Impact of misuse of antimicrobial agents on egg bitterness

Mohmed Karmi¹,
Asem M. Zakaria¹,
Marwa I. Khalifa¹

Abstract

A hundred raw eggs samples randomly selected from Aswan university residence restaurant due to students' complain of bitter taste presence in the table eggs served to them during breakfast. The samples were examined physically and chemically for antibiotic residues and vitamins A, D3 levels concentration by spectrophotometer. The result showed that all interior egg quality traits were normal except the presence of small blood spots in 5 eggs yolk, bitter taste were present in 18 eggs yolk, antimicrobial residues were detected in 19 eggs yolk and 4 eggs white. Antimicrobial residues positive samples were examined quantitatively by HPLC for doxycycline (DC) residues presence. The results showed that 100% of examined samples having DC residues with means concentrations of 35 ± 1.46 and 97 ± 4.46 ppb for eggs white and yolk, respectively. The mean levels of vitamin A and D3 in eggs yolk were 447 ± 5.49 $\mu\text{g}/100\text{g}$ and 11 ± 1.35 $\mu\text{g}/100\text{g}$ respectively. We conclude that the high concentrations of vitamins and doxycycline may be the cause of bitter taste of eggs.

¹ Department of Food Hygiene, Faculty of Veterinary Medicine, Aswan University, 81528 Sahary City, Egypt.

Contact information:

Marwa Ibrahim Khalifa.

Address: Department of Food Hygiene, Faculty of Veterinary Medicine, Aswan University, 81528 Sahary City, Egypt.

✉ mrwakhalifa@yahoo.com

Introduction

In late decades, there has been an expanding interest in functional nourishments, which is relied upon to keep on expanding later on. By 2050, the total world population is anticipated to achieve 9 billion individuals, with the most noteworthy population development rates happening in locales experiencing sustenance uncertainty. The International Egg Commission's vision is to provide an incessant and maintainable food supply, ensuring food self-sufficiency for the human population in the future [1]. To cob with the global demand by 2030, as much as 89 million tons of eggs will be required [2].

In Egypt, one of the most common demanded foods is chicken eggs; it is considered an economical source of high-quality protein and naturally contains most of the recognized vitamins (A, D, E, and

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B-vitamins). Moreover, eggs provide unique well-balanced nutrients for all people of different ages. Low caloric value and easy digestion makes eggs valuable in many therapeutic diets for adults [3]. Owing to global chicken eggs importance as being a functional food with nutritional significance, health benefits that affects nutrients, mass production and consumption patterns, so the evaluation of the external and internal quality of chicken eggs is important, and the improvements in egg quality and nutritional value may affect positively the daily nutrient consumption and accordingly the human health [4].

Egg flavor is considered one of the most important sensory properties that receive considerable interest within the poultry industry. Egg flavor can be affected by many factors. Those factors will vary depending upon the housing environment, genetics, drugs, storage area, fumigants or chemicals used in agriculture and feed ingredients such as fish oil and meal, soured milk, some vegetables including (onion, garlic, turnip, cabbage) and rapeseed or canola [5]. Egg bitterness is considered a flavor defect incompatible with consumer acceptance. Abnormal bitterness tends to be equated with dietary danger [6]. The unpleasant bitter taste can be found generally in rancid fats, decomposed proteins, plant-derived alkaloids and other toxins [7]. Some antibiotics as erythromycin, doxycycline, vancomycin, tetracycline, oxytetracycline, and bacampicillin have bitter taste specially when deposited in the yolk [8]. Residues of unchanged chemicals may remain in the commodity that may cause a physical and chemical alteration in its properties, thus resulting in flavor change, odor, taste, nutritional value and processing qualities [4]. Moreover, those residues can cause risks to the health of human, including allergic reactions, direct toxicity and increased bacterial tolerance to common antibiotics [9]. The two keys aspect of egg production and processing are egg quality and consumer acceptability.

The aim of this study was to investigate and determine the bitter taste causes which were present in table eggs served to students in Aswan university residence and its seriousness on the students' health.

Materials and Methods

Eggs sampling

A total of 100 raw brown hen's egg samples were randomly selected from the Student's Hostel Restaurant of Aswan University to be examined for determination of the cause of bitter taste. All samples were taken to the laboratory with a minimum of delay and kept in refrigerator until be examined.

According to the information obtained from the farm supplier and the University restaurant; the eggs were fresh, stored and transported under conditions that not affect the safety and quality of the egg. This information was confirmed through freshness testes and Haugh unit measurement which were normal.

Physical examination of eggs/ Exterior eggs quality traits

The external egg quality traits investigated were, shell cleanness, shell soundness, egg weight (g), shell color and shell texture. The shell color was assessed by naked eye, while shell texture was examined through palpation. The exterior egg quality is examined according to United State Department of Agriculture Food Safety and Inspection Service (USDA 2000) [10]. The egg weight was measured by a digital balance [11].

Interior raw eggs quality traits

A group of 30 raw eggs from the 100 randomly selected samples were examined physically for interior quality according to Nonga *et al.* [12] and Song [13]. The yolk and albumen heights were determined using a tripod micrometer, pH were measured

by pH meter, the color, and presence of blood spots or meat spots were inspected by naked eye also odor was smelled.

Organoleptical examination of boiled eggs

Thirteen eggs were boiled for 10 minutes, followed by slow cooling to be examined organoleptically for the presence of abnormal taste and odor in either egg white or yolk. Then 5 experienced panelists and 5 non-experienced persons were asked to test the boiled eggs for its taste and odor.

Antimicrobial residues detection/ Disc assay screening method

Disc assay was applied as initial screening test to determine whether there were antimicrobial residues (generally) in the eggs or not. Therefore, ciprofloxacin has been used as a positive control because it is not used in poultry farms, and can be helpful in judging the area of inhibition.

Thirteen eggs from the original 100 selected eggs samples were prepared for antimicrobial residues examination in white and yolk according to El Nasri *et al.* [14]. The indicator organism used was *Bacillus stearothermophilus*, discs were immersed into the eggs white and yolk individually. Then the discs were fixed on the surface of agar plates with 2 control discs of distilled water (negative control) and ciprofloxacin (positive control). The plates were then incubated at 37°C for 24 hours. The inhibition zone around the discs were measured and recorded.

Quantitative detection of doxycycline residues

Antimicrobial residues of positive samples were examined quantitatively for presence of DC residues by HPLC, Agilent series 1050 quaternary gradient pump with a reversed-phase column (C18) according to Furusawa [15].

Determination of vitamin A and D3 in eggs yolk

Vitamins A and D3 were determined in eggs yolk Spectrophotometrically according to AOAC official method [16].

Results and Discussion

Physical characterizations of eggs

The results in **Table 1** showed that all eggs were clean, having a normal shape with no shell cracks, but 45% of examined eggs have a rough shell with irregular pigmentation. The Exterior shell of the normal egg should be smooth, clean and free from cracks to avoid penetration by microorganisms, as per the guidelines set by USDA [10]. Rough shell or abnormal shell texture may be inherited, or due to some diseases as Newcastle disease (ND). Infectious laryngotracheitis (ILT) and Infectious bronchitis (IB), excessive use of antimicrobials as sulfa drugs, and tetracyclines, excess calcium consumption by the hens and copper deficiency [17].

Interior egg quality (albumin, yolk and overall quality) which is the most important to consumers starts to decline as soon as the egg is laid. Inspection of **Tables 1 & 2** showed that 30 (100%) examined raw eggs having good interior quality traits of both white and yolk as they have normal, odor, shape, color and size with mean Haugh unit of 69.8 ± 2.76 . Only small blood spots were present in eggs yolk of 16.7% of examined samples. These Blood spots may be due to ovarian hemorrhage which may be inherited, or ovarian diseases, ambient temperature, age of the hen (incidence is higher in older hens) deficiency of vitamin K, using sulfa drugs and antibiotics [18].

However consumer acceptance of eggs, depending on egg quality parameters such as Haugh units, yolk color, storage stability but also in particular the sensory traits [19]. By reviewing the data of organoleptic examination of 30 examined boiled

Table 1. Physical examination of exterior and interior raw egg quality traits.

Defect	No. examined samples	Positive samples	
		N	%
Tainted eggs	100	0	0
Rough shell with irregular pigmentation	100	45	45
Cracked shell	100	0	0
Mold growth	30	0	0
Discolored white	30	0	0
Embryonated egg	30	0	0
Small Blood spots	30	5	16.7
Meat spots	30	0	0
Stuck yolk	30	0	0
Bloody eggs	30	0	0

Table 2. Mean values of raw eggs characteristics.

Trait	No. examined samples	Mean value $\pm$ SE
Egg weight, g	100	58.2 $\pm$ 4.37
Albumen weight, g	30	32.3 $\pm$ 1.64
Albumen height, mm	30	5.2 $\pm$ 0.43
Yolk weight, g	30	17.6 $\pm$ 1.85
Yolk height, cm	30	1.22 $\pm$ 0.09
Haugh unit	30	69.8 $\pm$ 2.76
Albumen pH	30	9.2 $\pm$ 0.23
Yolk pH	30	6.2 $\pm$ 0.18

Table 3. Organoleptic examination of interior boiled egg contents.

Defect	No. examined samples	Positive samples	
		N	%
Abnormal odor of egg white	30	0	0
Bitter taste of egg white	30	0	0
Abnormal odor of egg yolk	30	0	0
Bitter taste of egg yolk	30	18	60

eggs recoded in **Table 3**. The following results were observed; 100% of examined eggs having a normal odor of both white and yolk, normal taste of eggs white and bitter taste in 60% of eggs yolk. Some of the causes of egg flavor problems have been thought to be traditional feed and feed additives [7]. Also some of the causes may be residues of unchanged chemicals as drugs, fumigants or chemicals used in agriculture and feed ingredients such as fish oil and meal, soured milk, some vegetables include, garlic, cabbage and rapeseed or canola [5].

Chemical analysis of eggs / Antimicrobial detection

Due to the multiplicity of the bitter taste causes, it was difficult to determine the specific cause of the egg bitter taste problem.

So, it was necessary to reviewing the veterinarian responsible for the farm supplier. The vet confirmed that the farm feeding system had not changed, but he had given a five days course of doxycycline (DC) for a mild respiratory manifestation present in the farm. Then a five days course of vitamins (AD3E) were given to improve production efficiency after antibiotic treatment.

Based on this information, 30 raw eggs were examined for the presence of antimicrobial residues in eggs white and yolk separately by disc assay method. The result revealed that 19 (63%) eggs yolk and 4 (13%) eggs white have antimicrobial residues as reported in **Table 4**. The sample is considered positive for antibiotic residues if it shows a zone of inhibition of about 2mm around the disc.

Table 4. Antimicrobial residues in the eggs white and yolk of examined samples.

Trait	No. examined samples	Positive samples	
		N	%
Egg white	30	4	(13)
Egg yolk	30	19	(63)

The sample is considered positive for antibiotic residues if it shows a zone of inhibition of about 2mm around the disc.

The excessive use of food additives and antibiotics in poultry farms and poor veterinary service lead to the appearance of antibiotics residues in eggs. The yolk has the longest development time of the three main egg components (yolk, albumen, and shell). Drugs linked with lipoprotein in the hen's liver. The egg yolk undergoes a stage of rapid growth before an egg is laid and speedy accumulation of lipoproteins drug complex in yolk occurs during this time and can appear in successive eggs following treatment for more than 10 days [20]. Albumen can also serve as a storage site for drug residues within eggs but the egg albumin contents produced within 24 hours after ovulation, so yolk potential for persistent drug residues is more than that of the albumen [21]. In the study carried out in Uganda, Sasanya *et al.* [25] recorded that 95% of the farmers never respect the withdrawal periods of drugs although 80% of them knew its importance and the eggs were sold before the end of withdrawal periods. In Khartoum State-Sudan; El Nasri *et al.*, [14] detected antibiotic residues in 49.6% of table egg.

According to disc assay, the antimicrobial positive samples were examined quantitatively for the presence of DC residues by HPLC. The number of positive samples supports to be enough to confirm our hypothesis about bitter taste cause.

The result recorded in **Table 5** confirmed that DC residues were detected in 100% of examined samples with means concentration of 35 ± 1.46 and 97 ± 4.46 ppb for eggs white and yolk respectively. DC is a yellow crystalline powder with a bitter taste [23]. DC bitterness may be the cause of bitter taste present in eggs as residues of unchanged chemicals

Table 5. Concentrations (ppb) of DC residues in the examined eggs samples.

Trait	No. examined samples	Positive samples		Mean value $\pm$ SE
		N	%	
Egg white	4	4	100	35 ± 1.46
Egg yolk	19	19	100	97 ± 4.46

may change the taste of the foods [24]. Because DC has high activity against a broad range of bacteria, it has been widely utilized in poultry to control chronic respiratory diseases, infectious synovitis, and fowl typhoid. Most farmers believed that there is no relation between antibiotics usage and their presence in eggs so they were selling eggs during administration to avoid economic loss that leads to presence of drug residues in tissues and eggs [12]. Residues of DC were detectable for a shorter period in egg white than egg yolk because the depletion period for egg yolk was longer than the white. The same result was reported by Gajda and Posyniak [25] who detected DC in the eggs of 12 days treated hens after withdrawal and 2 more days in egg yolk than in white. According to CODEX [26], there is no maximum residual limit (MRL) for DC in eggs and its use in laying hens is forbidden [27].

Many adverse biological effects may occur from DC residues in products for human consumption such as allergic reactions, autoimmunity, hepatotoxicity, and development of bacterial resistance. Bacterial resistance is the main cause of newly emerging infectious diseases traits, narrow the line of defense against microbial infections and increase health care costs [25].

Vitamins concentrations in eggs

The remained 10 eggs were examined spectrophotometrically for vitamin A and D3 levels in eggs yolk. The result reported in table 6 revealed that the mean level of vitamin A and D3 as indicated in Table 6 in eggs yolk were 447 ± 5.49 μ g/100g 11 ± 1.35 indicated in **Table 6** μ g/100g respectively which are higher than normal level that may be due to supplementation of vitamins (AD3E) to improve production efficiency after antibiotic treatment. The same conclusions were reported by Zang *et al.* [28] and Browning and Cowieson [29] that hens receiving more vitamins produce eggs with higher levels of vitamins. Thus hens gastrointestinal health is significantly affected by vitamin nutrition which

Table 6. Concentrations of vitamins A and D in eggs yolk.

Vitamin	No. examined samples	Normal control egg*	Mean $\pm$ SE**	USDA***
		level μ g/100g	μ g /100g	μ g/100g
Vitamin A	10	126	447 $\pm$ 5.49	160
Vitamin D/ D3	10	3.2	11 $\pm$ 1.35	2.0

*: The normal control level in the table refers to the level of vitamins in normal organic eggs. Obtained from organic farms and sensory examined. **: Mean of Vitamins in examined eggs (bitter taste). ***: The general levels of vitamins in eggs according to USDA 2018 [31].

will improve egg quality and reduces the number of dirty and cracked eggs produced, this could have positive marketing ramifications in eggs with a higher vitamin level and a better nutritive value. But the addition of a high level of Vitamin A and D in hen's diet may create off flavor of poultry products like meats and eggs [30].

Conclusion

The data obtained from this study showed that the DC residue may be one of the factors that causing the bitter taste in eggs. Eggs with antibiotic residues considered unfit for human consumption. Therefore there's a need to increase awareness of the farmers and veterinarians to prevent antibiotics misuse and how to respect the dose and withdrawal period of any drug used. In Egypt, routine screening test for the poultry products to detect antibiotics residues must be practiced. This approach would also slow the general development of antibiotics resistance.

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