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Heavy Metal and Biogenic Amines in Some Fresh Retailed Meat in Egypt

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Abstract
Background: *Biogenic amines (BAs) and heavy metal residues have been considered as one of the silent killers because of their significant health impacts on the consumers appeared after cumulative accumulation of ingested BAs and heavy metal residues following successive consumption of apparently wholesome foods suffered from rearing environment and post slaughtering storage conditionsfaults.*
Material and Methods: *A total of 120 random raw meat samples from fore and hind quarters of cattle, sheep and camel carcasses (20 each) were collected from different butcher shops in Tanta city, Gharbia governorate, Egypt. All samples were subjected to detection of heavy metals (lead, cadmium and arsenic), and determination of BAs content (histamine, putrescine, cadaverine and tyramine) by Atomic Absorption Spectrophotometry (AAS) and Liquid Chromatography techniques, respectively*.
Results: *The obtained results showed that mean values of heavy metal concentration of fore quarter samples were higher than those of hind quarter samples of cattle, sheep and camel carcasses which recorded 0.18, 0.27 and 0.12 (mg/kg) for lead concentration; 0.15, 0.20 and 0.09 (mg/kg) for cadmium concentration; 0.12, 0.39 and 0.08 (mg/kg) for arsenic concentration. On the other hand, results for determination of biogenic amines contents showed that mean values of fore quarter sample were higher than those of hind quarter samples of cattle, sheep and camel carcasses which recorded 11.7, 13.8 and 10.5 for histamine concentration & 15.2, 17.1 and 22.3 for putrescene concentration & 10.8, 12.2 and 10.1 for cadaverine concentration and 4.9, 6.7 and 3.9 for tyramine concentration. In addition, statistical analysis of variance revealed significant differences between meat cuts and species (P≤0.5).*
Conclusion: *Finally, results of this study declared that hind quarter samples had less concentration of heavy metal and BAs than those of fore quarter samples with special reference to camel meat samples which had the least levels of heavy metals and BAs content followed by cattle then sheep samples, respectively. Moreover, it is strongly recommended application of strict hygienic measures of rearing food animals; and proper hygienic storage and handling procedures.*
Keywords: *Heavy metals, Biogenic amines, Carcasses.*

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I. Introduction

The world-wide commercial meat production is a crossing-boundaries, well-developed trading industry; which is considered one of the largest suppliers of animal protein and good source of essential amino acids, vitamins and minerals for human consumption. Its significance is thought to be greater in some developing countries where green pastures and wide rural areas are available because of relatively cheap production costs, and providing an excellent source of high-quality animal protein due to its high meat yield, low shrinkage during cooking and low cost, but sometime meat production animals may accumulate heavy metals and other elements which may be naturally present in air, water, soil and feeding or can reach it as a result of human activities such as industrial and agricultural processes[1]**.** In addition, some handling and storage faults lead to production of biogenic amines (BAs), which are nitrogenous bases formed by the microbial decarboxylation of free amino acids. Its public health significance reflecting their toxic effects associated with high levels in meat and meat products. Owing to their consistent presence with microbial spoilage they are utilized as quality indicator in terms of spoilage/ freshness of meat and meat products. The reason for the formation of these amines is multi-factorial however the poor-quality meat is the most important one[2]**.**

Metals, such as iron, copper, zinc and manganese, are essential metals since they play important role in biological systems, whereas mercury, lead and cadmium are toxic, even in trace amounts. The essential metals can also produce toxic effects at high concentrations. Only a few metals with proven hazardous nature are to be completely excluded in food for human consumption. Thus, only three metals, lead, cadmium and mercury, have been included in the regulations of the European Union for hazardous metals[3].

Heavy metal toxicity after long period of accumulative consumption in contaminated foods found to be a cause of many health hazards. **Kickbusch et al.**[4]found that lead toxicity affects theblood, nervous, genital, urinary and gastric system and in experimental animals, it can cause carcinogenesis, mutagenesis and teratogenesis. While, cadmium is another toxic heavy metal which causes the high blood pressure, mutations, prostate cancer, decreases the glomerular filtration rate and affect bone growth.

On the other hand,BAs are produced in foods where high levels of protein are present, for example in meat. During fermentation or spoilage, the protein breakdown products, peptides and amino acids, represent precursors for amine formation; so, BAs levels are used as spoilage indicators[5]. The BAs that are often found in foods include cadaverine, putrescine, histamine, tyramine, serotonin, β-phenylethylamine, spermine and spermidine, wherecadaverine is formed from lysine; whileputrescine is formed from ornithine, and histamine from histidine[6].out of which, determination of the cadaverine concentration could be used to monitor spoilage in both red and white meat and also the tyramine concentration is a useful indicator to control red meat during storage[7].

The most common symptoms of histamine poisoning are due to the effects it has on different systems (cardiovascular, gastrointestinal, respiratory, etc.) producing low blood pressure, skin irritation, headaches, edemas, and rashes typical of allergic reactions[8]. Furthermore, histamine plays a role in the health problem known as histaminosis or histamine intolerance associated with the increase of histamine in plasma[9]. It is also important to point out that histamine is a mediator of allergicdisorders.Other amines, such as putrescine and cadaverine, are also associated with this illness, although both seem to have much lower pharmacological activity on their own but enhance the toxicity of histamine and decrease the catabolism of this amine when they interact with amine oxidases, thus favoring intestinal absorption and hindering histamine detoxification[10].

Therefore, this study aimed to investigate the levels of some heavy metals and biogenic amines in raw retailed meats of cattle, sheep and camel for improving hygienic measures during handling of meat and selecting more safer meats for human consumption.

II. Materials And Methods

2.1. Collection of samples:

A total of 120 fresh random fore and hind quartermeat samples of cattle, sheep and camel carcasses (20 of each) were collected from different butcher's shops located in Tanta city, Gharbia governorate, Egypt. Each sample was kept separately in an ice box and transferred as rapid as possible.The collected samples were subjected to determination of some heavy metalresidue levels (lead, cadmium and arsenic), all well as BAs contents (histamine, putrescine, cadaverine and tyramine).

2.3. Determination of heavy metals by using Atomic Absorption Spectrophotometer

The collected samples were examined for determination of their lead, cadmium and arsenic levels on the basis of wet weight (mg/Kg) following the next steps:

2.3.1. Washing procedures was conducted according to[11]

Washing of equipment were thoroughly cleaned with deionized water and soaked in hot diluted HNO_3 (10%) for 24 hours and raised several times with deionized water and dried to ascertain that all the equipmentwas metal free. On the other side, the digestion vessels were rinsed once with distilled water, once with the mixture (250 ml deionized water, 200 ml conc. HCl and 80 ml H_2O_2) and once with 10% HNO_3. Finally, all containers were thoroughly washed with deionized water and air-dried in incubator away any from contamination or dust.

2.3.2. Digestion technique was performed according to [12]

Accurately, 1 g of each sample was digested by 10ml of digestion mixture (60ml Nitric acid "65%" and 40ml Perchloric acid "65%") in screw capped tube after maceration by sharp scalpel. Tubes were allowed to stand over night at room temperature, and then they were heated for 4 h in water bath at 110^{o}C to ensure complete digestion of the samples, where the digestion tubes were vigorously shaken at 30 minutes intervals. The tubes were then left to cool at room temperature and diluted with 1ml deionized water (30%) as well as reheated in water bath at 70^{o}C to ensure complete digestion of the samples. At this point, all organic matrixes have been destroyed.

Each tube was diluted with deionized water till reach 25 ml and filtered with Whitman filter paper No. 42.The filtrates were collected in Pyrex glass test tubes capped with polyethylene film and kept at room temperature until analyzed for their lead, cadmium and arsenic concentrations.

2.3.3. Preparation of blank and standard solutions[13]

Instrumental procedures for various analyses were based on those suggested in the operator manual of the Flame Atomic Absorption Spectrophotometer (VARIAN, model AA240 FS, Australia). However, blank and standard solutions were prepared in the same manner as applied for wet digestion and by using the same chemicals.

Blank solution consisted of 10 parts of nitric acid and 1 part of H_2O_2 then was diluted with 25 parts of deionized water and filtered. Furthermore, the standard solutions using pure certified metal standards at different strengths were prepared by10 parts of nitric acid and 1 part of H_2O_2 then was diluted with 25 parts of deionized.

2.3.4. Analysis:

The digest, blanks and standard solutions were aspirated by Atomic Absorption Spectrophotometer and analyzed for their concentrations of such elements.

The apparatus has an auto sampler, digital absorbance and concentration readout capable of operating under the following conditions recommended by the instrument instruction:

Condition	Lead	Cadmium	Arsenic
Lamp wave length (nm)	217.0	228.8	193.7
Lamp current (mA)	5	2	7
Slit width (nm)	1.0	0.7	1.0
Used gas	AC/A	AC/A	AC/N2O

AC/N2O= Acetylene / Nitrous oxide

AC/A= Acetylene / Air

2.3.5. Quantitative determination of heavy metals:

Absorbency of lead, cadmium and arsenic was directly recorded from the digital scale and their concentrations were calculated according to the following equation:

$$C=R \times (D/W)$$

Where,

C= Concentration of the element (wet weight).

R= Reading of digital scale of AAS.

D= Dilution of the prepared sample.

W= Weight of the sample.

N. B. The concentration of each element in the blank solution was alsocalculated and subtracted from each analyzed sample.

2.4. Determination of BAs by using HPLC

Technique:

Four biogenic amines including histamine, cadaverine, putrescine andtyramine were determined in all examined samples based on the protocol recommended by [14]and[15]

2.4.1. Reagents preparation:

- Dansyl chloride solution: 500mg of dansyl chloride were dissolved in 100 ml acetone
- Standard solutions: Stock standard solutions of the tested amines were prepared as the following: add 25 mg of each standard pure amine (histamine-2HCl, cadaverine– 2HCl, putrescine-2HCl) were dissolved in 25 ml distilled water individually.

2.4.2. Extraction of samples

Twenty-five gm of each sample musculature were blended with 125 ml of 5% Tri chloro-acetic acid (TCA) for 3 min, followed by filtration using filter paper (Whatman, No. 1). Thus, 10 ml of the filtrate were transferred into test tube containing 4 gm sodium chloride and 1 ml of 50% sodium hydroxide. The filtrate was extracted 3 times using 5 ml of mixed solution of n-butanol and chloroform (1:1 v/v), and the upper clear layer was transferred to 100 ml separating funnel by using disposable Pasteur pipette. To combine the organic extracts (upper layer), 15 ml of n-heptane was added in separating funnel and extracted three times with 1.0 ml portions of 0.2 n-HCl, the HCl layer was collected in a glass Stoppard tube. Solution was evaporated just to dryness using water bath at 95°C with aid of a gentle current of air.

2.4.3. Formation of dansylamines

One hundred µl of each stock standard solution (or sample extract) were transferred to 50ml vial and dried under vacuum. About 0.5 ml of saturated NaHCO3 solution was added to the residue of the sample extract (or the standard). Vial was stoppered and carefully mixed to prevent loss-due to spattering. Carefully, 1.0 ml dansyl chloride solution was added and mixed thoroughly using vortex mixer. The reaction mixture was incubated at 55°C for 45 min. Actually, 10 ml of distilled water was added to the reaction mixture, then vial was stoppered and shake vigorously using vortex mixer, the extraction of dansylated biogenic amines was carried out using 5ml of diethyl ether for 3 times again, vial was stoppered, shake for 10 min. and the ether layers were collected in a glass tube using disposable Pasteur pipette. The combined ether extracts were carefully evaporated at 35°C in dry bath with aid of current air. The obtained dry material was dissolved in 1ml methanol and 10µl were injected in HPLC.

2.4.4. Apparatus HPLC conditions:

High performance liquid chromatography (HPLC) used for dansylamines determination was an Agilent 1100 HPLC system (Agilen Technologies, Germany) equipped with UV detector (Model G 1314A) set at 254nm wavelength. Data were integrated and recorded using Chemstation Software program.

Table no. 1: Gradient solvent program for separation of BAs by HPLC

Time /min.	Flow rate ml/min.	Solvent A%	Solvent B%	Solvent C%
0	1	60	20	20
10	1	20	40	40
15	1	15	35	50
20	1	60	20	20
25	1	60	20	20

A=0.02N acetic acidB=MethanolC= Acetonitrile

2.5. Statistical Analysis:

Analysis of Variance (ANOVA) test was applied for statistical evaluation of the obtained results for each parameter according to [16].

III. Results

From the achieved results in Table (2), fore quarter meat samples of different species revealed higher heavy metal residue than hind quarter samples. In addition, sheep meat samples showed the highest heavy metal concentrations than cattle and camel meat samples in both collected samples from fore and hind quarter samples. Moreover, lead was the most prominently detected metal residue in comparison with cadmium and arsenic levels in all meat species in both fore and hind quarter collected samples. Furthermore, significant differences were obtained ($P<0.05$) associated with the species and meat cuts.

Table no. 2: Concentrations of heavy metals (mg/Kg) in the examined fore and hind quarter examined meat samples (n=20 of each).

Cuts	Fore Quarter			Hind Quarter		
Meat Kind	Lead	Cadmium	Arsenic	Lead	Cadmium	Arsenic
Cattle meat	0.18 ± 0.01^{b}	0.15 ± 0.01^{b}	0.12 ± 0.01^{b}	0.14 ± 0.01^{b}	0.07 ± 0.01^{b}	0.06 ± 0.01^{b}
Sheep meat	0.27 ± 0.02^{a}	0.20 ± 0.01^{a}	0.17 ± 0.01^{a}	0.21 ± 0.01^{a}	0.14 ± 0.01^{a}	0.13 ± 0.01^{a}
Camel meat	0.12 ± 0.01^{c}	0.09 ± 0.01^{c}	0.04 ± 0.01^{c}	0.05 ± 0.01^{c}	0.04 ± 0.01^{c}	ND

* Means with different superscripts in the same rows were significantly differed ($P<0.05$)

Referring to the compatibility of the examined samples for human consumption in relation to the total number of the examined samples collected from fore and hind quarter (60 of each) based on their heavy metal concentrations, Table (3) showed that more hind quarter samples were recorded to be fit for human consumption than samples collected from fore quarter.

Table no. 3: Fitness of the examined meat samples for human consumption in relation to their heavy metal concentrations (n=60).

Heavy metal	MPL (mg/Kg)*	Accepted samples of fore quarter		Accepted samples of hind quarter	
		No.	%	No.	%
Lead	0.1	44	73.3	49	81.6
Cadmium	0.05	45	75.0	51	85.0
Arsenic	0.25	56	93.3	59	98.3

* Maximum Residual Limit stipulated by EOS[17]No.3602/2013.

From the recorded results of BAs levels in Table (4), fore quarter meat samples of different species revealed higher BAs levels than hind quarter's samples. In addition, sheep meat samples showed the highest BAs concentrations than cattle and camel meat samples in both collected samples from fore and hind quarter samples. Moreover, putrescine was the most prominently detected BA, followed by histamine, cadaverine and tyramine, respectively in all meat species in both fore and hind quarter collected samples. Furthermore, significant differences were obtained ($P<0.05$) associated with the species and meat cuts.

Table no. 4: Concentrations of BAs (mg%) in the examined of fore and hind quarter examined meat samples (n=20 of each).

Cuts	Fore Quarter				Hind Quarter			
Meat Kind	Histamine	Putrescine	Cadaverine	Tyramine	Histamine	Putrescine	Cadaverine	Tyramine
Cattle	11.7 ± 0.5^{b}	15.2 ± 0.8^{b}	10.8 ± 0.6^{b}	4.9 ± 0.3^{b}	9.9 ± 0.4^{b}	12.4 ± 0.6^{b}	9.8 ± 0.5^{b}	3.3 ± 0.3^{b}
Sheep	13.8 ± 0.9^{a}	17.1 ± 1.0^{a}	12.2 ± 0.8^{a}	6.7 ± 0.5^{a}	12.0 ± 0.7^{a}	14.7 ± 0.8^{a}	10.5 ± 0.6^{a}	4.6 ± 0.3^{a}
Camel	10.5 ± 0.6^{c}	11.9 ± 0.7^{c}	10.1 ± 0.7^{c}	3.9 ± 0.3^{c}	8.4 ± 0.5^{c}	9.3 ± 0.5^{c}	8.6 ± 0.5^{c}	2.5 ± 0.2^{a}

* Means with different superscripts in the same rows were significantly differed ($P<0.05$)

Referring to the edibility of the examined samples for human consumption in relation to the total number of the examined samples collected from fore and hind quarter (60 of each) based on their BAs concentrations, Table (5) showed that more hind quarter samples were recorded to have BAs within safe limits than samples collected from fore quarter. Moreover, referring to tyramine, all the examined samples (100%) were within the permissible limits; while putrescine had the lowest acceptability levels in comparison with histamine, cadaverine and tyramine.

Table no. 5: Acceptability of the examined samples of fore and hind quarter retailed meats based on their BAs levels (n=60).

BAs	MRL (mg%)*	Accepted samples of fore quarter		Accepted samples of hind quarter	
		No.	%	No.	%
Histamine	20	56	93.3	57	95.0
Putrescine		49	81.6	53	88.3
Cadaverine		54	90.0	57	95.0
Tyramine		60	100	60	100

*Egyptian Organization for Standardization "EOS" [17](2013). No.3602/2013 for fresh beef

IV. Discussion

Presence of heavy metal residues and/or BAs in a concentration exceeding the permissible limits have been considered a silent threat in the consumer's health. Not only for their bad impacts on the general health, but also because of the apparently wholesome foods; so, their detection in foods especially meats and meat products is essential to keep the consumer's health and safety.

A. Detection of heavy metals

A.1. Lead concentration

Referring to the recorded results in **Table (2),** the obtained mean values of lead concentrations were higher than those recorded by **Hassouba**[18] (0.002 mg/kg), and **Makanjoula**[19] (lead levels ranged from 0.01 to 0.02 mg/kg according to the locality of collection while some samples had not lead concentration); while were lower than those recorded by **Badis et al.** [20](0.77, 0.34 and 0.2 mg/kg for beef, sheep and camel meat samples collected from Algeria, respectively); **Sathyamoorthy et al.** [21](ranged from 2.4 to 4.6 mg/kg in the examined

cattle meat samples collected from India),and**El-Ghareeb et al.** [22] (2.26 and 2.71 mg/kg for camel and sheep meat samples collected from Al-Ahsa central slaughterhouse, Saudi Arabia, respectively; while came in line with the obtained results in the point of sheep meats revealed higher lead concentrations than camel meats).

A.2. Cadmium concentration

Concerningwith cadmium concentration (mg/kg) mean values as mentioned in **Table (2),**nearly similar results were obtained by **Badis et al.** [20] (0.15, 0.13 and 0.09 mg/kg for beef, sheep and camel meat samples collected from Algeria, respectively), while higher results were previously reported by **Sathyamoorthy et al.** [21] (5.1 to 6.6 mg/kg of the examined cattle meat samples collected from India); **El-Ghareeb et al.** [22] (0.29 and 0.44 mg/kg for camel and sheep meat samples collected from Al-Ahsa central slaughterhouse, Saudi Arabia, respectively; while came in line with the obtained results in the point of sheep meats revealed higher lead concentrations than camel meats), but**Hassouba et al.** [18] (0.005 mg/kg of the examined beef samples collected from Luxor city, Egypt)**,** and **Makanjoula**[19] (0.01 mg/kg in the examined cattle meats, while some samples had not lead concentration) reported lower results.

A.3. Cadmium concentration

Referring to the recorded results of Arsenic levels in the examined meat samples in **Table (2),**higher results were obtained by **Sathyamoorthy et al.** [21] (ranged from 3.7 to 5.6 mg/kg of the examined cattle meat samples collected from India), and **El-Ghareeb et al.** [22] (12.89 and 10.05 mg/kg for camel and sheep meat samples collected from Al-Ahsa central slaughterhouse, Saudi Arabia, respectively), while **Makanjoula**[19] did not record any positive cattle, sheep or camel examined meat samples for Arsenic contamination in their examined samples collected from Nigeria.

Contamination with heavy metals is a serious threat because of their toxicity, bioaccumulation and biomagnifications in the food chain [23]. Although contamination of animal feed by toxic metals cannot be entirely avoided given the prevalence of these pollutants in the environment, there is a clear need for such contamination to be minimized, with the aim of reducing both direct effects on animal health and indirect effects on human health [24]. Toxic effects of metals have been described in animals under relatively low levels of metal exposure; one of the earliest effects is the disruption of trace element metabolism [25].

The risk associated with the exposure to heavy metals present in food product had aroused widespread concern in human health. Improvements in the food production and processing technology had increased the chances of contamination of food with various environmental pollutants, especially heavy metals. Ingestion of these contaminants by animals causes deposition of residues in meat. Due to the grazing of cattle on contaminated soil, higher levels of metals have been found in beef and mutton [26]. **Gonzalez-Waller et al.**[27] also recorded the levels of toxic metals (lead and cadmium) in meat product exceeding recommended limits.

The risk of heavy metal contamination in meat is of great concern for both food safety and human health because of the toxic nature of these metals at relatively minute concentrations [28]. In other cases, contaminated animal feed and rearing of livestock in proximity to polluted environment were reportedly responsible for heavy metal contamination in meat [29].

Lead is a metabolic poison and a neurotoxin that binds to essential enzymes and several other cellular components and inactivates them enhanced its toxic effects which are seen on haemopoietic, nervous, gastrointestinal and renal systems [30]. In addition, food is one of the principle environmental sources of cadmium as cadmium moves through the food chain it becomes more and more concentrated lead totoxic effects of cadmium causes renal dysfunction, hypertension, hepatic injury and lung damage [31].

B. Biogenic amines

Biogenic amines (BAs) are important nitrogenous compounds formed mainly by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones. In food and beverages, BAs are formed by the enzymes of raw material and generated by microbial decarboxylation of amino acids. Therefore, the total amount and variety of amines strongly depend on the nature of the food and on the microorganisms present. Their presence is related to spoilage and fermentation processes. Toxicological characteristics of food poisoning are generally associated with histamine and tyramine. Other amines, such as putrescine, cadaverine, and phenylethylamine, are also important since they could intensify the undesirable effects of histamine. The other amines may form nitrosamines which are formed by the reaction of secondary or tertiary amines with a nitrosating agent. In foods, the nitrosating agent is usually nitrous anhydride and is produced from nitrite in acidic, aqueous solution. Thus, BAs are considered as a food hazard, although there is not a threshold for these biomolecules in European legislation, except for histamine in fishery products. When present in high concentrations, they could have toxicological effects, causing health problems in consumers, especially for sensitive individuals[32]**.**

The recorded results of BAs of the examined samples as illustrated in Table (4) were compared with some previously reported results by **Triki et al.** [7] (0.34 and 0.1 mg% for tyramine, 1.34 and 1.19 mg% for putrescine in the examined beef and sheep meats, respectively; while histamine and cadaverine were not detected);**Bogdanović et al.**[33](1.2, 1.9, 0.9 and 0.2 mg% for histamine, putrescine, cadaverine and tyramine in the examined meat samples collected from Croatian retail market, respectively), and **Tang et al.** [34] (0.25, 0.45 and 0.22 mg% for cadaverine, putrescine and tyramine in the examined camel meat samples collected from Egypt, respectively.

As well, BAs could be used as an index of food quality in meat, fish and cheese. In a lot of cases, increased levels of amines signal a decreased quality of the commodity earlier than it is possible to judge by sensory evaluation. However, in some fermented meat products there are high levels of amines connected with higher sensory acceptability[35]. Foodborne biogenic amines toxicity lead to dilatation of blood vessels, capillaries and arteries, causing headache, gastrointestinal distress and oedema. Also,tyramine causes the increase of noradrenaline concentration in blood as an indirect effect, acting vasoconstrictor causing hypertension and migraine[36].

Variation between the obtained results of the current study and the previously reported results may be attributed to the difference in the rearing quality, environment, season of collection, locality of sampling, and the hygienic quality of slaughtering and handling of fresh raw meat.

V. Conclusion

In conclusion, there were great fluctuation in the result among cattle, sheep and camel meat sample. Where camel samples recorded the least content of heavy metal and biogenic amines content especially hind quarters samples. Additionally, from the obtained results it is recommended to follow strict hygienic measures adapted during handling of meat at slaughter houses and local markets leading and promote sources of feeding for food animal rearing.

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