Effect of Selenium-Enriched Bacteria and Other Selenium Sources on Meat Quality Parameters of Mid-Producing Lohman Brown Laying Hens

Muhammad, A.I.[1,3], Dalia, A.M.[2], Samsudin, A.A.[1]*

[1]Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia; [2] Department of Animal Nutrition, Faculty of Animal Production, University of Khartoum, P.O. Box 321, Khartoum, Sudan; [3]Department of Animal Science, Faculty of Agriculture, Federal University Dutse. P.M.B. 7156, Jigawa State, Nigeria;

*Corresponding author: anjas@upm.edu.my

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Abstract

The use of toxic and less bioavailable inorganic selenium can now be substituted with an alternative organic source obtained from bacterial species to benefit human and animal nutrition. This study investigates the effects of selenium sources on laying hen's meat quality. A total of 144 Mid-Producing Lohman Brown Classic laying hens, aged 23 weeks, were divided into four experimental groups (each with 36 hens) based on the form of Se supplementation (no Se supplementation, 0.3 mg/kg of inorganic Se in the form of sodium selenite, 0.3 mg/kg organic Se from Sel-Plex, Alltech Inc., Nicholasville, KY), and 0.3 mg/kg organic Se from *Stenotrophomonas maltophilia* (bacterial organic Se). The trial period was seventeen weeks, after which the hens were slaughtered and breast muscles sampled at week 40. The results showed that dietary Se supplementation enhanced ($P<0.05$) cooking loss, drip loss, and meat tenderness of breast muscles significantly in the enriched bacterial Se group compared to the inorganic and basal diet groups. Se sources also had a significant impact ($P<0.05$) on the colour (lightness, redness, and yellowness) of the breast meat. Bacterial organic Se supplementation reduced cooking loss and drip loss, and it improved the tenderness and breast meat colour of Lohman brown laying hens.

Keywords: Selenium, meat quality, cooking loss, drip loss, meat tenderness, laying hens.

Introduction

Laying hens are primarily bred for egg production, although, some strains include the Noiler breed (Nweke-Okorocha *et al.*, 2020), Beijing-you chickens, Luhua chickens, and Jingbai cross-bred laying hens were bred for both eggs and meat (Fu *et al.*, 2015). As laying hens grow older and enter the late laying phase (over 40 weeks), their production and eggshell quality decrease (Xiao *et al.*, 2022). In addition, there is evidence that aged hens have poorer egg quality, thinner shells, and higher breakage rates which is linked to their ill-health (Gan *et al.*, 2020). Laying hen meat is preferred depending on the region; for instance, the Chinese prefer it because of its high amounts of essential amino acids, intramuscular fat, and polyunsaturated fatty acids (PUFAs), all of which improve the flavour and texture of meat (Zhang *et al.*, 2017). Hen meat is commonly used to make strongly aromatic broths and other savoury soups as a frequent eating habit due to the balanced nutritious

components and specific flavour and aroma (Kokoszyński *et al.,* 2016). Furthermore, hen soups are considered health-promoting and therapeutic in Asia, specifically in China, when consumed by those recovering from fatigue, surgery, and disease, special traditional French recipes of *bourride* (boiled chicken) made from spent hens over decades (Semwogerere *et al.,* 2019). Hen meat is frequently used in the production of processed meats including Mortadella sausages, surimi, and jerky-type products.

Lipid oxidation in meat affects the quality of raw and cooked meat and results in a deterioration in flavour, colour, odour quality and nutritive value (Descalzo *et al.*, 2008). Lipid peroxides and secondary lipid oxidation products can cause a chain reaction that results in free radicals that harm proteins, nucleic acids, and cellular membranes (Descalzo *et al.,* 2008). The softening of the myofibrillar structure by endogenous peptidases causes post-mortem improvements in meat softness (Herrera-Mendez *et al.,* 2006). Ageing is the process of preserving raw meat above the freezing point, and it is linked to a rise in tenderness, and about 50% of the tenderisation occurs within 24 h of the slaughter, and the other 50% takes place during ageing. It is widely accepted that endogenous enzymes' role in proteolysis, or tenderization is significant, and enzymes may lose activity or be redistributed between cellular compartments. The rate of ageing accelerates with greater temperatures and more rigour development, which also differs significantly between species.

The presence of reactive free radicals over the tolerable limits causes cell damage, which has an impact on the nutritional values of the products. Synthetic or natural antioxidants are used (dietary) in live cells to scavenge and reduce or limit the damaging effects of reactive species (oxidants), such as oxygen or nitrogen species (ROS or RNS), on normal physiological functions. Selenium (Se) is an important trace element that is only required in trace amounts in animal diets but plays a critical role in an enzyme that is involved in a specific antioxidant process as well as various physiological functions (Edens & Sefton, 2016a). It is primarily applied in poultry diets as an inorganic (sodium selenite) or organic form, however, the latter demonstrates superior beneficial characteristics and increased physiological systems functionality. Because of their higher bioavailability, Se-enriched animal products production, antioxidant activity, improved meat quality, and oxidative stability of animal products, organic Se such as selenium-enriched yeast, bacteria, and selenomethionine are used as a safer and better source of Se in many species (Dalia *et al.,* 2020). The mechanisms by which Se functions are carried out exclusively by Se-containing proteins.

Many authors have investigated the positive effects of selenium on the quality and stability of poultry meat and eggs (Dalia *et al.,* 2020). Selenium has an antioxidant effect on the physicochemical characteristics of meat by inhibiting lipid oxidation, as well as maintaining the colour stability of haem pigments and reducing quality loss meat from heat-stressed hens (Liu *et al.,* 2021). Dietary Se supplementation improves the organoleptic properties of meat such as juiciness, crispness, and appealing to consumers and, significantly decreases the meat drip loss, cooking loss, tenderness, and thiobarbituric acid reactive substances (TBARS) in broiler meat (Dalia et al., 2020), and heat-stressed hens (Liu *et al.,* 2021). Selenium can be used in combination with other antioxidants e.g. Vit E (tocopherol) and Vitamin C, therefore, dietary supplementation with Se is cardinal in the animal antioxidant system, including tissue oxidative stability, meat quality, and

prolonged shelf life (Michalczuk *et al.,* 2021).

The use of diverse species of microorganisms, such as bacteria, to supplement Se is a recent discovery in providing the nutritional benefits of microelements (Yang *et al.,* 2019). Various bacteria strains have been utilized in microbial reduction methods that can ingest and transform inorganic Se and retain it in their cells as organic Se and selenoprotein (Sumner *et al.,* 2019). Based on their propensity to collect absorbed Se, *Stenotrophomonas maltophilia* bacteria (ADS18) were chosen as selenoproteins with greater Se concentrations in their cells (Dalia *et al.,* 2017). Although Se may improve the quality of meat, there is little scientific information on the effect of this new organic Se source on layers (mid-producing laying hens), and no published studies on the effect of ADS18 bacterial organic Se on the meat quality of mid-producing laying hens. Consequently, the goal of this study was to evaluate how effective ADS18 bacterial organic selenium is as an alternative organic Se source on mid-producing hen meat quality when compared to inorganic Se.

Materials and methods

Preparation of organic selenium from bacteria (*Stenotrophomonas maltophilia*)

The preparation of *Stenotrophomonas maltophilia* (ADS18) was described by Dalia *et al.* (2017) and Dalia *et al.* (2020). The extraction of seleno- protein from Se-enriched bacterial cells was carried out using a dialysis technique. Briefly, the 30% glycerol stock culture of the ADS18 strain was used to prepare aliquots fresh culture (24 h) after reviving in nutrient broth media supplemented with 10 µg/mL sodium selenite. Then 24 hours of incubation at room temperature. After that, a single colony was sub-cultured using the spread plate method and incubated for 24 hours at the predetermined temperature. A single colony was inoculated into 10 mL inorganic Se-enriched nutrient broth and allowed to grow for 24 hours. An inoculum containing 1×10^6 isolated bacterial cells was inoculated into the same media and incubated for 24 h at stated temperature. The bacterial pellets were harvested and twice rinsed with deionized water to eliminate any inorganic Se that may have remained in the bacterial cells after the culture was centrifuged at 6000 rpm for 15 minutes. The selenium-enriched bacterial cells were collected, sonicated and lyophilized at −20 °C. The biomass (Se-enriched) was used as the Se source in the feeding trial.

Animal ethics

The Institutional Animal Care and Use Committee of Universiti Putra Malaysia (UPM/IACUC/AUP-R063/2018). All procedures were performed under the guidelines and regulations for the administration affairs concerning experimental animals as stipulated.

Experimental animals, design, and treatments

A total of one hundred and forty-four (n=144) 18-week-old Lohman Brown hens were purchased from a commercial farm in Kuala Selangor, Selangor, Malaysia. The layer birds were raised on A-shape two-tier stainless-steel two birds per cage with the size of 55 × 50 × 45 cm3 (width × depth × height) at the Poultry Unit, Universiti Putra Malaysia, Serdang. A corn and soya bean-meal basal pre-lay fed at 17 weeks of age and layer diet was prepared using FeedLIVE software based on the Lohman management guide (2018). Layer birds were fed *ad libitum* with formulated layer mash (Table 1) and water. The hens were randomly divided into

four homogenous groups (36 hens in each), with six replicates (6 hens per replicate) differing in the form of Se added to the basal diet beginning at 23 weeks of age. Birds in each group were fed with diet either without Se (Con), supplemented with 0.3 mg/kg of sodium selenite (Na_2SeO_3), Se-yeast SelPlex (Se-Yeast) (Alltech Inc., Lexington, KY, USA), and *Stenotrophomonas maltophilia* (bacterial organic Se, ADS18), for sixteen weeks commencing from 23rd weeks of age and continued up to 40th week.

Table 1. Ingredient composition and calculated nutrient levels of the basal diet (on Dry matter basis)

Ingredients	Pre-lay	Con	Na_2SeO_3	Se-Yeast	ADS18
Corn (QL)	49.50	44.00	44.00	44.00	44.00
Soybean Meal (QL)	23.00	29.00	29.00	29.00	29.00
Wheat Pollard (QL)	18.50	11.00	11.00	11.00	11.00
CPO (QL)	1.50	3.50	3.50	3.50	3.50
L-Lysine	0.10	0.10	0.10	0.10	0.10
DL-Methionine	0.17	0.25	0.25	0.25	0.25
Dicalcium Phosphate (18%)	2.00	2.00	2.00	2.00	2.00
Calcium Carbonate	1.60	7.70	7.70	7.70	7.70
Choline Chloride	0.15	0.10	0.10	0.10	0.10
Salt	0.35	0.35	0.35	0.35	0.35
Mineral Mix*	1.00	0.60	0.597	0.597	0.597
Vitamin Mix**	1.00	0.60	0.60	0.60	0.60
Antioxidant***	0.62	0.40	0.40	0.40	0.40
Toxin Binder****	0.62	0.40	0.40	0.40	0.40
Sodium Selenite	0.00	0.00	0.003	0.00	0.00
Se-Yeast	0.00	0.00	0.00	0.003	0.00
ADS18-Bacteria	0.00	0.00	0.00	0.00	0.003
Total	100	100	100	100	100
Calculated composition					
Metabolizable energy (Kcal/kg)	2760.14	2761.24	2761.24	2761.24	2761.24
Crude protein (%)	16.35	17.66	17.66	17.66	17.66
Fat (%)	3.48	5.3	5.3	5.3	5.3
Fibre (%)	4.27	3.98	3.98	3.98	3.98
Calcium (%)	1.32	3.65	3.65	3.65	3.65
Total Phosphorus (%)	0.10	0.88	0.88	0.88	0.88
Av. Phosphorus for poultry (%)	0.56	0.48	0.48	0.48	0.48
Analysed Se (mg/kg) *****	0.00	0.03±0.01	0.31±0.02	0.32±0.01	0.33±0.02

* Mineral premix supplied (per kg of diet): copper 15 mg, zinc 120 mg, iron 120 mg, manganese 150 mg, iodine 1.5 mg, and cobalt 0.4 mg.**Vitamin premix supplied (per kg of diet): Vitamin A (retinyl acetate) 10.32 mg, vitamin E (DL-tocopherol acetate) 90 mg, cholecalciferol 0.250 mg, vitamin K 6 mg, cobalamin 0.07 mg, thiamine 7 mg, riboflavin 22 mg, niacin 120 mg, folic acid 3 mg, biotin 0.04 mg, pantothenic acid 35 mg and pyridoxine 12 mg. ***Antioxidant contains butylated hydroxyanisole (BHA). ****Toxin binder contains natural hydrated sodium calcium aluminum silicates to reduce the exposure of feed to mycotoxins. Feed live International Software (Nonthaburi, Thailand) was used to formulate the diets. ***** The Se content measured using ICP.MS.

Slaughtering and meat sampling

Twelve hens were selected randomly from each dietary treatment (2 hens from each replication) and slaughtered at week 40 according to the Halal procedure described in the Malaysian Standard (MS: 2009), to collect breast meat samples. For further analysis, the right part of the breast meat was collected directly into a plastic bag and kept at −80 °C.

Measurement of meat physicochemical properties

The meat physicochemical parameters of the hen's meat were measured from the breast muscle, and taken from the carcass at an average of 45 min post-slaughter. Around 30 – 35 g of tissue was cut, labelled, vacuum packed, and stored in a 4 °C chiller. The remaining breast muscle part was cut into three equal parts each tagged for an ageing period of day 0, 1, and 5 post-slaughter.

Meat water holding capacity (MWHC) or drip loss

The samples of fresh breast meat were collected on day 0, weighed individually (approximately 30 g), and recorded as the initial weight (W_1). The samples were then vacuumed-packed in sealed polyethylene plastic bags and stored at 4 °C for 24 h. Samples were gently removed from the bags and dried after days 1 and 5 of storage and performed a second weighing as W_2. For each piece, the percentage drip loss was calculated and expressed as the percentage of differences in samples' initial weight. The sample weight after storage days I and 5 was divided by the initial weight of the sample (Honikel, 1998).

Drip loss (%) = $[(W_1 - W_2) \div W_1] \times 100$.

Cooking loss

The cooking loss of the breast meat was by the modified method of Abdulla *et al.* (2017). Frozen breast meat subsamples (stored at -80 °C) were removed on days 1 and 5 and transferred overnight to 4 °C chiller to thaw, weighed individually, and recorded as initial weight (W_1). The samples were then cooked for 15 minutes in a pre-heated water bath at 80 °C. After cooking, they were cooled under the running tap and then weighed as W_2. The cooking loss was determined using the equation below based on the differences between the initial (W_1) and the final (W_2) weight.

Cooking loss (%) = $[(W1 - W2)/W1] \times 100$.

Shear force

Subsamples for the study of shear force or texture were collected from breast muscle samples previously used for cooking loss determination. The meat sub-samples were cut (1 cm high x I cm width x 2 cm length dimension) by the Volodkevitch bite jaw attached to a texture analyser (TA.HD plus®, Stable Micro System, Surrey, UK) in the middle and perpendicular to the longitudinal direction of the fibres (Sazili *et al.*, 2005). Then, the shear force values were recorded for each sample as the mean of all subsamples and the results were expressed in kilograms.

Colour measurement

Samples were taken from the freezer at -80 °C and left at 4 °C overnight to thaw. The plastic packaging was removed from the samples and all the samples were allowed to bloom for 20 min in the air before measuring the colour by MINOLTA CR300 (Minolta Camera Co. Ltd, Osaka). Before use, the device was calibrated against black and white reference tiles. L* (lightness). a* (redness)

and b* (yellowness) were measured in triplicate rotation on each sample. Also, calculated were the ratios of hue angle [tan/1 (b*/a*)], and chroma saturation index $\sqrt{(a^2 + b^2)}$ (Önenç & Kaya, 2004).

Muscle pH measurement

The pH of the meat samples at three ageing periods (0 days, 1 day, and 5 days) was measured with a pre-calibrated portable pH meter (Mettler Toledo, AG 8603, Switzerland) as described by Kareem *et al.* (2015). Before each use, the pH meter was calibrated following the instructions of the manufacturer. Approximately 0.5 g of each sample was homogenized with 10 mL of 5 mM sodium iodoacetate, and 150 mM KCl solution using a homogenizer (Wiggen Hauser® D-500, Berlin, Germany) for 20 s to stop continuous glycolysis. An electrode connected to the pH meter was immersed in 10 mL to measure the pH of the homogenates. Every sample was measured in triplicates and the mean pH values were calculated for each treatment (Abdulla *et al.*, 2017).

Statistical analysis

Repeated measurement analysis was performed for meat parameters and tested for dietary treatments, ageing, and interaction using a mixed model of SAS software 9.4 Version (SAS Institute Inc., Cary, NC). In addition, an F test was used to evaluate the orthogonal contrasts between treatments and to estimate the linear effect of selenium sources for meat parameters. The data were analysed using one-way analysis of variance (ANOVA) by the General Linear Model (GLM) procedure. For the assumption of normality, the model's histogram distribution and Quantile-Quantile (Q-Q) plots were utilized. At a significance level of $P< 0.05$, the Duncan Multiple Range Test was performed to differentiate means. In all tables, the results were reported as mean ± SEM.

Results

Meat quality

The effects of dietary Se supplementation on hen's breast meat quality are presented in Table 2 and Table 3, over day 0, 1, and 5, respectively. Drip loss was significantly lower ($P< 0.05$) in hens fed with ADS18 bacterial protein compared to Se-yeast, sodium selenite, and the control group though measured at day 1 and 5 only. Cooking loss, drip loss, and shear force were significant ($P<0.05$) by the Se sources. Selenium supplementation with ADS18 resulted in lower ($P<0.05$) cooking loss compared to Se-yeast, Na_2SeO_3, and control, although similar to Se-yeast except for day 5, during the ageing period. Shear force values were higher in hens fed a basal diet and supplemented with Na_2SeO_3 compared to those fed a diet supplemented with Se-yeast and ADS18 ($P<0.05$). Regarding contrast comparison, significant ($P<0.05$) differences were observed between basal diet, inorganic, and organic sources of Se in terms of drip loss (24 and 120 h), cooking loss ((24 and 120 h), and shear force (0, 24 and 120 h) of breast muscle, where hens fed diets supplemented with organic sources of Se (ADS18 or Se-Yeast) had higher parameter values. Moreover, hens that received an unsupplemented or inorganic source of Se diet had higher ($P< 0.05$) drip and cooking loss and shear force than those fed organic sources over the ageing period.

Meat colour

The Se sources had a significant influence on the lightness, redness, and yellowness of the breast muscle ($P<0.05$; Table 3). The intensity of the red and yellow colour of the breast muscle was significantly ($P<0.05$) higher in hens fed the diet

supplemented with ADS18 or Se-Yeast source than in those fed diets unsupplemented or with Na_2SeO_3. However, the lightness of the breast meat was the reverse order among the treatment groups, i.e., as supplementation of Se at 0.3 mg/kg diet decreases lightness of the breast muscle of a particular treatment group over an ageing period, a concomitant increase in yellowness and redness was observed for the corresponding treatment group. However, Se supplementation had no significant ($P>0.05$) effect on pH values of breast muscle over the ageing time. Based on contrast comparison, there was a difference ($P<0.05$) between inorganic Se (Na_2SeO_3) and organic (ADS18 or Se-Yeast) sources in terms of redness and yellowness of the breast muscles, in which hens receiving supplemented Se source had higher parameters values. Significant ($P<0.05$) differences between treatments over the ageing period were noticed for the Chroma (C*) with no difference ($P>0.05$) observed for the hue (h*) index.

Discussion

The water-holding capacity of fresh meat is its ability to retain inherent moisture as it affects both the yield and quality of the end product (Edens & Sefton, 2016b). Many studies of different opinions were reported in line with the efficacy of dietary sources (inorganic and organic) and levels of Se. Selenium has been shown to play a vital role in the intra and extra-cellular antioxidant systems of the body (Surai, 2006). The maintenance of cell membrane integrity is linked with the improvement of antioxidant status, which may ultimately reduce moisture loss. Muscle drip loss in chickens supplemented with organic and nano-Se was found to be lower than, in pigs (Bakhshalinejad *et al.*, 2019). Drip and cooking losses were two important markers that reflect the quality of meat. In this study, regardless of the treatments, cooking loss increased as ageing advanced (0 to 5) with the same pattern observed in drip loss. As ageing progresses, the increase in drip loss may be due to the relaxed ability of the collagen and myofibrillar proteins matrix to hold the water molecules. This mechanism was explained by the role of the calpain enzyme in binding myofibrils to the cell membrane (Edens & Sefton, 2016b). This is achieved by activation of the calpain enzyme, resulting in rapid fragmentation (due to the contraction effect during rigour mortis) of intermediate protein filaments in the tissue (e.g., desmin, which binds myofibrils to the cell membrane), which in turn prevents the decrease of the overall tissue cell membrane. Consequently, for the calpain enzyme activity in drip losses to be maintained, a sufficient balance of antioxidants and adequate pH is required. Our findings are in accordance with those of Bakhshalinejad *et al.* (2019), who observed that broilers fed nano-Se had lower drip and cooking loss in their thigh muscle compared to those fed sodium selenite (SS) or selenomethionine (SM) diets. In other findings (Naylor & Choct, 2000), drip losses of 13.57 % and 24.92 % were shown for chest and thigh muscles, respectively. It is obvious, that dietary supplementation of Se via organic form resulted in a significant reduction in drip and cooking loss over the ageing time compared to inorganic Se. Its higher bioavailability and enhanced oxidation-reduction result in preserving cell membrane integrity. The influence of Se-supplemented hens on cooking and drip losses could imply some consumer preference by enhancing the juiciness of the meat as it minimizes the loss of water-soluble nutrients in relation to drip loss particularly. Mahan *et al.* (1999) clinched that Se of inorganic source acts as a tissue desolator pro-oxidant.

Table 2. Effect of different dietary selenium sources on meat quality of mid-producing Lohman Brown laying hens at 40 weeks of age.

Parameters	Ageing	Dietary treatments [1]				P-value	Trt*Days	Contrast, *P-values*		
		Con	Na_2SeO_3	Se-Yeast	ADS18			Unsuppl vs. Suppl	Inorg vs. Org	Se-Yeast vs. ADS18
Drip loss %	1	3.69±0.44^{aB}	3.06±0.29^{abB}	2.43±0.30^{bcB}	1.76±0.22^{cB}	0.0027	0.8599	0.0027	0.0234	0.1556
	5	5.22±0.28^{aA}	4.65±0.23^{abA}	3.87±0.26^{bA}	2.86±0.39^{cA}	0.0001	0.8599	0.0005	0.0021	0.0247
	P-value	0.0151	0.0016	0.0049	0.0324					
Cooking loss %	0	21.75±0.49^{a}	18.08±0.78^{abB}	17.88±0.59^{abB}	15.50±0.44^{bB}	0.0308	0.4362	0.0081	0.4114	0.2302
	1	24.34±0.35^{a}	24.34±0.23^{aA}	22.74±0.30^{bA}	19.86±0.58^{cA}	<.0001	0.4362	0.0002	<.0001	<.0001
	5	24.59±0.99^{a}	24.33±0.49^{aA}	21.34±0.67^{bA}	20.06±0.78^{bA}	0.0006	0.4362	0.0059	0.0008	0.2436
	P-value	0.3806	<.0001	<.0001	0.0001					
Shear force (kg)	0	1.58±0.08^{aA}	1.29±0.09^{bA}	0.92±0.13^{bA}	0.74±0.13^{b}	<.0001	0.1623	<.0001	0.0013	0.2634
	1	1.20±0.11^{aB}	0.90±0.09^{bB}	0.63±0.07^{cB}	0.52±0.08^{c}	<.0001	0.1623	<.0001	0.0043	0.4218
	5	0.99±0.08^{aB}	0.89±0.10^{aB}	0.81±0.05^{aAB}	0.51±0.04^{b}	0.0001	0.1623	0.0033	0.0114	0.0041
	P-value	0.0002	0.0055	0.0806	0.1255					
pH	0	5.54±0.10	5.53±0.06	5.65±0.04	5.59±0.02^{A}	0.5622	0.9077	0.5314	0.2746	0.5163
	1	5.36±0.66^{ab}	5.26±0.03^{b}	5.49±0.09^{a}	5.43±0.05^{abB}	0.0921	0.9077	0.668	0.0173	0.5009
	5	5.37±0.03	5.41±0.06	5.48±0.11	5.47±0.05^{AB}	0.5539	0.9077	0.2567	0.3897	0.9124
	P-value	0.1546	0.0021	0.3178	0.0466					

*Values in the same row with different superscripts (lower case) are significantly different ($p < 0.05$) and comparing the treatment effects whereas, values in the same column with different superscripts (upper case) are significantly different ($p < 0.05$) and comparing the ageing effect for each parameter. [1]Con: without Se; Na_2SeO_3: sodium selenite; Se-yeast: Selenium yeast; ADS18: *Stenotrophomonas maltophilia* $^{a-c}$. Meat quality characteristics data are means of the duplicated analysis of 0-, 1- and 5-day samples per treatment. Unsuppl: Unsupplemented; Suppl: Supplemented; Inorg: Inorganic; Org: Organic

Table 3. Effect of different dietary selenium sources on breast meat (*Pectoralis major*) pH and colour of mid-producing Lohman Brown laying hens laying hens at 40 weeks of age.

Colour	Ageing	Dietary treatments[1]				*P-value*	Trt*Days	*Contrast, P-values*		
		Con	Na_2SeO_3	Se-Yeast	ADS18			Unsuppl vs. Suppl	Inorg vs. Org	Se-Yeast vs. ADS18
L*	0	61.09±0.53^{aB}	58.66±0.70^{bC}	58.18±0.83^{bC}	57.58±0.12^{bB}	0.0209	0.0148	0.0029	0.4424	0.6113
	1	62.30±0.48^{bB}	64.42±0.54^{aB}	60.55±0.90^{bB}	58.33±0.54^{cB}	<.0001	0.0148	0.1075	<.0001	0.0166
	5	68.17±0.43^{aA}	66.86±0.78^{abA}	65.91±0.38^{bA}	63.76±0.92^{cA}	0.0002	0.0148	0.001	0.0161	0.0259
	P-value	<.0001	<.0001	<.0001	<.0001					
a*	0	3.30±0.32^{bB}	3.39±0.27^{b}	5.35±0.18^{aAB}	5.51±0.13^{a}	<.0001	0.2755	<.0001	<.0001	0.6345
	1	3.71±0.12^{bA}	3.74±0.15^{b}	5.67±0.22^{aA}	6.10±0.15^{a}	<.0001	0.2755	<.0001	<.0001	0.0697
	5	2.71±0.13^{dC}	3.42±0.19^{cb}	4.77±0.17^{bB}	5.70±0.25^{a}	<.0001	0.2755	<.0001	<.0001	0.0009
	P-value	<.0001	0.2821	0.0215	0.2487					
b*	0	16.54±0.32^{bA}	16.72±0.22^{bB}	19.39±0.48^{aA}	20.29±0.59^{aA}	<.0001	0.9513	<.0001	<.0001	0.2678
	1	9.08±0.27^{B}	8.82±0.36^{C}	9.99±0.52^{B}	12.02±0.47^{B}	0.5942	0.9513	0.7995	0.0011	0.8259
	5	18.98±0.44^{dA}	20.50±0.54^{cA}	22.03±0.36^{bA}	23.61±0.34^{aA}	<.0001	0.9513	<.0001	<.0001	0.011
	P-value	<.0001	<.0001	<.0001	<.0001					
C*	0	16.88±0.31^{bB}	17.07±0.25^{bC}	20.12±0.52^{aB}	21.04±0.64^{aB}	<.0001	0.0005	<.0001	<.0001	0.1652
	1	20.04±0.28^{bA}	19.05±0.35^{bB}	21.26±0.50^{aAB}	21.25±0.44^{aB}	0.0003	0.0005	0.3067	<.0001	0.982
	P-value	<.0001	<.0001	0.003	<.0001					
h*	0	-0.37±0.82	-0.38±0.49	-5.07±0.41	0.75±0.49	0.2798	0.1589	0.6474	0.5235	0.074
	1	-1.21±0.73	0.66±0.28	-1.11±1.04	0.24±1.26	0.3611	0.1589	0.2796	0.3305	0.2961
	5	-2.73±2.53	-5.46±4.15	0.40±0.29	1.38±0.67	0.1969	0.1589	0.5985	0.0386	0.7797
	P-value	0.572	0.1706	0.3202	0.6563					

*Values in the same row with different superscripts (lower case) are significantly different (P< 0.05) and comparing the treatment effects whereas, values in the same column with different superscripts (upper case) are significantly different (P< 0.05) and comparing the ageing effect for each parameter. [1]Con: without Se; Na2SeO3: sodium selenite; Se-yeast: Selenium yeast; ADS18: *Stenotrophomonas maltophilia* $^{a\text{ - }c}$ Meat quality characteristics data are means of a duplicated analysis of 0-, 1-, and 5-day samples per treatment. L*: lightness, a*: redness, b*: yellowness, C*: chroma, and h*: hue angle. Unsuppl: Unsupplemented; Suppl: Supplemented; Inorg: Inorganic; Org: Organic

A general decrease in shear force was observed concerning the texture of the breast meat as ageing progressed. The summation of the mechanical force of muscle tissue post rigour mortis by yielding a loss of structure in the course of postmortem storage is termed tenderness (Takahashi, 1996). Meat tenderness, while affected by several factors, is another criterion for determining meat quality with a supreme effect on consumer preference. An enzymatic breakdown of collagen substances holding the muscle fibre firmly may be due to the decreases in shear force values (while increasing tenderness) over the ageing period and through all the treatment groups. The results corroborate earlier findings of Bakhshalinejad *et al.* (2019), who observed an influence of shear force values with nano-Se on the breast and thigh muscles of the broiler at 42 days of age. Similarly, Cozzi *et al.* (2011) reported a significant decrease in shear force (3.69 against 4.22 kg/cm^2) of the longissimus thoracis muscle of Charolais young bulls fed after 6 and 11 days post mortem Se-enriched yeast strain (*Saccharomyces cerevisiae* NCYC R397). Therefore, the increased meat lightness and decreased shear force in hens that received the organic Se supplemented (particularly ADS18) could be because of changes in tissue likely stimulated by the increased accumulation of selenoamino acids. It can be observed, that prolonged ageing time contributes to lower shear force values, regardless of the effect of the treatment, confirming the findings by Lindahl *et al.* (2010).

Colour variations, measured as L* (lightness), a* (red), b* (yellow), chroma (saturation or colour intensity), and hue (colour tone) after 0, 1, and 5 days of refrigerated storage were assessed. Visual appearance (colour) is among the sensory characteristics that play a strong role in the acceptability of meat and meat products by customers. The addition of Se through organic form had a positive effect on the colour characteristics of breast tissue in hens. Furthermore, the influence was not limited to the early post-mortem (0 day) whereas the supplemented groups displayed a similar trend of colour attributes over the ageing period (5 days). Reports on the colour stabilization effect with a different dietary source of selenium are fewer in layers with the majority in broilers (Bakhshalinejad *et al.*, 2019). Meat colour and drip loss are key indices for the evaluation of meat quality and are linked to the oxidation status in muscles (Li *et al.*, 2017). The meat colour of broiler chickens is influenced by Se of different sources of either organic (Se-chitosan, Se-enriched yeast (SY), DL-selenomethionine (SM) and nano-selenium (NS)) or inorganic (sodium selenite (SS)) varying inclusion levels (0.30 mg to 1.0 g) of Se per kg of feed (Bakhshalinejad *et al.*, 2019). On the other hand, there was no positive or negative effect of either form of selenium on the colour of broiler chicken meat (Li *et al.*, 2017). Consequently, Se is assimilated into selenoenzymes or replaced methionine into general body proteins via the methionine transporter mechanism amid the absorption of the organic form, thus, a contrast to the inorganic form (Surai, 2006). In line with the present findings, (Bakhshalinejad *et al.*, 2019) reported a reduction in meat brightness in response to dietary organic compared to inorganic selenium form. The possible explanation for these results could be the relation between the paler colour observed in breast tissue and the increase in water-holding capacity of the hens supplemented with inorganic Se sources. However, no effect of selenium supplementation was observed on pH among all the treatment groups. Although not statistically significant, higher pH values were recorded in hens supplemented with organic (Se-Yeast or ADS18), perhaps by enzyme glutathione

peroxidase action in catalysing and depleting the hydrogen peroxide (H_2O_2).

Conclusion

In conclusion, dietary Se supplementation is critical for improving the quality of meat. The current findings show that both inorganic Se and organic Se, notably enriched bacterial proteins from ADS18 bacteria, have a significant impact on physio-chemical parameters (water holding capacity, cooking loss, and shear force) of hen's meat and colour. Therefore, enriched bacterial proteins from ADS18 bacteria could be a promising source of organic Se for laying hens, allowing them to produce Se-enriched products (eggs or meat) that benefit animals and humans via the food chain.

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