

ASSESSMENT OF MEAT PROTEIN QUALITY OF EXPERIMENTALLY BROILERS FED DIET SUPPLEMENTED WITH PROBIOTICS USED AS SUBSTITUTE TO ANTIBIOTICS IN LUXOR CITY

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Received: 25 September 2022; **Accepted:** 12 December 2022

ABSTRACT

It has been proven that including probiotics in the diet of poultry will increase the quality of the meat and protein. Therefore, the purpose of this paper was to evaluate the administration of antibiotics and probiotics to broiler chickens and to determine the contribution of probiotics to the prevention of *Salmonella typhimurium* infection in Ross broiler hens. Three groups of one-day-old Ross broilers each contained: group1(G1-50 chicks), group2 (G2-100 chicks), and group3(G3 -100 chicks). (G1-50 chicks)negative management (non-infected and non-treated chicks). (G2-100 chicks) gentamycin and colistin sulphate were administered to chicks at the age of one who had *S. typhimurium* infections for five days at a dose of 0.5 gm/25 liters of drinking water. G3 third group (100 chicks) probiotics (Guardizen M) mixed probiotics concentrate 5.6 g (1x10¹⁰ cfu) of *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, and *Bifidobacterium bifidum* were given to chickens who had *S. typhimurium* infections. It was supplementary to the water for five days in a row. Each chick in the experimentally infected groups received an oral inoculation of 1 mL of saline suspension containing 10⁹ CFU of *S. typhimurium* at the age of 6 days. After infection, the experiment's time frame was extended by three weeks. Take a swab for a Salmonella count to detect infection at 14, 21, and 28 days of age after the onset of symptoms. Results showed that group G3 had lower clinical symptoms and a lesser mortality level than group G2. The PM examination showed that the liver had hypertrophy, round borders, and white necrotic foci on its surface in addition to being discoloured. The bowel displayed enteritis with hemorrhagic patches, a bloody yolk sac that wasn't digested, petechial intestinal mucosal bleeding, and enteritis. In G2 treated with antibiotics, the *S. typhimurium* count was 4x10⁷, 3x10⁶, and 2x10⁶ CFU in the second, third, and fourth weeks, respectively. Antibiotics were used to treat the other group, G3. The counts of *S. typhimurium* in the second, third, and fourth weeks were, respectively, 3.6x10⁷, 2.3x10⁶, and 1.1x10⁶ CFU. In the meat of broilers nourished feed treated with probiotics and antibiotics, the percentage of protein content was 22,8% and 22,0%, respectively. The current analysis found that samples containing colistin sulphate had residue levels of 200 ppb that were higher than the permitted residue limits (150 ppb).

Keywords: probiotics , poultry, Salmonella typhimurium, Lactobacillus plantarum

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INTRODUCTION

In recent years, an increasing number of researchers have devoted time and energy to examining the function of probiotics in various situations. Live microscopic organisms known as probiotics can provide a host with a variety of health benefits when given in a prescribed dosage (FAO and WHO, 2002). Additionally, probiotics can improve nutrient absorption and digestion (Yirga, 2015). In the poultry business, maintaining a normal gut microflora through the competitive exclusion of gut microorganisms is the most crucial function of probiotics. They have an impact on them via their byproducts (such as organic acids and volatile fatty acids) and also reduce the pH of the gut environment, which generates an unfavorable environment that prevents the growth of harmful bacteria and fungi (Allou *et al.*, 2014). Additionally, adding probiotics to a broiler's diet could change the flavor of their meat while also compromising their safety (Wadoum *et al.*, 2019). Lactic acid bacteria (LAB), the most popular probiotic, has been successfully employed as a substitute for antibiotics in animal production. LAB strains have been effectively used in chicken feed as supplements to provide advantages including accelerating growth (Nafees 2018) and reducing enteric pathogen infectiousness (Wang *et al.*, 2018). *Salmonella spp.* are most common in poultry, particularly *S. typhimurium* (Liu *et al.*, 2019). Probiotic medication is an efficient technique to deal with pathogenic infections with few side-effects and a high safety profile because growth-promoting drugs are prohibited in many countries (Plaza-Diaz *et al.*, 2019; Shi *et al.*, 2020).

Compared to other animal production methods, commercial chicken production reported antibiotic usage more frequently. Antibiotics were utilized by farmers for both medicinal and preventative purposes (Chowdhury *et al.*, 2021). Antibiotics are used to either manage illnesses such as

intestinal infections and respiratory ailments or to encourage growth (Elbaz *et al.*, 2020). (Fasina *et al.*, 2016). The presence of "antibiotic residues" in poultry meat, one of the most significant effects of antibiotic use, compelled the European Union to outlaw the usage of antibiotics as growth promoters (European Union Commission 2005). Antibiotics are misused and/or overused, which leads to the accumulation of residues meant for human consumption in poultry tissues. Colistin has been used widely, and resistance has been growing quickly. Sorour and co. (2022). Researchers from a number of different nations have found "antibiotic residues" in poultry meat [NRP 2017 and Al-Mashhadany 2018]. In the meantime, "antibiotic residues" in poultry tissues may coexist, having a direct harmful effect on consumers and being one of the main factors in human infections developing antimicrobial resistance. Additionally, it has been linked to allergies in hypersensitive people (Muaz *et al.*, 2018). This study used the A-Clinical Signs, Mortalities, and PM assessment to assess the administration of probiotics and antibiotics to broiler chickens. Additionally, it is also done to check for "Colistin sulphate residue" in chicken meat.

MATERIALS AND METHOD

1. Probiotics:

It contains *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, and *Bifidobacterium bifidum* in 5.6 g (min. 11010 CFU) of mixed probiotics concentrate (Guardizen M). According to the manufacturer's recommendations, it was administered in the water to drink or in the feed at one day of age for five days in a row at a dose of 0.5 gm/25 liters of the drinking water or 1000 g/ton of feed.

2. Antibiotic drug:

At one day of age, gentamycin and colistin sulphate medications are given five days in a row at a dose of 0.5 gm/25 litres of drinking water. Gentamycin was bought from

Shams's Company and Expired date of gentamycin is 3 years.

About colistin sulphate were bought from fayser's Company and the expiry date of gentamycin is 3 years, whereas, Guardizon was bought from Geopharma's Company and the expiry date of gentamycin is 3 years.

3. Preparation of the *S. Typhimurium* strains:

Timms *et al.* (1990) approach was used to set the challenge inoculum (1990). *S. Typhimurium* strains were prepared in the Animal Health Research Institute, Doki previously recovered *S. Typhimurium* (Group B 1, 4, 5, 12 I 1, 2) strains from the Luxor province and identified them using serology. Using a 0.5 McFarland tube, sediment was collected, diluted with sterile buffer saline, and adjusted to have 109 CFU/ml.

4. Chickens:

250 total chicks from which hundred mixed-sex 1 day-old Saso broiler chicks were used to assess the effectiveness of probiotics and

antibiotics in preventing *S. typhimurium* infection. The chicks were from a breeder flock that was salmonellosis-free. A control group of 50 chicks was included in the random division of the chicks into two equal groups, each of which comprised 100 birds. At 6 and 17 days of age, respectively, all birds received the standard immunization regimen for broilers against illnesses like New Castle using live vaccine strains of the Hitchner B1 and La Sota viruses. At the age of 14, a live intermediate strain (228 E) used in the Gumboro disease vaccination was given. All vaccinations were administered by instilling ocular drops. The birds were kept in sanitary, well-ventilated, separate experimental rooms and floor pens.

5. Experimental Design:

We used 250 mixed-sex Saso broiler chicks that were just one day old. Ten chicks were selected at random and sacrificed on the first day. To demonstrate that the chicks had no *S. typhimurium* infections, the swap was removed and analyzed bacteriologically. The following table shows how the groups of chicks were randomly divided:

Table 1: Groups of chicks in experimental design.

Groups	Number
G1 Negative control (non-infected and non-treated chicks).	50 chicks
G2 (chicks infected with <i>S. typhimurium</i> and treated with antibiotics)	100 chicks
G3 (chicks infected with <i>S. typhimurium</i> and treated with probiotics)	100 chicks

6. Experimental infection:

Each chick in the experimentally infected groups received an injection of 1 ml of saline suspension containing 109 CFU of *S. typhimurium* at the age of 7 days old (Okamoto *et al.*, 2007). After infection, the experiment's time frame was extended by three weeks. A swab was taken to count the *S. typhimurium* after the onset of symptoms in order to identify the infection.

7. Detection and counting of *S. Typhimurium*:

Swab contents were extracted aseptically at the age of 14 days old and put into sterile

tubes with 9 ml of buffer peptone water before being cultured for 18 hours.

In accordance with ISO 6579 (2002) and AMD, the cells were next plated on XLD agar and cultured for 24 hours at 37°C (2020).

8. Evaluation of the Probiotics and Antibiotics Administration:

A. Clinical signs, mortalities, and PM examination:

All chicks were observed daily for clinical signs, mortality, and post-mortem lesions.

B. Determination of protein content:

The Kjeldahl formula was used to compute the protein. The protein is digested at a high temperature with concentrated sulfuric acid, sodium sulphate, and a metal catalyst to convert the meat's nitrogenous components into ammonium salts. The free ammonia is then produced by distilling the ammonium salts with steam and collecting it with either a solution of diluted boric acid or hydrochloric acid that has been suitably colored with pH indicators. During the hydrochloric acid treatment, surplus hydrochloric acid is back-titrated to neutral using a sodium hydroxide solution.

According to Hanai *et al.* (2018). 's instructions, colistin was extracted from muscle samples and calibration samples using solid-phase extraction (SPE), which was then followed by derivatization with FMOC-Cl solution. The drug was examined using reversed-phase Ki-netex® XB C18 columns and HPLC (Agilent 1200). (4.6 mm i.d., 250 mm, 5 μm). The column temperature was 40 °C, and all measurements were performed at "excitation" and "emission" "wavelengths" of (260 nm) and (315 nm), respectively. Acetonitrile, tetrahydrofuran, and water (50:14:20, v/v/v) in the "mobile phase" at a flow ratio of 1.6 ml/min.

9. Detection of "antibiotic residues":**RESULTS**

Table 2: Mortality rate in G1 control, G2 treated with antibiotics and G3 treated with probiotics.

Week	G1 Control (50 chicks)	G2 (100 chicks)	G3 (100 chicks)
2 nd week	5	9	8
3 rd week	3	6	4
4 th week	2	4	2

Table 3: Detection of *S. typhimurium* isolated from chicken meat.

Week	G2 treated with antibiotics	G3 treated with probiotics
2 nd week	detected	Detected
3 rd week	detected	Detected
4 th week	Not detected	Not detected

Table 4: Count of *S. typhimurium* isolated from intestinal content.

Week	G2 treated with antibiotics	G3 treated with probiotics
2 nd week	4×10^7 cfu/g	3.6×10^7 cfu/g
3 rd week	3×10^6 cfu/g	2.3×10^6 cfu/g
4 th week	2×10^6 cfu/g	1.1×10^6 cfu/g

Table 5: Determination of meat protein content using Kjeldahl method.

Meat protein content	G2 treated with antibiotics	G3 treated with probiotics
	22%	22.8 %



Fig. (1) liver of freshly dead birds with white necrotic foci on the surface.



Fig. (2) Intestine of freshly dead birds with haemorrhagic patches, bloody unabsorbed yolk sac and petechial hemorrhage of the intestinal mucosa.

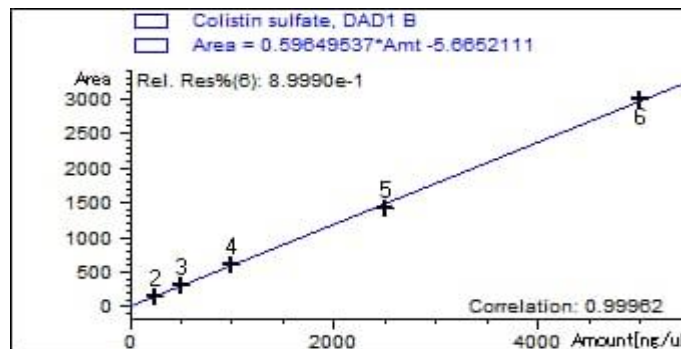


Fig. (3): HPLC standard calibration curves for Colistin sulfate standards at 200 ppb

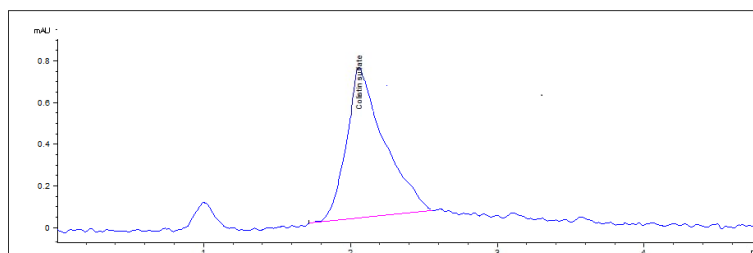


Fig. (4): Chromatograms showing 200 ppb of Colistin sulfate in water at a retention time 2.1 min.

DISCUSSION

Solid-phase extraction (SPE), followed by derivatization with FMO-CI solution, was used to extract colistin from muscle samples and calibration samples in accordance with the methodology reported by Hanai *et al.* (2018).

Using a reversed-phase Ki-netex® XB C18 column, the medication was analyzed by HPLC (Agilent 1200). (4.6 mm i.d., 250 mm, 5 m). All observations were made with the column temperature at 40 °C with excitation and emission "wavelengths" of (260 nm) and (315 nm), respectively. Acetonitrile, tetrahydrofuran,

and water (50:14:20, v/v/v), at a "flow rate" of 1.6 ml/min, make up the mobile phase. The broilers in the control treatment displayed worse than the treatments on day 21 after administration. By the second, third, and fourth weeks of the trial, 9, 6, and 4 chicks in G1 and 8, 4, and 2 in G2 died, respectively. (Table 2). The mortality rate was lowered to 2% with the addition of probiotics. According to Elsayed *et al.* this was the case (2011). When challenged orally with *Salmonella Pullorum* mixed culture with *Salmonella Gallinarum*, the probiotic mix group (G3) produced the lowest mortality rate of 2/20 (10%) dead chicks, compared to 4/20 (20%) dead chicks in the control negative group and 7/20 (35%) dead chicks in the positive control group. According to Khan *et al.* (2011), and Takam (2019) probiotics were fed to chickens, and this had a positive impact on their immune systems and decreased mortality rates. This is because probiotics may hasten the improvement of healthy microflora in chicks and boost their resistance to infection by some enteric bacterial pathogens.

The important organs in the probiotics-treated groups revealed very minor alterations during the PM test (Fig.1&2). The livers of the recently deceased birds displayed round borders, hypertrophy, and discolouration. On the surface of the liver, there are white necrotic foci (Fig. 1). Bloody unabsorbed yolk sacs, enteritis, petechial bleeding of the intestinal mucosa, and haemorrhagic patches were all seen in the intestine (Fig. 2). These results harmonize with those of Elsayed *et al.* (2011), who discovered that enteritis was the cause of PM lesions in the positive control group, enteritis and enlargement with necrosis of the liver, spleen, and kidneys were present in the second group, and enlargement of the liver, spleen, and kidneys were present in the third group (those who received probiotics).

Approximately 200 serovars of "Salmonella" have been isolated from poultry and poultry products, with *Salmonella enteritidis* and *Salmonella typhimurium* being the most frequently associated serovars with poultry outbreaks [Cadirci *et al.* (2021)] and Timms *et al.* (1990) *Salmonella* infection in poultry has long been classified as a zoonotic disease of economic importance in public health throughout the world [Antunes *et al.* 2016; Popa and Popa, 2021]. In the current investigation, *S.*

typhimurium was detectable in the intestinal content of chicks during the second and third weeks of the trial, but by the fourth week in G1 beside G2 chicks, it was no longer detectable (Table 3). In G2 treated with antibiotics, the *S. typhimurium* count was 4×10^7 , 3×10^6 , and 2×10^6 CFU in the second, third, and fourth weeks, respectively. A probiotic treatment reduced it in G3 to 3.6×10^7 , 2.3×10^6 , and 1.1×10^6 CFU in the second, third, and fourth weeks, respectively (Table 4). In G3, there was a decline in the number of *S. typhimurium* (infected and treated with the probiotic group).

Probiotics' ability to lower the number of *Salmonella spp.* was thoroughly researched by a number of studies. *Salmonella spp.* and other bacterial infections could be suppressed by *Lactobacillus sp.* treatment, according to Mohamed *et al.* (2019). The CFU in the group supplemented with probiotics and challenged with *Salmonella enteritidis* showed no change at 420×10^8 by the start of the second week, but it showed a noticeable decrease of 230×10^8 by the experiment's 30th day when compared to the group that received a standard diet and was challenged with *Salmonella* 10×10^8 and 712×10^8 via the end of the experiment. There are a number of hypotheses that explain how probiotics containing lactic acid bacteria prevent *Salmonellae* from colonizing birds. One of them is the production of lactic acid, which has an unfavourable pH for *Salmonellae* growth (Johanssen *et al.*, 2004); another is the competition between enteric bacteria and *Lactobacilli*, which is known as competitive exclusion (Heres *et al.*, 2003); and a third is the production of bacteria (Pascual *et al.*, 1999). By producing organic acids, hydrogen peroxide, and bacteriocins, Kaktcham *et al.* (2019) demonstrated that lactic acid bacteria might impede the activity and proliferation of harmful germs. There were two phases to bacteriocin's inhibitory mechanism. The first stage involved the target bacterial membrane cells' specific and nonspecific receptors absorbing bacteriocin. The bacteriocins grow more sensitive during this stage, particularly to proteolytic enzymes. Lethal alterations in the sensitive strains occurred during a second phase that was irreversible (Damayanti *et al.*, 2012).

In terms of meat protein content, broilers that nourished diets complemented with probiotics and antibiotics had meat with a protein content

of 22.8% and 22.0%, respectively (Table 5). This is in line with Suryadi *et al.* (2019) who discovered that feed supplemented with the highest concentration of probiotics produced meat with the highest protein content. According to Ibrahim *et al.* (2021), adding prebiotics to diets that contained 95, 90, and 85% of the protein needed to meet NRC requirements considerably enhanced the amount of protein in the broiler breast and thigh meat. In theory, the presence of "lactic acid bacteria" is what causes broilers fed with probiotics to have a high meat protein content. In addition to producing digestive enzymes like proteases, these bacteria may survive in the "digestive system" and attach to the intestinal walls (Widiyaningsih, 2011). He also advises using probiotics that contain lactic acid bacteria, which can produce other digestive enzymes that break down chemical bonds in nutrients, including protein, to make molecules smaller and easier to absorb, leading to an increase in the rate at which these nutrients, including protein, are absorbed. Meat has more protein because of increased protein absorption.

In order to increase the digestibility, growth, and health of animals, antibiotics have been added to animal feed ingredient mixes. On the other side, its usage as a growth promoter can encourage resistance (Krisnan *et al.*, 2019). The withdrawal time after taking antibiotics is another issue. Cardinal *et al.* (2019) discovered that raising production costs led to a rise in the cost of poultry meat as a consequence of eliminating antimicrobial growth boosters from the broiler diet. In poultry and livestock agriculture, almost 90% of antibiotics are administered at subtherapeutic levels (Sorour *et al.*, 2022). High levels of colistin resistance were found by Dawadi *et al.* (2021). Maximum residue limits (MRLs) in foods of animal origin have been regulated by the European Commission and are mentioned in the Commission Regulation (EU) in order to avoid exposing end consumers to "antibiotic residue" concerns (2010). The results of this analysis demonstrated that samples containing colistin sulphate had residual levels of 200 ppb (Figs. 3 and 4) that were higher than the permitted residue limits (150 ppb). Due to a deficiency of detection techniques and monitoring that restrict the amount of drug residue in foods through strict residue limits, there is a very high danger of residue in poor nations (Kebede *et al.*, 2014). Numerous studies have established that the main adverse effect on

human health is the presence of leftover antibiotics in meals derived from farm animals (Islam *et al.*, 2020). The colistin residue was mentioned in a number of research. Thuat *et al.* (2020), who discussed the "antibiotic residues" in chicken meat, demonstrated one of them. Additionally, they stated that colistin was shown to be a remnant of 13% of the 26 different antibiotics that were heavily utilized in the production of chickens. Using a "Thin Layer" Chromatography experiment, Bristy *et al.* (2019) discovered that the colistin residue in broiler meat was 50%.

CONCLUSION

The current study suggested employing probiotics in poultry since they can significantly improve flock health, have a positive impact on *S. typhimurium* counts, don't require a withdrawal time, and provide the highest broiler protein content.

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تقييم جودة ونسبة البروتين في دجاج التسمين تم تغذيته على الخمائر كبائل للمضادات الحيوية في مدينة الأقصر

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لقد ثبت أن تضمين البروبيوتيك في غذاء الدواجن سيزيد من جودة اللحوم والبروتينات. لذلك ، كان الغرض من هذه الدراسة هو تقييم إعطاء المضادات الحيوية والبروبيوتيك لدجاج التسمين وتحديد مدى مساهمة البروبيوتيك في الوقاية من عدوى ميكروب سالمونيلا التيفيموريوم في دجاج التسمين روس وتم تقسيمها الى المجموعات الاتية:- المجموعة الاولى G1 وتتكون من ٥٠ ككتوت روس وتسمى المجموعة السلبيه وتحتوى على الكتاكت غير المصابة وغير المعالجة. المجموعة الثانية G2 وتتكون من ١٠٠ ككتوت روس وتم معالجتها ب Gentamycin و colistin sulphate تلك الكتاكت مصابة بعدوى *S. typhimurium* وذلك لمدة خمسة أيام بجرعة ٥,٥ جم / لتر من ماء الشرب. المجموعة الثالثة G3 تتكون تلك المجموعة من ١٠٠ ككتوت روس تم إعطاءها في مياه الشرب لمدة خمسة أيام متتالية البروبيوتيك المختلط (Guardizen M) بتركيز ٥,٦ جم (١x10¹⁰ cfu) من *Lactobacillus plantarum* ، *Lactobacillus acidophilus* ، *Lactobacillus rhamnosus* ، *Lactobacillus bulgaricus* ، و *Bifidobacterium bifidum* تلك الكتاكت مصابة بعدوى *S. typhimurium* .. تلقى كل ككتوت في المجموعات المصابة تجريبياً تلقياً فموياً قدره ١ مل من محلول ملحي يحتوي على ١٠٩ زيمبايوي من *S. typhimurium* في عمر ٦ أيام. بعد الإصابة ، تم تمديد الإطار الزمني للتجربة لمدة ثلاثة أسابيع. تم أخذ عينة من السالمونيلا للكشف عن العدوى في عمر ١٤ و ٢١ و ٢٨ يوماً بعد ظهور الأعراض. أظهرت النتائج أن المجموعة G3 كانت لديها أعراض سريرية أقل ومستوى وفيات أقل من المجموعة G2. أظهر فحص الصفة التشريحية أن الكبد يعاني من تضخم وله حواف مستديرة وبؤر نخرية بيضاء على سطحه بالإضافة إلى تغير لونه. أظهرت الأمعاء التهاباً معوياً مع بقع نزفية ، وكيس محي دموي لم يتم هضمه، ونزيف مخاطي في الأمعاء والتهاب معوي. في G2 المعالج بالمضادات الحيوية، كان عدد ميكروب سالمونيلا التيفيموريوم 4x10⁷ ، 3x10⁶ ، 2x10⁶ CFU في الأسابيع الثاني والثالث والرابع على التوالي. تم استخدام المضادات الحيوية لعلاج المجموعة الأخرى G3. كان عدد ميكروب سالمونيلا التيفيموريوم في الأسابيع الثاني والثالث والرابع على التوالي ٣,٦x10⁷ و ٢,٣x10⁶ و ١,١x10⁶ CFU. في لحوم الدجاج اللحم المغذية المعالجة بالبروبيوتيك والمضادات الحيوية ، كانت نسبة البروتين ٢٢,٨٪ و ٢٢,٠٪ على التوالي. وجد التحليل الحالي أن العينات المحتوية على كبريتات الكوليسيتين تحتوي على مستويات متبقية من ٢٠٠ جزء في البليون أعلى من حدود البقايا المسموح بها (١٥٠ جزء في البليون).