Van-Heugten *et al.*, 2003; Gao *et al.*, 2008; Morales-López *et al.*, 2010; Cai *et al.*, 2014). (Chademana & Offer, 1990; Haddad & Goussous, 2005; Dias *et al.*, 2017; Zhang *et al.*, 2019) reported that supplementation of yeast culture can improve nutrient digestibility in sheep and lambs. However, Park *et al.* (2020) reported that brewer's yeast supplement can linearly increase nutrient digestibility of DM and N in laying hens. This variation happened due to the amount of the yeast, experimental animals, and yeast density diets.

## CONCLUSION

The present study will be the base of future research. The inclusion of yeast supplementation to HD and LD diets of laying hens has improved feed intake and egg quality. However, egg production and nutrient digestion were not affected by yeast supplementation in HD and LD diets. Therefore, further research with HD and LD diets with various levels of yeast supplementation is needed to understand laying hens on performance.

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