

Effects of conventional and organic rearing systems and hen age on oxidative stress parameters of blood and ovarian tissues in laying hens

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Summary: In this study, effects of hen age and rearing systems on some oxidative stress parameters in serum and ovarian tissues of hens are investigated. For this purpose, total of 48 Bovans White commercial laying hybrids from two different rearing systems (conventional cage and organic) at 30 and 60 weeks of age were used. Serum and ovarian tissues of the hens were collected following slaughter process and malondialdehyde (MDA), glutathione (GSH), glutathione peroxidase (GSH-Px) and catalase (CAT) levels of serum and ovarian tissues were determined. MDA levels of serum was found significantly higher in conventional system, both in 30 and 60 weeks old ($P<0.05$). GSH-Px activity was lower, conversely CAT activity was significantly higher in conventional system ($P<0.05$). Hen age didn't affect MDA, GSH, GSH-Px and CAT levels of serum ($P>0.05$). In addition, there weren't any significant interactions between rearing system and hen age in serum ($P>0.05$). MDA ($P=0.010$) and GSH ($P<0.01$) levels of ovarian tissue were found significantly higher in conventional system while GSH levels of ovarian tissue had increased in 60 weeks old ($P<0.01$). GSH-Px and CAT activity was similar between groups ($P>0.05$). Interaction between rearing system and hen age affected activity of CAT in ovarian tissue ($P<0.05$). As a result, conventional battery cages caused oxidative stress interrelated with increasing lipid peroxidation and MDA levels in laying hens. Antioxidant enzymes that play role in reduction of MDA were inadequate in this system. Oxidant/antioxidant balance can be established at 30 or 60 weeks of age by hen organism and therefore MDA levels of tissues would not be affected by age.

Keywords: Antioxidant activity, hen age, lipid peroxidation, rearing system.

Konvansiyonel ve organik yetiştirme sistemleri ile tavuk yaşının yumurta tavuklarının kan ve yumurtalık dokusundaki oksidatif stres parametreleri üzerine etkisi

Özet: Bu çalışmada yetiştirme sistemleri ve tavuk yaşının, tavukların serum ve yumurtalık dokusundaki bazı oksidatif stres parametreleri üzerine etkileri incelendi. Bu amaçla 30 ve 60 haftalık yaşta, iki farklı sistemden (konvansiyonel kafes ve organik) toplam 48 adet ticari yumurtacı hibrit olan Bovans White kullanıldı. Kesim işlemini takiben tavukların serum ve yumurtalık dokuları toplanarak, malondialdehid (MDA), glutatyon (GSH), glutatyon peroksidaz (GSH-Px) ve katalaz (CAT) düzeyleri belirlendi. Konvansiyonel kafeste yetiştirilen hem 30, hem de 60 haftalık tavukların serum MDA düzeyleri önemli derecede yüksek bulundu ($P<0.05$). Konvansiyonel kafeste GSH-Px aktivitesi düşük, aksine CAT aktivitesi önemli düzeyde yüksek tespit edildi ($P<0.05$). Tavuk yaşı serum MDA, GSH, GSH-Px ve CAT düzeylerini etkilemedi ($P>0.05$). Buna ek olarak, serumda, yetiştirme sistemi ve tavuk yaşı arasında önemli bir etkileşim bulunmadı ($P>0.05$). Konvansiyonel sistemdeki tavukların yumurtalık dokusunda MDA ($P=0.010$) ve GSH ($P<0.01$) seviyeleri önemli düzeyde yüksek bulunurken, GSH seviyesi 60 haftalık yaşta arttı ($P<0.01$). GSH-Px ve CAT aktiviteleri gruplar arasında benzer çıktı ($P>0.05$). Yetiştirme sistemi ve tavuk yaşı arasındaki etkileşim yumurtalık dokusunda CAT aktivitesini etkiledi ($P<0.05$). Sonuç olarak, konvansiyonel bataryalı kafesler, lipid peroksidasyon ve MDA düzeylerinin artmasıyla ilişkili olarak tavuklarda oksidatif strese neden oldu. Bu sistemde MDA'nın azalmasında rol oynayan antioksidan enzimler yetersiz kaldı. Oksidan/antioksidan dengesi 30 ve 60 haftalık yaşlarda tavuk organizması tarafından kurulabildi ve dolayısıyla dokuların MDA düzeyleri yaştan etkilenmedi.

Anahtar sözcükler: Antioksidan aktivite, lipit peroksidasyon, tavuk yaşı, yetiştirme sistemi.

Introduction

Since the hens had been domesticated from red jungle fowl, selective breeding for high productivity did not alter the hens' fundamental behaviors (10). Experiments have shown that they need some behaviors such as nesting, dust bathing, perching and foraging and

space allowance for different activities (12). However, many of these behaviors are restricted in conventional battery cages due to their unsatisfactory rearing environments (31). In these systems, 430-560 cm² floor areas are provided to per hen and 4-10 hens are confined in each cage compartment (47). Environment forces birds

to some health problems, behavioral and metabolic disorders such as keel bone deviation, cage fatigue, fear, fatty liver hemorrhagic syndrome etc (24).

European Union (EU) adopted that “animals are sentient beings” with Amsterdam Treaty in 1997. The Protocol implemented member states’ EU policies on agriculture to “pay full regard to the welfare requirements of animals” (17). Moreover, EU directive 74/99 gave minimum standards for protection of hens. In accordance with the directive, conventional battery cage systems were completely banned by January 2012 in member states of EU (4). The prohibition of this system was voiced in Turkey in 2015 (8), but it has been postponed to a future date. Well-being of animals is an increasing concern worldwide in the last decades. Consumers have important demand for food produced in animal-care production systems (2). As a result of this, many producers tend to establish well-managed alternative rearing systems which offer higher welfare standards for hens (23, 43).

Organic egg production is getting more popular among alternative systems during the last 15 years (30). The Council Regulation 1804/99 ruled organic farming in poultry production. Hens must be allowed to have access to outdoor areas (4m²/hen), kept at lower flock sizes of maximum 3000 hens/house in the each poultry house (18).

The conventional cages and organic systems have been compared in many researches in terms of most notable egg production, quality (external and internal), safety (microbiological and chemical), nutritional traits of eggs and welfare status. Hen-day egg production was found higher in conventional cages than litter one as well as dirty and cracked egg ratio was higher in non-cage systems. Eggs produced from non-cage systems were characterized by higher contamination ratios with aerobic bacteria and chemical contamination such as dioxins and heavy metals. Nutrition plays basic role on nutritional composition of eggs. But generally, amount of carotenoid is higher in the non-cage eggs. Lack of activity has negative effects on behavior of hens in conventional cages. On the other hand, all of these mentioned parameters above have caused various discussions about comparison of two rearing systems (15, 24).

Physiological and biochemical parameters are indicators to show welfare of animals in different rearing environments (29). Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals. Any free radicals involving oxygen can be referred to as reactive oxygen species (ROS). ROS is actually a natural by-product of the routine metabolism of oxygen in cells. However, level of ROS metabolites extremely increase under stress conditions even under environmental stress (41). Exceeded stress causes imbalance between production of ROS and ability of body to deactivate or

detoxify resulted oxidative stress (37). Malondialdehyde (MDA), a reactive aldehyde is a final product of lipid peroxidation in cells. Increase in MDA levels is a good indicator of oxidative stress in cells (35). Higher levels of ROS production damage cellular lipids, proteins and DNA by affecting their structures. Glutathione (GSH) is a tripeptide (L- γ -glutamyl-L-cysteinylglycine) with multiple functions as a carrier of an active thiol group in the form of a cysteine residue. GSH has a role as an antioxidant either directly by affecting mutually with ROS and electrophiles or by working as a cofactor for different enzymes in living cells (40). Glutathione peroxidase (GSH-Px) is a well-known selenoenzyme that acts as an antioxidant. It catalyzes the reduction of harmful peroxides by glutathione and protects lipid membranes and other cellular components against oxidative damage (48). Catalase is an intracellular antioxidant enzyme that catalyzes the reaction of hydrogen peroxide to water and molecular oxygen. Catalase is very effective in high-level oxidative stress and protects cells from hydrogen peroxide produced within the cell (5). Mechanism of oxidative stress is given in Figure 1 (46).

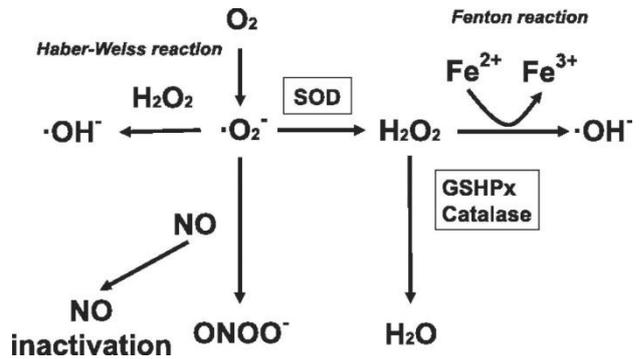


Figure 1. Mechanism of oxidative stress (46).

Şekil 1. Oksidatif stresin mekanizması (46).

Over free radical production and antioxidant system imbalance play a role in oxidative stress with aging (42). Long-lived cells tend to accrue relatively greater amounts of damage than those composed of short-lived cells (37).

In line with this information, present study aimed to determine the effects of rearing systems and hen age on some oxidative stress parameters in laying hens.

Materials and Methods

Experimental Design

The study was conducted at two conventional cages (C) and two organic (O) farms of contractual farmers with an integrated commercial company, with the approval of Fırat University Animal Researches Local Ethic Committee (FUHADYEK, verdict no: 2015/47). The Bovans White layers at 30 and 60 weeks old hens were chosen from each system. A total of 48 hens, 12 hens per

group were randomly selected in 30 and 60 weeks age within experimental groups. The groups were: 1) Conventional and 30 weeks old (C30), 2) Conventional and 60 weeks old (C60) 3) Organic and 30 weeks old (O30), 4) Organic and 60 weeks old (O60). The study was organized 2 (conventional and organic systems) x 2 (30 and 60 weeks old) factorial designs with two flocks (replicate) from per group, 6 hens per replicate. Samples were collected on two different days of the week in January-February 2016.

Cage system had the capacity of approximately 40000 hens in 4 storeys. Battery cages (70 x 55 x 50 cm; width x depth x height) were commercial wire cages containing 7 hens per cage, providing 550 cm² of floor space/hen. Rearing conditions were organized according to needs of the hens. Lighting regime was 16 hours light and 8 hours dark from age of 25 weeks to the end of laying period. Three nipple drinkers and 70 cm long feed trough was provided in cage units. Manure was removed with automatic belt system during the rearing period.

Organic system had both indoor and outdoor areas. The indoor area included total 3000 hens littered by wood shavings with 560 m² (5-6 hens/m²) space floor. Outdoor area with 12000 m² (4 m² per hen) covered with natural grass vegetation. All hens accessed to outdoor area via pop holes. Total pop holes were 15 m along to entire length of building. 25 birds per feeder and 10 birds per nipple drinkers were organized in the system. Lighting schedule was similar to cage system (16 h light/8 h dark). Hens were able access to outdoor area throughout 8 hours in daylight when the weather was good.

Feed and fresh water were automatically distributed in both systems. Diets were obtained from same commercial company and were in accordance with NRC (16.9-17.4% crude protein and 2800 ME kcal/kg) (31). Chemical composition of feed was determined according to the Association of Official Analytical Chemists (3). Organic crude materials were used in the organic diets. Compositions of the diets were given at Table 1.

Table 1. Ingredients and chemical composition of diet.
Tablo 1. Karma yemlerin içeriği ve kimyasal kompozisyonu.

Feed Ingredients	30 weeks of age		60 weeks of age	
	Conventional & Organic		Conventional & Organic	
Maize*	27.00		27.60	
Wheat*	20.00		24.85	
Wheat bran*	9.00		5.28	
Soybean meal* (48% Crude protein)	16.20		17.15	
Sunflower meal*(32% Crude protein)	6.64		5.90	
Maize gluten meal* (43% Crude protein)	4.76		3.00	
Vegetable oil*	5.60		5.35	
DL-Methionine	0.12		0.08	
L-Lysine hydrochloride	0.01		-	
L-Threonine	0.01		-	
L-Tryptophan	0.01		-	
Dicalcium phosphate	1.63		1.41	
Ground limestone	8.60		3.70	
Sodium bicarbonate	0.10		5.40	
Salt	0.16		-	
Vitamin-mineral mix**	0.16		0.28	
Nutritional composition, %				
Dry matter	90.9		90.8	
Crude protein	17.8		16.7	
Available phosphorus	0.38		0.34	
Calcium	3.76		3.90	
Sodium	0.15		0.15	
Methionine	0.73		0.65	
Lysine	0.84		0.84	
Threonine	0.67		0.65	
Tryptophan	0.24		0.24	
ME, Kcal/kg	2718		2741	

*Organic crude materials were used in organic diet.

**Mix supplied per 2.5 kg: 12000 IU vitamin A; 2500 IU cholecalciferol; 15000 IU vitamin E; 3 mg menadione; 3 mg thiamin; 2 mg pyridoxine; 15 mg vitamin B12, 1 mg folic acid; 25 mg niacin.

Mix supplied per kilogram: 80 mg Mn; 35 mg Fe; 50 mg Zn; 5 mg Cu; 2 mg iodine; 0.15 mg Se; 4 mg choline chloride.

Blood samples (n=48) of birds at 30 and 60 weeks age from each rearing system were collected into heparinized tubes at slaughter line during the neck cut. Following blood collection, ovarian tissues (n=48) were removed carefully. An amount of whole blood sample was reserved for GSH and GSH-Px determination. Sera were extracted from the remaining blood samples with 10 min centrifugation at 3000 rpm. Sera were used for MDA determination. Finally, erythrocyte suspensions were used for catalase (CAT) determination after washing 3 times with 0.9% NaCl. All samples were stored at -20°C until being analyzed (21).

Chemical Analysis

Lipid Peroxidation: Lipid peroxidation was measured by thiobarbituric acid reactive substances (TBARS) method (35), and was expressed in terms of the MDA content, which is served as for standard of 1,1,3,3-tetraethoxypropane. Formed MDA created a pink complex with thiobarbituric acid (TBA) and absorbance was read at 532 nm. Values of MDA were expressed as nmol/ml plasma for plasma and nmol/g tissue for ovarian tissues.

Reduced Glutathione (GSH): Concentration of reduced glutathione was assayed by the method of Chavan et al. (9) and was expressed as $\mu\text{mol/g Hb}$ for whole blood and $\mu\text{mol/g tissue}$ for ovarian tissues. The method is based on the capacity of sulfhydryl groups present in whole blood to react with 5, 5'-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent) and form a yellow dye with maximum absorbance at 412 nm.

Glutathione Peroxidase (GSH-Px): GSH-Px activity was assayed by the method of Matkovics et al. (27). GSH-Px activity was determined by using cumene hydroperoxide and reduced glutathione (GSH) as co-substrates and the loss of GSH following enzymic reaction at 37°C was measured spectrophotometrically (Schimadzu UV-1800, Japan) with Ellman's reagent at 412 nm. The enzyme activity was expressed as units per gr of Hb (U/g Hb) for whole blood and units per gr of protein (U/g protein) for ovarian tissues.

Catalase (CAT): CAT activity were determined according to the method of Aebi (1) and expressed as k/g Hb for erythrocyte and k/g protein for ovarian tissues. Decomposition of H_2O_2 can be directly followed by the decrease of absorbance at 240 nm. The difference in absorbance at 240 nm per time unit allows determining the CAT activity.

Haemoglobin (Hb) concentration was determined according to cyanmethaemoglobin method (19). Protein was measured by the method of Lowry et al. (26), with bovine serum albumin as standard.

Statistical Analysis

Experiment was designed by 2x2 factorial formations. Two-way analysis of variance was performed using General Linear Model (GLM) procedure with IBM SPSS Statistics 22 package program. The results were considered significant when P values were lower than 0.05 or equal (22).

Results

Mean values and standard errors of examined parameters were given in Tables 2 and 3. According to Table 2, MDA levels of serum was found significantly higher in conventional system, both in 30 and 60 weeks old ($P<0.05$). GSH-Px activity was lower, conversely CAT activity was significantly higher in conventional system ($P<0.05$). Hen age didn't affect MDA, GSH, GSH-Px and CAT levels of serum ($P>0.05$). There weren't any significant interactions between rearing system and hen age in serum ($P>0.05$).

According to Table 3, MDA ($P=0.010$) and GSH ($P<0.01$) levels of ovarian tissues were found significantly higher in conventional system. GSH activity of ovarian tissues were increased in 60 weeks old ($P<0.01$). GSH-Px and CAT activities were similar between groups ($P>0.05$). Interactions between rearing system and hen age affected activity of CAT in ovarian tissues ($P<0.05$).

Table 2. Effects of rearing system and hen age on oxidative stress parameters of serum in laying hens.

Tablo 2. Yetiştirme sistemi ve tavuk yaşının yumurtacı tavuklarda serumdaki oksidatif stres parametreleri üzerine etkileri.

	Rearing System (RS) (n=48)				P-Significance		
	Conventional (n=24)		Organic (n=24)		RS	A	RSxA
Age (A) Week	30 (n=12)	60 (n=12)	30 (n=12)	60 (n=12)			
MDA, nmol/ml	2.42±0.06	2.30±0.07	2.13±0.07	2.22±0.09	*	NS	NS
GSH, $\mu\text{mol/g Hb}$	12.80±0.41	14.54±0.70	13.59±0.41	13.81±0.73	NS	NS	NS
GSH-Px, U/g Hb	32.36±1.52	33.52±1.40	35.76±1.26	38.29±2.33	*	NS	NS
CAT, k/g Hb	31.31±2.28	30.66±2.82	25.24±0.94	26.81±1.86	*	NS	NS

MDA: Malondialdehyde; GSH: Glutathione; GSH-Px: Glutathione peroxidase; CAT: Catalase; NS: $P>0.05$ statistically not significant; *: $P\leq 0.05$ statistically significant.

Table 3. Effects of rearing system and hen age on oxidative stress parameters of ovarian tissue in laying hens.

Tablo 3. Yetiştirme sistemi ve tavuk yaşının yumurtacı tavuklarda yumurtalık dokusundaki oksidatif stres parametreleri üzerine etkileri.

	Rearing System (RS) (n=48)				P-Significance		
	Conventional (n=24)		Organic (n=24)		RS	A	RSxA
Age (A) Week	30 (n=12)	60 (n=12)	30 (n=12)	60 (n=12)			
MDA, nmol/g tissue	9.68±0.57	9.61±0.34	9.25±0.50	7.61±0.33	**	NS	NS
GSH, µ mol/g protein	1.03±0.08	1.23±0.06	0.71±0.10	1.07±0.04	**	**	NS
GSH-Px, U/g protein	29.40±1.83	32.30±1.08	29.80±2.14	32.20±1.34	NS	NS	NS
CAT, k/g protein	45.38±3.30	42.22±2.14	39.60±3.47	51.20±3.38	NS	NS	*

MDA: Malondialdehyde; GSH: Glutathione; GSH-Px: Glutathione peroxidase; CAT: Catalase; NS: P>0.05 statistically not significant; *: P≤0.05, **: P≤0.01 statistically significant.

Discussion and Conclusion

Oxidative stress resulting from increased production of free-radicals and ROS and/or decrease in antioxidant mechanism, does damage to biological macromolecules such as nucleic acids, lipids and proteins, and alters normal metabolism and physiology of living cells (7, 25). MDA is the most studied and well known indicator of lipid peroxidation and oxidative stress. Lipid peroxidation is a free-radical-mediated chain of reactions that results in oxidative deterioration of polyunsaturated lipids (20). Oxidant production increases with aging and it can damage nucleic acids, lipids and proteins of the cells, but a specific mechanism by which oxidants that causes the aging process has not been established (6). Cells contain various antioxidant defense mechanisms to deleterious effects of oxidant production; however, sufficiency of the antioxidants can decrease with increased age (33). Differently from researches mention about effects of aging on oxidative stress (6, 33), it was found that there wasn't any significant difference between age groups of the present study on MDA levels of each tissue. However, increasing MDA levels of serum and ovarian tissues were obtained in conventional battery system of the present study. Increased MDA levels of tissues can be associated with presence of stress in this rearing system. In accordance with these findings, Shini (39) observed that heterophile/lymphocyte ratio (H/L), an indicator of stress, was raised in hens kept in battery cages. In another study on immune response of laying hens in different rearing conditions with social stress, heterophile percentage and H/L ratio were lower and antibody production was higher in furnished caged hens compared to conventional caged hens (28). Mortality rate is a useful variant to describe rearing systems and general indicator of welfare of animals, some problems such as bumble foot lesions and keel bone deviation enhance the mortality in conventional caged hens (36, 44). Pohle and Cheng (36) reported that another indicator of stress, serotonin concentration was reduced while corticosterone concentration was increased in conventional battery cages. On the other hand, some researchers (43) reported that feather pecking, high

mortality levels and keel bone fractures were significant welfare problems in non-cage rearing systems for laying hens. Petrik et al. (34) compared two systems which are floor and cage. They reported that plumage condition was not affected by rearing system. Keel fracture prevalence was higher in floor-housed flocks compared to cage-housed flocks. Tactacan et al. (43) showed that plumage condition and immunological response parameters appear to be similar for hens housed in conventional and enriched cage systems. Enrichment of laying hen cages resulted in better bone quality, which could have resulted from increased activity. Another concern about welfare of hens in alternative systems is dust (38). David et al. (13) implied that polluted air may become an increased threat to health and therefore also a welfare problem when hens are kept with access to litter. Mugnai et al. (30) compared organic and conventional systems in terms of oxidative and immune positions of laying hens. Differently from present study results, researchers found about 30% higher ROS production of blood in organic system than conventional system, but accompanied by highest the antioxidant status. Erişir et al. (16) reported that rearing system did not change plasma levels of MDA, retinol and β-carotene in Pekin ducks and consequently did not cause any oxidative stress. Moreover, general situation of ovarian tissues significantly affect on performance of poultry and egg composition (14, 50). Wang et al. (50) implied that increased corticosterone concentration of ovarian tissues decreased follicular development was associated with reduced availability of yolk precursor. Another research stated that albumen corticosterone concentrations of egg in conventional cage, free range and barn systems were not significantly affected by farms structure, but the albumen corticosterone concentrations were high in the beginning of production period and remained stable during later stages of the period (14).

Antioxidant enzymes are considered to be first line of cellular defense against oxidative damage by suppressing formation of ROS, or opposing their action (46). GSH level of ovarian tissues and CAT activity of serum were found to be significantly higher in

conventional battery system, GSH-Px activity of serum was found inversely lower than organic system. Increase in antioxidant levels suggests enhancement of antioxidant potential of tissues to reduce oxidative stress and that, antioxidants can protect against oxidant impairment (41). Reduced GSH-Px activity of serum in conventional system could be associated with inadequate enzyme activity or another enzyme joined antioxidant metabolism in where CAT has a potential role. Reduced MDA level of ovarian tissues in organic system at age of 60 weeks could be associated with increased GSH levels in this group. Verma et al. (49) observed significantly higher activities of CAT and SOD enzymes in Vanaraja chickens housed in deep litter system as compared to cage and semi-intensive systems. GSH-Px activity was lower in deep litter as compared to two other systems, and GSH levels were found maximum in cage system. They also observed an increase in lipid peroxidation at 8th week as compared to 4th week of age, which was correlated with diminished activities of SOD and CAT. Cole et al. (11) reported that, based on theory of active oxygen, aging process of an organism and the subsequent decline of its functions can be primarily attributed to excessive expression of active oxygen, but can be reduced by strengthening the antioxidant system. Increasing antioxidant activity in 60 weeks old laying hens could be associated with the increasing metabolic activity to overcome in the current status of organism. Similarly, Ognik et al. (33) said that SOD and CAT enzyme activities decreased, but other antioxidants such as total antioxidant potentials (FRAP), vitamin C and total glutathione (GSSG+GSH) levels of blood increased in 9,12 and 15 weeks of age in turkey. In spite of the increasing levels of oxidants (H₂O₂) with age, MDA level of blood was not significantly affected from the age groups of the study.

As a conclusion of this study, conventional battery cages caused oxidative stress interrelated with increasing lipid peroxidation and MDA level in laying hens. Antioxidant enzymes were not able to decrease in MDA levels of hens in this system. However, oxidant/antioxidant balance could be established at 30 and 60 weeks of age by the antioxidant system, and any significance was not found between age groups in this study. Understanding of structures, advantages and disadvantages of the rearing systems used in egg production is important for the farmers who will be new participants and/or existing farmers of this sector. Further researches are necessary to improve the systems in terms of performance, environment protection, animal welfare and product quality.

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