Research Article

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Dietary live yeast supplementation alleviates transport-stress-impaired meat quality of broilers through maintaining muscle energy metabolism and antioxidant status

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Abstract

BACKGROUND: This experiment was to investigate the effect of dietary live yeast (LY, 1×10^{10} CFU g^{-1}) supplementation on serum metabolic parameters, meat quality as well as antioxidant enzyme activity of transported broilers. A total of 192 one-day-old broilers were randomly assigned to four treatments with six replicates and eight chicks per replicate: a basal diet without transportation (CON), a basal diet containing 0 (T), 500 (T + LY_{500}) and 1000 mg kg^{-1} (T + LY_{1000}) LY with 3 h of transportation after feeding for 42 days, respectively. The serum and muscle samples of broilers were collected immediately after 3 h of transportation.

RESULTS: A higher ($P < 0.05$) final body weight and average daily weight gain were observed in T + LY_{1000} group compared with CON and T groups. The T + LY_{1000} group reduced ($P < 0.05$) the serum lactate contents and improved ($P < 0.05$) the pH_{24h} and decreased ($P < 0.05$) the drip loss in muscles of transported-broilers. Also, the T + LY_{1000} group enhanced ($P < 0.05$) the total-antioxidant capacity and reduced ($P < 0.05$) the malondialdehyde in serum and muscles. Besides, the messenger RNA (mRNA) expression of avian uncoupling protein (avUCP) in muscles was down-regulated ($P < 0.05$) of T + LY_{1000} group compared with T group.

CONCLUSION: Dietary LY supplementation alleviates transport-stress-impaired meat quality of broilers through maintaining muscle energy metabolism and antioxidant status. Therefore, LY may serve as a potential protector for broilers under transport stress in the future.

Keywords: live yeast; broilers; transport stress; meat quality; antioxidant status

INTRODUCTION

Transport stress is a complex stress which is usually combined with other stressors like catching, uploading and offloading of birds into vehicles without food and water.[1] Transport stress has been considered as one of the important factors influencing broiler physiological and metabolic changes[1] and meat quality.[2,3] The entire poultry production system includes a series of phases and the meat quality is related with postmortem modifications including pre-slaughter period, slaughter and processing.[4] The reactive oxygen species (ROS) level can be dramatically increased, which could lead to oxidative stress.[5,6] As a result, body weight (BW) loss, lower feed conversion efficiency, higher physical injuries and increased disease susceptibility ultimately triggered a higher mortality of broilers.[7-9] Furthermore, oxidation is associated with adverse effects on flavor, color, nutritional value, and may lead to the emergence of toxic compounds in meat,[10] resulting in economic losses in the animal industry and reducing consumer acceptability.[11] The total yield and quality of meat might depend on environmental factors during transportation of poultry.[12] Transport stress of poultry is usually related to feed withdrawal, which might have a negative impact on the health and meat quality of poultry.[4] The reserve of glycogen in the body can be

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exhausted by prolonged feed withdrawal as related to transportation stress, which is responsible for the higher pH of muscle fiber, higher drip loss with paler meat color.[4] Also, transportation stress had a great influence on the live weight and hot carcass weight of pigs.[13] Currently, scholars and producers are looking for an effective way to minimize the stress response and enhance the meat quality of transported broilers. Previous research documented that dietary supplementation with *Forsythia suspensa* extract,[2] ascorbic acid,[14] oregano essential oil[11] were effective methods to alleviate the stress response and enhance the meat quality of transported-broilers.

Live yeast (LY) is one of the most promising microbial derived products, which is usually used in animal diets.[15] It is well-known that LY is a feed additive that contains high amounts of non-starch polysaccharides as well as mannose and glucan. Supplementation of LY in diets can contribute to the improvement of animal intestinal health by regulating intestinal structure and inhibiting enteropathogenic bacterium.[16-18] Ahiwe *et al.*[19] revealed that diets supplemented with 1.5 to 2 g kg^{-1} levels of whole yeast and yeast cell wall enhanced growth performance and production of meat in broilers. Moreover, the addition of 5 g kg^{-1} yeast culture to the diet showed a higher weight gain by the improvement of nutrient retention and intestinal morphology in pigs.[20] Besides, inclusion of 5 g kg^{-1} LY in diets has been noted to improve the meat tenderness and reduce the oxidation effects of broilers.[15] However, there is little research that has been documented regarding the role of LY in transported broilers. Hence, the current study was conducted to investigate the effect of dietary supplementation with LY (*Saccharomyces cerevisiae*) on serum metabolites, antioxidant enzyme activity and meat quality of broilers under transport stress, which may provide an alternate strategy for transported-broilers.

MATERIAL AND METHODS

Experimental product

The commercial product of LY (*S. cerevisiae*, strain CNCM I-4407, 10^{10} CFU g^{-1}) applied in our study was provided by the Lesaffre Feed Additives (Marcq-en-Baroeul, France).

Diets, birds and experimental design

A total of 192 one-day-old male chicks (Arbor Acres, weighted 45.2 ± 0.46 g, obtained by the Arbor Acres Poultry Breeding (Beijing, China)) were randomly distributed to four experimental diets, consisting of the non-transported broilers fed a basal diet without LY (CON), and the transported-broilers supplemented a basal diet with LY at 0 (T), 500 mg kg^{-1} ($T + LY_{500}$) and 1000 mg kg^{-1} ($T + LY_{1000}$), respectively. Each treatment involved six replicates of eight broilers each replicate.

All broilers were raised in wire-floored cages with automatic temperature control system for 42 days (first 3 days kept at 33 °C, then decreased by 3 °C weekly until the temperature reached 24 °C), the broilers were exposed to continuous light (10 to 20 lux) and allowed *ad libitum* food and water. The real-time monitoring of light and ventilation was performed during the experiment. Furthermore, broilers were vaccinated against Newcastle disease at the first and fourth weeks and inactivated infectious bursal disease vaccine at the second and third weeks. The experiment was divided into two phases: starter (day 1 to day 21) and grower (day 22 to day 42). The dietary composition and nutritional levels are summarized in Table 1, which satisfied or excelled the requirements of NRC (1994).

On the morning of day 42, all birds were fasted 8-h overnight before, BW and feed consumption on a replicate basis were recorded to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR; ADFI:ADG). Then, a total of 144 transported-broilers were placed into 18 crates (0.80 m × 0.80 m × 0.50 m), respectively (six crates per treatment). Each treatment involved six crates of eight chicks each. All 18 crates were randomly positioned in the same lorry (70 km h^{-1}, the inside temperature was maintained 25.5 ± 1.5 °C with 71.6 ± 2.8% relative humidity). The transport time was from 06:00 to 09:00 a.m. Neither feed nor water was available during the transportation.

Sampling and procedures

All chickens were weighed before and after transportation to calculate the live weight loss. After transportation, one bird with near mean BW were chosen (include CON) from each replicate and slaughtered immediately by bleeding through the left carotid artery. Approximately 5 mL of blood was collected into a 10 mL anticoagulant-free vacutainer tube (Greiner Bio-One GmbH, Kremsmunster, Austria). The serum was collected by centrifugation (3000 × *g* for 10 min at 4 °C), then kept at −20 °C until analysis. Additionally, samples of the entire right breast and right thigh muscles of slaughtered broilers were sampled for determination of pH, meat color and drip loss. Some of the muscle samples were immediately placed in RNAase-free tubes, frozen in liquid nitrogen, and preserved at −80 °C for the further measurement of antioxidant enzyme activity and messenger RNA (mRNA) expression related to the stress response.

Serum metabolites

Serum levels of glucose, uric acid and non-esterified fatty acids (NEFAs) were analyzed using colorimetric methods by automatic biochemistry analyzer (Hitachi 902 Automatic Analyzer; Hitachi, Tokyo, Japan). The level of serum lactate was determined using BioSystems kits. The level of corticosterone was analyzed using an enzyme immunoassay kit (Cusabio Biotech, Wuhan, China). The reagent kits were obtained from Nanjing Jiancheng Institute of Biological Engineering (Nanjing, China) and operated by strict following of the instructions of the kits.

Meat quality

Muscle pH was recorded at 45 min and 24 h after slaughter, respectively. The pH meter (Cyberscan PH310; EUTECH, Singapore) was pricked into the breast and thigh muscles (approximately 1 cm) and recorded. The pH meter was calibrated with standard buffers of pH 4.0 and pH 7.0 before use. Each sample was measured twice, then calculated the mean value and ΔpH ($\Delta pH = pH_{45min} - pH_{24h}$).[21] The lightness ($L^*$), redness ($a^*$), and yellowness ($b^*$) values were determined in the central part of the breast and thigh muscles using a colorimeter (Shanghai Precision Scientific Instruments, Shanghai, China). Each sample was measured three times for the same part and the mean value was calculated. The regular shaped breast and thigh muscles were placed in sealing bags, filled with nitrogen to expand the bags to avoid evaporation and oxidation (minimize the contact between the meat samples and the inner wall of the bags), then suspended in a refrigerator at 4 °C, and the samples were removed after 24 h.[22] The calculated formation of drip loss as follow:

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Table 1. Composition and nutrient levels of basal diets (%, as-fed basis)

Ingredients	Starter (days 1–21)	Finisher (days 22–42)
Corn	58.17	64.26
Soybean meal	30.44	24.05
Corn gluten meal	2.00	2.50
Fish meal	2.00	2.00
Soybean oil	3.38	3.60
Dicalcium phosphate	1.50	1.04
Limestone	1.30	1.35
Salt	0.30	0.30
L-Lysine	0.01	0.08
Methionine	0.14	0.04
Threonine	0.01	0.03
Chromium oxide	0.25	0.25
Premix[a]	0.50	0.50
Calculated nutrient levels		
Metabolizable energy (MJ kg^{-1})	12.76	13.17
Crude protein	21.00	19.00
Calcium	1.00	0.90
Standardized ileal digestible lysine	0.86	0.73
Standardized ileal digestible methionine	0.30	0.28
Standardized ileal digestible threonine	0.63	0.56
Standardized ileal digestible tryptophan	0.28	0.24
Analyzed nutrient levels		
Dry matter	88.20	87.31
Crude protein	21.34	19.42
Calcium	1.01	0.89
Phosphorus	4.56	3.47
Lysine	1.08	0.92
Methionine	0.53	0.39
Threonine	0.82	0.75
Tryptophan	0.29	0.24

[a] Provided g kg^{-1} of the complete diet: retinylacetate, 4500 IU; cholecalciferol, 1200 IU; DL-α-tocopherylacetate, 2500 IU; thiamin, 5000 mg; riboflavin, 20 000 mg; phylloquinone, 10 000 mg; niacin, 45 000 mg; pantothenicacid, 35 000 mg; biotin, 1500 mg; folicacid, 3000 mg; cyanocobalamin, 40 mg; zinc, 45 mg; manganese 50 mg; iron, 30 mg; copper, 4 mg; cobalt,120 g; iodine, 1 mg; selenium, 120 g.

$$\text{Drip loss} = [(\text{Initial weight} - \text{Final weight})/\text{Initial weight}] \times 100\%.$$

Glycolytic metabolite and antioxidant enzyme activity

Muscle glycogen and lactate levels were measured using the method described by Hambrecht *et al.*[23] and modified by Zhang *et al.*[21] Briefly, approximately 0.5 g of sample was put into a 10 mL centrifuge tube, and 0.85 mol L^{-1} perchloric acid ($HClO_4$) pre-chilled at 4 °C was added at a ratio of 9:1, then homogenized in an ice bath (25 000 × *g* for 1 min). The homogenate was centrifuged (3000 × *g* at 4 °C for 10 min) and the supernatant was extracted. The supernatant was neutralized with 10 mol L^{-1} potassium hydroxide (KOH) solution, and about 1 mL was preserved at −80 °C for lactic acid analysis. The remaining supernatant was incubated with

Table 2. Sequence for real-time polymerase chain reaction primers

Genes	Primer sequence (5′-3′)	Product size (bp)	GenBank No.
avUCP	F: GAGAAACAGAGCGGGATTTGAT R: GCTCCTGGCTCACGGATAGA	90	NM204107
avANT	F: GGAGCCACTTCCCTCTGCTT R: CGGTCCCCGAGACCAGAGAA	110	NM204231
avPGC-1α	F: CCTGGGTGGCAGTGTCAGAT R: GCTTATTCAGTTCAGCCCGAAT	145	NM001006457
β-actin	F: ATCCGGACCCTCCATTGTC R: ATCCGGACCCTCCATTGTC	120	NM-205518

F, forward primer; R, Reverse primer; avUCP, avian uncoupling protein; avANT, avian adenine nucleotide translocator; avPGC-1α, avian peroxisome proliferator-activated receptor-γ coactivator-1α; β-actin, avian β-actin.

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Table 3. Effects of dietary supplementation with live yeast (LY) on growth performance of broilers

Items	Treatments				SEM	*P*-Value
	CON	T	T + LY_{500}	T + $LY_{1,000}$		
BW at 1 day (g)	44.4	45.1	46.2	45.2	0.64	0.288
BW at 42 day (g)	2010^{b}	1985^{b}	2188^{ab}	2271^{a}	66.5	0.016
ADG (g)	46.8^{b}	46.2^{b}	51.0^{ab}	53.0^{a}	1.59	0.017
ADFI (g)	69.8	69.9	73.6	75.9	3.08	0.430
FCR	1.49	1.51	1.44	1.43	0.032	0.326

All measurements are presented by mean values and standard error of the mean (SEM) ($n = 8$).
Means within a row with different superscript lowercase letters differ significantly ($P < 0.05$).
CON, broilers fed the basal diet and no transport; T, T + LY_{500} or T + LY_{1000}, broilers were fed the basal diet supplemented with live yeast at 0, 500 or 1000 mg kg^{-1} respectively, and experienced 3 h pre-slaughter transport.
SEM are pooled SEM.
BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio (ADFI:ADG).

glucoamylase (A7420; Sigma, St Louis, MO, USA) incubation solution (pH 4.8) at 55 °C for 2 h to hydrolyze glycogen to glucose. The incubation solution was neutralized with 10 mol L^{-1} KOH solution and stored at −80 °C for glycogen analysis. The glycolytic potential (GP) was calculated as follows: GP = 2 × [glycogen] + [lactate].[24] The activities of hexokinase (HK), pyruvate kinase (PK) and lactate dehydrogenase (LDH) were determined using commercial kits, then calculated according to the methods of previous studies.[25-27]

The concentration of total antioxidant capacity (T-AOC), malondialdehyde (MDA), catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) in serum and muscles via spectrophotometry (PU 8720 UV/vis scanning spectrophotometer; Pye Unicam, Cambridge, UK). All commercial assay kits were provided by Nanjing Jiancheng Institute of Biological Engineering (Nanjing, China) and all protocols of the kits were followed.

Real-time polymerase chain reaction

The total RNA was extracted from frozen breast muscle samples referring to the instruction manual of Trizol Reagent, and the optical density (OD) values and RNA concentration were determined on a nucleic acid analyzer (NanoDrop ND-1000 spectrophotometer; Thermo Fisher Scientific, Waltham, MA, USA). The integrity of RNA was examined by 1% agarose gel electrophoresis, and then reverse transcription was performed. The amounts of mRNA were quantified by iCycler iQ multicolor real-time polymerase chain reaction (PCR) detection system (Bio-Rad Laboratories, Hercules, CA, USA). A 20-μL PCR mixture including 1 μL complementary DNA (cDNA), 1 μL primer (10 pmol mL^{-1}), 10 μL 2× SYBR Green quantitative PCR master mix, 7 μL double-distilled water. The PCR reaction: 95 °C for 10 min, 95 °C for 15 s (40 cycles), 60 °C for 60 s. The SYBR green fluorescence was determined at the end of each cycle. The melting curve is recorded at 60 °C. The primer sequence of avian uncoupling protein (avUCP), avian adenine nucleotide translocator (avANT) and avian peroxisome proliferator-activated receptor-γ coactivator-1α (avPGC-1α) are presented in Table 2 (**β**-actin as a housekeeping gene**),** which were designed by Ouyi Biotech (Shanghai, China) and synthesized by TsingKe Biotech (Beijing, China). All determinations were performed in triplicate and the mean values were recorded, then normalized with mRNA expression of β-actin and calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

The original values were initially processed by Excel (Microsoft, Redmond, WA, USA). Statistical analysis of the data was carried out by the SAS procedure (version 9.2; SAS Institute, Cary, NC, USA). All values were analyzed by using analysis of variance (ANOVA) and Tukey's *post hoc* test to compare treatments means. Data were expressed as mean and standard error of the mean (SEM). Differences were regarded as statistically significant at $P < 0.05$ unless otherwise noted.

RESULTS

Growth performance and body weight loss of transported broilers

Compared with CON and T groups, the T + LY_{1000} group had a higher ($P < 0.05$) BW on day 42 and a higher ADG of broilers (Table 3).

Table 4. Effects of dietary supplementation with live yeast (LY) on live body weight (BW) loss of transported-broilers

Items	Treatments				SEM	*P*-value
	CON	T	T + LY_{500}	T + $LY_{1,000}$		
BW before transport (g)	2010^{b}	1985^{b}	2188^{ab}	2271^{a}	66.5	0.016
BW after 3 h transport (g)	1976^{b}	1931^{b}	2140^{ab}	2224^{a}	64.9	0.014
BW loss (g)	35.6^{b}	54.0^{a}	48.1^{ab}	46.9^{ab}	3.99	0.025
BW loss/BW before transport (g g^{-1})	1.77^{b}	2.72^{a}	2.20^{ab}	2.07^{b}	0.173	0.004

All measurements are presented by mean values and standard error of the mean (SEM) (i = 8).
Means within a row with different superscript lowercase letters differ significantly ($P < 0.05$).
CON, broilers fed the basal diet and no transport; T, T + LY_{500} or T + LY_{1000}, broilers were fed the basal diet supplemented with live yeast at 0, 500 or 1000 mg kg^{-1} respectively, and experienced 3 h pre-slaughter transport.
SEM are pooled SEM.

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Table 5. Effects of dietary supplemented with live yeast (LY) on serum metabolite parameters of broilers experienced pre-slaughter transport

Items	Treatments				SEM	*P*-Value
	CON	T	T + LY_{500}	T + $LY_{1,000}$		
Corticoserone (ng mL^{-1})	29.2^{b}	38.1^{a}	32.4^{ab}	30.9^{ab}	1.45	0.004
Uric acid (umol L^{-1})	121	133	125	130	3.7	0.149
Glucose (mmol L^{-1})	7.93^{a}	5.73^{c}	6.38^{bc}	7.71^{ab}	0.38	0.002
Lactate (mmol L^{-1})	0.68^{ab}	0.88^{a}	0.69^{ab}	0.62^{b}	0.058	0.034
Non-esterified fatty acids (mmol L^{-1})	0.63	0.47	0.49	0.59	0.047	0.118
DPPH radical scavenging activity (%)	82.4^{a}	65.4^{b}	70.7^{ab}	77.0^{ab}	3.87	0.037

All measurements are presented by mean values and standard error of the mean (SEM) ($n = 6$).
Means within a row with different superscript lowercase letters differ significantly ($P < 0.05$).
CON, broilers fed the basal diet and no transport; T, T + LY_{500} or T + LY_{1000}, broilers were fed the b asal diet supplemented with live yeast at 0, 500 or 1000 mg kg^{-1} respectively, and experienced 3 h pre-slaughter transport.
SEM are pooled SEM.
DPPH, 2,2-diphenyl-1-picrylhydrazyl.

A higher ($P < 0.05$) BW loss was observed in T group compared with the broilers without transportation, the T + LY_{1000} group lowered ($P < 0.05$) the BW loss in transported-broilers (Table 4).

Serum metabolite parameters

Compared with CON group, the T group enhanced ($P < 0.05$) the contents of serum corticosterone and reduced ($P < 0.05$) the concentrations of glucose and the activity to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals. The T + LY_{1000} groups enhanced ($P < 0.05$) the glucose content and reduced ($P < 0.05$) the levels of corticosterone and lactate of transported-broilers (Table 5).

Meat quality

In breast muscle, a higher ($P < 0.05$) lightness, ΔpH and drip loss and a lower ($P < 0.05$) pH_{24h} were noticed in T group compared with the broilers without transportation, the T + LY_{1000} group reduced ($P < 0.05$) the lightness and ΔpH, and enhanced ($P < 0.05$) the pH_{24h} in transported-broilers.

In thigh muscle of broilers, a higher ($P < 0.05$) ΔpH and drip loss, and a lower ($P < 0.05$) pH_{24h} were observed in T group compared

Table 6. Effects of dietary supplemented with live yeast (LY) on meat quality of broilers experienced pre-slaughter transport

Items	Treatments				SEM	*P*-Value
	CON	T	T + LY_{500}	T + $LY_{1,000}$		
Breast muscle						
*L**	45.5^{b}	48.9^{a}	46.8^{ab}	46.1^{b}	0.54	0.003
*a**	4.05	3.51	3.87	4.01	0.174	0.141
*b**	13.9	12.3	15.7	13.7	1.35	0.388
pH_{45min}	6.43	6.38	6.35	6.38	0.076	0.901
pH_{24h}	5.86^{a}	5.49^{c}	5.66^{bc}	5.76^{ab}	0.072	0.011
ΔpH	0.56^{b}	0.87^{a}	0.68^{ab}	0.62^{b}	0.064	0.006
Drip loss (g g^{-1})	1.13^{b}	2.28^{a}	2.18^{a}	1.83^{ab}	0.301	0.056
Thigh muscle						
*L**	48.5	49.9	48.0	49.6	1.17	0.618
*a**	3.75	3.17	3.28	3.52	0.204	0.231
*b**	15.1	15.3	15.2	16.8	0.73	0.334
pH_{45min}	6.38	6.30	6.33	6.37	0.102	0.939
pH_{24h}	5.80^{a}	5.43^{b}	5.73^{ab}	5.81^{a}	0.068	0.005
ΔpH	0.59^{b}	0.87^{a}	0.60^{ab}	0.56^{b}	0.065	0.015
Drip loss (g g^{-1})	1.45^{b}	2.93^{a}	2.23^{ab}	1.96^{b}	0.272	0.012

All measurements are presented by mean values and standard error of the mean (SEM) ($n = 6$).
Means within a row with different superscript lowercase letters differ significantly ($P < 0.05$).
CON, broilers fed the basal diet and no transport; T, T + LY_{500} or T + LY_{1000}, broilers were fed the basal diet supplemented with live yeast at 0, 500 or 1000 mg kg^{-1} respectively, and experienced 3 h pre-slaughter transport.
SEM are pooled SEM.
*L**, lightness; *a**, redness; *b**, yellowness; ΔpH, $pH_{45min} - pH_{24h}$.

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Table 7. Effects of dietary supplemented with live yeast (LY) on glycolytic potential (GP) in muscle of broilers experienced pre-slaughter transport

Items	Treatments				SEM	*P*-Value
	CON	T	T + LY_{500}	T + $LY_{1,000}$		
Breast muscle						
HK activity (U g^{-1} of protein)	14.6^{b}	16.3^{a}	15.9^{a}	15.4^{ab}	0.25	0.014
PK activity (U g^{-1} of protein)	11.6^{b}	12.8^{a}	12.0^{ab}	11.8^{b}	0.24	0.012
LDH (U g^{-1} of protein)	3.14^{b}	4.03^{a}	3.84^{ab}	3.52^{ab}	0.182	0.018
Lactate (μmol g^{-1})	72.4	80.9	79.1	76.4	2.88	0.223
Glycogen (μmol g^{-1})†	32.4	28.6	30.5	31.1	1.25	0.217
GP (μmol g^{-1})‡	137	138	140	139	3.9	0.958
Thigh muscle						
HK activity (U g^{-1} of protein)	18.0^{b}	20.5^{a}	19.6^{ab}	18.8^{ab}	0.48	0.023
PK activity (U g^{-1} of protein)	8.05	9.06	8.77	8.57	0.321	0.201
LDH (U g^{-1} of protein)	2.64^{b}	3.23^{a}	2.91^{ab}	2.73^{b}	0.11	0.014
Lactate (μmo g^{-1})	55.1	62.1	59.3	53.3	3.39	0.285
Glycogen (μmol g^{-1})†	25.2^{a}	20.1^{b}	20.1^{b}	22.9^{ab}	1.26	0.033
GP (μmol g^{-1})‡	102	105	100	99.1	4.3	0.721

All measurements are presented by mean values and standard error of the mean (SEM) ($n = 6$).
Means within a row with different superscript lowercase letters differ significantly ($P < 0.05$).
CON, broilers fed the basal diet and no transport; T, T + LY_{500} or T + LY_{1000}, broilers were fed the basal diet supplemented with live yeast at 0, 500 or 1000 mg kg^{-1} respectively, and experienced 3 h pre-slaughter transport.
SEM are pooled SEM.
HK, hexokinase; PK, pyruvate kinase; LDH, lactate dehydrogenase.
† Includes glucose, glucose-6-phosphate, and glycogen.
‡ Glycolytic potential = 2 × [Glycogen] + [Lactate].

with the broilers without transportation, the T + LY_{1000} group reduced ($P < 0.05$) the ΔpH and drip loss, and increased ($P < 0.05$) the pH_{24h} in transported-broilers (Table 6).

Glycolytic potential

In breast muscle, T group increased ($P < 0.05$) the activities of HK, PK and LDH compared with the broilers without transportation, the T + LY_{1000} group reduced ($P < 0.05$) the activity of PK in transported-broilers.

In thigh muscle, higher ($P < 0.05$) activities of HK and LDH and a lower ($P < 0.05$) concentration of glycogen were noted in T group. The T + LY_{1000} group decreased ($P < 0.05$) the activity of LDH in transported-broilers. No difference was observed on lactate and glycogen in breast and thigh muscle of broilers between treatments (Table 7).

Activity of antioxidant in serum and muscle

In serum of broilers, T group enhanced ($P < 0.05$) the MDA content and lowered ($P < 0.05$) the levels of T-AOC and SOD compared with the broilers without transportation. Dietary supplemented with LY reduced ($P < 0.05$) the level of MDA in transported-broilers. Also, the T + LY_{1000} group increased ($P < 0.05$) the contents of T-AOC in transported-broilers.

In breast muscle of broilers, a lowered ($P < 0.05$) level of T-AOC and an enhanced ($P < 0.05$) level of MDA was observed by T group compared with the broilers without transportation. Dietary supplemented with LY reduced the MDA content of transported-broilers. Also, T + LY_{1000} group improved ($P < 0.05$) the content of T-AOC in transported-broilers.

In thigh muscle of broilers, lowered ($P < 0.05$) levels of T-AOC and GSH-Px and an increased ($P < 0.05$) level of MDA was observed by T group compared with the broilers without transportation. The T + LY_{1000} group increased ($P < 0.05$) the levels of T-AOC and GSH-Px and reduced ($P < 0.05$) the level of MDA in transported-broilers (Table 8).

Relative mRNA expression of avUCP and avANT in muscles

Compared with the CON group, the T group up-regulated ($P < 0.05$) the mRNA expression of avUCP in breast and thigh muscles and down-regulated ($P < 0.05$) the mRNA expression of avANT in thigh muscle of broilers. The T + LY_{1000} group down-regulated ($P < 0.05$) the mRNA expression of avUCP in breast and thigh muscles in transported-broilers (Table 9).

DISCUSSION

A number of researches in poultry have been conducted to evaluate the function of LY, which indicated that LY is an effective feed additive which can improve growth performance and health of broilers.[15,16] Although He *et al.*[28] reported that dietary LY supplementation had no significant effects on ADG and FCR during the whole experimental period, Sousa *et al.*[29] found that the ADG and FCR during the finisher period were increased in broilers fed diets containing 6% yeast. In the present study, we found that dietary LY supplementation (1000 mg kg^{-1}) in broilers beneficially increased final BW and ADG, which indicates the improved growth performance.

With the continuous implementation of intensive farming, the transportation process of broilers from farms to slaughterhouses is faced with a multi-factor integrated stress process. Factors, such as pre-slaughter feeding restriction, catching, crating and transportation, could trigger serious stress for broilers.[3,30] Moreover, the weight loss during transportation is strongly correlated with the transportation time and distance.[31] In the current study, 3 h transported-broilers increased the BW loss, which is in agreement with the findings of previous studies.[2,9] Meanwhile, dietary LY at

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Table 8. Effects of dietary supplementation with live yeast (LY) on antioxidant capacity of serum and muscle in broilers experienced pre-slaughter transport

Items	Treatments				SEM	*P*-value
	CON	T	T + LY_{500}	T + $LY_{1,000}$		
Serum						
T-AOC (U mL^{-1})	16.5^{a}	12.6^{b}	15.1^{ab}	16.0^{a}	0.80	0.014
GSH-PX (U mL^{-1})	758	645	693	730	47.3	0.391
SOD (U mL^{-1})	73.2^{a}	52.5^{b}	63.7^{ab}	60.4^{ab}	3.80	0.012
CAT (U mL^{-1})	51.1	43.8	45.5	54.4	3.42	0.150
MDA (nmol mL^{-1})	7.95^{b}	10.64^{a}	8.75^{b}	7.48^{b}	0.422	< 0.001
Breast muscle						
T-AOC (U mg^{-1} of protein)	1.15^{a}	0.85^{c}	0.95^{bc}	1.09^{ab}	0.038	0.001
GSH-PX (U mg^{-1} of protein)	10.6	8.8	9.6	10.0	0.47	0.098
SOD (U mg^{-1} of protein)	36.8	34.0	33.8	36.2	1.49	0.386
CAT (U mg^{-1} of protein)	3.68	2.95	3.49	3.50	0.204	0.097
MDA (nmol mg^{-1} of protein)	1.52^{b}	1.72^{a}	1.53^{b}	1.54^{b}	0.043	0.052
Thigh muscle						
T-AOC (U mg^{-1} of protein)	1.33^{a}	0.90^{b}	1.06^{ab}	1.25^{a}	0.079	0.005
GSH-PX (U mg^{-1} of protein)	12.1^{a}	8.50^{b}	10.3^{ab}	11.8^{a}	0.63	0.004
SOD (U mg^{-1} of protein)	42.6	40.8	43.5	44.1	1.81	0.613
CAT (U mg^{-1} of protein)	6.69	5.82	5.85	6.15	0.293	0.165
MDA (nmol mg^{-1} of protein)	1.03^{b}	1.49^{a}	1.37^{a}	1.04^{b}	0.062	< 0.001

All measurements are presented by mean values and standard error of the mean (SEM) ($n = 6$).
Means within a row with different superscript lowercase letters differ significantly ($P < 0.05$).
CON, broilers fed the basal diet and no transport; T, T + LY_{500} or T + LY_{1000}, broilers were fed the basal diet supplemented with live yeast at 0, 500 or 1000 mg kg^{-1} respectively, and experienced 3 h pre-slaughter transport.
SEM are pooled SEM.
T-AOC, total antioxidant capacity; GSH-PX, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; MDA, malonaldehyde.

1000 mg kg^{-1} was noted to alleviate the weight loss of transported-broilers. A study has revealed that the primary sources of weight loss during transport are as follows: emptying of gastrointestinal chyme, dehydration of broilers, as well as oxidative decomposition of body composition.[32] In the present study, broilers were fasted for 8 h without water deprivation before transportation, thus we speculated that the weight loss might mainly originate from the decomposition of body composition. Additionally, antioxidants could prevent the oxidative decomposition of serum lipoproteins, then contribute to the efficiency of nutrient utilization for broilers.[33] The LY has antioxidant properties.[28,34] Therefore, the mitigation of weight loss during transportation by LY may be closely associated with the antioxidant properties of LY.

Table 9. Effects of dietary supplementation with live yeast (LY) on the relative messenger RNA (mRNA) expression of avian uncoupling protein (avUCP), avian adenine nucleotide translocator (avANT) and avian peroxisome proliferator-activated receptor-γ coactivator-1α (avPGC-1α) in muscle of broilers experienced pre-slaughter transport

Items	Treatments				SEM	*P*-Value
	CON	T	T + LY_{500}	T + $LY_{1,000}$		
Breast muscle						
avUCP	0.68^{b}	1.18^{a}	0.83^{ab}	0.71^{b}	0.092	0.008
avPGC-1α	1.29	0.93	1.12	1.30	0.124	0.132
avANT	1.31	0.96	1.11	1.19	0.128	0.236
Thigh muscle						
avUCP	0.69^{b}	1.12^{a}	0.85^{ab}	0.79^{b}	0.083	0.014
avPGC-1α	1.44	1.05	1.29	1.39	0.157	0.343
avANT	1.39^{a}	0.92^{b}	1.29^{ab}	1.30^{ab}	0.116	0.038

All measurements are presented by mean values and standard error of the mean (SEM) ($n = 6$).
Means within a row with different superscript lowercase letters differ significantly ($P < 0.05$).
CON, broilers fed the basal diet and no transport; T, T + LY_{500} or T + LY_{1000}, broilers were fed the basal diet supplemented with live yeast at 0, 500 or 1000 mg kg^{-1} respectively, and experienced 3 h pre-slaughter transport.
SEM are pooled SEM.

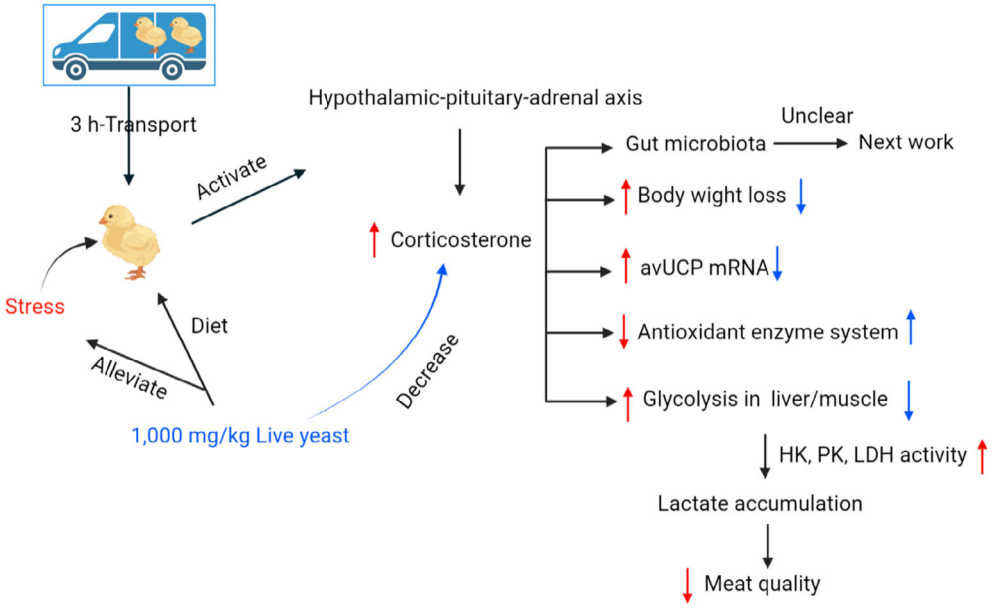

Figure 1. Summary of effect of dietary supplemented with live yeast (LY) for transported-broilers. Red arrows emphasize the effect of transport stress on broilers in our research. The blue arrows emphasize the effect of dietary supplementation with LY at 1000 mg kg^{-1} for transport-broilers. HK, hexokinase; PK, pyruvate kinase; LDH, lactate dehydrogenase.

The stress response of broilers can be characterized by serum metabolites. Serum corticosterone increased through the hypothalamic pituitary adrenocortical axis, which was activated by stressed-broilers.[32] Therefore, corticosterone is an essential indicator of the stress response in poultry.[35] In the current study, transportation induced a significant increase in serum corticosterone concentration in broilers, suggesting that transportation triggered a stress response in broilers. Glucose is the main source of energy for the body, and the liver is an essential organ for glycogen storage.[36] Under stress condition, hepatic glycogenolysis is enhanced, and the glucose produced enters the bloodstream to maintain the relative stability of blood glucose levels. When the liver glycogen stores are depleted, the catabolism of body lipids and body proteins are strengthened to maintain the energy requirements of the body, which is primarily reflected in the increased concentration of serum uric acid and decreased concentration of glucose.[37] Decreased muscle or serum glucose concentrations and increased lactate levels both indicate that the birds are under stress. Additionally, an increase in serum LDH activity was noticed [LDH reduces pyruvate produced by the glycolytic pathway to lactate and oxidizes NADH to produce adenosine triphosphate (ATP)], which further confirmed our hypothesis.[38] In the current research, dietary supplemented with LY at 1000 mg kg^{-1} decreased the level of serum corticosterone and lactate, indicating that LY could alleviate the transport stress of broilers. Furthermore, dietary LY and transport stress did not alter the levels of uric acid and free fatty acids in broilers, which may be attributed primarily to the adequacy of liver glycogen stores in broilers.[39]

Muscle pH is an essential parameter for meat quality determination.[40] A lower pH causes actin and myosin in the muscle to condense and contract into granules, destroying the spatial structure, increasing the free water, decreasing the water holding capacity, and ultimately having a direct effect on muscle color (especially lightness).[41] Under transport stress, the energy metabolism of the organism is strengthened, the energy supplying substances such as glucose and glycogen produce ATP through glycolysis to replenish energy .[9] With the prolongation of transportation time, the muscles contract violently and the anaerobic enzymatic reaction increases, which causes an accumulation of lactic acid produced in the muscles and leads to a decrease in the pH of muscles.[42] Also, excessive decrease in muscle pH can lead to protein denaturation, disruption of the fibrous chimeric structure of muscle proteins, and increased drip loss,[43] which was in accordance with our findings that the transported-broilers without LY decreased pH_{24h} as well as enhanced the drip loss of breast and thigh muscle. Moreover, the increased lightness of breast muscle was associated with the decreased pH value. Dietary supplemented with LY at 1000 mg kg^{-1} improved these parameters in transported-broilers, which is probably attributed to the fact that LY relieved stress in broilers, thus slowed down the rate of glycolysis in the muscle.

The glycolytic system is the process of converting muscle glycogen to lactate through a series of enzymatic reactions in animal muscle tissue under hypoxic conditions, and the process continues until glycolytic enzymes are inactivated or glycogen is depleted.[8] Transport stress accelerates muscle glycolytic metabolism by affecting muscle glycolytic enzyme activity and glycolytic potential.[44,45] The HK, PK and LDH are the key enzymes engaged in the glycolytic pathway.[46] Zhang *et al.*[24] revealed that the broilers experiencing 3-h transport had reduced the muscle glycogen content, enhanced the muscle LDH activity and lactate content, which was in similarity with our finding that transported-broilers without LY supplementation showed reduced glycogen content in thigh muscle. However, dietary supplemented with 1000 mg kg^{-1} LY lowered the levels of PK in thigh muscle but did not increase the glycogen content of transported-broilers, which is probably attributed to the insufficient amount of LY additive. Furthermore, transport stress is the result of multiple stressors acting together, the stress factors mainly include the age, breed, and health status of broilers, the formulation and nutritional level of the diet, fasting or not, and the catching method before transport, the temperature during

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transport, and the resting time after transport.[3,45,47] Therefore, the reports of transport stress on the metabolism of substances in broilers are inconsistent.

The ROS are normal metabolites inside the organism and serve a vital role in cell signaling. The accumulation of ROS in excess could trigger a lipid peroxidation reaction in the cell membrane, leading to massive MDA production.[48] Thus, MDA content can be regarded as a marker of transport stress-induced lipid oxidation.[49] Besides, our research evaluated the effects of transport stress on non-enzymatic (T-AOC) and enzymatic systems (SOD, GSH-Px and CAT), respectively, which indicated the antioxidant capacity of the organism.[48] In the current research, transport stress enhanced the level of MDA in serum and muscles of broilers, indicating that transport stress induced oxidative damage. The reduced T-AOC activity in serum and muscle of transported-broilers indicated that the free radical scavenging activity of the non-enzymatic system of the organism is lowered, which is in accordance with the decrease in DPPH radical scavenging rate induced by transport stress. Dietary supplementation of antioxidants could alleviate transport stress-induced oxidative damage.[2] In our findings, dietary supplemented with 1000 mg kg^{-1} LY reduced the MDA content in serum and muscles, indicating that dietary supplemented with LY could inhibit the increase of MDA content caused by transport stress and effectively avoid lipid peroxidation. Also, dietary supplementation of LY enhanced the concentration of T-AOC in serum and thigh muscle and GSH-Px in thigh muscle, suggesting that LY improved the antioxidant defense system of transported-broilers, which is probably achieved by modulating the secretion of mucosal sIgA and inhibiting the pathogenic bacteria colonization.[50]

The avUCP and avANT are collectively involved in the uncoupling mechanism at the inner mitochondrial membrane in skeletal muscle cells, and up-regulation of their expression can enhance the uncoupling effect to scavenge excessive ROS and reduce cell damage.[51] Previous studies have revealed that an up-regulation of avANT and avUCP mRNA expression in the skeletal muscle of birds were noted under cold acclimatization and fasting.[51-53] Pan *et al.*[2] and Wang *et al.*[49] indicated that an up-regulated mRNA expression of avUCP in breast muscle was noticed in transported-broilers, but the content of MDA was not decreased, which was in line with our study, suggesting that the up-regulated mRNA expression of avUCP was unable to avoid muscle oxidative damage caused by transport stress. Xu *et al.*[54] also demonstrated that up-regulation of mRNA expression of avUCP in skeletal muscle could only reduce the accumulation of ROS and lipid oxidation in broiler chickens 45 min post-slaughter but had no effects on oxidative damage in muscle after 24 h. In the current study, dietary supplemented with 1000 mg kg^{-1} LY down-regulated the mRNA expression of avUCP in breast and thigh muscles, indicating that LY may control the production of ROS in mitochondria via modulating the expression of muscle avUCP.

CONCLUSION

In conclusion, LY alleviates transport stress in broilers, ameliorates the BW loss and reduced meat quality triggered by transport stress by improving the antioxidant enzyme defense system and down-regulating the mRNA expression of avUCP in muscle (Fig. 1). Herein, dietary supplemented with LY maybe a potential protection for broilers under transport stress. In the next work, we will consider increasing the additive level of LY (more than 1000 mg kg^{-1}) to further verify the anti-stress effect of LY on broilers and focus on the microbial changes in the broiler intestine.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICS STATEMENT

This research was approved by the Institutional Animal Care and Use Committee of China Agricultural University (No. AW02801202-1-2, Beijing, China).

AUTHOR CONTRIBUTIONS

Tengfei He: conceptualization, methodology, software, data curation, writing – original draft preparation. Tengfei He, Jiayu Ma, Shad Mahfuz, Yuhui Zheng, ShenFei Long and Jian Wang: validation, formal analysis. ShenFei Long, Shad Mahfuz, Yuhui Zheng and Xiangshu Piao: writing – review and editing. Xiangshu Piao: supervision, project administration, funding acquisition.

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