

Trace Mineral Content of Conventional and Free-Range Broiler Chickens Analyzed by Inductively Coupled Plasma Mass Spectrometry

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The study is intended to explore the effects of different feeding modes on the trace elements in Chinese local chicken breast meat and leg meat. The experiment was conducted with the adoption of 600 San Huang chicks after a 21-day brooding and then 500 healthy breeding chickens in close weight were randomly assigned to two processing modes, that is, indoor floor and free-range, with 10 replicates per system and each of them contains 25 chickens. On the sixty-third day of breeding, 20 chickens were randomly selected from two different feeding modes, slaughtered after corona, carcass dissected after cooling with a viewing to collecting the samples of chest muscle and leg muscle.

Then the method of inductively coupled plasma mass spectrometry is applied to determine in samples the contents of zinc, copper, selenium, manganese, iron, chromium, lead, cadmium and mercury. The results showed that the free-range breeding way is more favourable as the contents of zinc, iron, manganese and chromium in leg meat is significantly higher than those in indoor-floor rearing system ($P < 0.01$), while the content of selenium in breast is also significantly higher than that of in indoor-floor rearing system ($P < 0.05$). Instead, the content of copper in breast in indoor-floor rearing mode is significantly higher than free-range mode ($P < 0.05$). However, the cadmium content (0.0016 mg/kg) in leg meat in free-range chickens is significantly higher than that indoor-floor breeding mode (0.0010 mg/kg). That is far less than the content of the maximum allowable chicken sample cadmium content (0.1 mg/kg) in the value of China's food limited standard in pollutants.

1. Introduction

In recent years, broiler rearing system has attracted the attention of a number of researchers from different perspectives. In terms of broiler meat quality and consumers' interests, among numerous non-genetic factors that may have a considerable effect on some meat quality traits, a broiler rearing system has been recognized over the past years by a large number of authors as being particularly important (Bogosavljevic-Boskovic et al., 2015). Free-range broiler production has increased substantially as a result of the greater demand for the so-called natural products (Parisi et al., 2015), which avoids the use of dietary animal by-products and antibiotics for poultry growth. Many consumers believe these products have superior sensory qualities and report that they "taste better".

In the United States, small farmers have adopted a free-range poultry production method that promotes pasture intake, which has been termed the pastured poultry system. Compared with conventional free-range and organic systems, the pastured poultry alternative is likely to induce considerably greater levels of pasture consumption. Pasture may constitute a source of energy and protein for growing broilers. In addition, the presence of a range of bioactive compounds in the forage, such as xanthophylls and several hypocholesterolemic and anticarcinogenic compounds, may lead to improvements in meat quality. At the same time, the housing systems might affect breast meat color and fatty acid contents (Husak et al., 2008). Therefore, demands to organic and free-range reared broiler meat have also become increasingly available to

consumers (Inci et al., 2015). Consumer interest in specialty poultry products derived from free-range or organic production systems has been steadily increasing in the United States and Europe (Fanatico et al., 2006). Free-range poultry has become highly popular with consumers as evidenced by its increasing presence in the marketplace.

From the perspective of animal welfare, more and more researchers conduct studies to improve well-being by evaluating the alternative rearing system (Barbosa et al., 2005). Consumer preference for poultry specialty products is related to a perceived greater quality and security of meat derived from such systems coupled with high standards of animal welfare. Under free-range or organic systems, chickens have access to an outside area with no limits, decreasing pressure, increasing comfort, promoting foraging like eating grass and insects, and free activity and thus theoretically improving the welfare of the chickens. The breeding density directly influences in the traditional rearing mode the production index and welfare; conventional confined systems of keeping chickens lead to chickens stress, resulting in physiological and behavioral responses, a decline in production performance and lower welfare levels.

From the point of nutrition, meat poultry is a valuable source of proteins, minerals and vitamins. The levels of trace elements in chicken products and various parts of chicken samples have been widely reported in the literature (Bohrer et al., 2007). However, the data on the trace element levels in chicken products derived from free-range or Conventional confined production systems are very limited. Furthermore, Consumers often demand information regarding the nutrient composition of food and the quality of products consumed. Therefore, it is beneficial to researching and comparing trace element levels in meat from broilers of different breeds raised under different housing systems.

2. Materials and Methods

2.1 Chickens, housing and diet

The study was conducted at the poultry unit of the Teaching and Research Farm, Northeast Agricultural University, Acheng (longitude 126° 38', latitude 45° 45') from June to September, 2012. All procedures used in the present study were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University.

The test material used in this study included a total of 500 fast-growing Sanhuang broiler strains in china. On 1-d-old, chicks were kept growing until day 21 of life; on day 22, 500 healthy birds of similar body weight (mean 354 g) were randomly selected and assigned to one of two raising systems (indoor floor and free-range) with ten replicates of 25 chickens in each system (i.e., a total of 250 chickens per system). During the first 3 weeks, the broilers were reared in the poultry house (indoor floor). On day 22, they were assigned to two groups: Group I, including broilers reared in indoor floor system in the poultry house at a stocking density of 10 chickens/m², and Group II, comprising free range broilers that were provided with a grass range of 4m²/chicken, besides the identical usable area in the poultry house. Group I broilers were reared in a deep litter consisting of wood shavings. The litter was not refreshed during the fattening period so as not to disturb the test broilers, that is, to avoid the potential stress effect.

Natural lighting system was used. Optimal microclimate conditions were provided by naturally ventilation system (naturally ventilated) with side and roof openings. Apart from the natural environment (grass, fresh air and sunlight, and higher roaming potential), the free range system employed in this study also involved the intake of natural food through foraging and feeding from the range. In addition, two production systems supply food and water, which makes it possible for the chickens to eat and drink freely. Poultry feed nutrition standards according to the NRC (1994) could be divided into three poultry breeding cycles (brood, breeding and fattening) as shown in table 1.

Upon fattening (on day 63), 20 broilers were randomly selected from each group of broilers: Group I (indoor floor) and Group II (free-range) broilers. The chickens selected were slaughtered after corona and cooled in a cooling chamber for 24 hours at 0 to 4°C. Thereafter, the dressed cold carcass of each broiler was dissected into primal cuts (breast and thigh) with 20 grams chosen following the method prescribed by the Regulation on Poultry Meat Quality. The samples were stored in a deep freezer at -21 °C until the analysis was completed within one month.

2.2 Trace element analysis

The instrument for the determination of trace elements is inductively coupled plasma mass spectrum which is short for ICP-MS (7500 a, the Agilent company). Instrument parameters are shown in table 2. Measurement method is that test samples (0.5 g) were soaked in concentrated nitric acid (65%, 5 ml) and heated in the microwave digestion device (MARSX, CEM companies in the United States) with the temperature controlled by a control panel and diluted to 50 ml using ultrapure water treatment system (900) South Korea Human up. Tubes with seals do not produce volatile loss, from the standard solution to the high standards of purity of liquid (Agilent companies in the United States, zinc, copper, selenium, manganese, iron, chromium, lead,

cadmium and mercury) and used to get the calibration curve, use of several standard reference material verification analysis programs; the standard elements are from national standard substance research center and all chemicals used are at the optimal level as for purity.

Table 1: Broilers' diet and nutrient levels in each phase

Ingredients	Phase 1 (1-21 d)	Phase 2 (22-42 d)	Phase 3 (43-63 d)
Corn(%)	58.81	63.60	68.95
Soybean meal(%)	35.40	30.0	24.40
Soybean oil (%)	1.20	2.00	2.70
Limestone(%)	1.00	1.00	1.00
CaHPO ₄ (%)	2.00	1.90	1.50
Nacl(%)	0.30	0.30	0.30
Premix(%)	1.00	1.00	1.00
DL- Methionine(%)	0.19	0.12	0.10
L-Lysine(%)	0.10	0.08	0.05
Nutrient levels			
ME(MJ/kg)	12.16	12.59	12.98
CP(%)	20.90	18.82	17.42
Met(%)	0.52	0.45	0.34
Met+Cys(%)	0.91	0.78	0.64
Lys(%)	1.09	1.00	0.88
Calcium(%)	0.96	0.92	0.80
Available phosphorus(%)	0.48	0.40	0.38

Table 2: ICP-MS instruments working conditions and parameters

Test Item	Parameters
Reflect power (kW)	1.30
Sampling depth (mm)	6
Torch-H (mm)	-0.3
Torch-V (mm)	-0.3
Carrier gas (L/min)	0.65
Nebuliser Pump (rps)	0.1
S/C temperature (°C)	2 °C
Oxide(CeO/Ce)	≤1.0 %
Doubly Charged (Ce ²⁺ /Ce)	≤1.0 %
Nebulizer type	Babington

2.3 Statistical analysis

The differences in the trace elements content of the two experimental groups through variance analysis are analyzed, and the results are calculated by means of mean + / - standard deviation (\pm S) with the statistical analysis software (SAS, SAS Institute company release 9.0) adopted.

3. Results

Trace element levels in the chicken products from the different housing systems are shown in table 3 and table 4 through the ICP-MS analysis. According to these data, zinc has the highest concentration in the investigated samples and followed by iron, copper and manganese. The concentrations of trace elements in leg muscle were far higher than those in breast muscle (Table 3, Table 4) in two different rearing systems (indoor floor and free range), except for Cr and Hg whose concentrations did not differ between breast muscle and leg muscle. In other words, the results showed a considerable difference between trace elements concentrations amongst the meat from the broilers reared under the different systems. The concentrations of trace elements in the leg muscle were affected more by husbandry systems than those in breast muscle. In

leg muscle, the trace elements values in free-range broilers such as zinc, manganese, iron and chromium were higher than those in indoor floor broilers with the differences being significant ($p < 0.01$). In breast muscle, the value for copper and iron from indoor floor broilers was higher than that from free-range broilers, and the difference is significant ($p < 0.01$). On the contrary, the selenium value in breast muscle from the broilers reared in the free-range mode was higher than that from the indoor floor broilers with the differences in the rest trace elements being non-significant.

Table 3: The trace elements content of chicken in the indoor floor production mode unit: mg/kg

Elements	Breast meat				Leg meat			
	Minimum	Maximum	Mean	SD	Minimum	Maximum	Mean	SD
zinc	5.3340	7.0590	6.1964	0.7372	18.6700	19.3900	19.0300	0.2920
copper	0.4066	0.8855	0.6461	0.2108	0.6375	0.8435	0.7405	0.0966
selenium	0.0851	0.0880	0.0864	0.0013	0.1023	0.1099	0.1061	0.0032
manganese	0.1338	0.1440	0.1389	0.0037	0.1847	0.1950	0.1899	0.0039
iron	6.1140	8.2550	7.1845	1.0349	10.1650	10.8900	10.5550	0.3430
chromium	0.1796	0.2169	0.1983	0.0172	0.1860	0.1950	0.1905	0.0043
lead	0.0112	0.0194	0.0153	0.0036	0.0110	0.0252	0.0181	0.0066
cadmium	0.0001	0.0004	0.0002	0.0001	0.0004	0.0021	0.0010	0.0007
mercury	0.0005	0.0010	0.0008	0.0002	0.0002	0.0008	0.0007	0.0003

Table 4: The trace elements content of chicken in the free-range production mode unit: mg/kg

Elements	Breast meat				Leg meat			
	Minimum	Maximum	Mean	SD	Minimum	Maximum	Mean	SD
zinc	5.8765	6.1940	6.0765	0.1482	18.6300	21.9100	20.2700	1.3575
copper	0.4010	0.4901	0.4456	0.0350	0.7576	0.9177	0.8377	0.0680
selenium	0.0866	0.0923	0.0894	0.0026	0.1064	0.1155	0.1105	0.0042
manganese	0.1296	0.1582	0.1439	0.0139	0.2254	0.2398	0.2326	0.0054
iron	5.7800	6.6240	6.2020	0.3214	12.9400	16.7800	14.8600	1.6278
chromium	0.1789	0.1943	0.1866	0.0069	0.1999	0.2411	0.2205	0.0166
lead	0.0145	0.0175	0.0160	0.0013	0.0143	0.0266	0.0204	0.0048
cadmium	0.0006	0.0009	0.0007	0.0001	0.0013	0.0018	0.0016	0.0002
mercury	0.0007	0.0008	0.0008	0.0000	0.0004	0.0012	0.0008	0.0003

4. Discussion

Zinc is known to be involved in most metabolic pathways in humans and zinc deficiency can lead to loss of appetite, growth retardation, skin changes and immunological abnormalities. The lowest and highest zinc values were found 5.334 $\mu\text{g/g}$ in breast muscle and 21.91 $\mu\text{g/g}$ in leg muscle. In the literature zinc values have been reported 2.87 $\mu\text{g/g}$ in chicken. The level of zinc in breast muscle samples was found lower than that in leg muscle and zinc value from free-range Sanhuang broilers is significantly higher than that from indoor floor broilers ($P < 0.05$).

Copper is known to both vital and toxic for many biological systems and may enter the food materials from soil through mineralization by crops, food processing or environmental contamination. (Ferreira et al., 2005) detected copper mean value in chicken meat thigh 0.07 and breast 0.04(mg/100g) and copper values in chicken samples investigated in the presented work were found to be nearly at the same level as that found in the above-mentioned literature. But copper values in leg muscle from free-range broilers were significantly lower than those from indoor floor broilers with no significant differences between leg muscles.

Iron is a mineral essential for life and for our diets. It is known that adequate iron in a diet is very important for decreasing the incidence of anemia (Ghaedi et al., 2006). Iron values both in breast muscle and leg muscle from free-range broilers were found significantly higher than those from indoor floor broilers ($P < 0.05$) in this study.

The lowest and highest manganese concentrations were found 0.1296 $\mu\text{g/kg}$ in breast muscle and 0.2398 $\mu\text{g/kg}$ in leg muscle from the products of free range broilers. Manganese values in leg muscle from free-range broilers were found significantly higher than those from indoor floor broilers ($P < 0.05$). The Institute of Medicine recommends that the intake of manganese from food, water and dietary supplements should not exceed the tolerable daily upper limit of 11 mg per day (National Research Council Recommended Dietary Allowances, 1989).

Selenium is recognized as an essential micronutrient in animal and humans, playing important biological roles as antioxidant. The highest selenium concentrations were found 115.5 $\mu\text{g}/\text{kg}$ in leg muscle from free range broilers, significantly higher than those from indoor floor broilers ($P < 0.05$). In the literature, selenium levels in chicken samples have been reported in the range of 97–154 ng/g in Slovenian (Smrkolj et al., 2005) and 76.3–82.4 ng/g in Greek (Pappa et al., 2006). Selenium values in investigated chicken samples are basically identical with the values in Slovenian and Greek literature.

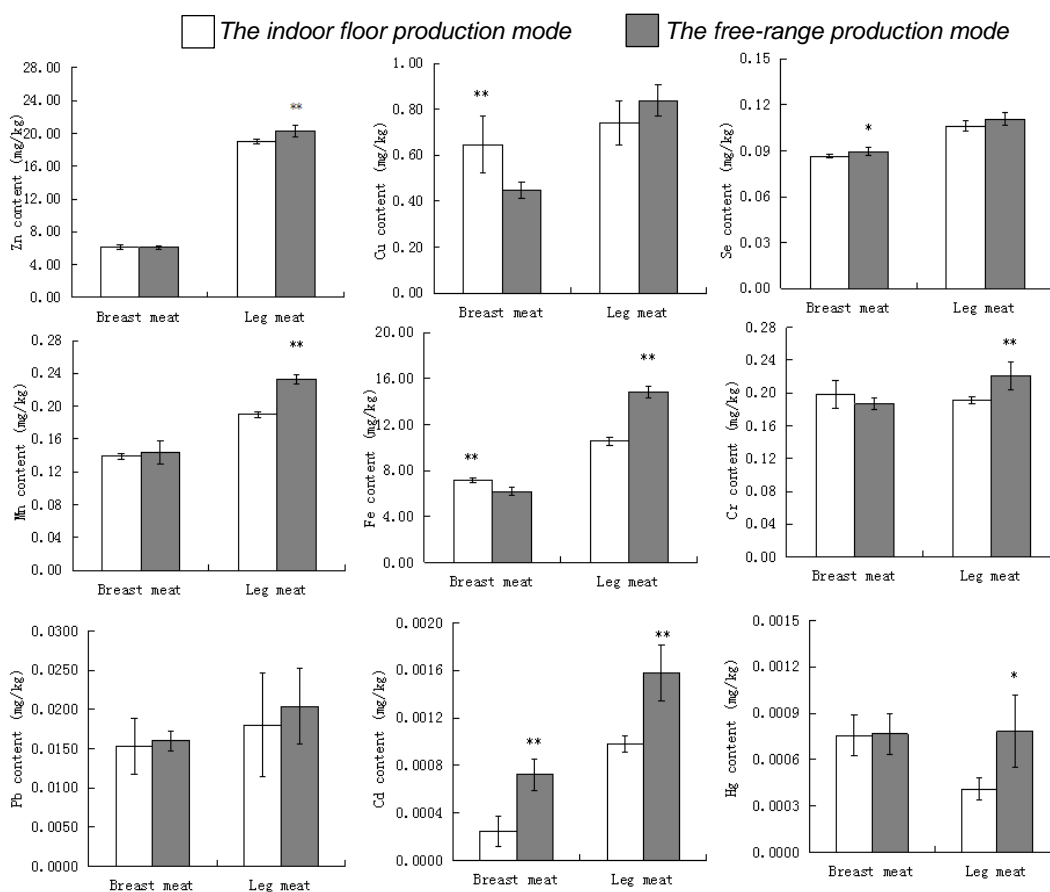


Figure 1: The content of trace elements in two kinds of three yellow chicken breast and thigh in the feeding mode

Chromium is considered as an essential trace element. The recommended daily intake of chromium is 50–200 μg . Chromium levels in breast muscle and leg muscle samples were found 0.1789–0.2169 mg/kg and 0.1860–0.2411 mg/kg respectively. Chromium values in leg muscle from free-range broilers were found significantly higher than those from indoor floor broilers ($P < 0.05$). Chromium content detection values are 0.0018 to 0.027 mg/kg . Chromium values both in breast muscle and leg muscle from free-range broilers were found significantly higher than those from indoor floor broilers ($P < 0.05$) in this study.

Cadmium may accumulate in the human body and may induce kidney dysfunction, skeletal damage and reproductive deficiencies (Kabaivanova et al., 2015). The minimum and maximum cadmium contents of the samples were found 0.10 $\mu\text{g}/\text{kg}$ in breast muscle samples and 2.14 $\mu\text{g}/\text{kg}$ in leg muscle samples in extensive indoor rearing systems. Cadmium levels in chicken samples have been reported in the range of 0.05–0.09 mg/kg in Nigerian (Onianwa et al., 2000). The maximum cadmium level permitted for chicken samples is 0.1 mg/kg according to China's Food Contaminants Limited Standard (GB2762-2005). Cadmium levels in chicken products from the two rearing systems were lower than permitted levels in this study. The difference is not significant as for lead and mercury content in the samples from the two rearing systems and far less than the maximum allowable pollutant in the food according to the above-mentioned standard in China.

5. Conclusion

It has been proved by the experimental research feeding mode affects the composition of trace elements in the chicken and the differences in feeding mode mainly consist in the varieties and quantities of chickens' freely gathering food (grass, insects), soil, exercise and other factors. The experiment proved the influence of feeding mode (indoor floor and free-range) on trace elements content in chicken with specific conclusions shown as follows:

Under the two feeding systems, the content of zinc, selenium, iron and manganese in chicken leg muscle is significantly higher than that in the breast ($P < 0.05$).

Under the free-range mode, the content of zinc, selenium, iron, manganese, chromium and cadmium in chicken leg muscle is significantly higher than that from the indoor floor mode ($P < 0.05$).

In short, our research suggests that the several kinds of trace elements content of chicken can be easily measured by ICP-MS method. And important conclusions are that feeding system can affect the composition of some trace elements in the chicken and compared with the indoor floor mode, some trace elements values of chicken products raised under the mode of free-range are much higher.

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