Study of The Effect of Folic Acid on The Phenotypic Appearance of Local Chicken Embryos

***Fatma Ali Gliwan Mona Hassan Abu Hajar Fatima Mustafa Al-Shaqmani**

Department of zoology, Misurata University
*Corresponding author: f.glewan @sci.misuratau.edu.ly

Submission data: 16.10.2024 Acceptance data: 6.2.2025 Electronic publishing data:8.2.2025

Abstract: Folic acid is a type of vitamin essential for poultry health. It plays an important role in the formation of DNA and cell division. It also helps reduce the risk of neural tube defects in the fetus. It is important in the formation of red blood cells and helps them grow and perform their functions in a healthy manner. In addition, it is considered essential for healthy embryonic development.

In this study, the effect of folic acid on the external appearance of local chicken embryos was studied. 90 fertilized eggs of equal sizes were collected and divided into three groups, each containing 30 eggs. The first group was injected with folic acid at a concentration of 20 micrograms, the second group with folic acid at a concentration of 40 micrograms, and the third group was not injected and then placed in incubators.

This study concluded that there was no difference between uninjected embryos and embryos injected with two different concentrations of folic acid 20 and 40 micrograms at the age of 7 days. It was noted that folic acid enhanced the growth of injected embryos at the age of 14 days, and at the age of 21 days, egg hatching was normal and no abnormalities were observed in the chicks.

Keywords: Folic acid, Phenotypic, chicken, embryo, concentration.

Introduction:

Folic acid is an essential nutrient from the B-vitamin group. Folic acid is involved as a cofactor in many intracellular reactions (Donnelly, 2001). It is an essential element required for a range of enzymatic reactions in amino acid synthesis and is given to pregnant women. There are studies that have shown that folic acid has negative effects on fetal development (Greenberg *et al*., 2011). Acquired folic acid deficiency is very common and is associated with malnutrition, obesity and kidney failure (Shulpekova *et al.,* 2021). Folic acid can overcome health problems in chicken embryos as it is available in feed and water, which leads to increased development of the reproductive system, folic acid content in eggs, hatchability, and weight gain. Ingested folic acid is absorbed through the intestinal lumen and transported to the liver via blood vessels (Pertiwi *et al.,* 2022). It also has positive effects on the nutrition of chicken embryos and on growth performance and blood component levels and increased blood glucose, folic acid, and phosphorus levels, and reduced levels of cholesterol and triglycerides in chicken eggs injected with folic acid ((Nouri *et al.,* 2018).

Some researchers have indicated that folic acid increases the growth rate of chicken embryos and thus increases body weight [1]. Folic acid has several effects, including in case of folic acid deficiency, malnutrition, chronic diseases, depression, and lack of concentration. Positive effects are beneficial for treating depression, treating Alzheimer's disease, treating lack of concentration, preventive treatment during pregnancy, and reducing the incidence of neural tube defects (Reynolds, 2002).

Classification of chickens:

Chickens are classified in the animal kingdom according to Al-Nasser *et al.* (2007).
Kingdom: Animalia
Phylum: Chordata
Subphylum: Vertebrata
Class: Aves
Order: Galliformes
Family: Phasianidae
Genus: *Gallus*
Species: *Gallus gallus.*

Hebert *et al.* (2005) studied the effect of adding folic acid to chicken diet and the aim of folic acid performance in chicken eggs. A significant effect of folic acid was observed in egg plasma, which increased its improvement, and was not affected in the total weights of eggs and egg yolks. The researcher in [2] conducted a study to determine the effect of injecting chicken eggs with folic acid at different concentrations. The study concluded that there was an increase in embryonic development and egg hatching rate length and a high increase in embryo, yolk weight and a decrease in the weight of the white chicken eggs (Kahraman *et al.,* 2008). studied the effect of folic acid and adding high levels to chicken feed. The aim was to study the effect of high levels of folic acid on the performance and quality of chicken eggs. The study concluded that adding folic acid at high levels to the diet had a positive effect on the weight of chicken embryos and egg production and quality. Tactacan *et al.* (2010) studied the effect of folic acid in eggs and the activities of enzymes dependent on folic acid. It aimed to study the concentration of folic acid in the blood levels of chicken embryos. The study concluded that folic acid has an effect in improving egg performance in chickens, and folic acid supplements have an Effective effect on chicken embryo enzymes,

Hebert *et al.*(2011) conducted two studies to determine whether the types of grains in chicken diets could affect the folic acid content of chicken eggs. In the first study, the type of grains in the diet significantly affected the total folic acid content. In the second study, the productive performance of chickens was not affected by the type of grain. The study concluded that the type of grains in the diet may affect the concentration of folic acid in eggs; however, food supplements do not affect the concentration of folic acid in eggs. Tactacan *et al.*(2012) conducted a study to evaluate the effect of increasing folic acid on chicken embryos. The study concluded that folic acid does not affect the production performance of chicken embryos and an increase in the concentration of folic acid was observed in the plasma of chicken eggs. Oosterbaan *et al.*(2012) studied the effect of folic acid on the growth and morphology of chicken embryos. The aim is the effect of folic acid on the development of blood vessels in chicken embryos. The study concluded that there are no negative effects during development and on the morphology and blood vessels in chicken embryos, the researchers [3, 2012] studied the effect of injecting chicken eggs with different levels of folic acid, the levels were (25-30-35 micrograms/egg) egg hatching, deformities, productive performance and age of chicken embryos. The aim is the effect of injecting chicken eggs with folic acid on productive performance. We conclude that injecting chicken eggs with folic acid led to an improvement in the hatching rate, productive performance and age of chicken embryos. Chicken embryos and no abnormalities in the embryos.

(Yu *et al.*, 2014) conducted a study to determine whether folic acid affects the level of brain cells in the body of chickens, the results showed that folic acid increased the proliferation of brain cells but reduced the accumulation of fat in each cell, which affects mental differentiation, Aruna Adepu and Sagar Narala, (2016) studied the protective effect of folic acid in chicken embryos for two days. Folic acid was injected into chicken eggs. The study concluded that folic acid is effective in preventing congenital abnormalities in chicken embryos. Liu *et al.* (2016) conducted a study to find out whether folic acid enhances the embryonic growth of newly hatched chicken embryos. The aim is to investigate the effect of injecting folic acid into eggs on the growth of chicken embryos. The study concluded that folic acid improved the morphology of chicken embryos. Said *et al.* (2017) The researchers studied the protective effect of folic acid on improving eggs during hatching. The study aimed to evaluate the effect of injecting eggs with folic acid on growth performance and egg production. The results indicated that chicken embryos hatched from eggs increased in the improvement of the morphology of chicken embryos. Nouri *et al.* (2018) studied the effect of injecting chicken eggs with folic acid on growth performance and blood component levels in chickens. An increase in glucose levels was observed, while cholesterol, triglycerides, and calcium levels in eggs decreased. It had positive effects on growth performance and blood component levels of chicken embryos.

Janist *et al.*(2019) studied the effect of folic acid on egg yolk concentration and egg quality and examined the productive performance of chicken embryos at different levels (0-0.02-0.04) micrograms of folic acid. The results indicated that folic acid had no effect on productive performance, egg quality, or egg yolk concentration in chicken embryos. Turgut *et al.* (2019) studied the dose-response relationship of the effect of folic acid on chicken embryos. The first group was not injected with folic acid, and the second group was injected with folic acid. The study concluded that there was no effect of folic acid on chicken embryos. Liu *et al.* (2020) studied the effects of folic acid regulation in the liver cells of chicken embryos. The results showed that folic acid increased significantly within the liver cells of injected chicken embryos compared to the group not injected with folic acid. The study concluded that folic acid helps to regenerate liver cells in chicken embryos. By studying folic acid during boiling eggs, folic acid, this study compared the contents of folic acid in chicken eggs, a significant difference in the content of folic acid in eggs was observed, the percentage was found to be 15%, and the color of organic egg yolk was more abundant after adding 60 grams of folic acid dose (Czarnowska *et al.*, 2021). Sun *et al.* (2021) This study was conducted to measure the percentage of folic acid in chicken eggs. When folic acid was added to the system at different levels for chickens from (0 to 10 mg/g), the study found that folic acid in egg yolk was found at 91-98% of the total folic acid in chicken eggs, Gonzales *et al.*(2023) studied the effect of folic acid injection on the weight and morphology of body organs in chicken embryos, the study found that folic acid increases the improvement of the morphology and improves the weight gain performance in chicken embryos, Oladokun *et al.* (2023) studied the effect of folic acid in feed and hatching performance Chicken and body growth, the study concluded that folic acid helped improve embryonic development and hatching performance in chickens.

Objective of the study:

This study aims to know the extent of the effect of different concentrations of folic acid on the phenotype in local chicken embryos, and whether it has any negative effects or deformities in the embryos when injected with different concentrations of folic acid.

Materials and methods:

Materials used:

Samples used: 90 fertilized eggs from fertilized chickens were used.

Apparatus and materials used:

. Egg incubator.

. Fertilized chicken eggs.

. Gloves.

. Paraffin wax.
. Injection needles.
. Alcohol.
. Folic acid at two concentrations of 20 micrograms and 40 micrograms.
2.4 Preparation of folic acid concentrations:
- To prepare a concentration of 20 micrograms, add 0.1 ppm of folic acid to 200 ml of distilled water.
- To prepare a concentration of 40 micrograms, add 0.2 ppm of folic acid to 200 ml of distilled water.

Method of work:

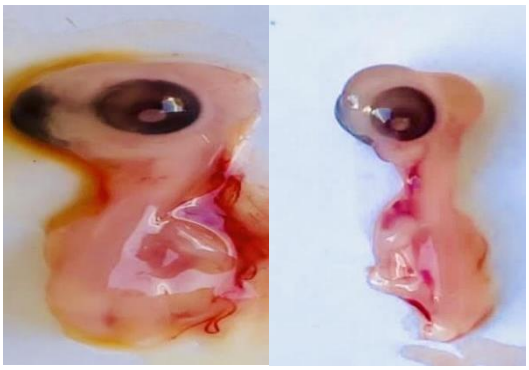
Experimental Design:
The fertilized eggs were taken and divided into 3 groups, each group containing 30 eggs as follows:
- The first group: not injected (control sample).
- The second group: was injected with a concentration of 20 micrograms folic acid .
- The third group: was injected with a concentration of 40 micrograms folic acid (Liu *et al*., 2016).
1- It was punctured the eggs with a needle.
2- It was injected the eggs with the appropriate concentrations.
3- It was closed the hole with paraffin wax). (Liu *et al*., 2016)
4- Then the eggs were placed in the incubator and the temperature was set to (37.5 degrees Celsius).
30 eggs were examined at 7 days of age, then another 30 eggs were examined at 14 days of age, then 30 eggs were examined after hatching at 21 days of age, where 10 eggs were taken from each group and the groups injected with different concentrations were compared with the control sample.

Statistical analysis:
The SPSS program was used to conduct the statistical analysis to study the difference between the groups of eggs injected with folic acid and those not injected, using the analysis of variance (ANOVA) test.

Results and Discussion:

Results of sample examination:
90 samples of fertilized eggs were examined through different stages of this study at the ages of 7 days, 14 days, and 21 days, and the difference between chicken embryos injected with folic acid and uninjected embryos (control sample) was identified, and observations and observations were recorded in the following time periods:

Embryos aged 7 days: It was observed in chicken embryos not injected with folic acid that the growth of the embryo and the appearance of the limbs were clearly formed as in Figure (1-A). When comparing embryos injected with two different concentrations of 20 micrograms and 40 micrograms of folic acid with uninjected chicken embryos, it was observed that there were no differences or deformities in the external appearance as in Figure (1-B-C).



(A) (B)

(C)

Figure (1): A 7-day-old fetus (A-Fetus not injected with folic acid B-Fetus injected with a concentration of 20 micrograms of folic acid C-Fetus injected with a concentration of 40 micrograms of folic acid).

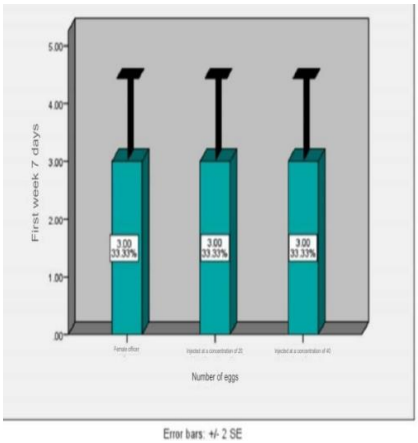


Figure (2) Number of eggs not injected with folic acid and injected with a concentration of (20-40) micrograms at the age of 7 days.

From Figure (2), we notice that the three groups are equal and there is no difference when comparing eggs injected with folic acid and eggs not injected with folic acid, and the p-value = 1.000) was greater than the alpha value, which indicates that there are no significant differences.

Embryos aged 14 days: It was observed in chicken embryos not injected with folic acid that the growth of the fluff and limbs was clearly formed as in Figure (3-A), and in chicken embryos injected with a concentration of 20 micrograms of folic acid, the body shape was larger in size and the growth of the fluff was greater compared to the uninjected embryos as in

Figure (3-B), and in embryos injected with a concentration of 40 micrograms of folic acid, it was also observed that the growth of the fluff was greater compared to the uninjected chicken embryos and those injected with a concentration of 20 micrograms and the body was larger in size as in Figure (3-C), evidence that folic acid enhanced the growth of the injected embryos.

(A) (B)

(C)

Figure (3) : a 14-day-old embryo (A-Embryo not injected with folic acid B-Embryo injected with 20 micrograms of folic acid C-Embryo injected with 40 micrograms of folic acid).

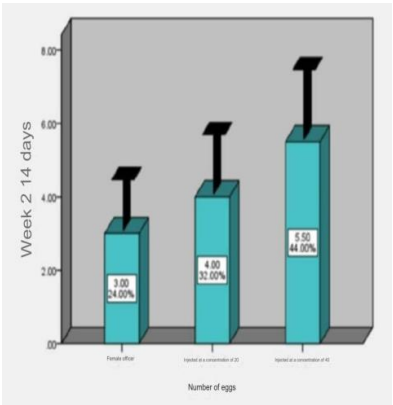


Figure (4): Number of eggs not injected with folic acid and injected with two different concentrations of 20 and 40 micrograms at 14 days of age.

From Figure (4), we notice that there is an increase in the growth of embryos injected with folic acid at a concentration of 20 micrograms compared to embryos not injected with folic acid, and the value (p-vale=0.000) was less than the alpha value, which indicates the presence of significant differences. We also notice that the embryos injected with a concentration of 40 micrograms had greater growth compared to the embryos not injected and the embryos injected with a concentration of 20 micrograms, and the value (p-vale=0.000) was less than the alpha value, which indicates the presence of significant differences.

21-day-old embryos: The eggs were hatched in the three groups (non-injected embryos - embryos injected with two different concentrations of 20 and 40 micrograms of folic acid), and no negative effects or deformities were observed in the morphology of the chicken embryos.

(A) (B)

(C)

figure (5): 21-day-old embryos (A- Embryos not injected with folic acid B- Embryos injected with a concentration of 20 micrograms of folic acid C- Embryos injected with a concentration of 40 micrograms of folic acid).

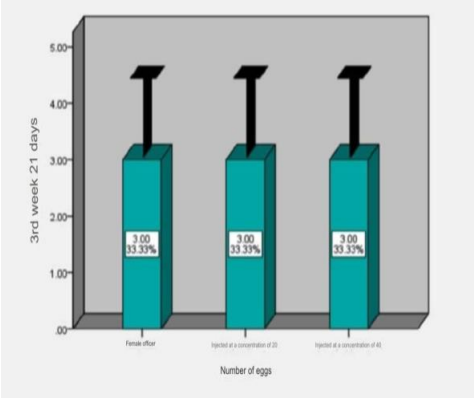


Figure (6): shows the number of eggs not injected with folic acid and injected with two different concentrations of 20 and 40 micrograms at the age of 21 days.

From Figure (6), we notice that the three groups are equal and there is no difference when comparing the eggs injected with folic acid and the eggs not injected with folic acid, and the p-value = 1.000)) was greater than the alpha value, which indicates that there are no significant differences.

Discussion:

The results of our study agreed with Liu *et al*. (2016) that injecting fertilized chicken eggs with folic acid at concentrations of 20-40 micrograms improved the morphology of the injected embryos and there were no negative effects or deformities during the incubation period.

It also agreed with Gonzales *et al*.(2023) that injecting chicken eggs with folic acid led to an improvement in the morphology and an increase in the weight of chicken embryos, and it also agreed with El Said (2017) that the protective effect of folic acid on growth and egg production led to an improvement in the morphology and enhanced the growth of chicken embryos.

It also agreed with the authors[3] when injecting chicken eggs with different levels of folic acid and its effect on embryos, an improvement in the morphology was observed and there were no deformities in chicken embryos.

There is no study that did not agree with our study.

Conclusion:

We conclude from this study:

1- When injecting chicken eggs with folic acid, it has a positive effect on embryonic formation in the early stages and increases growth and improves the external appearance.

2- The effect of folic acid solution with a concentration of 20 and 40 micrograms does not improve the hatching of chicken eggs and their hatching is normal.

3- There is a relationship between increasing the concentration of folic acid and improving the performance of egg hatching and the growth of chicken embryos.

Recommendation:

We recommend through this study to conduct such studies on embryos of other animals to know the effect of folic acid on embryonic formation and to use folic acid in different doses to clarify the effect of folic acid, we recommend the importance of estimating the cumulative dose of folic acid for pregnant women.

References:

[1] **الجاف، فرح خالد عبدالكريم، (2005)**: تأثير حقن بيض التفقيس بمستويات مختلفة من محلول حمض الفوليك في التطور الجنيني والصفات الانتاجية و الفسيولوجية. للفروج الناتج، رسالة ماجستير، كلية الزراعة، جامعة بغداد.

[2] **خلدون عبد اللطيف، فرح الجاف(2007):** تأثير حقن بيض الدجاج بمحلول حمض الفوليك بتراكيز مختلفة في الطور الجنيني ونسبة الفقس للبيض الكلي والمخصب، قسم الثروة الحيوانية – مجلة علوم الدواجن، كلية الزراعة -جامعة بغداد، العدد (2: 159 - 168) العراق.

[3] **الشمري ناجح جابر ، عبد الهجو نادية (2012)**: تأثير حقن بيض التفقيس بحمض الفوليك في الصفات الانتاجية والنوعية ذبائح فروج اللحم، قسم الثروة الحيوانية، كلية الزراعة جامعة بغداد، العدد -4(2):76-88 العراق.

[4] **Al-Nasser, A., Al-Khalaifa, H., Al-Saffar, A., Khalil, F., Albahouh, M., Ragheb, G., ... & Mashaly, M. (2007).** *Overview of chicken taxonomy and domestication. World's Poultry Science Journal, 63(2), 285-300.*

[5] **Aruna Adepu, Sagar NaralaPublished 2016** *Medicine, Biology TLDR Folic acid in combination with the inositol can be used to prevent the neural tube defects caused by cotinine and has shown better efficacy when compared with their individual treatments. Corpus ID: 212611515.*

[6] **Czarnowska-Kujawska, M., Draszanowska, A., Gujska, E., Klepacka, J., & Kasińska, M. (2021)**. *Folate content and yolk color of hen eggs from altered farming systems. Molecules, 26(4), 1034.*

[7] ***Donnelly, J. G. (2001).*** *Folic acid. Critical reviews in clinical laboratory sciences, 38(3), 183-223.*

[8] **El Said, E. A. (2017*).*** *Effect of fortification of eggs with folic acid during incubation and post hatch ascorbic acid supplementation on productive performance, blood constituents and immune status of broiler chicks. Egyptian Journal of Nutrition and Feeds, 20(2), 277-285.*

[9] **Greenberg, J. A., Bell, S. J., Guan, Y., & Yu, Y. H. (2011).** *Folic acid supplementation and pregnancy: more than just neural tube defect prevention. Reviews in obstetrics and gynecology, 4(2), 52.*

[10] **Gamboa Gonzales, N. F., Alves Leão, A. P., Ribeiro Alvarenga, R., & Zangeronimo, M. G. (2023).** *The effects of in ovo injection with sulfur amino acids and folic acid on the gene expression, relative organ weights, hematologic parameters, performance, and carcass characteristics of broiler chickens. Animal Biotechnology, 34(7), 2207-2218.*

[11] **Hebert, K., House, J. D., & Guenter, W. (2005).** *Effect of dietary folic acid supplementation on egg folate content and the performance and folate status of two strains of laying hens. Poultry science, 84(10), 1533-1538.*

[12] **Hebert, K., Tactacan, G. B., Dickson, T. M., Guenter, W., & House, J. D. (2011).** *The effect of cereal type and exogenous enzyme use on total folate content of eggs from laying hens consuming*

diets supplemented with folic acid. Journal of Applied Poultry Research, 20(3), 303-312.

[13] **Janist, N., Srichana, P., Asawakarn, T., & Kijparkorn, S. (2019).** *Effect of supplementing the laying hen diets with choline, folic acid, and vitamin B12 on production performance, egg quality, and yolk phospholipid. Livestock Science, 223, 24-31.*

[14] **Kahraman, R., Abas, I., Eseceli, H., & Toker, N. (2008)**. *The effect of high levels of folic acid on performance and egg quality of laying hens fed on diets with or without ascorbic acid from 28 to 36 weeks of age. J Anim Vet Adv, 7(4), 389-395.*

[15] **Liu, Y., Zhi, L., Shen, J., Li, S., Yao, J., & Yang, X. (2016).** *Effect of in ovo folic acid injection on hepatic IGF2 expression and embryo growth of broilers. Journal of animal science and biotechnology, 7, 1-9.*

[16] **Liu, Y., Zhao, J., Wang, F., Zhou, J., & Yang, X. (2020).** *Comparative proteomic analysis of chicken primary hepatocytes with folic acid free or supplementation culture mediums. Journal of World's Poultry Research, 10(1), 1-11.*

[17] **Nouri, S., Ghalehkandi, J. G., Hassanpour, S., & Aghdam-Shahryar, H. (2018).** *Effect of in ovo feeding of folic acid on subsequent growth performance and blood constituents levels in broilers. International Journal of Peptide Research and Therapeutics, 24, 463-470.*

[18] **Oladokun, S., & Adewole, D. (2023).** *An investigation of the effect of folic acid and its delivery routes on broiler chickens' hatch and growth performance, blood biochemistry, anti-oxidant status, and intestinal morphology. Journal of Animal Science, 101, skad083.*

[19] **Oosterbaan, A. M., Steegers, E. A., & Ursem, N. T. (2012).** *The effects of homocysteine and folic acid on angiogenesis and VEGF expression during chicken vascular development. Microvascular research, 83(2), 98-104.*

[20] **Pertiwi, H., Nur Mahendra, M. Y., & Kamaludeen, J. (2022).** *Folic Acid: Sources, Chemistry, Absorption, Metabolism, Beneficial Effects on Poultry Performance and Health. Veterinary medicine international, 2022.*

[21] **Reynolds, E. (2002).** *Benefits and risks of folic acid to the nervous system. Journal of neurology, neurosurgery, and psychiatry, 72(5), 567.*

[22] **Sun, D., Jin, Y., Zhao, Q., Tang, C., Li, Y., Wang, H., ... & Zhang, J. (2021).** *Modified EMR-lipid method combined with HPLC-MS/MS to determine folates in egg yolks from laying hens supplemented with different amounts of folic acid. Food Chemistry, 337, 127767.*

[23] **Shulpekova, Y., Nechaev, V., Kardasheva, S., Sedova, A., Kurbatova, A., Bueverova, E., ... & Ivashkin, V. (2021).** *The concept of folic acid in health and disease. Molecules, 26(12), 3731..*

[24] **Tactacan, G. B., Jing, M., Thiessen, S., Rodriguez-Lecompte, J. C., o'Connor, D. L., Guenter, W., & House, J. D. (2010).** *Characterization of folate-dependent enzymes and indices of folate status in laying hens supplemented with folic acid or 5-methyltetrahydrofolate. Poultry science, 89(4), 688-696.*

[25] **Tactacan, G. B., Rodriguez-Lecompte, J. C., & House, J. D. (2012).** *The adaptive transport of folic acid in the intestine of laying hens with increased supplementation of dietary folic acid. Poultry science, 91(1), 121-128.*

[26] **Turgut, U., Kazan, S., Cakin, H. A. K. A. N., & Ozak, A. H. M. E. T. (2019).** *Valproic acid effect on neural tube defects is not prevented by concomitant folic acid supplementation: Early chick embryo model pilot study. International Journal of Developmental Neuroscience, 78, 45-48.*

[27] **Yu, X., Liu, R., Zhao, G., Zheng, M., Chen, J., & Wen, J. (2014).** *Folate supplementation modifies CCAAT enhancer-binding protein α methylation to mediate differentiation of preadipocytes in chickens. Poultry Science, 93(10), 2596-2603.*