Egg production, egg quality, organ weight, bone ash, and plasma metabolites in 30-week-old Lohmann LSL lite hens fed corn and soybean meal-based diets supplemented with enzymatically treated yeast[1]

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ABSTRACT Highly prolific modern hens are susceptible to metabolic disorders that could be modulated by functional feedstuffs such as enzymatically treated yeast (**ETY**). Therefore, we assessed the dose-response of ETY on hen-day egg production (**HDEP**), egg quality attributes, organ weight, bone ash, and plasma metabolites in laying hens. A total of 160 thirty-week-old Lohmann LSL lite hens were placed in 40 enriched cages (4 birds/cage) based on body weight (**BW**) and allocated to 5 diets in a completely randomized design for a 12-wk trial. The diets were isocaloric and isonitrogenous corn and soybean meal based supplemented with 0.0, 0.025, 0.05, 0.1, or 0.2% ETY. Feed and water were provided ad libitum; HDEP and feed intake (**FI**) were monitored weekly, whereas egg components, eggshell breaking strength (**ESBS**), and thickness (**EST**) were monitored biweekly, and albumen IgA concentration was measured on wk 12. At the end of the trial, 2 birds/cage were bled for plasma and necropsied for liver, spleen, and bursa weight, ceca digesta for short chain fatty acids (**SCFA**) and tibia and femur for ash content. Supplemental ETY reduced HDEP quadratically ($P = 0.03$); the HDEP was 98, 98, 96, 95, and 94% for 0.0, 0.025, 0.05, 0.1, and 0.2% ETY, respectively. However, ETY linearly and quadratically ($P = 0.01$) increased egg weight (**EW**) and egg mass (**EM**). Specifically, EM was 57.9, 60.9, 59.9, 58.9, and 59.2 g/b for 0.0, 0.025, 0.05, 0.1, and 0.2% ETY, respectively. Egg albumen increased linearly ($P = 0.01$), and egg yolk decreased linearly ($P = 0.03$) in response to ETY. In response to ETY, the ESBS and plasma Ca increased linearly and quadratically ($P \leq 0.03$). Plasma concentration of total protein and albumin increased quadratically ($P \leq 0.05$) with ETY. Diets had no ($P > 0.05$) effects on FI, FCR, bone ash, SCFA, and IgA. In conclusion, 0.1% or higher ETY reduced egg production rate; however, linear improvement in EW and shell quality linked to larger albumen and higher plasma protein and Ca suggested modulation in protein and calcium metabolism.

Key words: enzymatically treated yeast, laying hen, egg production and quality, gastrointestinal health and metabolism

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INTRODUCTION

Egg consumption as a protein choice has increased in popularity over time (Conrad et al., 2017). From 2002 to 2012, the average American egg consumption increased by 2.5 g/day, from 23.0 g/day to 25.5 g/day, respectively (Conrad et al., 2017). Global trends have seen egg consumption steadily grow over the past 50 yr. Current forecasts indicate that egg consumption will continue to escalate for years without accounting for the predicated population growth (OECD/FAO, 2021). Given the current estimates, egg farmers globally are searching for ways to increase egg production, egg quality, and hen longevity to supply the growing demand for eggs while improving overall profitability.

The modern laying hen has been genetically selected to optimize egg production, feed conversion, and longevity (Underwood et al., 2021). However, high productivity presents hens with various challenges, such as increased stress, metabolic disorders, compromised immunocompetence, skeletal issues, and disease susceptibility (Sonntag et al., 2019). Antimicrobial growth promoters (**AGP**) have previously been used to overcome these challenges and maintain high production levels.

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Still, the progression of antimicrobial resistance has placed restrictions on the indiscriminate use of AGP in animal production, necessitating research on alternatives such as postbiotics (Van Boeckel et al., 2015; Salim et al., 2018). Postbiotics are preparations of inanimate microorganisms and their components that potentiate health benefits on the host (Salminen et al., 2021), and yeast (*Saccharomyces cerevisiae*) has been a postbiotic candidate through different preparation methods (Patterson et al., 2023). Enzymatically treated whole yeast (**ETY**) contains functional components within the cell, such as enzymes, nucleotides, and peptides. In addition, yeast cell wall components, such as β-glucans, α-mannans, glycoproteins, and chitin, have gained interest as previous studies demonstrated their viability as alternative to AGP (Yitbarek et al., 2012; Anwar et al., 2017). Supplementing enzymatically hydrolyzed yeast products may impact nutrient digestion, absorption, and overall production performance (Shurson, 2018; Ogbuewu et al., 2019). Additionally, yeast β-glucans and α-mannans have been found to stimulate gut microbiome function, modulate immunocompetence and enteric pathogens (Kiarie et al., 2019; Lu et al., 2019; Perricone et al., 2022).

The ETY product evaluated in the current study has been reported to improve growth performance, indices of gastrointestinal health and function and metabolism in broiler chickens (Kiarie et al., 2022; Maina et al., 2022) and piglets (Alagbe et al., 2022; Christensen et al., 2022). However, the efficacy of ETY in laying hens has not been investigated. Considering that previous studies elucidated the potential benefits of yeast derivates on poultry production and health, it is necessary to investigate potential benefits of ETY in laying hens. We hypothesized that the inclusion of ETY in layer diets would positively impact overall egg production and quality, indices of gastrointestinal health and metabolism. Therefore, this study aimed to assess the effects of supplementing diets for 30-wk-old Lohmann LSL lite hens with various doses of ETY on egg production, egg quality, plasma metabolites, lymphoid organ weights, and long bone ash content.

MATERIALS AND METHODS

All research followed the guidelines and was approved by the Animal Care Committee at the University of Guelph (protocol #4518). In addition, guidelines set forth by the Canadian Council on Animal Care were adhered to (CCAC, 2009).

Experimental Birds and Housing

This trial used 160 Lohmann LSL Lite laying hens that were 30 wk of age (**WOA**) at the start of the study. The hens were sourced as day-old chicks from Archer's Hatchery (Brighton, Ontario, Canada) and reared at the Arkell Poultry Research Station (Arkell, Ontario, Canada) through to 30 WOA. All hens were housed in the same room at the Arkell Poultry Research Station for the entire trial and kept in FDI Enriched Cage Systems (FDI Poultry Equipment, Mitchell, Ontario, Canada). Each cage measured 61 cm wide by 66 cm deep and 46 cm high and had a stocking density of four hens each. Birds were randomly assigned to cages, where the initial total cage weights had a covariance under 4% across all cages. The room was consistently kept between 18°C and 20°C throughout the entire trial. The birds received 14 h of light per day (3:00−17:00), with no humans in the room within the first 4 h to allow for an undisturbed lay period for the hens. Light intensity was kept at 15 lux for the study. All management housing conditions met the requirements for Lohmann LSL-Lite laying hens and followed the guidelines set by the Arkell Poultry Research Facility (Lohmann Breeders, 2020).

Experimental Diets

The experimental diet (Table 1) was a pelleted corn-soy-based laying hen diet formulated to meet or exceed all breed requirements for Lohmann LSL Lite hens (Lohmann Breeders, 2020). The yeast was an enzymatically treated non-GMO *Saccharomyces cerevisiae* containing 40% cell wall components (β-1-3 and 1-6 glucans and mannan oligosaccharides) and 36% crude protein (Livalta Cell HY40, Livalta, Ab Agri Ltd., Cambridgeshire, Peterborough, UK). The description of the enzymes used for preparation of ETY and complete chemical composition has been reported (Alagbe et al., 2022). The Control diet consisted of a 0% inclusion of ETY, whereas the other experimental diets had an inclusion rate of 0.025, 0.05, 0.1, and 0.2% ETY. Incorporating ETY into the diets replaced a small amount of corn in the formulation.

Experimental Procedures and Sample

Before the initiation of the study, the hens were fed a commercial antibiotic-free pelleted corn and soybean-based diet (Floradale Feed Mill Limited, Floradale, Ontario, Canada). A completely randomized design was used to allocate the diets to cages, allowing for 5 treatments and 8 replicate cages per diet. Hens were provided with ad libitum feed and water. In wk 1 and 2, feed spillage was extremely high, resulting in this data not being recorded. As a result, the feed troughs were fitted with a wire mesh covering to minimize spillage and feed intake was monitored from the beginning of wk 3. The feeders were weighed weekly to determine the weekly feed consumption per cage. Egg production was recorded daily. Egg weights and egg quality characteristics were assessed once a week; 2 eggs per cage were randomly selected for eggshell-breaking strength (**ESBS**) and eggshell thickness (**EST**) in wk 2, 4, 6, 8, 10, and 12 with the procedures set forth by Mwaniki et al. (2018). Briefly, 2 eggs were weighed individually and sorted into their respective marketable weight classes; peewee, <42 g; small, 42≤ to >49 g; medium, 49≤ to >56 g; large, 56≤

Table 1. Experimental diets composition, as-fed basis.

Item	ETY inclusion, %				
	0	0.025	0.05	0.10	0.20
Corn	54.8	54.8	54.8	54.7	54.6
Wheat	10.0	10.0	10.0	10.0	10.0
Soybean meal	14.7	14.7	14.7	14.7	14.7
Corn gluten	5.00	5.00	5.00	5.00	5.00
Pork meal	3.00	3.00	3.00	3.00	3.00
Soy oil	0.66	0.66	0.66	0.66	0.66
L-Lysine HCL	0.12	0.12	0.12	0.12	0.12
DL-Methionine	0.12	0.12	0.12	0.12	0.12
Limestone fine[3]	3.04	3.04	3.04	3.04	3.04
Limestone coarse[3]	6.08	6.08	6.08	6.08	6.08
Monocalcium phosphate	1.58	1.58	1.58	1.58	1.58
Salt	0.33	0.33	0.33	0.33	0.33
Sodium bicarbonate	0.07	0.07	0.07	0.07	0.07
Vitamin and trace mineral premix[1]	0.50	0.50	0.50	0.50	0.50
ETY[2]	0.00	0.025	0.05	0.10	0.20
Ingredient total	100.0	100.0	100.	100.0	100.0
Calculated provisions					
AMEn, kcal/kg	2,799	2,799	2,799	2,799	2,799
Crude protein, %	17.5	17.5	17.5	17.5	17.5
SID Lys, %	0.68	0.68	0.68	0.68	0.68
SID Met, %	0.38	0.38	0.38	0.38	0.38
SID Met+ Cys, %	0.61	0.61	0.61	0.61	0.61
SID Thr, %	0.48	0.48	0.48	0.48	0.48
Calcium, %	4.10	4.10	4.10	4.10	4.10
Available phosphorous, %	0.44	0.44	0.44	0.44	0.44
Sodium, %	0.18	0.18	0.18	0.18	0.18
Analyzed provisions					
Dry matter, %	90.1	91.2	89.4	90.2	89.7
Gross energy, kcal/kg	2,940	3,020	2,882	2,943	2,900
Crude protein, %	16.6	15.4	16.5	17.2	17.3
Calcium %	3.82	4.19	3.85	3.70	4.04
Phosphorus %	0.71	0.70	0.74	0.67	0.73
Sodium %	0.18	0.17	0.20	0.17	0.18

[1]Provided per kilogram of diet: vitamin A, 8,800.0 IU; vitamin D3, 3,300.0 IU; vitamin E, 40.0 IU; vitamin B12, 12.0 mg; vitamin K3, 3.3 mg; niacin, 50.0 mg; choline, 1,200.0 mg; folic acid, 1.0 mg; biotin, 0.22 mg; pyridoxine, 3.3 mg; thiamine, 4.0 mg; calcium pantothenic acid, 15.0 mg; riboflavin, 8.0 mg; manganese, 70.0 mg; zinc, 70.0 mg; iron, 60.0 mg; iodine, 1.0 mg; copper, 10 mg; and selenium, 0.3 mg.

[2]Enzymatically treated whole non-GMO *Saccharomyces cerevisiae* strain, 40% β-1.3/1.6 glucans and mannan oligosaccharides and 36% crude protein (Livalta^{TM}Cell HY40, Livalta, Peterborough, UK). This included replacing a small amount of corn.

[3]fine, <0.595 mm; course, ≥2 mm.

to >63 g; XL, 63≤ to >70 g; jumbo, 70≥ g (CFIA, 2021). The ESBS was completed with an Egg Force Reader (OKRA Food Technology LTD, West Bountiful, UT). An Egg Shell Thickness Gauge (OKRA Food Technology LTD, West Bountiful, UT) was used to determine EST. In wk 4, 8, 10, and 12, one egg/cage was used to determine the weight of egg components. The eggs were cracked, and the yolk and shell were weighed, with these values subtracted from the whole egg weight to derive albumen weight. Egg components were presented as absolute or relative to egg weight. One egg per cage was collected randomly on wk 12 eggs and transferred to the laboratory; egg albumin was isolated and stored at −80° C until required for IgA analyses.

At the end of the trial, all hens were group-weighed on cage basis and divided by cage population to derive the final body weight. Two hens per pen were selected randomly, individually weighed, bled via cardiac puncture, and euthanized via cervical dislocation. The birds were dissected, liver, spleen, and bursa were removed, blotted dry with a paper towel, and weighed. Blood was collected in heparinized blood tubes (Becton, Dickison and Company, Franklin Lakes, NJ), placed on ice, and transported to the laboratory. The left tibia and femur were excised to determine ash content. Ceca digesta samples were collected from the 2 birds, pooled into one sample, and stored at −20°C until required for SCFA analyses.

Sample Processing and Laboratory Analyses

Samples for the diets were finely ground using a grinder (CBG5 Smart Grind, Applica Consumer Products Inc., Shelton, CT) and thoroughly mixed for chemical analyses. Dry matter was determined by weighing samples before and post-oven drying at 100°C for 12 h. Gross energy was determined using a bomb calorimeter (IKA Calorimeter System C 5000; IKA Works, Wilmington, NC) (AOAC, 2005). Nitrogen content was determined using a CNS-2000 Carbon, Nitrogen, and Sulfur Elemental Analyzer (LECO Corporation, St. Joseph, MI) via the combustion method (AOAC, 2005). These nitrogen values were multiplied by 6.25 to estimate CP levels. Concentration of calcium, phosphorous and sodium in the diet was determined in a commercial laboratory (SGS Canada Inc, Guelph, ON, Canada). Blood samples were centrifuged using Beckman Coulter

Centrifuge, rotor JS-4.2 with a radius of 184 mm, at 4°C, at 2,520 *g*, for 15 min to separate the plasma. Plasma samples were submitted to Animal Health Laboratory (Guelph, Ontario, Canada) for analyses of a select panel of avian biochemistry profiles with the methods described by Greenacre et al. (2008). Excess flesh was removed from the tibia and femur and oven dried at 60°C for 72 h. Once dried, the tibia and femur were weighed as one, ashed together in a furnace at 550°C for 10 h, and ash weight recorded.

The concentration of cecal digesta short-chain fatty acid (**SCFA**) was determined as described by Leung et al. (2018). Briefly, SCFA were derivatized to the corresponding phenyl esters using phenyl chloroformate reagent and analyzed using a gas chromatograph (Agilent Technologies, Santa Clara, CA); pivalic acid (Sigma-Aldrich, St. Louis, MO) was the internal standard. The chromatograph had a glass column packed with Carbopack B-DA/4% Carbowax in the ratio of 2:3 in the stationary phase. Helium was used as the carrier gas and the flame ionization detector. The quantified metabolites were acetic, propionic, butyric, valeric, and lactic, and all expressed in mmol/kg. Undiluted egg albumen samples were used to determine IgA concentration using chicken-specific IgA ELISA quantification kits per manufacturer protocol (Bethyl Laboratories Inc., Montgomery, TX), as illustrated by Lu et al. (2019) and Bi et al. (2020).

Calculations and Statistical Analyses

Hen-day egg production (**HDEP**, %) was calculated weekly; the total weekly egg count was divided by the number of hens. The EW (g/bird/d), EM (g/bird/d), FI (g/bird/d), and FCR (FI/EM) were calculated as described by Mwaniki et al. (2018). Eggshell calcium deposition rate (%) was calculated by using diet-analyzed calcium content, eggshell weight, and FI as follows:

$$Eggshell\ \ Ca\ \ deposition = \frac{Egg\ shell\ weight_g \times\ 40\%}{FI_g\ \ \times\ \ analyzed\ \%\ Ca\ in\ feed}$$

Where:

a) It was assumed that the eggshell was 40% calcium (Brun et al., 2013; Lee et al., 2021).

Crude protein intake was calculated as follows:

$$CP\ intake\ per\ day_g = Daily\ hen\ FI_g\ \times\ \frac{Diet\ \%CP}{100}$$

Organ weights were expressed relative to body weight.

The BW change throughout the trial was calculated as follows

$$BW\ \ change, \% = \frac{(Final\ BW - Initial\ BW)}{Initial\ BW} \times 100$$

The bone ash (pooled tibia and Femur) was calculated as described by Akbari Moghaddam Kakhki et al. (2019) using the following equation

$$\text{Ash content, } \% = \frac{\text{Ash weight (g)}}{\text{Dried weight (g)}} \times\ 100$$

All statistical analysis was completed using SAS Statistical Software version 9.4 using GLIMMX procedures. For this experiment, the cage was treated as the experimental unit. For egg production (HDEP), egg quality, egg parts, ESBS, EST, FI, FCR, protein intake, and calcium deposition, a model based on diet, time, diet by week, and associated interactions was used. The fixed effect of diet was the model used to determine the effects on organ weights, tibia/femur ash, plasma metabolites, cecal metabolites, egg albumen IgA, and body weight change. Statistical significance was declared at $P < 0.05$. LS means for diet were separated with a dose-response curve and ST with Tukey's test. Linear and quadratic contrasts were analyzed to determine the different responses to ETY. Significance was declared at $P < 0.05$, and trends at $P \leq 0.10$ were discussed. A post-experimental statistical power analysis on the diet, week and their interacting effects was carried out using GLM power analysis methods of SAS with the diet, week, and associated interactions as main effects. Analyzed actual powers for HDEP (n = 400) were 93.5, 89.9, and 84.4% for diet, week, and their interactions, respectively.

RESULTS

The analyzed chemical composition of experimental diets is displayed in Table 1. The CP was 16.6, 15.4, 16.5, 17.2, and 17.3%; the gross energy was 2,940, 3,020, 2,882, 2,943, and 2,900 kcal/kg for 0.0, 0.025, 0.05, 0.10 or 0.20% ETY diets, respectively. There was no diet and week interaction on any response criteria ($P > 0.05$). Supplemental ETY tended ($P = 0.07$) to linearly increase FI (Table 2); the FI was 101.9, 104.6, 104.0, 103.4, and 103.5 g/b/d for 0.0, 0.025, 0.05, 0.10, or 0.20% ETY, respectively. The ETY reduced HDEP quadratically ($P = 0.029$) with the value of 97.8, 98.1, 95.9, 94.6, and 94.0% for 0.0, 0.025, 0.05, 0.10, and 0.20% ETY diets, respectively. Hen age had no effect ($P > 0.05$) on HDEP. The EW and EM experienced ($P < 0.05$) a linear and quadratic increase as ETY supplementation increased. There was a noticeable increase in EW ($P = 0.011$) as the hens aged from 60.8 g at 33 WOA to 62.6 g at 42 WOA; however, there was no hen age effect ($P = 0.999$) on EM. There was no ($P > 0.05$) diet effect on FCR (Table 2). There was a quadratic increase ($P = 0.026$) in CP intake in response to ETY on CP intake (Table 2). The CP intake was 16.9, 16.1, 17.1, 17.8, and 17.9 g/b/d for the 0.0, 0.025, 0.05, 0.10, or 0.20% ETY diets, respectively.

The proportion of albumen, yolk, eggshell, ESBS, EST, and egg albumen IgA and calcium deposition are displayed in Table 3. Although a linear response was not observed ($P > 0.05$), there were quadratic effects of

Table 2. Effects of feeding enzymatically treated yeast on egg production, feed intake, and FCR in LSL-lite hens (from wk 33 to wk 42) of age.

ETY[1] inclusion, %	Feed intake, g/d	HDEP, %	Egg weight, g	Egg mass, g	FCR[2], g/g	Crude protein intake, g/d
0	101.9	97.8	59.3	57.9	1.76	16.9^{b}
0.025	104.6	98.1	62.1	60.9	1.73	16.1^{c}
0.05	104.0	95.9	62.3	59.9	1.75	17.1^{b}
0.1	103.4	94.6	62.2	58.9	1.77	17.8^{a}
0.2	103.5	94.0	63.0	59.2	1.77	17.9^{a}
SEM	1.009	0.984	0.265	0.689	0.023	0.165
Sampling time, week						
3	106.1	96.3	60.8^{b}	58.6	1.83	17.9
4	104.0	97.7	61.3^{ba}	59.9	1.74	17.2
5	104.6	97.2	61.3^{ba}	59.6	1.77	17.4
6	104.4	96.8	61.3^{ba}	59.3	1.77	17.3
7	102.2	96.6	61.9^{ba}	59.8	1.72	17.0
8	103.1	95.7	61.9^{ba}	59.3	1.75	17.1
9	103.1	94.5	62.3^{ba}	58.9	1.77	17.1
10	101.9	94.8	62.3^{ba}	59.0	1.74	16.9
11	101.1	95.2	62.1^{ba}	59.2	1.72	16.8
12	104.2	96.0	62.6^{a}	60.0	1.75	17.3
SEM	1.427	1.391	0.375	0.974	0.032	0.233
P-value						
Diet	0.418	0.008	<0.001	0.034	0.533	<0.001
Week	0.358	0.807	0.011	0.990	0.409	0.328
Diet × Week	0.990	1.000	1.000	1.000	0.999	0.988
Response to ETY						
Linear	0.073	0.354	<0.001	0.008	0.403	0.435
Quadratic	0.104	0.029	<0.001	0.032	0.794	0.026

[1]Enzymatically treated whole non-GMO *Saccharomyces cerevisiae* strain, 40% β-1.3/1.6 glucans and mannan oligosaccharides and 36% crude protein (Livalta Cell HY40, Livalta, Peterborough, UK). This included replacing a small amount of corn.
[2]Calculated by dividing feed intake by egg mass.LS means with different superscript letters within a column differs, $P < 0.05$.

ETY on the proportion of albumen ($P = 0.006$) and yolk ($P = 0.026$). Albumen increased as the level of ETY increased, with 57.4% of the egg weight being albumen for 0% ETY and 59.1% in the 0.20% ETY. As the albumen increased, the yolk decreased as a percentage of whole egg contents. The yolk was 27.7% in the 0% ETY and decreased to 26.3% in the diets containing 0.2% ETY (Table 3). A significant time effect was observed for the proportion of egg albumen, yolk, and eggshell ($P < 0.01$). Eggshell decreased significantly ($P < 0.01$) over the sampling period. Eggshell breaking strength had a linear ($P = 0.010$) and quadratic ($P = 0.033$) response to ETY. There were no ($P > 0.05$) diet effects on the concentration of egg albumen IgA. There was a

Table 3. Effects of feeding enzymatically treated yeast on egg quality attributes in LSL-lite hens (from wk 31 to wk 42) of age.

	Egg components, %				Eggshell quality		
ETY[1] inclusion, %	Albumen	Yolk	Shell	Egg albumen IgA, ng/60 g egg	Breaking strength (ESBS), kgF	Thickness (EST), mm	Calcium deposition rate in eggshell[3], %
0	57.4	27.7	14.9	4,487.4	4.70	0.416	94.9
0.025	57.9	27.8	14.3	4,318.1	4.95	0.424	83.1
0.05	58.2	27.3	14.5	4,644.4	4.94	0.422	93.9
0.1	58.6	26.8	14.6	4,416.8	4.85	0.416	97.2
0.2	59.0	26.3	14.6	4,383.8	4.84	0.421	91.3
SEM	0.360	0.300	0.220	127.4	0.080	0.030	2.09
Sampling time (Week)							
2[2]	-	-	-	-	4.92	0.421^{ab}	-
4	58.9^{a}	26.3^{b}	14.9^{a}	-	4.99	0.419^{ab}	93.3
6[2]	-	-	-	-	4.99	0.427^{a}	-
8	57.2^{b}	27.5^{a}	15.3^{a}	-	4.75	0.413^{b}	94.2
10	58.4^{ab}	27.5^{a}	14.1^{b}	-	4.77	0.420^{ab}	90.6
12	58.5^{a}	27.4^{a}	14.0^{b}	-	4.75	0.420^{ab}	90.1
SEM	0.320	0.270	0.200		0.080	0.003	1.87
P-values							
Diet	0.014	0.002	0.440	0.443	0.141	0.225	<0.001
Week	0.002	0.002	<0.001	-	0.075	0.052	0.327
Diet × Week	0.942	0.934	0.798	-	0.813	0.934	0.649
Response to ETY							
Linear	0.150	0.596	0.102	0.763	0.010	0.079	0.079
Quadratic	0.006	0.026	0.135	0.690	0.033	0.165	0.135

[1]Enzymatically treated whole non-GMO *Saccharomyces cerevisiae* strain, 40% β-1.3/1.6 glucans and mannan oligosaccharides and 36% crude protein (Livalta Cell HY40, Livalta, Peterborough, UK). This included replacing a small amount of corn.
[2]Indicates data was not collected for that sampling period.LS means with different superscript letters within a column differs, $P < 0.05$.
[3]Based on the analyzed calcium concentration in experimental diets

Table 4. Effects of feeding hens enzymatically treated yeast (ETY) on egg weight classification[1] distribution (%).

	Egg categories[1]			
ETY[2] inclusion, %	Medium	Large	Extra large	Jumbo
0.00	8.30	71.9	19.8	0.000
0.025	3.10	55.2	41.7	0.000
0.05	3.10	45.8	50.0	1.00
0.10	3.10	43.8	50.0	3.10
0.20	0.000	42.7	55.2	2.10
SEM	0.280	0.990	1.060	0.190
P-values				
Diet	0.041	0.013	0.007	0.316
Response to ETY				
Linear	0.046	0.068	0.027	1.000
Quadratic	0.003	0.003	<0.001	0.550

[1]Egg classifications according to the Canadian Food Inspection Agency: peewee, <42 g; small, 42≤>49 g; medium, 49≤>56 g; large, 56≤>63 g; XL, 63≤>70 g; jumbo, 70≥ g (CFIA. 2021). No eggs laid were under 49 grams to be classified in the peewee or small categories.

[2]Enzymatically treated whole non-GMO *Saccharomyces cerevisiae* strain, 40% β-1.3/1.6 glucans and mannan oligosaccharides and 36% crude protein (Livalta Cell HY40, Livalta, Peterborough, UK). This included replacing a small amount of corn.

numerical ($P = 0.075$) but no statistical decrease in ESBS over the ST as the hens aged. There was no linear or quadratic response ($P > 0.10$) to ETY inclusion on EST. However, EST did peak at 0.427 mm during the 6-wk sampling period displaying a week effect ($P = 0.052$) of ETY. In terms of the eggshell calcium deposition rate, there were no linear or quadratic responses ($P > 0.05$) amongst the ETY inclusion rates and no differences ($P > 0.10$) among ST. The average marketable sizes of the eggs across the diets improved with incorporating ETY. Supplemental ETY decreased the number of medium ($P = 0.041$) and large eggs ($P = 0.013$) and increased the proportion of extra-large eggs laid ($P = 0.007$; Table 4). The number of eggs laid in the medium and large categories both experienced quadratic decreases ($P = 0.003$), while the quantity of extra-large eggs linearly increased ($P = 0.027$) and quadratically ($P < 0.001$).

The effects of the ETY inclusion on BW change, spleen, liver, and bursa of Fabricius weight and bone ash content are shown in Table 5. There was no mortality recorded during the trial. There was a quadratic tendency ($P = 0.06$) for reduction in BW loss in response to ETY. The BW change was −4.84, −3.48, −0.44, −1.05, and +0.08% for 0.0, 0.025, 0.05, 0.10, and 0.20% ETY, respectively (Table 5). There were no ($P > 0.10$) ETY effects on spleen weight. There was a tendency for a linear increase in the liver ($P = 0.09$) and a decrease in bursa ($P = 0.07$) weights in response to ETY. Diet had no ($P > 0.05$) effects on bone ash content. In terms of blood plasma metabolites (Table 6), the plasma calcium levels increased ($P < 0.01$) linearly and quadratically in response to ETY. Total protein and albumin showed quadratic increases to ETY of $P = 0.034$ and $P = 0.049$, respectively. There were linear ($P = 0.02$) and quadratic ($P = 0.01$) decreases in the plasma glucose levels. Plasma concentration of aspartate aminotransferase tended to increase linearly ($P = 0.06$) in response to ETY. The ETY had a tendency ($P \leq 0.09$) for a quadratic increase in the plasma concentration of uric acid and a decrease in the plasma concentration of potassium. The inclusion of ETY in the layer diets had no effects ($P > 0.05$) on cecal SCFA concentration (data not shown).

DISCUSSION

According to Lohmann LSL Lite standards, the HDEP is 96.2% at 30 WOA and 95.9% at 42 WOA (Lohmann Breeders, 2020). The present study observed HDEP of 97.8, 98.1, 95.9, 94.6, and 94.0 for 0.0, 0.025, 0.05, 0.10, to 0.20% ETY, respectively. This showed that increasing ETY in laying hen diets up to 0.05% could keep the hens at or above breed standards. Previous studies showed up to 0.05% YCW in hen diets slightly improved or had no effect on HDEP compared to the Control diet (Koiyama et al., 2018). Little research has looked at the impact of higher inclusion of yeast metabolites on HDEP. However, it has been found that inclusion levels of 0.09% negatively affected HDEP (Koiyama et al., 2018) which is in line with our current

Table 5. Effects of feeding enzymatically treated yeast on body weight change, organ weights, and pooled tibia and femur ash content in LSL-lite hens at 42 wk of age.

	Body weight		Organ weight, g/kg BW			
ETY[1] inclusion, %	Initial, kg/bird	Change, %[2]	Spleen	Liver	Bursa	Bone[3] ash content, %
0.00	1.67	−4.84	0.910	21.0	11.1	46.1
0.025	1.71	−3.48	0.950	22.5	9.54	45.8
0.05	1.75	−0.440	0.900	23.6	8.99	45.5
0.1	1.70	−1.05	0.960	22.4	10.4	45.2
0.2	1.69	0.080	0.860	21.4	10.0	45.1
SEM	0.020	1.77	0.040	1.05	0.860	0.820
P-values						
Diet	0.148	0.243	0.466	0.449	0.412	0.902
Response to ETY						
Linear	-	0.133	0.934	0.094	0.071	0.504
Quadratic	-	0.060	0.889	0.300	0.168	0.405

[1]Enzymatically treated whole non-GMO *Saccharomyces cerevisiae* strain, 40% β-1.3/1.6 glucans and mannan oligosaccharides and 36% crude protein (Livalta Cell HY40, Livalta, Peterborough, UK). This included replacing a small amount of corn.

[2]Calculated as the difference between final and initial body weight expressed as a percentage.

[3]Pooled tibia and femur.

Table 6. Effects of feeding hens enzymatically treated yeast (ETY) on plasma metabolites in 42 wk old LSL-Lite hens after 12 wk of feeding.

	ETY[1] inclusion, %						*P*-value		
							Diet	Response to ETY	
Item	0.00	0.025	0.05	0.10	0.20	SEM		Linear	Quadratic
Total protein, g/L	51.4	59.6	54.3	56.1	56.9	2.15	0.110	0.091	0.034
Albumin (A), g/L	18.0	20.6	19.5	19.6	20.0	0.850	0.282	0.084	0.049
Globin (G), g/L	33.4	39.0	34.8	36.5	36.9	2.05	0.373	0.282	0.148
A: G ratio	0.550	0.550	0.570	0.550	0.550	0.040	0.996	0.761	0.892
Glucose, mmol/L	14.1	13.1	13.4	13.1	13.4	0.280	0.057	0.022	0.009
Cholesterol, mmol/L	2.66	2.91	2.28	2.44	2.30	0.280	0.471	0.622	0.498
Total Bilirubin, μmol/L	0.250	0.250	0.380	0.250	0.000	0.150	0.519	0.661	0.663
Bile Acid, μmol/L	34.1	53.8	35.9	35.1	40.6	5.27	0.127	0.253	0.246
Uric Acid, μmol/L	213.0	270.0	238.0	239.0	283.0	23.6	0.284	0.255	0.088
Gamma Glutamyl transferase, U/L	114.0	110.0	94.1	109.0	113.0	22.1	0.969	0.602	0.813
Aspartate Amino transferase, U/L	145.0	275.0	237.0	180.0	166.0	41.5	0.190	0.060	0.232
Creatine Kinase, U/L	1,109.0	2,280.0	1,921.0	1,236.0	1,111.0	572.0	0.483	0.200	0.518
Amylase, U/L	276.0	289.0	291.0	267.0	303.0	20.7	0.778	0.589	0.544
Lipase, U/L	9.00	9.13	10.4	9.25	8.38	1.07	0.762	0.476	0.934
Lactate Dehydrogenase, U/L	231.0	798.0	570.0	254.0	243.0	209.0	0.235	0.137	0.450
Calcium, mmol/L	6.45	9.08	8.68	7.98	9.33	0.650	0.027	0.007	0.002
Phosphorus, mmol/L	2.03	2.27	2.06	1.98	2.16	0.190	0.826	0.673	0.666
Sodium, mmol/L	150.8	150.1	151.4	151.4	151.9	1.31	0.897	0.899	0.699
Potassium, mmol/L	6.81	6.49	6.14	6.01	6.06	0.310	0.324	0.157	0.068
Chloride, mmol/L	113.6	112.9	115.9	114.5	115.1	1.12	0.363	0.378	0.403
Bicarbonate, mmol/L	17.5	17.9	16.6	18.3	18.5	0.850	0.572	0.670	0.644

[1]Enzymatically treated whole non-GMO *Saccharomyces cerevisiae* strain, 40% β-1.3/1.6 glucans and mannan oligosaccharides and 36% crude protein (Livalta Cell HY40, Livalta, Peterborough, UK). This included replacing a small amount of corn.

findings where 0.1% and 0.2% ETY negatively (quadratic response) affected the HDEP. Additionally, the decrease in the HDEP with increased ETY inclusion could correlate to the increase in EW in response to ETY due to the increased metabolic demands of larger EW, which likely caused the decrease in overall egg production. A known negative relationship exists between EW and egg production, and a genetic correlation links the 2 factors (Akbas and Takma, 2005). The decreased HDEP through the inclusion of ETY contradicts the current literature surrounding yeast products fed to laying hens, as yeast autolysate and yeast cell walls included in laying hen diets increased egg production (Hashim et al., 2013). Studies show that YCW at a 0.05% inclusion increased EW by 2 g in the early lay period and 1 g in the late lay period over the Control diets (Gurbuz et al., 2011). As EM is the relationship between EW and HDEP, EW increases as HDEP decreases with increased levels of ETY supplementation, causing the EM to remain relatively stable. Since both EW and HDEP were improved with ETY at 0.025%, this related to an increased EM. This is in line with the current literature, which shows that the inclusion of YCW at 0.045% led to a peak in HDEP and a quadratic relationship (Koiyama et al., 2018). The findings of the present study were also in agreement with Koiyama et al. (2018), as HDEP peaked at 0.025% ETY and decreased quadratically with further supplementation.

Supplemental ETY slightly increased dietary CP, as ETY has greater CP levels than the corn it replaced (Patterson et al., 2023). Consequently, it cannot be concluded that the increase in EW directly responds to ETY inclusion, as it also increased FI and overall CP consumption, which impacts EW. Laying hens fed the same yeast strain (*Saccharomyces cerevisiae)* showed increased CP digestibility by roughly 3%, with the inclusion of 0.2%, which further increased the CP available for egg production (Hameed et al., 2019). As the CP intake levels increased with ETY inclusion, this may explain the differences in egg results found in the present study. Robinson and Kiarie (2019) reported direct increases in EW linked to higher concentrations of CP, with the CP increasing the albumen content. It was found that decreasing CP led to decreases in overall EW, HDEP and a 2% decrease in the proportion of albumen (Zhou et al., 2021). As a result, the observed increase in EW may be a side-effect of the ETY increasing the overall CP intake. Moreover, ETY may have impact on amino acids metabolism as indicated by egg albumen, liver weight, the plasma concentration of total protein, albumin, aspartate transferase, and uric acid.

As reported in previous studies feeding yeast bioactives (Zhang et al., 2020), ETY did not negatively impact the eggshell quality and, in fact, increased EST and ESBS numerically. Despite the decreased yolk content noted with ETY inclusion, the actual yolk weight in grams did not differ between treatments. An increase in albumen weight associated with decreased yolk weight has previously been reported with YCW inclusion (Koiyama et al., 2018). The albumen increase could be due to the increased CP intake across ETY treatments (Zhou et al., 2021), resulting in increased amino acid (**AA**) assimilation. The ETY contains functional proteins, and the amount of albumen correlates with increases in total immunoglobulins, such as IgA, found in egg albumen (Lu et al., 2019). Although Lu et al. (2019) reported that yeast bioactives increased yolk-bound IgA in breeder eggs, the present study analyzed for albumin-bound IgA, which may have

influenced the results. Since ETY contains high levels of β-glucans (Lu et al., 2019) and β-glucans have been reported to stimulate and support the immune system in poultry (Lu et al., 2019; Zhen et al., 2020), it was expected that the diets would have impacted both the bursa and spleen. Previous studies have shown that immune-stimulation with β-glucan and yeast inclusion results in either a larger bursa, spleen, or both in both broilers and breeder birds (Wang et al., 2022). The lack of ETY effects on spleen weight and a tendency for reducing bursa weight in the present study suggested ETY did not trigger an immune response in laying hens or, due to the length of the study, the hens adapted to the ETY inclusion. The tendency for heavier livers in ETY hens aligned with trends for increased feed intake and maintenance of BW.

Tibia and femur have both cortical and medullary bone properties (Robison and Karcher, 2019). The present data indicated ETY had no impact on these medullary bones and the calcium needed in egg production. The bone ash also correlates with bone-breaking strength in laying hens (Robison and Karcher, 2019; Alfonso-Carrillo et al., 2021; Hanlon et al., 2022). As hens kept in cages are at risk for cage layer fatigue and osteoporosis, this can be a significant animal welfare concern for the birds. The symptoms of these issues are weak and brittle bones, which increase producers' culls and mortality (Riddell et al., 1968; Attia et al., 2020). As the ash content was uniform across all ETY treatments, this indicates uniform bone health, and no symptoms of cage layer fatigue and osteoporosis were experienced. This helps to conclude that ETY does not increase the prevalence of cage layer fatigue or osteoporosis in laying hens (Riddell et al., 1968; Attia et al., 2020).

The increased plasma calcium concentration with ETY supplementation was unexpected, as there was no statistical difference in calcium deposition rate into the eggshell. Furthermore, the diets were formulated to meet or exceed calcium requirements, so the eggshell calcium deposition and bone ash did not differ. This indicates that additional calcium is absorbed from the diet rather than the medullary bones (Robison and Karcher, 2019). Since the ETY inclusion increased the calcium in the bloodstream, the ETY directly increased calcium absorption, altered the gut to improve calcium absorption, or was an effect of the increased FI. Since a positive relationship exists between excess calcium and uric acid formation (Jarrar et al., 1996), the increased blood calcium levels with ETY were likely responsible for the corresponding increase in uric acid levels. In addition, uric acid can also become elevated due to dietary nucleic acids, which indicates a toxicological effect (Kiarie et al., 2020). Interestingly, there has been a proven relationship in poultry with higher CP levels decreasing the blood uric acid levels linearly, which was not the case in this trial (Kuritza et al., 2022). Thus, the increased uric acid levels observed under ETY diets may result from increased calcium absorption and improved absorption of nucleic acids.

Cecal fermentation is highly dependent on the availability of luminal products of digestion that reach the ceca, exposing them to microbial activities. In addition, such fermentation may result in short-chain fatty acids, which enhance calcium uptake in the hindgut and provide energy when emptied into the lumen. Studies in broilers and pigs using ETY products tested in the present study have shown no effects on cecal fermentation (Christensen et al., 2022; Kiarie et al., 2022; Maina et al., 2022). The results of the present study may also be highly attributed to the physiological stage and age of the study hens; peak-producing hens utilize all available energy for production purposes, and therefore there may be no available cecal metabolites due to the high utility factor at the time of sampling.

With an estimated CAD 5/kg for the ETY, inclusion resulted in added production costs of \$1.25, \$2.50, \$5.00, and \$10.00/ton of feed for the >0.025% ETY. Given the FI of 101.9, 104.6, 104.0, 103.4, and 103.5 g/bird per day for 0.0, 0.025, 0.05, 0.10, and 0.20% ETY, respectively. If ETY was fed for 10 wk, this would cost producers an additional \$9.15, \$18.20, \$36.19, and \$72.45 per 1,000 hens housed, for 0.0 or 0.025, 0.05, 0.10, or 0.20% ETY, respectively. In Ontario, Canada (as of 17 July 2022), the producer price per dozen Grade 'A' eggs is \$1.95 for small, \$2.33 for medium, and \$2.63 for large, extra-large, and jumbo eggs (EFC. 2022; EFO. 2022). Therefore, there is the potential for producers to economically benefit from the added feed costs due to increases in EW and price points for the various egg classes based on their current market. These market classes are set for different weight ranges and vary among regions of the world, along with different price premiums for size. Consequently, based on these market factors, producers and nutritionists would have to decide whether ETY supplementation would benefit their overall profitability and individual ideal inclusion level. These additional profits to consumers do not factor in the potential for more eggs to reach the market due to the numerical increases that ETY has on ESBS and EST, as there is a positive correlation between these factors and a reduction in cracked eggs (Bain, 2005; Mertens et al., 2006). However, the decreased HDEP due to high levels of ETY inclusion must also be factored into producer decisions on the possible economic benefits.

In conclusion, although higher (>0.1%, quadratic response) doses of ETY reduced egg production rate, this was within the expected production for hens of this age and breed. Furthermore, the data showed that ETY supplementation could positively affect egg weight and eggshell quality linked to protein and calcium metabolism modulation. The 0.025% ETY diets resulted in the highest EM and indices of eggshell quality (ESBS and EST), with 96.9% of eggs falling in the highest paying >56 g classification range, suggesting fewer damaged and cracked eggs and more eggs reaching the market. However, given that the ETY was only fed for 12 wk, the long-term effects of ETY on the longevity of laying hens should be further studied.

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DISCLOSURES

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. H. Schulze is an employee of AB Agri Ltd.

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