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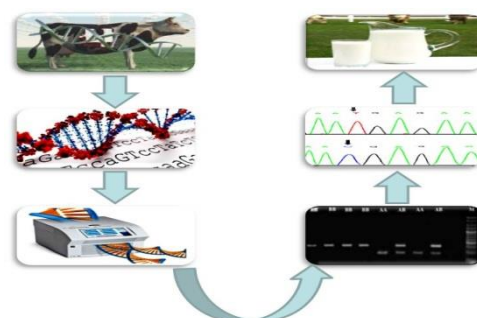
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Korevaar H (1992). The nitrogen balance on intensive Dutch dairy farms: a review. In: A. A. Jongebreur et al. (Editors), Effects of Cattle and Pig Production Systems on the Environment: Livestock Production Science, 31: 17-27.

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AOAC (1990). Association of Official Analytical Chemists. Official Methods of Analysis, 15th Edition. Washington D.C. pp. 69-88.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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|---|---|---|---|
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| **Molar** | mol/L | **Mililitre** | ml |
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6. In the English articles, a decimal point should be used instead of a decimal comma.

7. In chemical formulae, valence of ions should be given, e.g. $Ca2+$ and $CO32-$, not as $Ca++$ or $CO3$.

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# Identification of Locally Isolated *Clostridium difficile* from Rabbits

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## ABSTRACT

*Clostridium difficile* is one of the most important pathogens causing diarrhea and enteritis in rabbits as it causes pseudomembranous colitis that leads to intestinal damage and deaths. In this study, screening of rabbit farms from different localities in Egypt had shown rabbits suffered from diarrhea and enteritis to detect *Clostridium difficile* by ELISA, it revealed that five out of 50 samples (10%) were positive for it. These samples were further identification by cultivation and culture characters, microscopical examination, agglutination test, pathogenicity test and Polymerase Chain Reaction (PCR) by using specific primers for toxins genes (tcdA and tcdB). The results showing that three out of five isolates were confirmed as *Clostridium difficile* and concluded that these isolates causing pseudomembranous enterocolitis in rabbits and this disease unable to be treated by antibiotics, so it used for preparation of vaccine against the disease in rabbits.

**Keywords**: *Clostridiunm difficile*, Rabbits, Enteritis

## INTRODUCTION

There is a strong and continuing interest in the development of rabbit industry in Egypt. Rabbit industry as one of the small livestock has a unique commercial role in solving the shortage in the meat after poultry industry (Mohamed et al., 2013). To achieve this purpose a special light should be thrown on dangerous rabbit diseases which may affect the industry. Enteritis complex is one of the important causes of disease and economic losses in younger rabbits. Many causes lead to enteritis and can result in health problems for the rabbit (Songer, 1996).

Clostridia species are the cause in the induction of enteritis problem in rabbits with high occurrence percentages (Hong et al., 2017). Different types of Clostridial species found in weaned rabbit farms at Egyptian governorates. Clostridial enterotoxaemia refers to enteritis caused by toxogenic microorganism of the genus Clostridium which characterized by diarrhea and may be sudden death. The main etiological agentsare *Clostridium Perfringens; Clostridium spiroforme; Clostridium Butyricum,*and *Clostridium difficile* (Khalefa et al., 2012; El-Helw et al., 2014)

*Clostridium difficile* (*C. difficile*) is a gram positive, spore forming,anaerobic, toxin producing bacillus, catalase negative bacterium (Bruce et al., 1999). Pathogenic strains of *C.difficle* produce two potent toxins , enterotoxin A and cytotoxin B, these toxins are the virulence factors of the organism and thereisa global problem which caused by ingestion of vegetative organisms and spores , most likely the latter which survive exposure to gastric acidity and germinate in the colon (Doosti and Mokhtari, 2014). *C. difficile* recognized as a major nosocomial pathogen responsible for antibiotic related diarrhea and is etiological agent of perforation of the colon, pseudomembranous colitis or toxic megacolon and even death in humans (Hung et al., 2012). *C. difficile* also is an important factor for enteric disease in other species; it cause enterocloitis associated with diarrhea in horses specially foals (Taha, 2014), it cause chronic diarrhea in dogs (Andrea et al., 1994), and had been isolated from calves, pigs, rabbits and cats (Rodriguez Palacios et al., 2006). In rabbits, cases of clostridiosis caused by *C. difficile* have been reported from 35 to 55 days of age, in which enteroclitis with liquid caecal content was observed. *C. difficile* was first isolated from faeces of four often healthy newborn infants in 1935; it was named *Bacillus difficilis* because of difficulty in isolating and studying these bacteria. (Hall and O`Toole, 1935)

The aim of this study is to isolate and identify *C. Difficile* and confirm it as a causative bacterial agent of diarrhea from naturally infected rabbits and as a primarily step for preparation of vaccine from locally isolated strain.

## MATERIALS AND METHODS

### Ethical approval
All procedures performed according to Egyptian ethical standards of the National Research Committee.

### Screening for detection of *C. difficile* infection in rabbits

Fifty rabbits aged between one to four months were recently dead where suffered from abdominal distension and diarrhoea, obtained from different farms in Egypt. Screening was done by using RIDASCREEN® *C. difficile* toxin A/B kit (R-Biopharm AG, Dermstadt, Germany). This Kit using monoclonal antibodies against *C.difficile* toxins A and B used as screening test for detection of *C. difficile* toxins. Faecal and ceacal samples (approx. 100µl) were aspirated and 1ml RIDASCREEN® *C. difficile* sample dilution buffer were added, samples were homogenized, blend in votex mixer, suspension left stand a short period of time for the coarse stool particles to settle, and clarified supernatant of samples suspension used in the test. The assay was done according to procedure of ELISA Kit manual. The optical density of samples; positive, and negative control were recorded after reading by ELISA reader. Calculation the cut-off for the negative control, an assessment of specimen as positive if extinction rate is more than 10% higher than calculated cut-off value.

### Isolation of the causative agent

Positive faecal samples and/or ceacal contents from rabbits were collected and alcohol shocked is used (Saverio and Pauline, 1980), then pellets were inoculated on *C. difficile*agar base (CM0601, Oxoid LTD, England) with *C. difficile* selective supplement (SR0096E, Oxoid LTD, England) and incubated for 48 hours anaerobically at 37 °C (Saverio and Pauline, 1980). Suspected colonies were harvested and stained with Gram stain. Microscopical examination, culture characters (culture morphology, odour and effect of long wave length ultraviolet) and biochemical tests (catalase and oxidase tests) were carried out (Hafiz and Oakely, 1976).

### *Clostridium difficile* agglutination test

*Clostridium difficile* latex agglutination kit (Oxoid Ltd. DR1107, England) where used for detection of *C. difficile*. A loopful from the suspected colonies, positive control provided (*C.difficile* cell wall antigen) and negative control (0.85 % isotonic saline) were tested using the specific reagent provided (latex particle coated with IgG antibodies specific for *C. difficile* cells wall antigen) on a reaction card, latex particles agglutinate in large visible clumps within two minutes in positive control as well as in positive samples (Kelly et al., 1987).

### Pathogenicity test

Selected colonies were suspended in 1% peptone saline, and then was adjusted to $9\times10^{10}$ cells /ml, and inoculated intramuscularly in each of three rabbits aged between 30-40 days. The isolation of organism was done again (Hutton et al., 2014).

### Toxin preparation

Thioglycollate broth with 1% glucose was prepared and seeded with *C.difficile* suspected colonies and incubated anaerobically for 72 h, the supernatant collected after centrifugation at 3500 rpm for 30 min then filtered with Seitz filter and the toxognecity test was done by injecting the prepared crude toxin 0.2 ml IV (intravenous) and its three double fold dilution in three mice each (0.2ml /IV /mice) to investigate the minimum lethal dose (MLibby et al., 1982).

### Polymerase Chain Reaction

DNA was extracted from the suspected colony of the positive samples transferred into a 0.6-mL microcentrifuge tube containing 100 µl of sterile water and was boiled at 100°C for 10 min. After boiling, the sample was centrifuged at low speed (3000 rpm) to remove cell debris. The supernatant containing the DNA was used for amplification reactions (Perkins et al., 1995). Toxin A and B genes were amplified as described (Stuart et al., 2000) and the sequences of the primers used shown in table 1.

## RESULTS

Infected rabbits showed abdominal distension and diarrhea as in figure 1. Specific ELISA kit was used for detection of *Clostridium difficile* toxin A/B for the 50 faecal samples and ceacal contents and the obtained results confirm 5 positive samples representing 10% of tested samples, table 2.

Isolation of the positive faecal and ceacal samples on specific medium revealed greyish regular smooth colonies (Figure 2) with manure characteristic odour, and the gram stain of the suspected colonies showed gram positive bacilli as in figure 3 revealed. These obtained agreed with authors who stated that *C. difficile* colonies on blood agar were greyish in colour with characteristic distinctive manure odour, and the organism is gram positive rod measuring 0.5X 3 – 6 um (Mohamed et al., 2013). The isolated colonies produce a pale green fluorescence under long wave length ultraviolet as shown in figure 4. Furthermore, ELISA was done using RIDASCREEN® *C. difficile* Toxin A/B kit (R-Biopharm, Germany) as shown in table 2.

2

**Table 1.** Sequence of the primers used for polymerase chain reaction of the isolates designed for *Clostridium difficile*

| Gene target | Primer Name | Sequence (5`-3`) | Amplicon size (bp) |
|---|---|---|---|
| tcd A | F-YT-28 | GCATGATAAGGCAACTTCAGTGG | 602 |
| | R-YT-29 | GAGTAAGTTCCTCCTGCTCCATCAA | |
| tcd B | F-YT-17 | GGTGGAGCTGCTTCATTGGAGAG | 399 |
| | R-YT-18 | GTGTAACCTACTTTCATAACACCA | |

**Table 2.** Optical Density readings at 450 nm wave length five positive samples from the fifty infected rabbits *Clostridium difficile*, 2018 in Egypt

| Samples | Mean of O.D.(Absorbance) |
|---|---|
| 1 | 0.465± |
| 2 | 0.785± |
| 3 | 0.692± |
| 4 | 0.474± |
| 5 | 0.545 |
| Negative control | 0.128± |
| Positive control | 1.242± |
| Cut-off (negative control+0.15) | 0.278 |



**Figure 1**. Abdominal distension in rabbits infected with *Clostridium difficile*
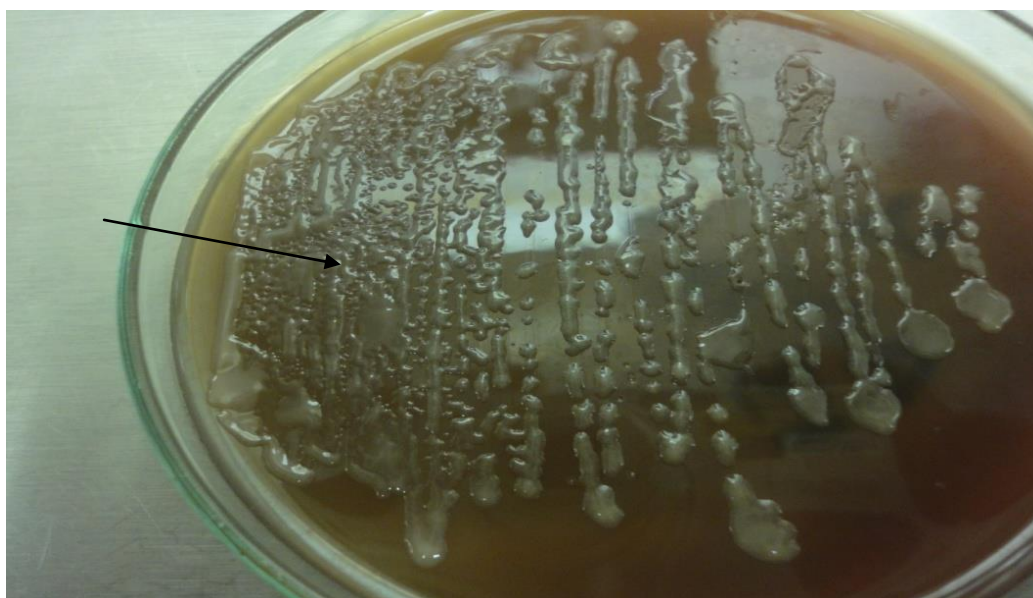


**Figure 2**. Suspected colonies of *Clostridium difficile* after cultivation on the specific medium

3
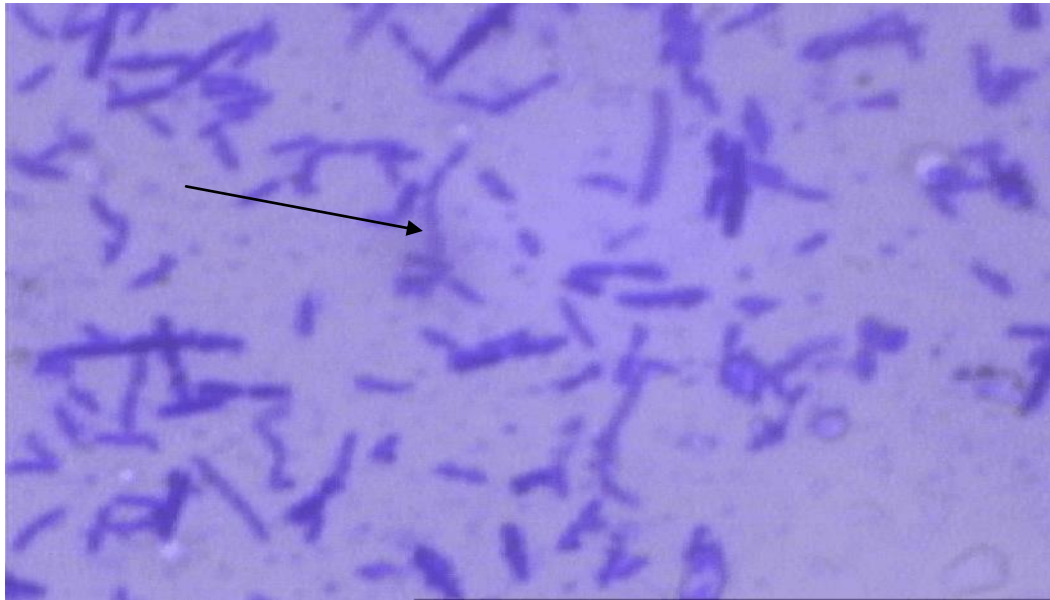
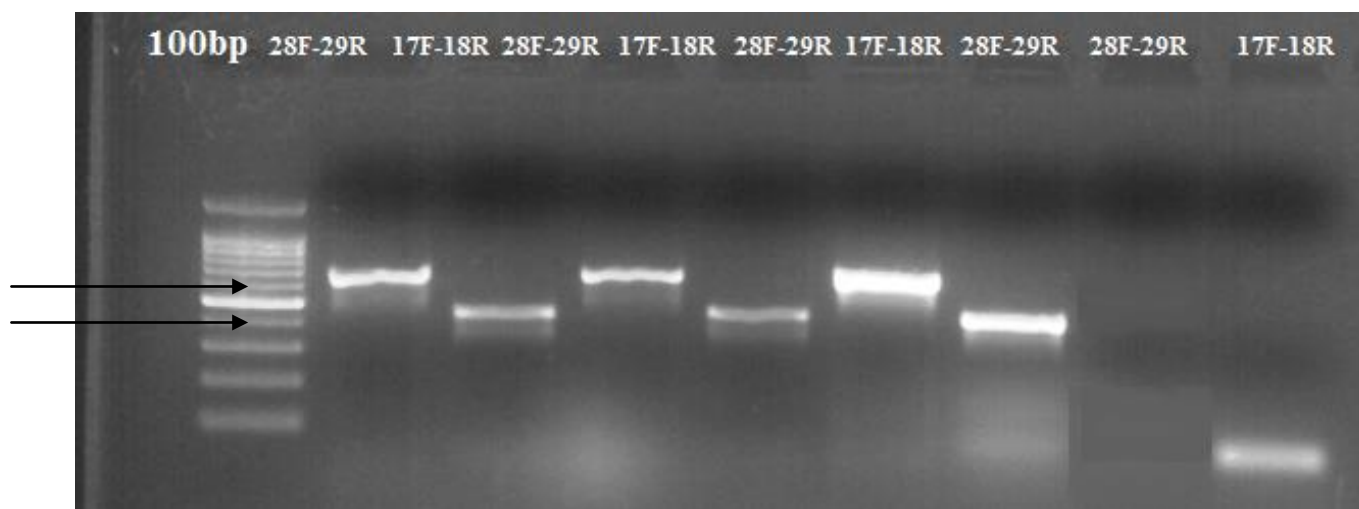**Figure 3.** Gram stain of the suspected colonies from the specific agar medium
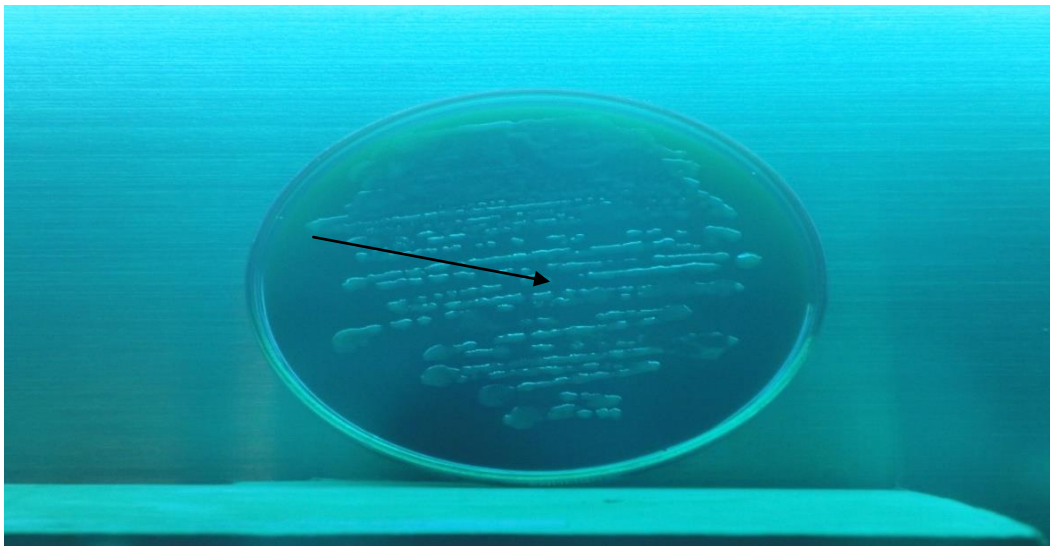


**Figure 4.** Pale green fluorescence colonies under long wave
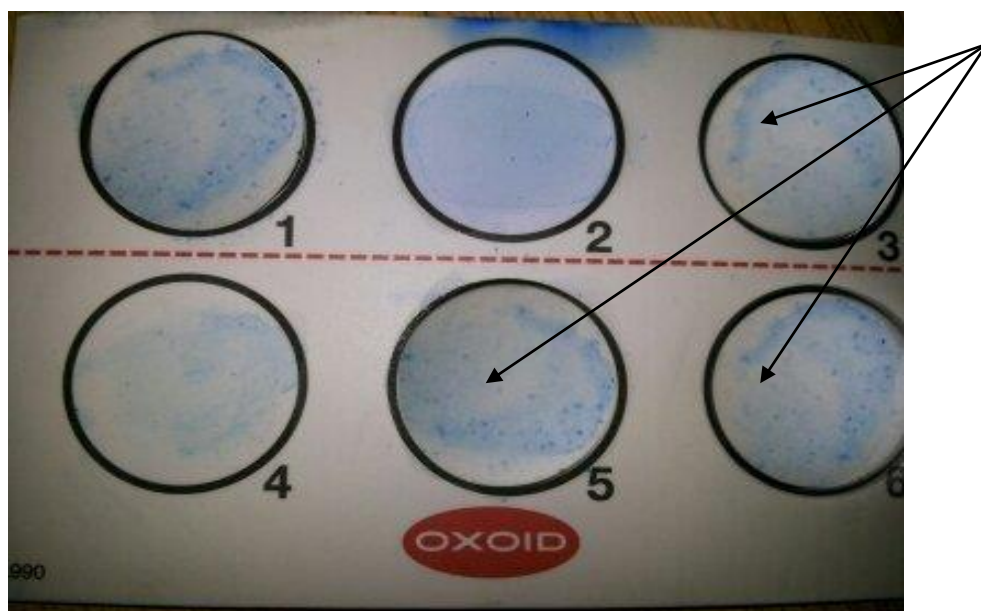


**Figure 5.** Latex agglutination test using the isolated *Clostridium difficile*. 1: positive control, 2: negative control. 3, 5, 6: positive samples and 4: negative samples

4

**Figure 6.** Polymerase chain reaction for the isolates of *Clostridium difficile* for tcdA and tcdB genes lane 1 :molecular markers 100-1000, lanes 2,4,6,8: for the four isolates with tcd A gene , lanes 3,5,7,9: the four isolates with gene tcd B, 2018

## DISCUSSION

*Clostridium difficile* is a confirmed pathogen in a wide variety of mammals, but the incidence of disease varies greatly in relation to host species, age, environmental density of spores, administration of antibiotics, and possibly of other factors. Lesions vary as well, in severity and distribution within individuals, and in some instances, age groups, of a given species. The cecum and colon are principally affected in most species, but foals and rabbits develop severe jejunal lesions (Keel and Songer, 2006).

   *C. difficile* is usually a harmless environmental bacterium and the normal intestinal microflora has to be disturbed before *C. difficile* can become established and produce its toxins. There is an initial disruption of the normal colonic bacterial floraby antibiotic treatment, allowing *C. difficile* from endogenous or exogenous origins to express itself in the colon then proliferate and produce toxins A and B simultaneously, these protein toxins bind to specific receptors on the luminal aspect of colonic epithelium transported to the cytoplasm by receptor mediated endocytosis. Thus, *C. difficile* toxins cause themucosal injury in the colon as a result damage to the cytoskeleton and inhibition of the functioning of tight junctions and cause fluid secretion, inflammation and mucosal damage, which by its turn lead to diarrhea and psudomembranous colitis.

   The isolates gave catalase negative using hydrogen peroxide (as no bubbles of liberated oxygen formed)and oxidase negative when tetramethyl-p-phenylendiamne added where no change in colour occurred , these results agreed with those who reported that *C. difficile* is catalase and oxidase negative (Perkins et al., 1995). *C. difficile* latex agglutination test for the colonies revealed 3 positive samples out from five samples, in comparing to the positive and negative controls provided as shown in figure 5. ELISA done revealed three positive sample as recorded in table 2. Toxin A/B was prepared from one of the positive colonies and inoculated IV in two mice each, the four mice (two of the toxins as it is and two with double fold dilution. of the toxin) were all dead within 48 hours which revealed that the isolated colony is toxogenic.

   PCR results using tcdA and tcdB genes primers as showed in figure 6, revealed that bands at 602 bp and 399 bp respectively for three positive samples, while other sample was negative for both genes ,that agreed completely with (Stuart et al., 2000) who detected four positive samples using PCR for the genes of toxins A and B from 10 samples. Also authors stated that 62% of samples revealed positive samples for *C.difficile* using multiplex PCR and they stated that tcdA and tcdB genes confirmed that isolate is toxogenic (Leond et al., 2000).

## CONCLUSION

All the results obtained from present study indicated that *C. difficile* which have been isolated is toxogenic and could be used for production of vaccine against pseudomembranous colitis in rabbits which in turn help the rabbit industry by providing the specific vaccine.

## DECLARATIONS

   **Author`s contribution**
   TMM and EHA isolate *Clostridium difficile* from infected rabbits and perform ELISA, EFE contributes agglutination test HES and YAA perform PCR for the isolated *C. difficile* and AAE provide the infected rabbits from the farms.

5

**Consent to publish**

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**Competing interests**

All authors have no conflict of interest.

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# Foodborne Diseases Related to the Consumption of Flesh Foods in Morocco (2010-2016)

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## ABSTRACT

The current study aimed to determine the epidemiological profile of foodborne diseases associated with flesh foods during 2010-2016 in Morocco. A retrospective study of foodborne diseases caused by flesh foods recorded by the Moroccan anti-poison and pharmacovigilance center during 2010-2016. During this period, 2963 foodborne diseases related to flesh foods were declared to the center, in which 24.83% were registered in 2015, and 20.75% in 2013. Diseases occurred mostly in urban areas (67.06%). The major affected group's ages were adults (33.81%) and children (14.44%). The average patient's age was $25.09 \pm 15.37$ years. Male were the most vulnerable to infection (54.80%) with a sex ratio (male / female) of 1.72. The most incriminate flesh foods were respectively chicken (47.35%), aquatic products (30.94%) and red meat (16.57%). The high incidence rate was related to chicken skewers (3.55 per 100000 people), while the high fatality rate was associated with giblets (3.33%). Diseases due to the restauration outside home accounted for 58.15%. The majority of cases were collective (84.27%) and occurred significantly in spring (18.49%) and summer (14.51%). clinical symptoms were present in 67.19 % of cases, mostly moderate (81.77%) with four death cases corresponding to fatal condition. The high incidence rates were recorded in the regions of Sahara. Foodborne diseases are spreading progressively in Morocco, especially in summer and hot climates. The majority of these diseases are due to the consumption of contaminated flesh foods. Therefore, the responsible of food safety in Morocco must ensure the quality control of these foodstuffs.

**Key words**: Epidemiology, Foodborne diseases, Meat, Morocco

## INTRODUCTION

The term flesh foods include all types of red meat, poultry, fish and their products (WCRF, 2018). They are among the major consumed foodstuffs around the world. During the period between 1961 and 2014, the global consumption of red meat and poultry has developed from 20 to 43kg/person/year (Ritchie et al., 2018). The Food and Agriculture Organization of the United Nations (FAO) has announced that the global consumption of fish and aquatic products has increased from 130 to 151.2 million tons during 2011-2016 and reach for the first time 20.1 kg/person/year in 2015 (FAO, 2018). In Morocco, according to the Ministry of Agriculture Fisheries Rural Development Water and Forests (MAPMDREF), the production of red meat destined for consumption has increase from 400000 to 550000 tons between the years 2008 and 2016, while the production of poultry has developed from 440000 to 620000 tons (MAPMDREF, 2017). Regarding the consumption of fish and aquatic products, it was doubled from 33717.2 to 66871.88 tons during 2000-2013 (FAO, 2013).

Because of their richness in micronutrients needed for the growth of pathogenic germs that are harmful to humans (WCRF, 2018; Nohr et al., 2007), flesh foods represent an important source of foodborne diseases worldwide (Heredia et al., 2018). Actually, poultry is considered as the first source of Salmonella (SfAM, 2014), while this germ is known as the major zoonotic bacterium responsible for death related to foodborne illnesses in the united states (Mead et al., 1999) and one of the public health problems worldwide (WHO, 2018). During 1980-2015, Salmonella was notified also as the second cause of foodborne outbreaks related to meat and its products globally (Omer et al., 2018), and one of pathogenic bacterium present mostly in fish and aquatic products (Novoslavskij et al., 2016). In USA, during 2009-2015, 4860 foodborne illnesses were associated with poultry, 2288 caused by fish and aquatic products, and 1984 by red meat (except the pork meat) (Dewey-Mattia et al., 2018). In France, the national institute for health surveillance has declared that red meat was the first responsible food for collective foodborne diseases during the years 2014-2015, followed by fish and poultry (Invs, 2014; Invs, 2015). In Morocco, according to the Moroccan Anti-Poison and Pharmacovigilance Center (MAPPC), from 17896 foodborne diseases cases declared during 1989-2008, fish and aquatic products were the

second incriminated (20.3%), followed by meat products (18.2%) (MAPPC, 2010). Moreover, meat products were in the first position as the most incriminated foods in diseases cases in Morocco from 2013 to 2015 respectively with 40%, 24% and 21.7%, while fish and aquatic products were in the third position in 2014 (14.7%) and 2015 (8.7%) (MAPPC, 2013; MAPPC, 2014; MAPPC, 2015).

Taking into account this bibliographic overview, our study aims to highlight the epidemiological characteristics of foodborne diseases caused by flesh foods in Morocco, to describe the demographic and clinical characteristics of cases recorded by the MAPPC during 2010-2016.

## MATERIALS AND METHODS

We proceeded to establish a retrospective descriptive epidemiological study of foodborne diseases associated with red meat, poultry and aquatic products registered by the MAPPC from 1 January 2010 to 31 December 2016 in Morocco. At this national institute, poisoning and diseases cases declared via the toxicological information service which was set up in 1980, are received and recorded in the declaration card which is completed by professionals in the health delegations and hospitals of Morocco. This center also records the statements from telephone records and /or monthly data of the cases declared by the provinces (MAPPC, 2009). The analysis of data is based on the characteristics study of affected population during the time interval of this study. For this reason, we took into account the age using. The INTOX classification method (WHO, 2000), sex and middle of residence. The second factor considered in this study concern the involved flesh food in disease case. Subsequently we assess the degree of severity, symptomatology and evolution of contracted diseases.

Poisoning Severity Score (PSS) was used to determine the severity of diseases in which the grade 0 corresponds to the absence of functional or physical sign, the grade 1 manifested by minor, transient and spontaneously regressing symptoms, the grade 2 is marked by persistent symptoms, the grade 3 shows severe or life-threatening symptoms, and the grade 4 corresponding to fatal poisoning (Persson et al., 1998).

The SPSS software (IBM SPSS Statistics, version 20) was used for data analyses, while the creation of maps was conducted using Microsoft excel 2016. The incidence and mortality rates were calculated based on the high commissioner for planning's data, and the variables were compared using the chi-square test with a significance level of p value <0.05.

## RESULTS

The census of data records by the MAPPC during 2010-2016, showed a total of 2963 foodborne diseases cases related to flesh foods. The high frequencies of these diseases were registered in 2015 and 2013 respectively 736 (24.83%) and 615 (20.75%) cases. However, 1987 (67.1%) cases have been detected in urban areas and 674 (22.7%) in rural middle (Figure 1).

Regarding the responsible types of flesh foods for these diseases, the figure 2 shows that chicken represents a rate of 47.35% in which chicken skewers were the most incriminated (83.89%), fish and aquatic products in the second position with 30.94%, minced and red meat shows respectively 9.78% and 6.78%, turkey corresponds to 3.78% in which turkey skewers were the most implicated (54.46%), giblet 1.02% and delicatessen 0.33%. The most responsible foods for hospitalization were chicken and minced meat (35.08%), while chicken, aquatic products, minced meat and giblet were incriminated in the four recorded death cases (Figure 2).

Concerning the characteristics of studied population, male was the most affected by foodborne diseases related to flesh foods with a percentage of 54.80% and a sex ratio (male /female) of 1.72. The average patient's age was 25.09 ± 15.37 years, while the age group most involved in this problem corresponds to adults with a percentage of 33.81%, followed by that of children 14.44%, and adolescents 6.54%. With regard to the characteristics of recorded diseases, the majority of cases were collective representing approximately 84.27%. The majority of declarations occurred first in spring with 18.49% and in summer with 14.51%. Clinical symptoms are manifested in 67.19 % of cases. The severity of diseases was mostly moderate rated at grade 2 in 81.77%. Four recorded deaths corresponded to grade 4 with a 100% mortality rate. The application of chi-square test showed that P<0.001 for all studied characteristics (Table 1).

The study of disease's severity due to flesh foods consumption according to seasons showed that the moderate grade (grade 2) was dominant during all seasons. Among 48.4% of diseases cases recorded during the four seasons, 68.20% were declared during spring and summer, in which 92.53% are classified between moderate and severe severity. However, among 46,511% of recorded hospitalization during the different seasons, 41.25% were detected in autumn, with two fatal cases were registered in autumn and summer seasons (Figure 3).

The high incidence of diseases was detected in 2015 (0.0215% people) and 2013 (0.0186% people), while the high fatality rate was registered in 2012 (0.36%) and 2014 (0.21%). Concerning flesh foods, the high incidence of diseases was related to the consumption of chicken skewers (0.0355% people) and aquatic products (0.0205‰ people), however

9

the high fatality rate was related to giblets (3.33%) with a hospitalization percentage of 43.33%, and minced meat (0.34%). 58.15% of diseases were caused by consumption of food out of home (restaurants, educational institutions, public place, work), while 31.85% were related to the consumption of food at home (Table 2).

Regarding the distribution of these cases on the Moroccan territory, during 2010-2014 (before the territorial redrawing of 2015), the regions of Oriental and Meknes-Tafilalet (north- central of Morocco) have recorded the largest number of diseases respectively 527 and 360 cases, while the regions of Settat-Casablanca and Rabat-Sale-Kenitra (northeast of Morocco), have known the high frequency of diseases in 2015-2016 respectively 77 and 66 cases. In regard to the incidence rates of diseases, the high incidence registered during 2010-2014, was in the region of Oued ed Dahab-Lagouira region situated in Sahara (1.78% people) and oriental region (0.26% people). However, in 2015-2016, the highest incidence rate recorded the regions situated in Sahara two, Laayoune-Sakia El Hamra (0.11% people) and Guelmim- Oued Noun (4.35% people) (Figure 4).
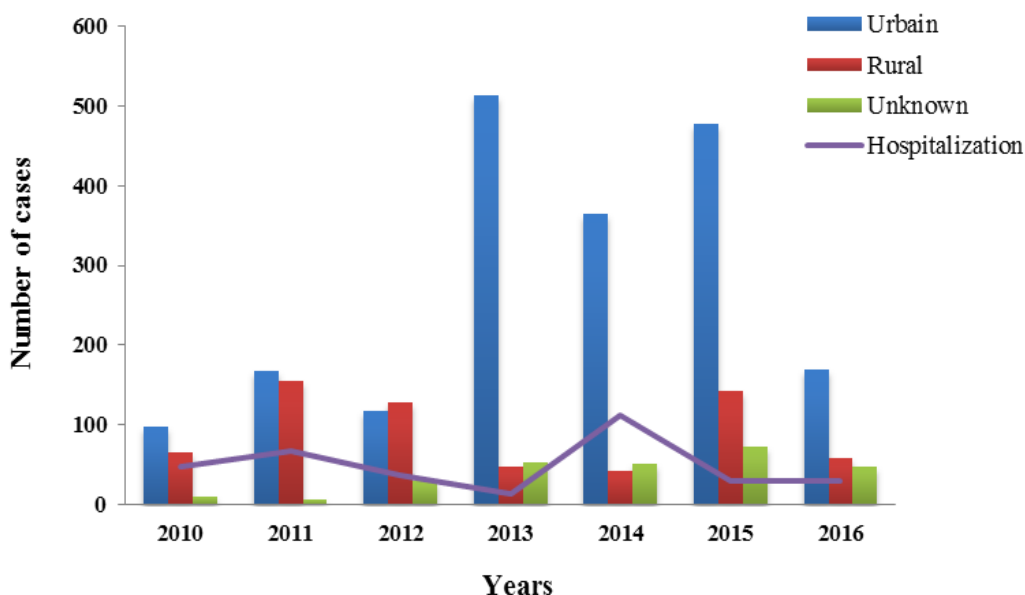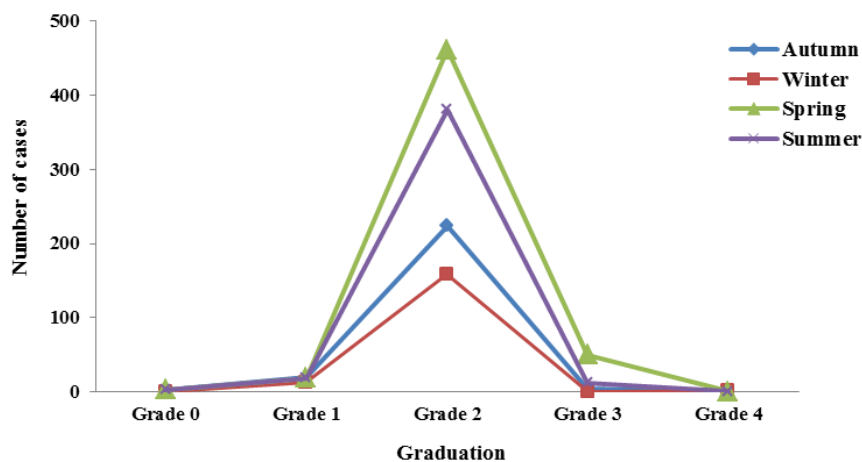


**Figure 1.** Foodborne diseases related to flesh foods according to years, middle of residence and hospitalization during 2010-2016 in Morocco.
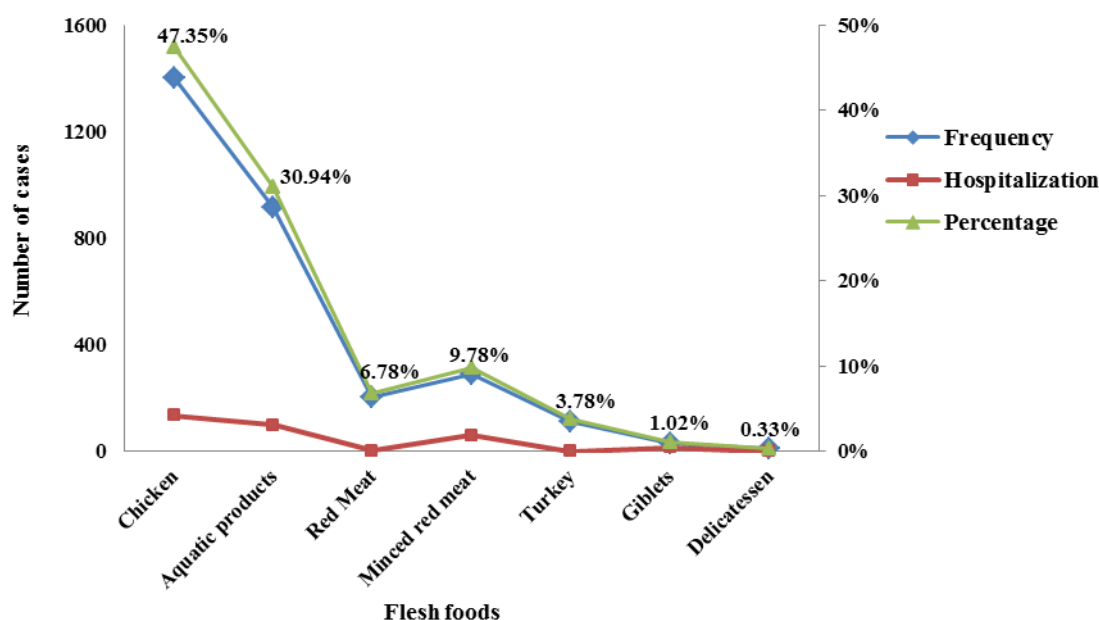


**Figure 2.** Distribution of diseases and hospitalization cases according to flesh foods types during 2010-2016 in Morocco

**Figure 3**. Severity of diseases according to the corresponding season during 2010-2016 in Morocco. Grade 0: Absence of symptoms. Grade 1: Symptoms slight. Grade 2: Prolonged Symptoms. Grade 3: Serious Symptoms. Grade 4: Death.

**Table 1.** Characteristics of foodborne diseases related to flesh foods in Morocco during 2010-2016

| Items | | Cases * (%) | Hospitalization* | Healing* | Death* | P-value |
|---|---|---|---|---|---|---|
| **Age groups** | Newborns (<4weeks) | 15 (0.50) | 0 | 12 | 0 | |
| | Nursling (4weeks- 12 moth) | 5 (0.16) | 1 | 2 | 0 | |
| | Toddlers (1-4 years) | 64 (2.16) | 17 | 51 | 0 | |
| | Children (5-14 years) | 428 (14.44) | 51 | 392 | 1 | <0.001 |
| | Adolescents (15-19 years) | 194(6.54) | 30 | 914 | 1 | |
| | Adults (20-74 years) | 1002 (33.81) | 105 | 849 | 1 | |
| | Elderly (>74 years) | 7 (0.23) | 1 | 4 | 1 | |
| | Unknown | 1248 (42.1) | 16 | 369 | 0 | |
| **Sex** | Female | 944 (31.85) | 167 | 765 | 1 | |
| | Male | 1624 (54.80) | 170 | 1463 | 3 | <0.001 |
| | Unknown | 395 (13.33) | 7 | 365 | 0 | |
| **Type of diseases** | Collective | 2497 (84.27) | 312 | 2322 | 4 | |
| | Isolated | 455 (15.35) | 30 | 261 | 0 | <0.001 |
| | Unknown | 11 (0.37) | 2 | 10 | 0 | |
| **Season** | **Autumn** | 271 (9.14) | 66 | 228 | 1 | |
| | **Winter** | 185 (6.24) | 12 | 158 | 1 | |
| | Spring | 548 (18.49) | 50 | 527 | 0 | <0.001 |
| | Summer | 430 (14.51) | 32 | 412 | 0 | |
| | Unknown | 1529 (51.60) | 184 | 1268 | 2 | |

*Number of cases
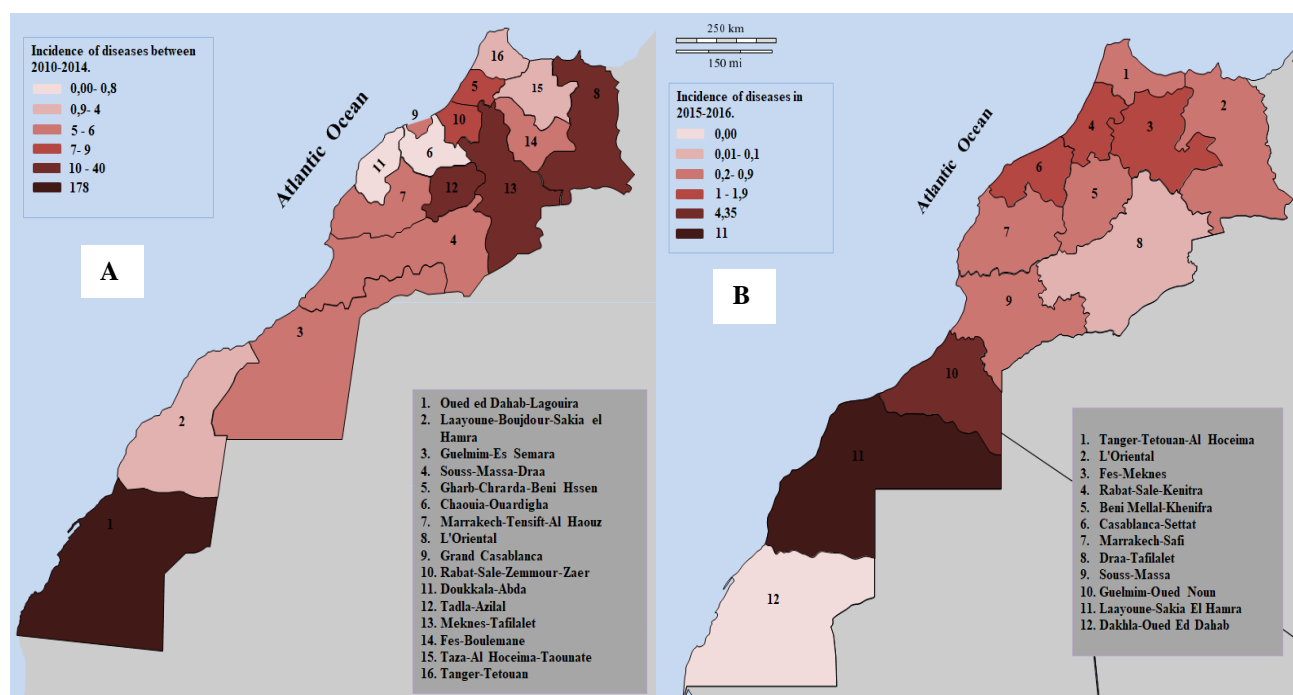


**Figure 4.** Incidence rates of foodborne diseases related to flesh foods by Moroccan regions during 2010-2014 (A) and during 2015-2016 (B)

11

**Table 2.** Evolution, incidence and fatality rate of diseases in Morocco according to year and responsible flesh food

| Items | Cases* | Hospitalization* | Healing* | Death* | Incidence (per 100000) | Fatality rate (%) |
|---|---|---|---|---|---|---|
| **Years** | | | | | | |
| 2010 | 173 | 48 | 142 | 0 | 0.54 | 0 |
| 2011 | 330 | 68 | 309 | 0 | 1.02 | 0 |
| 2012 | 276 | 37 | 215 | 1 | 0.84 | 0.36 |
| 2013 | 615 | 14 | 597 | 1 | 1.86 | 0.16 |
| 2014 | 458 | 113 | 418 | 1 | 1.35 | 0.21 |
| 2015 | 736 | 33 | 636 | 1 | 2.15 | 0.13 |
| 2016 | 375 | 31 | 276 | 0 | 1.08 | 0 |
| **Flesh foods** | | | | | | |
| Chicken | 226 | 48 | 179 | 0 | 0,68 | 0 |
| Chicken skewers | 1177 | 86 | 1101 | 1 | 3,55 | 0.08 |
| Turkey | 51 | 0 | 47 | 0 | 0,15 | 0 |
| Turkey skewers | 61 | 0 | 57 | 0 | 0,18 | 0 |
| Red Meat | 201 | 1 | 143 | 0 | 0,61 | 0 |
| Minced red meat | 290 | 59 | 241 | 1 | 0,87 | 0.34 |
| Giblets | 30 | 13 | 27 | 1 | 0,09 | 3.33 |
| Delicatessen | 10 | 0 | 7 | 0 | 0,03 | 0 |
| Fresh Aquatic products | 680 | 94 | 556 | 1 | 2,05 | 0.14 |
| Frozen fish | 1 | 0 | 1 | 0 | 0,00 | 0 |
| Canned fish | 234 | 4 | 233 | 0 | 0,71 | 0 |
| Sushi | 2 | 0 | 1 | 0 | 0,01 | 0 |
| **Place** | | | | | | |
| Home | 944 | 165 | 710 | 2 | 2,82 | 0.21 |
| Restaurants | 614 | 12 | 713 | 1 | 1,83 | 0.16 |
| Educational institutions | 557 | 27 | 469 | 0 | 1,66 | 0 |
| Public | 494 | 29 | 463 | 1 | 1,47 | 0.20 |
| Work place | 58 | 0 | 54 | 0 | 0,17 | 0 |
| Unknown | 296 | 88 | 207 | 0 | 0,88 | 0 |

*Number of cases

## DISCUSSION

During 2010-2016, 17076 cases of foodborne diseases were detected in Morocco in which 2963 (17.35%) were associated to flesh foods. The majority of cases were occurred in urban areas with a rate of 67.06% because of the overcrowding in urban middle which represents 59.54% of the Moroccan population during the study period (HCP), the availability of restaurants, work and study institutes representing 41.47% of cases and also the proximity to health facilities. The average age of affected population was $25 \pm 15.37$ years, adults represents the age group seriously affected by this problem (33.81%), followed by children (14.44%), and adolescents (6.54%). Adults and children are the age groups most often affected by foodborne diseases in Morocco (Talbi et al., 2006; MAPPC, 2015). In the world, 96000 children die every year because of the foodborne diarrheal diseases that are responsible for more than the half of the global food borne disease cases, and related mainly to meat consumption (WHO, 2018). Men were the most affected with 54.80% in which 45.13% are contracted in public places and restaurants, this can be explained on the one hand by the change of eating habits in Morocco whereas the consumption out of home (fast food) becomes very common mainly for young between 25- 40 years, and in the other hand by the sociological changes allowing the development of women's work (Ouazzani, 2018; Allali, 2017). The majority of cases were collective (84.27%) because of the consumption in group or of the same meals. Chicken as the most accessible meat in Morocco (Sarter, 2004) was the major involved flesh product in diseases cases (47.35%). In USA, chicken is the most responsible for poultry-associated outbreaks (64%) (Chai et al., 2017).

The second leading cause of foodborne diseases related to flesh foods in Morocco during the period of study is attributed to fish and aquatic products (30.94%). However, even though Morocco is considered as one of the major producer countries of marine captures worldwide, whereas it was classified as the 17th producing country in 2014 and the 13th in 2016 (FAO, 2016; FAO, 2018), the consumption of fish by Moroccans is low compared to other types of meat (ITC, 2015). Apart chemicals toxics mainly histamine and mercury that are known as the major toxins causing foodborne diseases related to fish consumption (Colombo et al., 2018; WHO, 2007) and which have been detected in low levels in fish in Morocco (El Hariri et al., 2017; MAPPC, 2017). These economic and health damages are due to the lack of respect for the hygiene standards during the transport and marketing stages in non-refrigerated holds by artisanal fishermen (which insures the largest part of maritime products in the Moroccan market), what causes deterioration of hygienic and organoleptic quality of products even before arriving at the port (DSFF, 2008).

Concerning diseases related to the red meat consumption, cattle, sheep, goats and camels are the types mainly produced in Morocco (MAPMDREF, 2015). In this study, they were responsible for 491 (16.57%) cases. The microbial quality of meat depends on the slaughter conditions, chilling temperatures during the slaughter and transportation process (Gram et al., 2002), while in Morocco, there is just six red meat's slaughterhouses recognized by the National Food Health Safety Product Office (ONSSA, 2017).

The high fatality rate observed in this study was associated with giblets (3.33%). Edible viscera including internal organ (liver, kidneys, thymus, heart and gizzard), and entrails (intestines) of a butcher animal are very popular, mainly poultry giblets (internal organs) because poultry is the most common consuming in Morocco (420000 tons/year) (HCP, 2016), however 92% of poultry meat and giblets produced by illegal slaughterhouses where the bright chicken, fresh meat and giblets are cohabited (AgriMaroc, 2017). The intestines of cattle, sheep, and goats are also largely used to make sausages using the traditional method in general, as a consequence, in Meknes city (Morocco), 80.77% of analyzed sausages were unfit for consumption, and 21.79% were showed the presence of *Salmonella* (Ed-Dra, 2017).

In USA, during 1998-2008, 13405 foodborne disease outbreaks were registered by the centers for disease control and prevention (CDC), in which the most responsible foods were poultry (18.9%), fish (18.6%) and beef (11.9%). Moreover, *Clostridium perfringens* and *Salmonella* were the most responsible pathogenic bacteria for illnesses caused by these foods (Gould, 2013).

Regarding the seasonal effect on the frequency of declared diseases, among 48.4% of recorded cases during the four seasons, 38.21% were detected in spring, 29.98% in summer, 18.89% in autumn and just 12.90% in winter, which indicate that the increase of temperature during spring and summer seasons has a very important role in the flesh foods spoilage, because of the availability of humidity and the optimum temperature for the multiplication of the majority of foodborne bacteria (Al-Jasass, 2013). This result is confirmed by the study that was conducted in Meknes city (Morocco), and which shows the important impact of the seasonal variation on the retail beef meat's hygienic quality (Boukili, 2019). The degree of severity for the larger part of cases was considered moderate during all seasons (81.77%), however it was severe during spring and summer respectively in 9.12% and 2.79% of cases.

According to the MAPPC, since 1980, foodstuffs in general are among the first three responsible agents for poisoning in Morocco. During 1999-2008, they were considered as the first cause of poisoning (13638 cases), which constitutes a worrying health problem.

## CONCLUSION

The frequency of foodborne diseases reported by the MAPPC is increasing which indicates that the center provides more effort to better assess the situation. However, foodborne diseases recorded in the region of Oued ed Dahab-Lagouira during 2010-2014 were not declared to the MAPPC until the year 2015, 306 cases corresponding to this region were declared by the press media. Moreover, during the two last years of this study, the MAPPC has not received any cases from the region of Dakhla- Oued Eddahab (same location), which indicate that the responsible for health must provide more effort at this level. Furthermore, the responsible institutes for food safety in Morocco must also ensure more certified slaughterhouses, and establish monitoring and control systems to manage the artisanal fishing sector.

## DECLARATIONS

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# Effect of Dietary Organic Selenium Supplementation on Growth Performance, Carcass Characteristics and Antioxidative Status of Growing Rabbits

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## ABSTRACT

Total of 45 weaned male New Zealand White (NZW) rabbits about six weeks old with an average initial body weight 618.11±10.01g were randomly allotted to three dietary groups; the first group fed the basal diet without organic Se, the second fed basal diet +0.2 mg Se-yeast, the third fed basal diet +0.2 mg Se-algae. The obtained results showed that supplementation rabbit diets with Se-yeast and Se-algae have no impact on final body weight and average daily body weight gain. Se-algae supplementation tended to increase (P<0.05) average daily feed intake. Rabbits group fed diet supplemented with Se-yeast achieved better (P<0.05) FCR than that group fed Se-algae (5.06 g feed/g gain). Supplementation of Se-algae at 0.2 mg was the highest (P<0.05) in total protein, albumin, and globulin concentration (7.94, 4.16 and 3.78 g/dl). Diets supplemented with Se-yeast or Se-algae significantly reduced plasma creatinine levels compared to the control group. All recorded values of creatinine and urea concentrations were within the normal ranges. Dietary supplementation with 0.2 mg Se-yeast or Se-algae resulted in a significant (P<0.05) decrease in the activity of AST enzyme. Plasma total cholesterol and plasma LDL levels were significantly decreased (P<0.05) with dietary supplementation with Se-yeast or Se-algae. There was a significant (p<0.05) decrease in plasma MDA level in rabbits fed diets supplemented with Se-yeast or Se-algae. While Catalase activity was significantly (P< 0.05) increased. Rabbits fed diet supplemented with Se-algae was the lowest (P<0.05) group in ether extract meat content while dietary supplementation of Se-algae significantly increased (P<0.05) Se content of rabbits meat of hind leg. Conclusively, Se-yeast and Se-algae can be used as selenium sources in growing rabbit diets without causing any adverse effects on growth performance. Besides, their beneficial effects in improving the antioxidative status.

**Key words**: Anti-oxidative status, Carcass, Growth, Organic selenium, Rabbit

## INTRODUCTION

Selenium (Se) supplements are commonly added to animal feedstuffs. Selenium is an important micronutrient in animals as well as human and its deficiency has various negative impacts (Kieliszek and BłaŻejak, 2016). Diseases such as white muscle, liver degeneration, exudative diathesis, impaired reproduction, and poor immunity have been associated with selenium deficiency. Se is a constituent of the enzyme Glutathione (GSH) peroxidase which plays a role in the detoxification of peroxides formed during metabolic processes (Mateos et al., 2010). The $H_2O_2$ is the most toxic molecule to the cells, and it is detoxified by GSH and catalase. Thus, the inhibition of GSH causes the inhibition of the activities of its dependent enzymes (Safhi et al., 2018). It is also important for a number of physiological processes including regulation and function of the immune system through its incorporation into selenoproteins. Also, Se is involved in the regulation of oxidative stress, redox mechanisms, and other crucial cellular processes involved in innate and adaptive immune response (Dalgaard et al., 2018). Body weight gain, feed conversion efficiency and antioxidant capacity of growing rabbits were improved when offered supplementary dietary Se at a level of 0.24 mg Se/kg Dry Matter (DM) (Zhang et al., 2011). While, Syvyk et al., (2018) mentioned the best dose of selenium for rabbits is 0.2 mg/kg of DM. This dose of Se seemed to be optimal for young rabbits for fattening.

Feedstuffs are routinely supplemented with various Se sources, but organic forms of Se like as selenized yeast, selenomethionine and selenium enriched algae are better utilized due to higher bioavailability and less toxic than the inorganic forms as selenites and selenates which can be toxic at increased dietary concentration (Suraï, 2002; Dunshea and Uglietta, 2008; Douch et al., 2009; Hassan et al., 2015). Papadomichelakis et al. (2017) reported that dietary organic Se supplementation at 0.5mg/kg improves meat fatty acids composition and oxidative stability of growing rabbits, whereas at 2.5mg/kg may induce pro-oxidant effects. Se-enriched microalgae may benefit from the presence of specific

bioactive compounds such as antioxidant, pigments, fatty acids, polysaccharides and immune active substances (Douch et al., 2009; Kouba et al., 2014). Dietary fortification with Spirulina platensis microalga seems promising in improving the oxidative stability of rabbit meat, besides, adding functional ingredients (Dalle Zotte et al., 2011). Chen and Wong (2008) stated that Se with phycocyanin (Spirulina platensis) is a promising organic Se and antioxidant agent. Selenium enriched spirulina supplementation improved growth performance and anti-oxidative status of growing rabbits under hot conditions (Hassan et al., 2015). Yeast enriched with Se has recently become commercially available, and research suggests that it may be an efficacious source for the production of Se enriched animal products (Shini et al., 2015).

The objective of this study was to evaluate the effects of dietary supplementation of organic Se forms such as Se-yeast and Se-algae on growth performance, carcass characteristics, blood biochemical parameters and antioxidant status of growing rabbits.

## MATERIALS AND METHODS

### Experimental region
The present study was carried out in rabbit research unit at Sakha research station located in Alexsanderia governorate, Egypt, belongs to Animal Production Research Institute (APRI), Agricultural Research Center (ARC).

### Ethical approval
This experiment was conducted after obtaining the ethical approval of the Animal Production Research Institute (APRI), Egypt.

### Experimental design and application
Forty-five (six weeks of age) New Zealand white male rabbits were divided randomly into three homogeneous groups (n=15 each) with 618.11±10.01 g average live body weight. Each group has five replicates of which three rabbits. The treated groups were, control (basal diet without any supplementation), the second one was supplemented by selenium enriched yeast (Se-yeast) at 0.2 mg/kg diet and the third group was supplemented with selenium enriched algae Spirulina (Se-algae) at 0.2 mg (Figure, 1). Se enriched yeast (Se-yeast) is produced by growing strain of yeast (*Saccharomyces cervisiae*) in a Se-enriched media, (Sel-Plex®, was obtained from Alltech Inc, Nicholasville, KY, USA). Selenium enriched algae is produced by growing strain of Spirulina platensis (*Arthrospira platensis*), algae containing 1 mg Se/g algae. This algae strain was obtained from agricultural microbiology department, National Research Centre (NRC), Giza, Egypt.

Throughout the experimental period, body weight was determined every four weeks (at 6, 10 and 14 weeks of age) and average body weight gain was calculated. During the whole experimental period, the feed intake was determined precisely and is given as grams per rabbit per week. From each cage, feed residuals were collected daily, weighed and taken into consideration for the calculation of feed intake and feed conversion ratio (FCR) was calculated as a ratio of gram of feed per gram of gain.
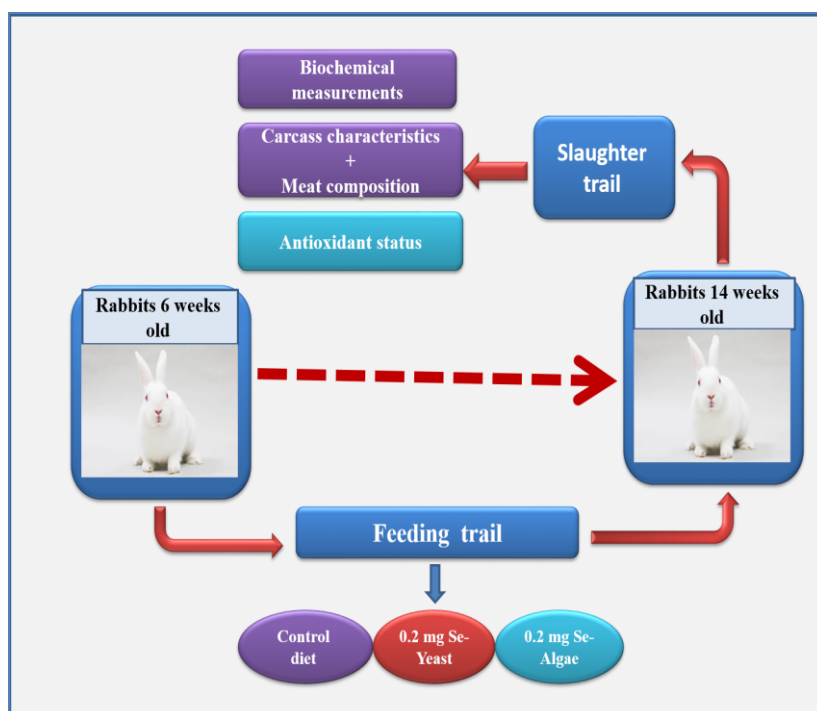


**Figure 1.** Experimental design and feeding trail of New Zealand white rabbit (6-14 weeks old) under Egyptian conditions

#### Experimental diets and housing

The experimental diets were pelleted and formulated to meet recommended nutrient requirements of growing rabbits according to Lebas (2013). Ingredient and chemical composition of the basal diet is presented in table 1. The control diet, thus containing only the endogenous Se contained in the ingredients of the diet (0.08mg Se/kg diet). Rabbits were housed individually in stainless steel cages (35 ×35 ×60 cm³) provided with feeders and automatic nipple drinkers. Diet and water were offered *ad libitum*. All rabbits were kept under the same management, hygienic and environmental conditions.

**Table 1**. Feed ingredients and chemical analysis of the basal diet on the dry matter basis for New Zealand white rabbit (6-14 weeks old) during January 2018

| Feed Ingredient | (%) | Chemical analysis (% dry matter basis) | (%) |
|---|---|---|---|
| Soybean meal (44% CP[a]) | 19.60 | Dry matter (DM) | 89.87 |
| Barley | 17.10 | Organic matter (OM) | 90.70 |
| Yellow corn | 7.00 | Crude protein (CP) | 17.86 |
| Wheat bran | 25.08 | Crude fiber (CF) | 13.33 |
| Clover hay | 24.50 | Ether extract (EE) | 2.350 |
| Molasses | 3.00 | Nitrogen free extract (NFE) | 58.44 |
| Limestone | 1.08 | Ash | 8.020 |
| Di- calcium phosphate | 1.71 | Methionine[b] | 0.670 |
| DL-Methionine | 0.08 | Methionine+cysteine[c] | 0.760 |
| Sodium chloride | 0.35 | Lysine[d] | 0.980 |
| Vit.-Min. premix[b] | 0.30 | Calcium[e] | 1.290 |
| Anti-coccidiosis | 0.10 | Available Phosphours[f] | 0.510 |
| Anti-Fungi | 0.10 | Digestible energy (Kcal/Kg DM) [g] | 2708.14 |
| Total | 100 | Selenium (mg/kg DM) [h] | 0.08 |

[a] CP: Crude Protein, [b]Vit.-Min. premix: Mineral and vitamin mixture supplied per kg of diet: Vitamin A 10000 IU, Vitamin D3; 1800 UI, Vitamin E; 15 mg, vitamin K3; 4.5 mg, Vitamin B1; 0.5 mg, Vitamin B2; 4 mg, Vitamin B12; 0.001 mg, Folic acid; 0.1 mg, Pantothenic acid; 7 mg, Nicotinic acid; 20 mgm I; 1 mg, Mn; 60 mg, Cu; 5.5 mg, Zn; 75 mg, Fe; 40 mg, Co; 0.3 mg, Robenidine; 52.8 mg, (b,c,d,e,f,h) Calculated on the basis of the ingredients composition. (g) Digestible energy (DE) was calculated according to Lebas (2013).

#### Carcass traits

At the end of the experimental period (14 weeks old), five rabbits from each treatment were randomly kept off feed for 12h, weighed and slaughtered for carcass characteristics and meat analysis. Carcass characteristics were measured according to Blasco and Ouhayoun et al. (1996). After complete bleeding, the skin, viscera, and tail were removed and the hot carcasses and its components were weighed as edible parts (liver, kidneys and heart) and the non-edible parts including lung, spleen, stomach, large intestine, small intestine were also weighed. Dressing percentage was calculated by dividing the hot dressed carcass weight by pre-slaughter weight and expressed as a percentage according to Blasco and Ouhayoun et al. (1996).

#### Biochemical measurements and antioxidant parameters

Blood samples (5ml from each rabbit) were collected during slaughtering to determine blood plasma components. Plasma was separated by centrifugation at 3000 rpm for 10 min and stored at −20°C until analyzed. Plasma total protein, albumin, total cholesterol, Low Density Lipoprotein (LDL-cholesterol), creatinine, urea, Aspartate Transaminases (AST), Alanin Transaminases (ALT), Malondialdehyde (MDA) and Catalase (CAT) were colorimetrically determined using commercial kits (purchased from Bio-Diagnostic, Cairo, Egypt, according to the manufacturers' instructions). The concentration of globulin (g/dl) was calculated by subtracting albumin values from total protein values, thereby we calculated Albumin/ Globulin ratio (A/G ratio).

#### Chemical analysis

Chemical analyses of the experimental diets, hind leg meat was carried out according to AOAC (2000) for Crude Protein (CP), Ether Extract (EE), Crude Fiber (CF) and ash. Total selenium of meat determination according to Shaltout et al. (2013), the analytical procedure was performed using an inductively coupled plasma mass spectrometry (ICP-MS) (Perkin Elmer-SCIEX, Thornhill, Canada), the concentration of total selenium was read from the calibration curve.

#### Statistical analysis

The differences among experimental groups were statistically analyzed using the general linear model procedures of SAS (2001), applying a One-Way Analysis of Variance (ANOVA). The significant differences among treatments means of treatments were compared using Duncan's multiple range test (P< 0.05) (Duncan 1955). All results were analyzed using the statistical model was: Yij= μ+ Ti+ eij, Where: Yij= the observation of ij; μ = Overall mean; Ti= Effects of i (treatments) and eij= Experimental random error.

## RESULTS

### Growth performance

The effect of dietary of Se-yeast and Se-algae on growth performance of growing rabbits is presented as shown in table 2. The obtained results showed that supplying rabbits diets with Se-yeast and Se-algae had no significant effect on the average of both final body weight and daily body weight gain during the different experimental periods compared to the control group. However, rabbits group fed diet supplemented with Se-algae was the highest group in final body weight (1918.66g) followed by rabbits group fed diet supplemented with Se-yeast (1888.66g) while, the lowest one was with that rabbits group fed the control diet (1880.66g). On the other hand, Se-algae supplementation tended to increase (P<0.05) average daily feed intake during the first, second and the whole periods compared to the rabbits group fed the control diet and the rabbits fed diet supplemented with Se-yeast, while, the supplementation of Se-yeast recorded the lowest (P<0.05) average daily feed intake during all the experimental periods.

The results also revealed that FCR values were not significantly (P>0.05) influenced by dietary treatment during the first period. It is worthy to notice that rabbits group fed diet supplemented with Se-yeast achieved better (P<0.05) FCR (4.33 g feed/g gain) than that group fed Se-algae (5.06 g feed/g gain) while the control group recorded better FCR than Se-algae group during both the second and the whole periods.

### Carcass characteristics

Effects of Se-yeast and Se-algae supplementation on carcass characteristics are summarized in table 3. The results indicated that dietary supplementation of Se-yeast and Se-algae did not significantly (P>0.05) affect the pre-slaughter weight, carcass weight, heart, and dressing percentages. However, there were significant differences (P<0.05) between rabbits group fed diets supplemented with Se-yeast and rabbits group fed diet supplemented with Se-algae in liver%, edible giblet%, and spleen%. While, there were significant (P<0.05) differences between the control group and rabbits group fed Se-algae diet in liver% and edible giblets. Moreover, Se-yeast supplementation increased (P<0.05) spleen% compared to the control group. The obtained results also showed that feeding rabbits on the tested experimental diets including the control had no effect on cecum weight, length and intestine length.

### Chemical composition of meat

Data concerning the effects of Se-yeast and Se-algae on the chemical compositions of rabbit meat are shown in table 4. It could be noticed that supplementation of dietary Se-yeast and Se-algae significantly decreased (P<0.05) DM and EE content compared to the control group. Rabbits fed diet supplemented with Se-algae was the lowest (P<0.05) group in EE content. On the other hand, it could be observed that there was a significant (P<0.05) increase in CP and ash meat content with supplementing Se-yeast and Se-algae compared to the control group. Regarding Se content, the dietary supplementation of Se-algae significantly increased (P<0.05) Se content of rabbit's meat of hind leg compared to the other tested rabbits' groups.

### Plasma constituents and antioxidative status

Data of plasma biochemical constituents are shown in table 5. Dietary supplementation of Se-yeast and Se-algae significantly (P<0.05) increased plasma total protein concentration. It is worthy to note that rabbits group fed diets supplemented with Se-algae at 0.2 mg was the highest (P<0.05) group in total protein, albumin and globulin concentration (7.94, 4.16 and 3.78 g/dl, respectively). While no significant (P>0.05) differences in albumin and globulin levels were observed between the rabbits group fed Se-yeast diet and rabbits group fed the control diet. The albumin/ globulin (A/G ratio) was not affected significantly (P>0.05) by the dietary treatments.

To assess the condition of the kidneys, the following parameters were taken into account creatinine and urea. Feeding diets supplemented with 0.2 mg Se-yeast or Se-algae significantly (P<0.05) reduced plasma creatinine levels when compared to the control group. As well as there were significant (P<0.05) differences in urea concentrations between the control group and rabbits fed diets supplemented with Se-yeast and Se-algae. Dietary supplementation with 0.2 mg Se-yeast or Se-algae resulted in a significant (P<0.05) decrease in the activity of AST enzyme while, the activity of ALT was not affected significantly (P<0.05) by the supplemental feeding of Se-yeast but it was lower (P<0.05) for the rabbits group given Se-algae diet compared to the rabbits fed the control diet.

Plasma total cholesterol and plasma LDL levels were significantly decreased (P<0.05) with dietary supplementation with Se-yeast or Se-algae. Regarding blood antioxidative status as shown in table 5. A significant (P<0.05) decrease of plasma MDA level was observed in rabbits fed diets supplemented with Se-yeast or Se-algae. An opposite effect was noticed in CAT activity whereas the values were significantly (P< 0.05) increased.

**Table 2.** Effect of dietary supplementation of Se-yeast and Se-algae on growth performance of New Zealand white rabbit (6-14 weeks old) during February 2018 in Egypt

| Item / Experimental diets | Control | Se-yeast | Se-algae | ±SEM | P value |
|---|---|---|---|---|---|
| Average body weight (g/rabbits) | | | | | |
| Initial body weight (g) | 618.33 | 617.66 | 618.33 | 17.75 | 0.999 |
| Final body weight (g) | 1880.66 | 1888.66 | 1918.66 | 36.36 | 0.739 |
| Average daily weight gain (g/day/rabbit) | | | | | |
| Weeks 6-10 | 27.33 | 25.97 | 27.59 | 0.99 | 0.475 |
| Weeks 10-14 | 17.75 | 19.42 | 18.84 | 0.93 | 0.439 |
| Weeks 6-14 | 22.54 | 22.69 | 23.22 | 0.59 | 0.702 |
| Average daily feed intake (g/day/rabbit) | | | | | |
| Weeks 6-10 | 77.25[b] | 74.55[c] | 83.06[a] | 0.65 | 0.0001 |
| Weeks 10-14 | 82.47[b] | 79.43[b] | 94.16[a] | 1.41 | 0.0001 |
| Weeks 6-14 | 79.85[b] | 76.99[c] | 88.62[a] | 0.92 | 0.0001 |
| Feed Conversion Ratio (g feed/g gain) | | | | | |
| Weeks 6-10 | 2.89 | 2.92 | 3.07 | 0.12 | 0.520 |
| Weeks 10-14 | 4.78[ab] | 4.33[b] | 5.06[a] | 0.24 | 0.106 |
| Weeks 6-14 | 3.58[ab] | 3.42[b] | 3.84[a] | 0.10 | 0.016 |

Se-yeast 0.2 mg selenium yeast/kg diet; Se-algae, 0.2 mg selenium algae/kg diet;SEM: Strandard Error Mean, [a,b,c] Means values with the same letter within the same row did not differ significantly (P>0.05)

**Table 3.** Effect of dietary supplementation of Se-yeast and Se-algae on carcass characteristics of New Zealand White (NZW) rabbits

| Item / Experimental diets | Control | Se-yeast | Se-algae | ±SEM | P value |
|---|---|---|---|---|---|
| Pre-slaughter weight (g) | 2033.3 | 2031.7 | 2061.7 | 28.67 | 0.720 |
| Carcass weight (g) | 1056.67 | 1056.67 | 1101.67 | 27.43 | 0.456 |
| Dressing (%) | 51.96 | 52.01 | 53.42 | 0.97 | 0.521 |
| Liver (%) | 3.08[a] | 3.08[a] | 2.08[b] | 0.23 | 0.034 |
| Heart (%) | 0.39 | 0.31 | 0.35 | 0.03 | 0.373 |
| Kidney (%) | 0.70[ab] | 0.80[a] | 0.60[b] | 0.039 | 0.036 |
| Spleen (%) | 0.80[b] | 1.23[a] | 0.63[b] | 0.07 | 0.004 |
| Edible giblet%[1] | 4.18[a] | 4.19[a] | 3.03[b] | 0.22 | 0.015 |
| Total edible parts%[2] | 56.15 | 56.21 | 56.45 | 1.03 | 0.975 |
| Cecum weight (g) | 88.23 | 85.67 | 84.33 | 9.11 | 0.959 |
| Cecum length (cm) | 31.67 | 31.33 | 31.00 | 0.92 | 0.880 |
| Intestine length (cm) | 286.00 | 283.67 | 291.00 | 13.61 | 0.927 |

Notes: Se-yeast, 0.2 mg selenium yeast/kg diet; Se-algae, 0.2 mg selenium algae/kg diet.  SEM: Strandard Error Mean. a,b Means values with the same letter within the same row did not differ significantly (P>0.05). (1) Edible Giblets %= (liver+ kidney + heart) / Pre-slaughter weight (g)*100 (2) Total edible parts %= (carcass wt. + edible giblets wt.) / Pre-slaughter weight (g)*100

**Table 4.** Effect of dietary Se-yeast and Se-algae supplementation on the chemical composition of New Zealand white rabbit's meat (6-14 weeks old) during April 2018 in Egypt

| Experimental diets / Item | Control | Se-yeast | Se-algae | ±SEM | P value |
|---|---|---|---|---|---|
| Moisture | 71.57[c] | 72.68[b] | 73.17[a] | 0.103 | 0.0001 |
| DM | 27.38[a] | 27.35[b] | 27.02[c] | 0.102 | 0.0001 |
| CP | 22.09[c] | 22.55[b] | 22.91[a] | 0.086 | 0.0017 |
| EE | 3.73[a] | 3.56[a] | 3.16[b] | 0.048 | 0.0004 |
| Ash | 1.20[c] | 1.24[b] | 1.31[a] | 0.004 | 0.0001 |
| Se (µg/g) content of hind leg | 0.091[b] | 0.098[b] | 0.12[a] | 0.003 | 0.0018 |

Se-yeast, 0.2 mg selenium yeast/kg diet; Se-algae, 0.2 mg selenium algae/kg diet; SEM: Strandard Error Mean. [a,b,c] Means values with the same letter within the same row did not differ significantly (P>0.05)

**Table 5.** Effect of dietary supplementation of Se-yeast and Se-algae on plasma constituents and blood antioxidative status of New Zealand white rabbit (6-14 weeks old) during April 2018 in Egypt

| Item \ Experimental diets | Control | Se-yeast | Se-algae | ±SEM | P value |
|---|---|---|---|---|---|
| **Plasma proteins** | | | | | |
| Total Protein (g/dl) | 6.25[c] | 6.78[b] | 7.94[a] | 0.11 | 0.0001 |
| Albumin (g/dl) | 3.23[b] | 3.54[b] | 4.16[a] | 0.12 | 0.0050 |
| Globulin (g/dl) | 3.02[b] | 3.25[b] | 3.78[a] | 0.12 | 0.0133 |
| A/G ratio | 1.07 | 1.09 | 1.11 | 0.07 | 0.0926 |
| **Kidneys functions** | | | | | |
| Creatinine (mg/dl) | 1.09[a] | 0.90[b] | 0.72[c] | 0.04 | 0.0039 |
| Urea (mg/dl) | 25.74[c] | 28.51[b] | 31.36[a] | 0.50 | 0.0008 |
| **Liver functions** | | | | | |
| AST (IU/l) | 49.94[a] | 40.61[b] | 37.77[c] | 0.80 | 0.0001 |
| ALT (IU/l) | 56.72[a] | 43.13[ab] | 31.73[b] | 4.15 | 0.0154 |
| **Plasma lipids** | | | | | |
| Cholesterol (mg/dl) | 200.5[a] | 182.97[b] | 169.27[c] | 3.90 | 0.0038 |
| LDL (mg/dl) | 64.98[a] | 57.43[b] | 50.21[c] | 1.60 | 0.0018 |
| **Antioxidative status** | | | | | |
| MDA (mmol/ml) | 3.85[a] | 3.23[b] | 2.62[c] | 0.10 | 0.0006 |
| Catalase (U/L) | 129.87[c] | 162.20[b] | 201.47[a] | 7.40 | 0.0015 |

Se-yeast, 0.2 mg selenium yeast/kg diet; Se-algae, 0.2 mg selenium algae/kg diet; SEM: Strandard Error Mean, [a,b,c] Means values with the same letter within the same row did not differ significantly (P>0.05), MDA: Malondialdehyde

## DISCUSSION

### Growth performance

The present study revealed that supplementation of rabbit diets with 0.2 mg Se-yeast and 0.2 mg Se-algae have no impact on final body weight and average daily body weight gain. The results agreed herein with Amer et al. (2018) who demonstrated that feeding rabbits diets supplemented with 0.3 or 0.6 mg Se-yeast did not have an improving effect on body weight and body weight gain. Besides, Hassan et al. (2015) showed that incorporation of 0.2 mg Se-algae in rabbit diet had no significant effect on average final body weight at 14 weeks old and daily body weight gain at the whole period. Moreover, Syvyk et al. (2018) noted that rabbits fed diets contained Se at levels of 0.1, 0.2, 0.3 and 0.4 mg/kg DM showed similar productivity and conversion of feed and found that a level of 0.2 mg Se/kg of DM was the best dose of selenium for fattening young rabbits.

Supplementation rabbit diet with 0.2 Se-algae increased (P<0.05) feed intake. This increase may be due to the significant impact on the nutrient status of the rabbit because of the profile of nutrient composition of Spirulina which considered as a source of essential bioactive compounds for organisms. They provide nearly all essential vitamins such as A, B6, B12, C, E, nicotinamide, biotin, folic acid, and pantothenic acid, polyunsaturated fatty acids and β-carotene (Spolaore et al., 2006; Dufosse et al., 2005). While rabbits fed diet contained 0.2 mg Se-yeast significantly (P<0.05) reduces feed intake. These findings are in accordance with the results of Saleh et al. (2013) reported that 0.3 ppm organic selenium reduced significantly feed intake of rabbits. Besides, Hassan et al. (2015) suggested that rabbits fed diet contained 0.2 mg Se-algae recorded the lowest feed intake (64.06 g/d) compared to rabbits fed the control diet (72.31 g/d).

Se-yeast supplementation at 0.2 mg/kg diet recorded the best (P<0.05) FCR. This result is consistent with findings of Hassan et al. (2015) who reported that rabbit fed diets included Se-algae at 0.05, 0.1, 0.2, 0.4 and 0.5 mg Se-algae/kg diet recorded better FCR than the control group and the best FCR (2.28 g feed/g gain) was achieved by 0.2 mg Se-algae. Furthermore, body weight gain, feed conversion efficiency and antioxidant capacity of growing rabbits was improved when offered supplementary dietary Se at a rate of 0.24 mg Se/kg DM (Zhang et al., 2011). A possible reason for this could be attributed to nutritional yeasts as selenium yeast which has unique effects on metabolism, improving growth performance and health status of animals due to its content of high protein, amino acids, energy and B vitamins (Shurson, 2018; Mahan, 1999). Conversely, Amer et al. (2018) in rabbits, where feed conversion ratio not significantly (P>0.05) different with 0.6 mg Se-yeast /kg diet compared to the other groups. Also, Ebeid et al. (2013) who found that addition of organic Se in the form of yeast at 0.3 mg/kg diet reduced FCR in growing rabbits. In addition, Marounek et al. (2009) did not show a significant (P>0.05) effect of Se supplementation at 0.4 mg/kg on feed conversion of rabbit diet.

It could be observed that there was no depression found after supplementing diets with Se-yeast and Se-algae, a reason for this may be that organic selenium sources could cross through the intestine and enter into the blood by active transport so organic Se has high absorption efficiency (Schrauzer, 2001). Moreover, organic Se build Se reserves in the body in the form of selenomethionine which can be used to help additional Se-protein production (Surai and Fisinin,

2014). It has been shown that selenium contributes to normal cell growth and has an important role in modulating the action of transcription factors and cell signaling systems (Kieliszek and BłaZejak, 2016). Furthermore, it was known that Se enhances the metabolism of the thyroid hormones, which is important for normal growth and metabolism because of selenoenzymes may modulate or control many aspects of thyroid hormone metabolism (Parchami and Fatahian, 2012; Mehdi and Dufrasne, 2016). Therefore, in this research, a combination of better absorption efficiency and thyroid hormone activation by Se-yeast and Se-algae availability may explain the improved feed efficiency of the rabbits. Thus, it is necessary to include selenium in rabbit diets.

Further confirmation of no indication of adverse effects on rabbits health associated with the use of Se-algae has been obtained from spirulina which can enhance the productive performance as well as lowering the problems of different animal diseases. These properties could be attributed to some natural constituents such as phycocyanin, beta-carotene, tocopherols, linolenic acid, minerals, vitamins and phenolic compounds that had been shown to have strong antioxidant properties which promote growth and maintain health (Alvarenga et al., 2011; Farag et al., 2016).

### Carcass characteristics

In present experiment, the results of carcass characteristics are in agreement with other results indicated no significant (P>0.05) effect of Se-algae supplementation on carcass yield of rabbits (Dokoupilovà et al., 2007). Also, Marounek et al. (2009) reported that no significant (P>0.05) difference in hot carcass weight, chilled carcass weight, and dressing percentage were observed with Se-selenite, Se-yeast and Se-algae supplementation in rabbit diets. Moreover, Payne and Southern (2005) found that carcass traits were not affected by Se sources or levels supplementations for chickens fed sodium selenite or Se-enriched yeast. Besides, Downs et al. (2000) stated that carcass and deboned meat yield of broiler were not influenced by the addition of 0.3 mg/kg Se to diets in the form of selenite and Se-enriched yeast. Dietary supplementation with 0.3 mg/kg inorganic and organic selenium had no significant (P>0.05) effect on carcass traits of turkeys (Mikulski et al., 2009). Recently, Amer et al. (2018) found that carcass traits were not affected by organic Se supplementation in rabbit diets at 0.6 mg /kg diet. In contrast, Hassan et al. (2015) found that supplying rabbit diets with Se-algae at 0.05, 0.1, 0.2, 0.4 and 0.5 mg/kg increased hot carcass, dressing%, and total edible parts% compared to the control group, and rabbits fed diet contained 0.4 mg Se-algae achieved the best hot carcass, dressing, edible giblets, and total edible parts. Shourrap et al. (2018) reported that dressing % was increased when chicks received 0.3 and 0.4 ppm/kg selenium enriched yeast.

### The chemical composition of meat

Dietary supplementation of Se-yeast or Se-algae decreased (P<0.05) EE content of meat. Rabbits fed diets supplemented with Se-algae was the lowest (P<0.05) group in EE content. Similar results were reported by Dalle Zotte et al., (2012) who stated that 5% spirulina supplementation seems promising in reducing the cholesterol content of fattening rabbit meat. In addition, Hassan et al. (2015) indicated that the content of EE of growing rabbit meat significantly (P<0.05) decreased when rabbit fed diet supplemented with 0.05, 0.1, 0.2, 0.4 and 0.5 mg Se-algae/kg DM. In this connection, Marounek et al. (2009) revealed that the hind leg meat of rabbits fed Se-algae (Chlrella) at 0.40 mg/kg diet contained less fat than that of control rabbits. While no effect observed on fat concentration of loin meat of rabbits.

Present results regarding DM, CP and EE content of rabbit meat, are in contrast to the findings reported by Dokoupilová et al. (2007), who indicated that the content of DM and fat in rabbit meat was not significantly (P>0.05) affected by dietary Se-enriched yeast supplementation. Also, Ebeid et al. (2013) who found that dietary Se-yeast supplementation at 0.3 mg/ kg diet had no significant (P>0.05) effect on meat composition of rabbits. Marounek et al. (2009) revealed that Se supplements had no effect on the DM and protein content of rabbits.

The present study was revealed that dietary Se-algae supplementation increased (P<0.05) Se content and ash content in the hind leg. Present findings are in agreement with Amer et al. (2018) who evaluated the inclusion of selenium yeast at 0.3 and 0.6 mg in the NWZ rabbit diets and found that selenium can be deposited in the meat and other tissue of rabbits and improved meat quality, which positively reflects on human acceptance. Similar results were reported by Dokoupilová et al. (2007), who found that lion and hind leg meat of rabbits fed diet supplemented with Se- enriched yeast contained four times more Se than the meat of rabbits fed the basal diet. Additionally, Marounek et al. (2009) reported that, Se concentration increased in meat of rabbits fed diets supplemented by Se-algae in which the Se concentration in the meat was doubled. Also, Boiago et al. (2014) observed highest Se concentration in muscles of broilers fed diets enriched with organic Se. Additionally, the organic selenium (Se-algae) is more deposited into the muscle tissue and animal organs than inorganic one (Behne et al., 2009). Papadomichelakis et al. (2017) concluded that dietary selenium yeast supplementation at 0.5 mg/kg level improved rabbits meat fatty acids composition and oxidative stability, whereas at 2.5 mg/kg may induce pro-oxidant effects.

Most research conducted in recent years on rabbit meat quality has focused on incorporating bioactive compounds in meat for the benefit of human health. Moreover, rabbit meat consumption could become a good way to provide bioactive compounds to human consumers (Hernández, 2008), since manipulation of rabbit diet is very effective in increasing the levels of selenium is also responsive to dietary supplementation (Lynch and Kerry, 2000). Shini et al. (2015) reported that tissue enrichment with Se may enhance an animal's resilience to stress and disease challenge. Organic Se, in the form of yeast enriched with selenomethionine, has obvious implications for the production of Se enriched animal products.

Therefore, it would be premature to conclude that the organic selenium sources used in present study have beneficial effects on carcass composition due to their positive effects on reducing the fat of content meat and a noticeable increase in meat protein content.

**Plasma constituents and blood antioxidative status**

The results of present study showed an increase in plasma total protein level which may be due to Se-yeast and S-alga supplementation. In this connection, Kovàcs et al. (2016) stated that increase of plasma total protein probably could be attributed to the synthesis of proteins related to immune response, and found that 5% Spirulina supplementation in rabbit diets increased plasma total protein by 13% as compared to the control group. Selenium is assumed to be built into the protein structure similarly as it is into Se-enriched yeast (Machat et al., 2005). Furthermore, Selenium is present in two biologically active forms, Se-containing enzymes and Se-containing proteins in animals (Zhang et al., 2011). Additionally, higher plasma albumin and globulin levels were achieved by dietary of Se-algae. These results were in line with Hassan et al. (2015) who observed that rabbit fed diets contained Se-algae caused a significant increase (P<0.05) in total protein and globulin. Also, Ebeid et al. (2013) postulated that Se-yeast supplementation at 0.3 mg/kg rabbit diet increased globulin concentration. It is clear to know that globulins are carrier proteins for steroid and thyroid hormones and play a vital role in natural and acquired immunity to infection (Ganong, 2005). Moreover, the reduction observed in creatinine concentrations depending on the protein content of the experimental diets. Creatinine content has been shown to depend on the quantity and quality of dietary protein (Esonu et al., 2001). The urea concentration was ranged normally with the dietary supplementation of Se-yeast and Se-algae. In addition, all recorded values of creatinin and urea were within the normal physiological ranges according to Brown (2002).

As previously mentioned, the activity of hepatic enzymes ALT and AST decreased, the enzymes levels remained within the physiological ranges as was reported by Harcourt-Brown (2002). The decline in total plasma cholesterol (P<0.05) and LDL-cholesterol may be related to the effect of selenium as it has an anabolic role on fat deposition. Also, the supplementation of Se as organic forms in yeast or algae could modulate the fatty acids composition in the whole body. Previous studies have demonstrated that dietary Se supplementation increased LDL receptor activity but decreased 3-hydroxy 3-methylglutaryl coenzyme A (HMG-CoA) reductase expression in rat, which can lead to decrease in serum LDL and cholesterol levels (Dhingra and Bansal 2006; Yang et al., 2010).

In agreement with present study, Amer et al. (2018) reported that rabbits fed diets contained organic Se in form of yeast decreased significantly (P<0.05) serum total cholesterol and LDL-cholesterol and showed hypolipidemic effect organic selenium. In addition, Hassan et al. (2015) found that dietary treatments with Se-algae significantly reduced total cholesterol and LDL-cholesterol in rabbits. In contrast, Ebeid et al. (2013) stated that supplementation rabbits' diet with 0.3 mg Se-yeast did not influence serum total cholesterol and LDL-cholesterol.

A serum concentration of MDA is an index of lipid peroxidation and oxidative stress, and its levels depend upon the antioxidants (Saffari et al., 2018). In present findigs regarding antioxidant status, plasma MDA decreased with the supplementation of Se-yeast and Se-algae. A reduction in oxidation level by the dietary supplementation of organic Se was associated with the higher (P<0.05) activity of catalase enzyme. The antioxidant enzymes play a key role in cellular defense against oxygen free radicals and oxidative stress (Bernabucci et al., 2002). Das (2011) found that CAT enzyme detoxified hydrogen peroxide $H_2O_2$ to $H_2O$ and $O_2$ which increased in production by the increase dismutation of $O_2$ by superoxide dismutase. Selenium is an essential element necessary for the function of several identified selenoproteins, including glutathione peroxidases, reductases, and deiodinases, as several essential enzyme functions with regard to normal immune function (Hall, 2018). In this connection, Zhang et al. (2011) found that that rabbit fed diet contained 0.24 mg/kg Se has greatest serum CAT activity. Also, reducing the lipid peroxidation expressed as serum MDA was with the inclusion of 0.15 and 0.3 ppm Se for growing rabbits diets (Ebeid et al., 2012). It could be concluded that organic Se in form of yeast and algae enhanced the antioxidative status of rabbits by minimizing lipid peroxidation and increase the activity of catalase as an antioxidant enzyme.

## CONCLUSION

Present findings demonstrated that organic selenium sources based on yeast or algae have the potential to be used as selenium sources for growing rabbits without causing any adverse effects on growth through their effect on improving antioxidant status of rabbits. It can be concluded that selenium does not play a direct role in promoting growth in rabbits. However, it helps to remove all constraints that may delay or inhibit growth performance. Moreover, feeding on Se-yeast or Se-algae resulted in the valuable deposition of Se in rabbit meat.

## DECLARATIONS

### Consent to publish
All the authors approved and agreed to publish the manuscript.

### Author's contributions
Dr. Fawzia Amer Hassan designed the study and drafted the manuscript, Dr. Noha Mahmoud Abdel-Azeem performed the statistical analysis and tabulation of the experimental data, Dr. Samah Mohamed Abdel-Rahman participated the chemical analysis and reviewed the manuscript, and Dr. Hamdy Farouk Amin participated the chemical analysis and practical part of the study and Dr. Lamiaa Fathy Abdel-Mawla performed the practical part of the experiment. All the authors approved the final manuscript

### Competing interests
The authors clarify that, they have no competing interest, and with respect to this search, all the authors are in agreement with each other and have no conflict with authorship or article publication, all authors approved the publishing of paper.

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# Effect of Environmental Heat Stress on Performance and Carcass Yield of Broiler Chicks

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## ABSTRACT

Environmental heat stress is one of the most challenging conditions which have adverse effect on the poultry industry. Broiler chickens are sensitive to heat stress mainly due to not having sweat glands. The current study was conducted to observe the effect of heat stress on performance of Ross-308 broiler chickens. 1600 Ross-308 broiler day old chicks were obtained from local hatchery and randomly divided into two groups, the heat stress group A (n=800) and heat free group B (n=800). Group A was reared in high temperature ($101^0$F) whereas group B was reared in ideal temperature. To evaluate the physiological stress indicators blood glucose levels and total blood cell count were checked on day 21 and 28. The parameters observed were; feed intake, body weight gain, feed conversion ratio, water intake and carcass yield. The results indicated that feed intake, weight gain, water intake, feed conversion ratio and carcass yield were significantly higher in group B compared to group A. It was concluded that heat stress has deleterious effect over the performance of broiler Ross-308 chicken.

**Key words:** Broiler Ross-308, Carcass yield, Environmental, Heat stress, Performance

## INTRODUCTION

Poultry is the one of the fastest growing industry of the animal production (Yousaf et al., 2017). About 1.3 billion people are employed directly or indirectly on livestock industry and accounts for 40% of agriculture Gross Domestic Product (GDP) (Anonyms, 2011). In Pakistan it is the second largest industry, which play key role in GDP of country (Yousaf et al., 2017). Poultry products are rich source of protein and good source of income. Importance of poultry is further emphasized by its demand and production ability in the country (Hussain et al., 2015).

Heat stress is one of the most challenging environmental conditions which have adverse effect on poultry industry. Broiler chickens are sensitive to heat stress (Yousaf et al., 2019, Yousaf et al., 2018). Poultry birds do not have sweat glands for heat releasing factor, if panting failed to reduce the high internal body temperature, birds become inactive, exhausted and mortality occurred because of the circulatory, respiratory and electrolytes imbalance (Swayne, 2017). Heat stress induces hormonal changing's which increased corticoid secretion (Brown et al., 1973). It has been investigating the effect of high environmental temperature on the performance of different poultry species, including broilers (Dozier et al., 2007) and has found that high environmental temperatures have deleterious effects on productive performance. Poultry birds increase body temperature (Altan et al., 2000), water consumption (Arce-Menocal et al., 2009) and decrease the feed consumption due to higher ambient temperature (Dozier et al., 2007).

Temperature and humidity play a key role which is one of the most important environmental factors during poultry house (Lourens et al., 2005). Broilers expose to ambient temperature, increase the body temperature (Reddy, 2000) consequently released corticosterone into the blood circulation to help the metabolism (Arce-Menocal et al., 2009). This hormone might cause cell mediate and humoral immunity failure because of the changing's in the plasma concentrations of corticosteroids and Adrenocorticotropic Hormone (ACTH) affect the lymphoid tissues, reduce the mass of spleen, thymus and bursa (Havenstein et al., 2003). Heterophils are present in the blood, formed leukocytes in the bone marrow, which are phagocytic in nature and shield the body of the bird against harmful micro-organisms and leukocytes play a key role in keeping immunity higher (Hussain et al., 2018). Heat exposure release excessive glucocorticoids, cause dissolution of lymphocytes which may cause lymphopenia (Yousaf et al., 2018). However, more heterophils are release in the blood circulation but heir phagocytic and bactericidal activities may decrease (Dozier et al., 2007). Heat stress is thought to have deleterious effect on an organism through the production of free radicals and Reactive Oxygen Species

(ROS) within the body (Bruskov et al., 2000). Free radicals and ROS are compound generated naturally within an organism during normal biological processes and are essential for several body processes, included immune function (Valko et al., 2007). Increased production of free radicals and ROS compounds can cause damage to the constituents of various biological tissues including lipids, proteins, and deoxyribose nucleic acid (Fang et al., 2000). This research was practiced to observe the effect of heat stress on performance of Ross-308 broiler chickens.

## MATERIALS AND METHODS

### Ethical approval
This experiment was performed according to all ethics and animal rights of Sindh Agriculture University Tandojam, Pakistan.

### Selection of breed/site
This experiment was conducted during the year 2018 to determine the effect of heat stress on the performance of Ross-308 broiler chickens. To determine the effect of environmental stress (n=1600), broiler chicks were purchase from commercial broiler hatchery.

### Group classification
On the arrival of birds to poultry house all the chicks were divided into two experimental groups group A served as the control (n=800 chicks) and group B served as the heat stress (n=800 chicks).

### Environmental condition poultry house
Group A, containing chicks were reared in high temperature $101^0$F whereas group B chicks were reared on ideal temperature $95^0$F at control poultry house. All conditions were remaining same of both groups expect the temperature. High temperature was providing during whole experiment to the group A chick. Humidity and ventilation were remaining same as group A. Poultry house condition of both chicks' groups are as table 1.

### Housing management
The house was completely cleaned, washed and sanitized using disinfect (formalin) before arrival of the day old chicks. It was painted with lime stone and dried for 24 hours. One $Ft^2$/bird floor space under deep litter housing system was provided to each and every chick in the house (Hussain et al., 2018). Continuous light was applied during the whole study. For ventilation viper touch (Big Dutchman, Holland) system was installed. Rice husk was used as litter at four-six inches' depth. Turning of litter practiced twice a day to minimize the gas production in the shed and maintained proper ventilation.

### Feeding
At farm chicks were offered water and feed immediately. All the chicks were feed and watered ad libitum on proprietary broiler started and finisher diets. The chickens were fed with starter diets from one to 10 days (3010 Kcal ME/kg, 22% crude protein), grower diets from 11 to 20 d (3175 Kcal ME/kg, 20% crude protein) and finisher diets from 21 to 35 d of age (3227 Kcal ME/kg, 18% crude protein). Water and feed were supplied ad libitum. The diet was formulated according to the recommendations of the NRC using windows user-Friendly feed formulation software program. Intake of feed and water was taken daily, while body weight and total feed consumed was recorded on weekly basis. Carcass measurements were also taken at the end of study.

### Vaccination schedule at poultry house
The vaccination schedule which was practiced during this experiment trial is mentioned in table 2.

### Live performance parameters
At the end of each and every experimental week, all birds and feed residues were weighed to determine the live performance parameters such as, average feed intake, average body weight, daily weight gain and Feed Conversion Ratio (FCR).

### Physiological stress indicators
To evaluate the heat stress in birds the blood glucose levels and Total Blood Cell Counts (TBCC) were used as measures (Walberg et al., 2001; Arruda et al., 2016).
**Blood glucose levels.** Blood glucose levels were checked for the both groups (A and B) on day 21 and 28. Ten birds from each pen were randomly selected and collected two ml blood from each bird from brachial vein by using

insulin syringe. Birds were kept for one hour of fasting in the morning time then blood collection procured was performed. Birds was handled very calmly to avoid any physical stress, then easily blood was collected within 30 seconds, then blood sample immediately poured collected blood sample in vecutainer contains an anti-clotting agent (EDTA) and label it. Collected blood store in low temperature ($4^0$C) and submitted it in laboratory for further analysis. Blood glucose was measure for individually and recorded it.

**Total blood cell count.** To analysis the TBCC total RBC's and WBC's were manually counted in a Neubauer chamber according to (Walberg et al., 2001) at 1/200 dilution. The correction factors for WBC's and RBC's were the number of counted cells multiplied by 50 and 10000 /mm$^3$ actively. Packed cell volume was determined using the micro-hematocrit technique, total blood protein by the refractometry method, and hemoglobin levels by the cyanmethemoglobin method. Wintrobe's indices, including Mean Cell Volume (MCV), mean corpuscular hemoglobin concentration, and mean corpuscular volume, were determined according to Jain (1993) and Pierson, (2000). Differential WBC's counts were performed on blood smear slides stained with hematoxylin-eosin hematological stain. Heterophils, lymphocytes, eosinophils, monocytes, and basophils were counted, and the heterophil to lymphocyte ratio (H:L) was calculated.

### Carcass yield and quality

To find out the quality and carcass yield on 30th day chicks were remaining for fasting up to 8 hours, 10 birds per each and every pen were randomly selected and weight it on digital scale then euthanized the birds to analysis the carcass yield. Yield was calculated as carcass weight relative to final body weight. Carcass quality was determined as a function of the presence or absence of dermatitis (breast blisters), dermatoses (scratches), arthritis, and bruising. Femoral degeneration was scored (0-2 scale) by the visual evaluation of the proximal epiphysis of the femur of both legs, according to the method described by Almeida Paz (2008).

### Measurements of performance indicators

All birds were weighed individually upon arrival. Thereafter, the birds of each group were individually weighed and the feed refusals of the group were recorded on the same day every week. Such recordings took place on days 7, 14, 21, 28 and 35 respectively. Feed refusals were weighed and average feed consumption together with feed consumption ratios were calculated for each group. Mortality in each group was also recorded at the before mentioned days.

### Statistical analyses

The raw data was tabulated in Microsoft excel then analyzed in ne-way analysis of variance (ANOVA) with JMP, software 7.0 Version and significant differences were compared through Student's comparison test. Result were considered significant if P < 0.05.

## RESULTS

During 35d trail period, mortality, feed intake, weight gain and FCR was recorded and results were presented in table 3 and weekly in table 4. Mortality was reduced significantly (P<0.05) in control B (3.46±0.03) as compare to A (7.25±0.05) group. Weight gain (g/bird) was significantly better (P<0.05) in B (2026±150) then A (1780±176). FCR was found significantly better (P≤0.05) in B (1.3±0.05), than A group (1.75±0.05). However, feed intake (g/bird) was also recorded better (P≤0.05) for group B (2745±86), then A (3121±76) the reason behind this due to heat stress decreased efficiency of feed utilization with increased environmental temperatures. The water intake (ml/bird) was significantly (P<0.05) higher in group A (8105±87) and lowest for group B (97399±65), table 3 and weekly presented in table 4.

## DISCUSSION

The experimental model used in this research to study effect of environmental heat stress upon the performance of broiler. Interestingly, the effect of heat stress on broilers performance recorded a record variation in term of results. A portion of the negative effects seen during hyperthermia may be related to the increased production of free radicals and ROS (Abdel-azeem et al., 2015). ROS are known to have deleterious effects on the constituents of biological tissues (protein, amino acids, lipids, and DNA), leading to cell damage and ultimately death (Tong et al., 2013). ROS production linked to heat stress has been reportedly associated with poor performance in broiler chickens (Abdel-azeem et al., 2015). High ambient temperature and relative humidity are major environmental stressors that influence performance of broilers by reducing feed intake, feed efficiency, nutrient utilization and feed conversion ratio (Jabbar and Yousaf, 2017). While the ideal environmental temperature enhanced growth rate and feed consumption (Tong et al., 2013). As the result shows that the control group A of Ross-308 was high meat yield then heat stress group B due to ideal temperature. The obtained result of present study revealed that heat free group intake lower feed resultantly consumed lowered level of

water, while ambient temperature caused painting in birds which was observed during this research trial and previous studies resulted painting when body temperature observed higher in the heat stress exposure birds, heat stress consequently increased body temperature, while broilers maintain their body temperature by increasing water consumption.

## CONCLUSION

From the present study, it is concluded that under tropical climatic conditions, especially in Pakistan, heat stress-condition impact harmfully and adversely on the productivity of Ross-308 broiler chicken.

## DECLARATIONS

### Author's contribution

Dr. Adnan Yousaf and Dr. Adnan Jabbar was the main researcher of this article, Dr. Nasir Rajput was research coordinator, Dr. Azizullah Memon was research supervisor, Dr. Rehana Shahnawaz and Dr. Farhan Farooq revised the article, Dr. Nasir Mukhtar contributions in statistics, Dr. Muhammad Abbas and Dr. Rabia Khalil assisted in results analysis and other activities related to the research.

### Competing interests
The authors have declared that no competing interest exists.

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# Study and Modelization of Veterinary Drug Residue Kinetics on Sawdust as Absorptive Biomaterial in Eastern Algeria during January 2017 to June 2018

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## ABSTRACT

Sawdust, an affordable resource, is being investigated as an adsorbent to eliminate residual contaminants from water. Wood processing residues such as bark and sawdust have been widely studied for some years for their adsorption and removal properties of toxic metals contained in contaminated effluents. The aim of our study is to know the source of chemical contamination of water by using spectrophotometry methods in field ultraviolet, the kinetic study of sodium salicylate adsorption on sawdust was modelized according to two kinetic models (pseudo first and second order), to determine the most optimal pattern to describe this phenomenon, was based in the comparison on the correlation coefficient ($R^2$) between the absorbance and the wavelength for both models and evaluate the optimal time of contact solid/liquid. Present results indicated that the correlation coefficient ($R^2$=0.999) for the pseudo-second order, which means that this model is the best for future studies in the kinetics of adsorption of sodium salicylate by sawdust, with a time of contact solid /liquid optimal is 44.5 minutes.

**Key words:** Residual contaminants, Sodium salicylate, Spectrophotometry, Veterinary drugs

## INTRODUCTION

Water is being one of the most basic constituents of life and essential resource for the development of the world, during the last century, the pressure growing on the water resources due to the demographic and economic growth resulted in considering various approaches for the stock management out of water (Meadows, 2012). Today, huge advance has been made in agriculture to meet the global nutritional needs of the population. This requires a contribution of fresh water to about 70% of global consumption (Derelanko, 2017) the use of water in agriculture faces social, economic and environmental problems that cannot be easily reconciled (Figuière et al., 2018; Juan et al., 2018; Toillier, 2018). The presence of the primary pollutants (residues of drugs) in the aqueous environmental matrices has become a subject of concern the fields of the environment and the public health (Jones et al., 2004). Because the analytical techniques improved the chemical products were identified in the water resources with concentrations varying from some Nano-Grams (ng) to several hundred micrograms per liter (Charuaudet al., 2018), consequently, it became essential to carry out an evaluation of the environmental and medical risks of the complex mixtures of organic micro-pollutants particularly in aquatic environments (Harris, 2010).

However, it would not be realistic to put forward repeated measures indefinitely, in time and space, to seek, identify and quantify all the residues of drugs present in the environment. Therefore, it is important to try a model to evaluate its principal families of medicines residues, minimizing the present risks at low dose during chronic exposures (Limbuet al., 2018). The adsorption of organic molecules on modified materials has proven to be a very effective treatment technique, but this efficiency makes the cost of the operation excessive (Gupta, 2011). Over the last two decades, many researchers have focused on the preparation of certain adsorbents from natural fruit tree waste (Arami et al., 2005; Forgacs et al., 2004), palm trees (Hazourli et al., 2007) or sawdust (Pekkuz et al., 2008). These natural absorbents, which are available at very low cost, have been shown to be effective against organic molecules at the laboratory scale, for example wood studies (Ho and Mckay, 1998), tree fern (HO et al., 2005) and palm fibres (Ofomaja, 2007). For the valorization of these adsorbents, the research focused on the study of the mechanisms governing the fixation of dye molecules on the grains fibres of the adsorbents (Aloulou et al., 2004). Wood is a building material whose industrial transformation generates by-products and saw dusts, used for other purposes such as energy valorization like fuel and clean adsorbent for the water treatment worn. However, the valorization of the sawdust-like support adsorbing in the purification of used water, requires knowledge of structure and texture of the material (Kyzas, 2013). The capacity of the sawdust to fix adsorbents

such as pollutants can be largely improved while making it undergo a chemical treatment. The modification of wood can be made using sulfuric acid, phosphoric acid or by enzymes (Zhang, 2006).

Sodium salt of the salicylic acid, also called Sodium Salicylate (SS), obtained by action of the sali-cylic acid on sodium bicarbonate, it is presented in the form of colorless and odorless crystals. It is used as analgesic and antipyretic, and is employed for the treatment of the rheumatic effects. Adsorption with the interface solid water / solution is a physical or chemical phenomenon by which molecules or ions present in a liquid waste "adsorbed" fixes at the interface of an "adsorbent" solid (Board, 2004). In the last three decades, the drug residue problems have emerged. Some of these molecules have genotoxic or hormono-mimetic properties and can potentially have an environmental impact on aquatic ecosystem (Benchouala, 2016; Emmanuel et al., 2008; Lavilleet al., 2008). Contaminate water is a vector for many diseases, water being one of the most basic constituents of life, cleanliness of water is essential for the sustainable health of the humans, aquatic and wildlife (Bano et al., 2018; Le Pimpec, 2002; Forbes, 1997).

In this work, we used sawdust from carpentry rejects to absorb salicylic acid in its normal state without any modification. We were particularly interested in studying the kinetics of salicylic acid adsorption to predict the best kinetic model of this responded material and to valorize it and benefit from its depolluting properties.

## MATERIALS AND METHODS

### Ethical approval
The experiment was carried out according to the national regulations on animal welfare and institutional animal ethical committee.

### Experimental design
The study was carried out in North-Eastern Algeria through the period from January 2017 to June 2018. The reason we chose to study spruce sawdust, is its availability in private carpentry factories in the Souk Ahras area and the enormous quantities of this wood waste that is thrown into public landfills. The samples were collected randomly from carpenters in the study area by collecting sawdust remains in a plastic sack and then mixed before the experiments. Three solutions with concentrations; 15, 10 and 5mg/l starting from the solution mother of SS of concentration 20 mg/ were prepared, then we measured absorbance (A) of each solution and we will define here the calibration curve to be a function $f$ (c)the calibration curve A= $f$ (c).

In a Becher one weighs m=50 Mg of the collected sawdust and addition volume of 50 ml of the SS solution (C=20 mg/l) then we started the stop watch and we took to each five minute, then we filtered and determined the concentration of the filtrate obtained, the operation was stopped when the value of the concentration of balance is constant.

We traced the quantity (Q) retained according to time (t) with the function $f$ (t): $Q = f(t)$.

One determines the variations of the adsorbed quantity:

$Q = (C_0 - C_t)$. V/m according to time (Zupanc, 2013).

With $Q$: the quantity adsorbed, $C_0$: initial concentration, $C_t$: concentration at the moment t, V volume of SS, m: weight.

### Sodium salicylate kinetic models
In our study we based on two kinetic models, pseudo first order and pseudo-second order, to determinate the best kinetic model for the adsorption of sodium salicylate; These mathematical models were chosen on the one hand for their simplicity and on the other hand for their application in the field of adsorption of organic or inorganic compounds on the various natural and synthetic adsorbents.

### Pseudo-first order
The equation of this model was introduced initially by Lagergren (Simonin, 2016) is form: $\frac{DQ}{DT} = K1(Qe - Qt)$

With Q the amount of adsorbed solute, $Q_e$ its value at equilibrium, $K_1$ the pseudo-first order rate constant and $t$ the time. After the integration and application of the condition limit (Q = 0 for T = 0 and Q = Qe for t=t) the equation becomes: $ln (Q - Qe) = ln (Qe) - K_1 t$

With Qe: the quantity of the adsorbed residue at equilibrium, $Q_t$: quantity adsorbed at the moment t, $K_1$: constant the kinetics of the reaction.

### Pseudo-second order
This model is form expressed by the equation (HO and MCKAY, 1999): $\frac{DQ}{DT} = K2 \times (Qe - Q)$

$Q_e$: quantity at equilibrium, Q: quantity adsorbed at the moment T, $K_2$ (constant): kinetics of the reaction.

After the integration and application of the same conditions limit preceding, the preceding equation becomes:

$$\frac{T}{Q} = \frac{1}{K2 \times Qe^2} + \frac{T}{Qe}$$

Q: Amount of adsorbed solute, $Q_e$: Value at equilibrium, Q: Quantity adsorbed at the moment T, $K_2$ (constant) kinetics of the reaction.

### Statistical analysis

The collected data are logged and processed using the program (Microsoft Excel software and Graph Pad Prism) to perform the description and evaluation.

The correlation qualities about adsorption kinetics, the relations for $K_1$ and $K_2$, present as follows:

Y=aX +b (a and b are parameters)

## RESULTS

### Visible ultraviolet absorption spectrophotometry

Spectrophotometry is a technique of analysis al-lows, inter alia identifying a chemical substance and of the concentration of an aqueous solution in a solution deter-mines, by the interaction of the electrons of the molecules of the aqueous solution (called chromophoric) with the light (Zupanc, 2013).

The determination of the residual concentration of SS is carried out by proportioning spectrophotometry in the UV screw field, by using the law of Beer-Lambert the wavelength of the maximum absorption of SS is 230 nm (Figure 1). From the different solutions, we obtained the following calibration curve (Figure 2). Figure 3 shows that the rate of adsorption is higher at the beginning of the processes and then becomes increasingly slow in the course of the time of agitation to reach balance. The time of balance of adsorption of this residue is 44.5 min the quantity selected is 19, 91 mg/g.

The layout of Ln (Qe-Q) according to time for residue SS gives a linear form shown in figure 4. The value of $K_1$was calculated starting from the slope of this line. $K_2$ (mg/g. min) is the constant kinetics determined starting from the slope of straight lines 1/ $Q_t$ according to time (Figure 4).

From the set of kinetic parameters provided by these graphs, it is clearly seen that the value of the coefficient of correlation $R^2$=0.999for the model of pseudo-second order was closer to 1 (Figure 5) and the coefficient of correlation was$R^2$=0,997for the model of pseudo-first order (Figure 4). Hence the kinetic model pseudo-second order gives a better description of the kinetics the reaction of adsorption SS on the sawdust compared to the pseudo first-order model.
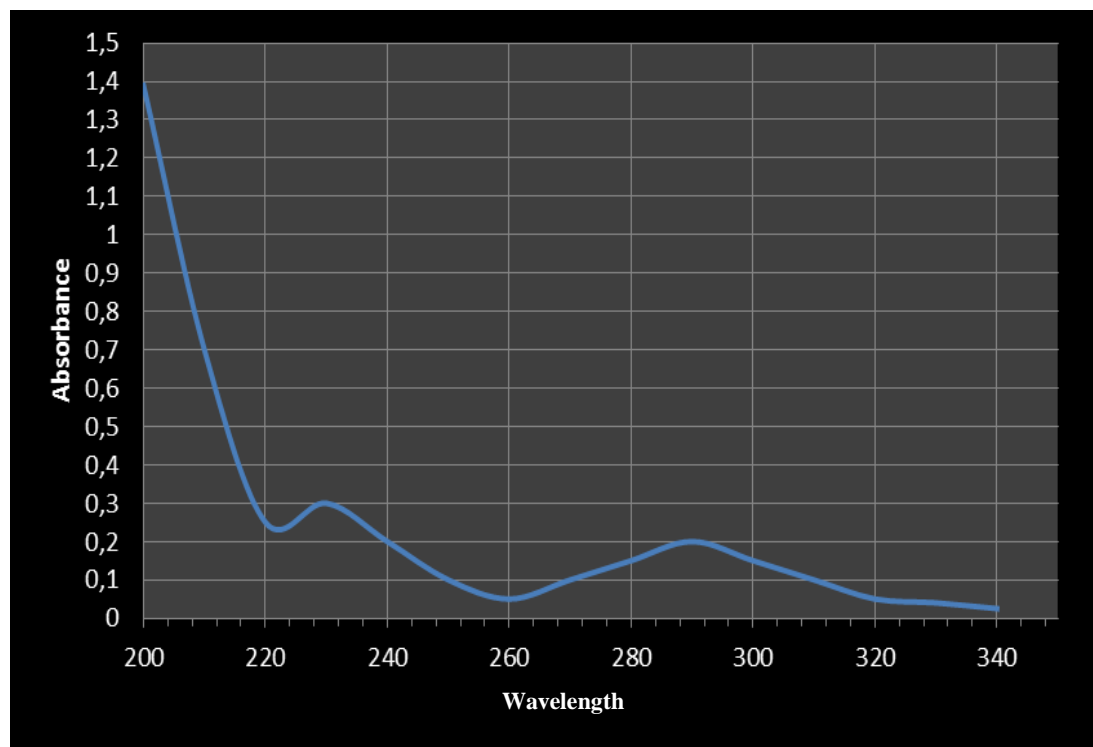


**Figure 1.** Absorption spectra of sodium salicylate in the ultraviolet field, Eastern Algerian during January 2017 to June 2018
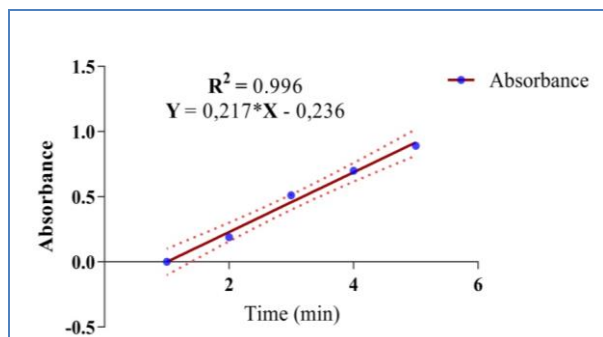
**Figure 2.** The calibration curve of sodium salicylate, Eastern Algerian during January 2017 to June 2018. $R^2$**:** the correlation coefficient, Y: Absorbance, X: time.



**Figure 4.** Linear form of the kinetic model pseudo-first order of adsorption of sodium salicylate on the sawdust, Eastern Algerian (January 2017 to June 2018). $R^2$: Correlation coefficient, Y: Ln $(Q_0-Q_t)$, X: time



**Figure 3.** Kinetics of adsorption of sodium salicylate on the used sawdust, Eastern Algerian during January 2017 to June 2018
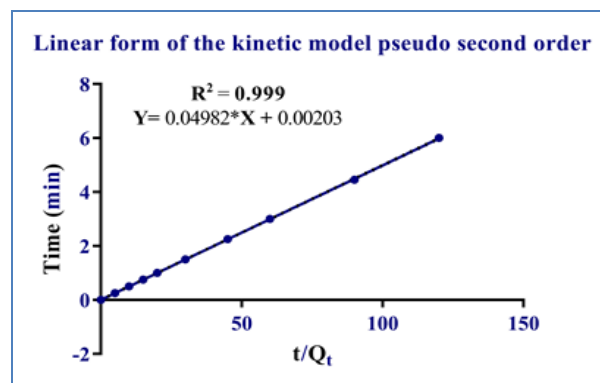


**Figure 5.** Linear form of the kinetic model pseudo second order of adsorption sodium salicylate (SS) on the sawdust, eastern Algerian (January 2017 to June 2018). $R^2$: Correlation coefficient, Y: time, X: $T/Q_t$.

## DISCUSSION

Many studies on the adsorption of dyes, toxic salts and oil from water using sawdust as an adsorbent have been reported, this material has proven that is not only abundant, but it is really an efficient and economic adsorbent (Shukla et al., 2002; Nag, 1995). The components and complexing properties of the sawdust explains the adsorption mechanism, as the cell walls of sawdust which are mainly composed of cellulose, lignin, and many hydroxyl compounds, such as tannins or other phenolic compounds (Suemitsu et al., 1986).

Most of the current literature on liquid/solid adsorption kinetics compares the respective capacities of first-order and second order pseudo-kinetics to describe the data (Simonin, 2016). It is preferable to use the correlation coefficient ($R^2$) in the comparison of method data to determine the most appropriate models (Lin and Wang, 2009). As a result of the linear models obtained from the kinetic parameters in this study, the value of the correlation coefficient ($R^2$) is 0.999 for the pseudo-second order model is closer to 1 and for the pseudo-first order model is 0.997.

The study of the adsorption kinetics of salicylic acid demonstrated the values and benefits of its depolluting properties but the choice of the most appropriate kinetic model for this material will facilitate the study and give more reliable results (Adib et al., 2018; Giraldo et al., 2018; Mekhalef et al., 2018).

## CONCLUSION

We have introduced a new assay of polluted Kinetic models. Several models are given in the literature to describe the kinetics of adsorption. In our study, we used kinetic laws of the pseudo-first order and of the pseudo-second order establish by Lagergren. Water treatment, as an example we worked on the absorption of SS from sawdust; The comparison of the results obtained by the two models kinetics shows that the pseudo-second order model is the best than kinetic model pseudo-first order for conducting studies of adsorption of SS by sawdust, with a time of contact solid/liquid optimal of 44,5 minutes.

## DECLARATIONS

### Competing interests
The authors have no competing interests to declare.

### Consent to publish
All authors gave their informed consent prior to their inclusion in the study.

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### Author`s contributions
Berghiche created the idea of the study, Berghiche and Boulebda participated in the design of study, Berghiche collected samples. Slimi and Boudis performed the experiments and collected Data, Berghiche, Khenenou and Labiad were involved in the collection of data, statistical analysis and drafting of the manuscript. Khenenou, Boulebda critically revised the manuscript, Khenenou, Boulebda and Berghiche read and approved the final manuscript.

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# Hormonal Changes in Relation to Productivity of Pregnant Rabbit Does

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## ABSTRACT

Pregnancy is a critical period for animals where it undergoes many physiological changes including hormones, which are not received a great attention in rabbit. Therefore, a total number of 25 New Zealand White pregnant rabbit does were used, to assess the changes in concentration of relevant hormones in relation to rabbit productivity. Blood samples were collected on 14, 21 and 28 days of pregnancy and on kindling day to quantify six maternal hormones. Litter size and weight, in addition to average of weekly and total milk yield were determined. Concentrations of the six hormones during the second half of pregnancy ranged between 3.2 to 4.0 ng/ml for progesterone (P4), 47.3 to 89.0 pg/ml for estrogen (E2), 2.0 to 3.7 ng/ml  for prolactin (PRL), 114.5 to 136.8 ng/ml for insulin like growth factor-I (IGF-I), 36.0 to 39.2 ng/ml for thyroxine (T4) and 1.9 to 2.2 ng/ml for triiodothyronine (T3). The corresponding values on kindling day were 1.6 ng/ml, 26.8 pg/ml, 4.6 ng/ml, 131.6 ng/ml, 37.4 ng/ml and 0.9 ng/ml, respectively. At day 14, maternal P4, E2, PRL, T4 and T3 were the lowest, whereas IGF-I was the highest compared to the other two days of pregnancy. At day 28, levels of E2, PRL, T4 and T3 were the highest in comparison with days 14 and 21 of pregnancy. On kindling day, P4 and T3 showed the minimal levels, whilst PRL exhibited the maximal level compared to the levels at all the gestational days. The relationship among the different hormones had various trends. The average of total milk yield (129.9 g/d) was negatively correlated with both P4 and E2, and positively associated with the PRL, IGF-I, T4 and T3 hormones. Furthermore, litter size was positively related with P4, E2 and PRL, and negatively with T4, T3 and IGF-I. Whereas, litter weight was negatively correlated with P4, E2 and T4. We recommend giving more attention to the rabbit doe reproduction, particularly close to parturition to achieve good economical return. However, further studies are urgently needed in this area.

**Key words:** Hormones, Litter size, Litter weight, Milk production, Pregnancy, Rabbits

## INTRODUCTION

In developing countries, rabbit production may play an important role in surmounting meat shortage especially that, rabbit's meat is characterized by highly nutritive value including low fat, cholesterol and increased protein level (Wahab et al., 2016). Despite of this distinctiveness, rabbit farming in Egypt is facing many constraints such as diseases, heat stress and poor feed quality (Ashour et al., 2018). Therefore, to improve this valuable industry, rabbit breeders need to understand their reproductive biology that considered fundamental step in rabbit farming success (Ashour et al., 2018).

During pregnancy, the endocrine network has an important and vital role in coordination of mammary gland (MG) development and the reproductive state of the animal (Neville et al., 2002). Through its direct action on MG development via the reproductive hormones (Progesterone; P4, Estrogen; E2 and Prolactin; PRL) (Neville et al., 2002) and indirect action through the metabolic hormones, such as thyroid hormones, that alter MG responsiveness to reproductive hormones and regulate milk synthesis (Neville et al., 2002).

Additionally, these hormones working on maintaining the pregnancy, this critical period needs the network of endocrine system (P4, E2, etc.) and the availability of the nutrient (lipids, glucose and proteins) to be continued successfully. The P4 hormone play a vital role during early and late pregnancy through its actions on implantation, myometrium and may regulate cytokine network in the uterus (González-Mariscal et al., 2009) Prior to labor, P4 tended to decrease meanwhile E2 increases to stimulate synthesis of prostaglandin (González-Mariscal et al., 2009). During late pregnancy in rabbits, the changes in P4, E2 and PRL levels are controlling the nest building to prepare the MG to nurse the newborn litter (González-Mariscal et al., 2009). Additionally, Insulin-Like Growth Factor –I (IGF-I) has an important role in the fetal growth and maternal metabolism during pregnancy. Thereby, regulating the availability of nutrient that required for the fetus growth (Sferruzzi-Perri et al., 2011). From the fore-mentioned studies, it is clear that several hormones are strongly interacted together in controlling reproductive functions, energy balance, growth and metabolism (Sferruzzi-Perri et al., 2011). However, the endocrinology of rabbit reproductive physiology is not received great attention (González-Mariscal et al., 2009). Nevertheless, the higher reproductive efficiency of rabbit does need

further understanding of all possible physiological mechanisms that regulate reproduction. So, the hormonal profiles should be examined either individually or in a relation to one another.

## MATERIALS AND METHODS

### Ethical approval

The present work has been conducted in accordance with guidelines of the ethical committee of Faculty of Agriculture – Cairo University, in cooperation with Animal Production Research Institute, Agricultural Research Center. The experimental fieldwork was carried out at rabbitry unit of Animal Physiology Laboratory. Whereas, the hormonal analyses were executed at Cairo University Research Park, Faculty of Agriculture, Egypt.

### Experimental animals and diets

A total number of 25 New Zealand White (NZW) rabbit does were checked for pregnancy (using hand palpation on the abdominal area) at day 14 after natural insemination. The pregnant does aged between 7-8 months with average body weight (BW) of 3252.58±18.60 g were used. Before starting the experiment, the does were put in individual cages with males and naturally mated. After that, they were checked for pregnancy (at day 14) which was considered the beginning of the experiment. The pregnant does were healthy and we did not notice any abnormal signs (such as scabies or inactive movement). The experiment started at mid of gestation and lasted until the weaning age of the kits. The animals were housed individually in galvanized wire batteries in well-ventilated indoor pens, and providing them *ad libitum* feed (fed commercial pelleted diet). The fresh and clean water was available during the experiment. The rations (Table 1) satisfied the nutrient requirements of the does during pregnancy and lactation according to Lebas (2004).

**Table 1.** Feed ingredients and chemical analysis of the basal diet (% dry matter basis) provided to the pregnant does during the experimental period

| Feed Ingredient | (%) | Chemical analysis (%DM basis) | |
|---|---|---|---|
| Soybean meal (44% CP) | 8.50 | Dry matter | 89.00 |
| Barley | 30.0 | Organic matter | 90.88 |
| Yellow corn | 9.50 | | |
| Wheat bran | 23.0 | Crud protein | 18.00 |
| Clover hay | 16.0 | Crud fiber | 10.60 |
| Corn gluten (60%CP) | 9.70 | | |
| Molasses | 0.30 | Ether extract | 2.50 |
| Limestone | 1.50 | Nitrogen free extract | 59.78 |
| Di- calcium phosphate | 0.50 | Ash | 9.12 |
| DL-Methionine | 0.20 | Lysine | 0.98 |
| Sodium chloride | 0.30 | Methionine+ cystene | 0.70 |
| Vit.-Min. premix[a] | 0.30 | Calcium | 0.95 |
| Anti-coccidiosis | 0.10 | Phosphorus | 0.64 |
| Anti-Fungi | 0.10 | Digestible energy(kcal/kgDM) | 2750 |
| Total | 100 | | |

[a]Each 1 kg contain contains: 12000 IU vit.A; 2200 IU vit. D3; 13.4 mg vit. E (determined); 2.0 mg vit. K3; 1.0 mg vit. B1; 4.0 mg vit. B2; 1.5 mg vit. B6; 0.0010 mg vit. B12; 6.7 mg vit. pantothenic; 6.67 mg vit. B5; 0.07 mg B8; 1.67 mg B9; 400 mg choline chloride; 133.4 mg Mg; 25.0 mg Fe; 22.3 mg Zn; 10.0 mg Mn;1 .67 mg Cu; 0.25 mg I and 0.033 mg Se, DM = Dry Matter, CR = Crude Protein

### Blood sampling and plasma hormonal analyses

From the vena behind the rabbit does ear, about 3ml of blood samples were collected from each doe at pregnancy days (14, 21 and 28, Figure 1) and on kindling day. We choose these specific pregnancy days, because rabbit placentation is occurred at the day 13 and fetal growth occurred from the days 21 to 28 of pregnancy according to Wahab et al. (2016). The samples were collected in heparinized tubes and were centrifuged at 3000 rpm for 20 minutes to get blood plasma that stored under -20°C until hormonal analyses, which have been done according to the manufacturer procedure. In blood plasma, we assayed hormones, P4, E2, IGF-I, T4 and T3 using ELISA kits obtained from Sino Gen Clon Biotech, No.9 BoYuan Road, YuHang District 311112, HangZhou, China. While, PRL hormone was determined by using ELISA kit from Glory Science Co. (G1725, USA). The measured hormones P4, PRL, IGF-I, T4 and T3 are expressed in nanogram per milliliter (ng/ml) except the E2, which expressed in picogram per milliliter (pg/ml). Hormones were quantified by using a specific antibody for each hormone, which make solid phase. The combine hormone antibody with labeled horseradish peroxidase to form antibody-antigen-enzyme-antibody complex. After many steps and forming the blue color, the stop solution added and the color change is measured spectrophotometrically at wave length 450nm.
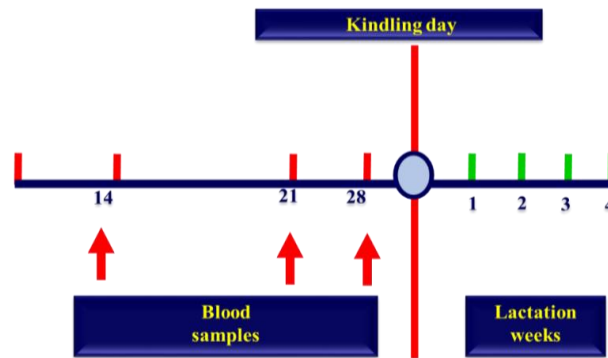
**Figure 1.** Time of collecting blood samples during gestation days and kindling day in New Zealand White rabbits.

### Body weights, litter size and milk production

The litter weights were recorded concurrently with collecting milk samples to determine the amount of produced milk (daily milk yield, DMY g/d) by calculating the difference in kit´s body weight before and after suckling. The dams continued in lactation for four weeks and the average of total milk yield (TMY, g/d) was calculated.

### Statistical analyses

The collected data were subjected to one-way analysis of variance to detect the effects of time of collecting blood samples (sample date, SD) on hormonal changes during gestation period and its impact on subsequent lactation, litter size and weight, using the General Linear Model (GLM) procedure of XLSTAT (2018).

The statistical model used was as follows: $Y_{ij} = \mu + SD_i + e_{ij}$. Where: $Yijk$= the individual observation, $\mu$ = the overall mean, $SD_i$= the effect of time (i=1, 2, 3, 4), $e_{ij}$= random error associated with the individual.

The differences among time means were separated according to Duncan's multiple range test (Duncan, 1955). The significance level was set at 5% (P< 0.05), and the correlation coefficient among hormones, milk yield, litter size and weight at birth was calculated.

## RESULTS AND DISCUSSION

### Hormonal changes in pregnant rabbit does

**Progesterone hormone:** As shown in table 2 and figure 2, P4 level at the mid of gestation was gradually increased reaching to the highest level (4.00 ng/ml) at day 21 of pregnancy. Then, decreased slightly prior to labor by two days. However, the differences among gestation days were insignificant (P>0.05). Thereafter, a drop and rapid reduction in P4 level have been recorded at delivery day. It could be noticed that, this level at kindling day was markedly and significantly (P<0.05) differed than that at all gestational days. In the present study, the level of P4 during the second half of pregnancy (14 − 28 days) is ranged between 3.16 to 4.00 ng/ml. This finding agree with that of Kelden et al. (2017) who found that P4 level was 3.1 ng/ml during pregnancy period in rabbits.

In contrast, our values of P4 level are lower than those reported in other studies. González-Mariscal et al. (2009) found that the maximal level was observed on day 14 (11±3 ng/ml) after which P4 concentration gradually declined. Szendro et al. (2010) reported that P4 level was 9.4 ng/ml in pregnant rabbits. Also, Kirat et al. (2015) observed that P4 level at 10th, 20th and 30th days of pregnancy were 9.9, 5.3 and 4.1 ng/ml, respectively. Additionally, the present data disagree and lower than those of Alfonso (2016) who found that, the highest level (12 ng/ml) of P4 was at day 14 of pregnancy, after that its level started to decrease at days 21 and 28 of pregnancy (10 and 8 ng/ml, respectively). On the other side, it is much higher than that (1.5 ng/ml) obtained by Bostanci et al. (2012). Generally, the level of P4 hormone in rabbits during pregnancy is lower than that in other species, Neville et al. (2002) stated that P4 in rat is ranged between 6-130 ng/ml, and reaching to 200 ng/ml in human.

These contradicted results concerning the changes in P4 levels and patterns in the above mentioned studies could be attributed to many factors particularly genetic makeup, parity, managerial and environmental conditions. It is well known that P4 level in rabbits remain high throughout pregnancy period (Szendro et al., 2010). This elevation in P4 level is essential for both, the first wave of MG differentiation, which called lactogenesis 1 that starting at mid-gestation and alveolar proliferation (Neville et al., 2002). Furthermore, it is essential for the maintenance of pregnancy in rabbits and is crucial for keeping the uterus in a quiescent state to prevent premature onset of labor (Kirat et al., 2015). Also, it helps the fetus implantation and maintain continuation of pregnancy (Kelden et al., 2017).

39

**Table 2.** Hormonal changes during the second half of gestational days and at kindling day in New Zealand White rabbit does

| Hormones (ng/ml) | Pregnancy days | | | Delivery day |
|---|---|---|---|---|
| | **14** | **21** | **28** | |
| P4 | $3.16 \pm 0.30^b$ | $4.00 \pm 0.32^b$ | $3.94 \pm 0.32^b$ | $1.58 \pm 0.30^a$ |
| E2 (pg/ml) | $47.31 \pm 4.99^b$ | $56.33 \pm 5.34^b$ | $88.99 \pm 5.34^c$ | $26.75 \pm 4.99^a$ |
| PRL | $1.97 \pm 0.75^a$ | $2.35 \pm 0.80^{ab}$ | $3.68 \pm 0.80^{ab}$ | $4.64 \pm 0.75^b$ |
| IGF-I | $136.81 \pm 6.77^b$ | $114.46 \pm 7.33^a$ | $118.69 \pm 7.33^{ab}$ | $131.57 \pm 6.77^{ab}$ |
| T4 | $36.00 \pm 1.72$ | $38.28 \pm 1.82$ | $39.20 \pm 1.82$ | $37.41 \pm 1.82$ |
| T3 | $1.87 \pm 0.12$ | $2.00 \pm 0.13$ | $2.19 \pm 0.13$ | $0.91 \pm 0.12$ |

[a, b,c] Means in the same row with different superscripts are significantly different (P<0.05), P4= progesterone hormone; E2= Estrogen hormone; PRL= Prolactin hormone; IFG-I= Insulin like growth factor–I; T4 = Thyroxine; T3 = Triiodothyronine
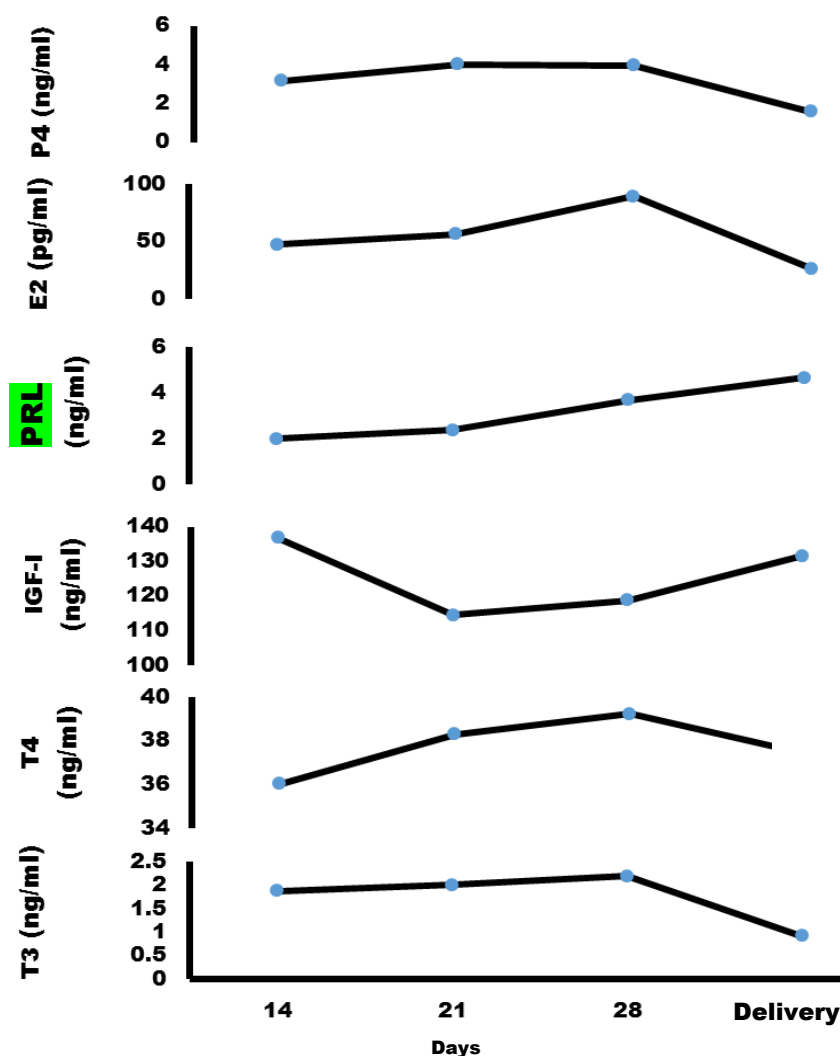


**Figure 2.** Hormonal changes during the second half of gestational days and at kindling day in New Zealand White rabbit does.

**Estrogen hormone**: During the second half of pregnancy days, E2 level increased progressively (Table 2 and Figure 2). It was significantly (P<0.05) increased overall the pregnancy days from the lowest level at day 14 to day 21 and continuing in increasing reaching to the highest level at day 28 before kindling. At day 28 of pregnancy, E2 level was markedly higher (P<0.05) than that at the previous levels at 14 and 21 days, which showed no significant (P>0.05) differences between them. This pattern of changes is similar to those reported by Al-Atawi et al. (2004) and Kirat et al. (2015).

Before labor by two days at day 28, E2 level obviously increased, whereas, P4 level slightly decreased. During pregnancy days, it could be noticed that the lowest levels of P4 and E2 were observed at day 14. Meanwhile, the highest levels were obtained at day 21 for P4 and at day 28 for E2 levels. Whereas, at delivery day, E2 level dramatically dropped (P<0.05) to lower level than that at all gestational days (Table 2). Nevertheless, both P4 and E2 levels showed

the minimal values at kindling day than those at days of pregnancy. These findings are in harmony with that of Garfield et al. (1998) who stated that, in rabbits, the corpus luteum is the major source of peripheral P4 and parturition is preceded by luteolysis and a sudden P4 withdrawal with a concomitant increase in E2.

The current level is ranged between 47.3 to 89.0 pg/ml during the second half of pregnancy days. This level is slightly lower than that (98.1 pg/ml) found by Bostanci et al. (2012). Conversely, our present level is much higher than other studies. González-Mariscal et al. (2009) reported that this level in pregnant does was 24±6 pg/ml on pregnancy 21 d. In addition, Kirat et al. (2015) showed levels of 5.5, 10.5 and 16.8 pg/ml on days 10, 20 and 30, respectively of rabbit pregnancy.

Both P4 and E2 have synergistic relationship, as confirmed in the present study by their positive correlation (+0.69; P<0.05), because during the second half of rabbit pregnancy the luteal P4 synthesis is dependent upon the E2 level. This means that E2 is considered a vital factor in maintaining P4 level during gestation period (Bostanci et al., 2012). Furthermore, Neville et al. (2002) found that, E2 stimulates P4 receptors in the luminal epithelial cells of MG. However, it has negative impact on milk production, because E2 accelerate MG involution. In the same context, Bostanci et al. (2012) mentioned that both P4 and E2 are important in maintaining uterine via regulation of blood flow and synthesis of cell surface receptors. Additionally, they cleared that E2 may indirectly influences the antioxidant system, thereby reducing the free radicals that caused oxidative stress, but the way of interacting of E2 and the antioxidant system is not yet clear.

**Prolactin hormone:** As seen in table 2 and figure 2, level of PRL hormone showed a continuous and pronounced increase during the studied gestational days. It was insignificantly (P>0.05) increased from the lowest level at day 14 to the highest level at day 28. There were no significant (P>0.05) differences among the three times of gestation. During the second half of pregnancy days, maternal PRL level showed the same pattern of E2, both hormones continuously increased to reach the maximum values at day 28. Meanwhile, at that day P4 hormone started to decrease. This means that P4 and PRL hormones had a negative relationship (-0.46; P<0.05) as recorded in the present study.

On kindling day, PRL hormone substantially increased to reach to the maximal level. This level was greatly higher than that at all the gestational days. This increase was significant (P<0.05) in comparison with the level at day 14 and insignificant (P>0.05) with that at 21 and 28 days. The trend was opposite to that of both P4 and E2 hormones, they were lower at delivery day than the other days of gestation (Figure 2). During the second half of pregnancy, PRL is ranged between 2.00 to 3.7 ng/ml. This concentration is higher than that (1.97 ng/ml) obtained by Kelden et al. (2017) in pregnant rabbits. Fortun et al. (1999) stated that, P4 levels were lower in lactating – pregnant rabbits than pregnant only without lactation. Moreover, they attributed these results to the increased level of PRL (Hyperprolactinemia) in the lactating – pregnant groups, which leads to inhibit P4 secretion. Additionally, suckling caused oxytocin release (has luteolytic role) which caused inhibition in P4 secretion. Also, they mentioned that, when the rabbits are pregnant and lactating at the same time, they consume more feed which have negative impact on P4 secretion. Level of PRL during gestation is essential for the second phase of MG differentiation (lactogenesis 2). This phase beginning around parturition, to prepare MG for colostrum secretion and then milk. In rats, a surge of PRL was reinitiated before labor by 24 h (Neville et al., 2002).

**Insulin like growth factor – I**: Concerning the IGF-I concentration during the second half of pregnancy, it was declined (P<0.05) from the highest level at day 14 to the lowest level at day 21. It is clear that, this hormone reduced in the trimester than that at mid gestation. There were no significant (P>0.05) differences in its level between days 21 and 28 (Table 2 and Figure 2). This reduction in IGF-I in late pregnancy is important to reduce maternal anabolic activity to increase the availability of nutrients and deliver to the uterus (Sferruzzi-Perri et al., 2011). These changes in IGF-I concentrations are in harmony with Sferruzzi-Perri et al. (2011) who mentioned that, IGF-I in rabbit increased during the first half of pregnancy that caused by the increases of mRNA expression in liver. Whilst in late pregnancy, IGF-I concentration decreased because of the decline in both liver and fat mass and at the same time the expression of IGF-I mRNA. In addition, Sferruzzi-Perri et al. (2011) cleared that the main IGF-I sources in pregnant rabbits are, all the body tissues (fat and liver) that increased in mass in parallel with increasing body mass.

These alterations in IGF-I level may consider as a factor that change the maternal endocrine system such as steroid hormones to support pregnancy. As mentioned by Sferruzzi-Perri et al. (2011) and Restiad et al. (2017) this hormone has the ability in enhances P4 secretion from placenta and stimulate estradiol production from ovary. In the current study, IGF-I was negatively correlated with both P4 and E2 (-0.21 and -0.15, P<0.05, respectively) and positively with PRL (0.002, P<0.05). In the same context, this hormone binds with rabbit embryonic coats (that consist of four layers formed by granulosa cells, oviduct, uterine secretions and the embryo itself) at day three of pregnancy via specific binding proteins, to help the embryo in development (by 83%). This hormone cannot cross placenta in sufficient quantities, so its effects on fetal growth is indirect effect via its impact on enhances maternal metabolism, nutrient partitioning and

development of placenta (Sferruzzi-Perri et al., 2011), as well as increases embryonic diameter and cell proliferation (Herrler et al., 1998).

Our current study revealed that, on kindling day, IGF-I level was insignificantly elevated than that during late pregnancy at days 21 and 28. During the second half of pregnancy, IGF-I concentration varied between 114.5 to 136.8 ng/ml. According to Bielohuby et al. (2014) who stated that, few studies determined concentration of IGF-I in rabbits and it is need further evaluation to describe the changes in IGF-I level in rabbits. Therefore, circulating IGF-I in rabbits varied widely among studies. Our current level is comparable to that (149 ng/ml) reported by Costa et al. (1998). In contrast, the present level is much lower than that 371 ng/ml (Thakur et al., 2000) and that 285.8 ng/ml (Bielohuby et al., 2014). Also, our finding completely disagree with that of Alfonso (2016) who stated that, IGF-I level in pregnant rabbits increased gradually with pregnancy advancement recording its lower level (200 ng/ml) at day 14 and the highest levels were at days 21 and 28 (290 and 300 ng/ml, respectively).

**Thyroid hormones:** There were no significant (P>0.05) differences in T4 and T3 levels among gestation days. Levels of T4 and T3 progressively and insignificantly (P>0.05) increased with the advancement of pregnancy from low levels at day 14 to the high levels at day 28 (Table 2 and Figure 2). Therefore, concentrations of T4 and T3 increased during the third period of pregnancy than that at the mid gestation. The present trend agrees with that of Cardinalli et al. (2009) who recorded increases in T3 level during the days 21 and 27 of rabbit pregnancy. On delivery day, T4 level slightly reduced than that at late pregnancy. Whereas, T3 level insignificantly (P>0.05) dropped to the minimal level than that observed at all gestational days (Figure 2). During the second half of pregnancy, concentrations of T4 and T3 hormones ranged between 36.0 to 39.20 and 1.9 to 2.2 ng/ml, respectively. As expected, there was a positive correlation (0.162; P<0.05) between T4 and T3 hormones.

The peripheral T4 is converted to the bioactive form (T3), and this conversion is one of the factors that affecting the bioavailability of thyroid hormones for the target tissues. The other factors are, releasing thyroid stimulating hormone, activity of hypothalamus-pituitary axis and the ability of thyroid hormones to bind with their receptors to activate the cellular process (Forhead and Fowden, 2014). During pregnancy, the maternal levels of thyroid hormones are very important for the fetus growth. Rabbits placenta is characterized with its highly permeability for thyroid hormones (via their binding protein receptors such as Transthyretin Receptor, TTR), because if there is any deficiency in fetal thyroid hormones the dam can compensate this deficiency and help them to grow well (Forhead and Fowden, 2014). Thyroid hormones are considering as growth stimulator for the fetus, through their direct and indirect actions. The direct one is their impact on fetal metabolism (increasing $O_2$ and glucose consumption) and the indirect through their relationships with the endocrine system, one of these hormones is IGF-I that expressed widely in fetus tissues. These findings, to some extent, support our present positive relationship between maternal T4 and T3 with IGF-I which was +0.17 and +0.25 (P<0.05), respectively. It is well known that thyroid hormone affected the reproductive functions.

The present study clarified the positive correlation between T4 and both P4 and E2 (+0.15 and +0.09, P<0.05, respectively). The same was recorded for the correlation between T3 and P4 which was+0.002 and +0.04 with E2.

**Productive performance of NZW rabbit does**

**Milk production:** The rabbit does lactate for four consecutive weeks and the average of total milk yield (TMY) was 129.94 g/d. This is in harmony with that of Ashour et al. (2018). During the weeks of lactation, rabbits produced 91.66 g/d in the first week (Figure 3). Then, the produced milk continued in increasing reaching to the maximum amount (185 g/d) at the third one. At the fourth week, the DMY declined to 113.11 g/d, this reduction attributed to MG involution and the decline in hormones level, mainly PRL. Furthermore, at end of lactation, the kits stopped suckling, which stimulates milk secretion via the sending signals to release oxytocin (Neville et al., 2002).

The data revealed a negative correlation between average of TMY and both P4 and E2 (- 0.21 and – 0.04; P<0.05, respectively). As mentioned previously by Brisken (2002) and Neville et al. (2002), P4 hormone has an important role in proliferation phase of alveolar morphogenesis. However, it has an inhibition effect on milk secretion during lactogenesis 1. Additionally, they cleared that P4 receptors in MG are consider absent during lactation and returned to be highly expressed at stage of MG involution. The same was for E2 hormone that caused a decrease in milk production and found to be as stimulator factor for MG involution (Neville et al., 2002). Furthermore, average of TMY was positively associated with the T4, T3, IGF-I and PRL hormones (0.22, 0.09, 0.01 and 0.39; P<0.05, respectively). During the transition period (from late pregnancy and lactation), the activity of enzyme five deiodinase (that covert the T4 hormone to the active form T3) is decreased in liver and increased in MG cells and found to be correlated with lactation intensity (Capuco et al., 2008). Also, during the transition period, thyroid hormones are necessary for galactopoietic responses to PRL (Capuco et al., 2008). The PRL hormones as described previously, increased at late pregnancy (stimulated by rising level of E2) and the expression of PRL receptors mRNA is increasing about 4-10 fold between late pregnancy and the day of kindling, because its importance for lactogenesis 2 (Neville et al., 2002).
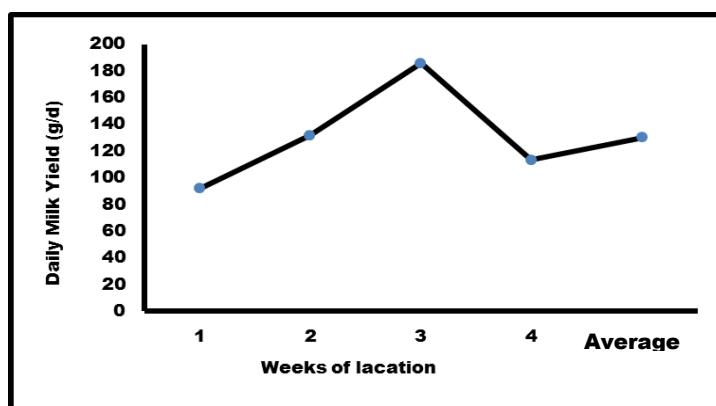
**Figure 3.** Daily milk yield in New Zealand White rabbit does throughout the lactation period.

**Litter size and weight:** At birth, the average of litter size (LS) was 6.33, this is closely to those reported by Kelden et al. (2017) and Ashour et al. (2018) who found that LS at birth was 6.8 and 5.67 in rabbit pups. The average of pup's birth weight was 325.56 g, which is mainly associated with their LS. In the present study the LS at weaning was 4.64, which was lower than that recorded at birth, and the average of BW increased eight folds higher than that at birth and reached to 2621g (Table 3). This is agreeing with Ashour et al. (2018) who found that LS and LW were 4.33 and 2261g, respectively. Furthermore, there was a positive relationship (0.03, 0.13 and 0.19; P<0.05) between LS and P4 (P4 has a role in helping the fetus in implantation, as mentioned previously), E2 and PRL, respectively. Moreover, a negative correlation between LS and T4, T3 and IGF-I (- 0.35, - 0.17 and – 0.25; P<0.05, respectively) was recorded.

The BW is often used as a proxy for fetal growth and development as well as for fetal nutritional status. The present data recorded that, BW was negatively correlated with P4, E2 and T4 (- 0.21, - 0.08 and – 0.29; P<0.05, respectively). Medici et al. (2013) supported our finding and stated that, when the dam had higher free T4 during early pregnancy this will lead to lower fetal growth and birth weight. Furthermore, BW was in a positive link with T3, IGF-I and PRL (+0.18, 0.27 and +0.29; P<0.05, respectively) this may attribute to both hormones (T3 and IGF-I) which considered as growth stimulator and enhances fetal growth and nutrient partitioning as mentioned previously by Sferruzzi-Perri et al. (2011) and Forhead and Fowden (2014). To the best of our knowledge, most of the research articles have been studied the impact of some feed additives or specific treatment on hormonal changes during pregnancy period such as Kelden et al. (2017). Also, few research articles concerned to clarify the changes of these hormones such as González-Mariscal et al. (2009).

In our study, we concerned to display and describe the changes in reproductive and metabolic hormones during pregnancy period in relation to doe's productivity. In addition, we recommend to give great attention for the female rabbit reproduction and understand deeply all the physiological factors during pregnancy period and their impact on milk production which is the main and nourishment source for the new born that depends on dam milk for about 18 days. In order to breed them in right way and gain the good return through LS and LW that considered the main economical traits in rabbit farming.

**Table 3.** Averages of litter size and litter weight from birth until weaning day in New Zealand White rabbit.

| Kits performance | Average | Standard deviation |
|---|---|---|
| **At birth** | | |
| Size (No) | 6.33 | 0.86 |
| Weight (g) | 325.56 | 48.95 |
| **At weaning** | | |
| Size (No) | 4.67 | 1.00 |
| Weight (g) | 2621.00 | 408.87 |

No = Number

**CONCLUSION**

The current study confirmed the relationship between the hormonal changes and rabbit productivity. However, further studies are urgently needed to verify our results and better understanding of reproductive rabbit's doe to achieve higher economical return.

## DECLARATIONS

### Author's contributions

Dr. Gamal Ashour (Professor of Animal Physiology) designed the plan of study and providing the experimental animals and tools, writing and revision of the research article, Dr. Samah Mohamed Abdel-Rahman applied the particle part of the study, laboratory and statistical analysis and tabulation of the data.

### Competing interests

The authors have no competing interest, and we are with respect to this search and in agreement with each other. In addition, we have no conflict with authorship or article publication.

### Consent to publish

All the authors approved and agreed to publish the manuscript

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# Mixed Mammary Carcinosarcoma in Domesticated Asian Palm Civet (*Paradoxurus hermaphroditus*)

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## ABSTRACT

A female Asian palm civet (*Paradoxurus hermaphroditus*), three years old was carried for a medical checkup to Ruddy animal's clinic in Sidoarjo, East Java, Indonesia. The civet suffers enlargement of abdominal mammary glands, painless lump, asymmetric size (4.1 and 8.4 cm in diameter), and lacerated wound on the large one with severe haemorrhage. The unilateral mastectomy was conducted under anaesthesia to handles both haemorrhage and tumour mass. Following the surgery, the tumour mass was stored in 10% neutral buffer formalin for histopathology using Hematoxylin & Eosin (H&E) staining and immunohistochemistry against antibody, anti-CD4+ and CD8+, further, a blood sample collected before and after surgery (on days: 0, 7, 30, and 60) for representing the healing progress. The chemotherapy was given using the combination of oral cyclophosphamide and intravenous injection of vincristine. According to laboratory results, the final diagnosis was mixed mammary carcinosarcoma with minimal expression of CD8+, notwithstanding, it showed the better prognosis after surgery and chemotherapy.

**Key words:** Asian palm civet, CD4+, CD8+, Mixed mammary carcinosarcoma, Therapy

## INTRODUCTION

Mixed mammary carcinosarcoma is an uncommon type of neoplasm in the animals because of consist malignant of differentiated cells and aggressive behaviour (Nunes et al., 2019). It has a high vascularisation sustains the abnormal proliferative cells to receive nutrition (Gal et al., 2008). Moreover, it raises the malnutrition of the host body as the compensatory effects of metastases (Al-Mansour et al., 2018). However, the blood vessels of a mixed mammary tumour arranged as the temporary duct with high fragility, with an incomplete layer, so that facilitate the haemorrhage emergence (Forster et al., 2017). That case frequently reported in the bitch (>4 years old). The contraceptive therapy also associated with the prevalence of mammary carcinoma in zoo animals especially canids (Moresco et al., 2009). The further theory stated that the failure of cytotoxic T-cell generates the proliferation of mammary carcinoma (Wei et al., 2008). Even though, the gene factor also contributes (Goebel and Merner, 2017).

In the clinic, a simple method to analyse and diagnose the mammary tumours in both human and animal is a cytological examination. However, this procedure is not satisfactory in canids because of the complex morphology of cells mammary tumours (Dolka et al., 2018). The histopathology and immunohistochemistry are known to be sufficient to analyse cells morphology and glycoprotein markers on the canine mammary tumours (Carvalho et al., 2016). The correct diagnoses facilitate the clinicians to provide precision therapy. Commonly, the surgical procedure is the principal treatment for mammary tumours. Mastectomy and chemotherapy are suggested to increase host survival rates (Dias et al., 2016). Gemcitabine, cyclophosphamide, vincristine, carboplatin and other anticancer drugs were demonstrated in the previous study (Karayannopoulou and Lafioniatis, 2016).

Among Indonesian civet, the mixed mammary carcinosarcoma hasn't reported due to the civet in Indonesia domesticated as the cultivation animal (to produce fermented coffee beans) and pet animal recently. This case provided the first report regarding mixed mammary carcinosarcoma in the Asian palm civet and supported by laboratory examination results that describe metastases phenomenon and its prognosis of healing.

## CASE REPORT

### Ethical approval
This experiment was performed according to the all ethics and animal rights in Ruddy animal's clinic in Indonesia.

**Anamnesis and clinical examination**

On July 21, 2018, a female Asian palm civet, three years old was carried for a medical checkup to Ruddy animal's clinic in Sidoarjo, East Java, Indonesia. Based on the owner anamnesis, the mammary glands enlargement occurs within last two months ago and developing rapidly. The lacerated wound is caused by itself bites. The civet was fed a diet with chicken's head, raw meat, dry cat food and bananas. The drinks were given using the mineral water ad libitum and milk once a day at night. The owner reported that the civet routinely injected using the progestin contraceptive without a clear understanding of its side effects. The clinical appearance showed that civet suffers the enlargement of abdominal mammary glands in both left and right sides, asymmetrical size (4.1 and 8.4 cm in diameter), a painless lump, with a severe lacerated wound on the large one. The severe haemorrhage has also been identified. The structure of ribs and waistline indicates that the civet suffers chronic thinness. Another clinical finding showed the pale colour of the mucous membrane, however, with normal pulse, respiratory rates, and body temperature (39.12º C).

**Premedication and anaesthesia**

The surgery is performed after the haematology analysis shows the standard value. Prior the anaesthesia, the premedication was conducted using the subcutaneous injection of atropine sulfate (0.03 mg/kg of body weight; V-Tropin®; Peru). Further, the dissociative anaesthetic was performed intramuscularly (Dugassa and Fromsa, 2018). It is utilising the combination of ketamine hydrochloride (15 mg/kg of body weight; Ketamil®; Australia), and xylazine hydroxylate (1.5 mg/kg of body weight; Xyla®; Holland). The abdominal hair surrounding the tumour mass was shaved using a hair clipper and disinfected using a 10% iodine tincture. Upon the civet under the anaesthesia, the sodium chloride inserted via the intravenous route using pediatric infuse set (25 drops/ minute; Infusion Set Child Gea®; Indonesia).

**Mastectomy**

Prior mastectomy, the ligation was performed on the blood vessels of the wound to prevent the blood loss. The unilateral mastectomy was conducted by elliptical skin incision surrounding the tumour mass, with a borderline within 2 cm from the central area. The blunt dissection was performed if the underlying muscle attached to the tumour mass. Dissection plane directed to the next healthy fascia to encounter the tumour invasion. Again, the haemorrhage is controlled by the ligation of the blood vessels. Warm saline was given to lavage the excision area. Closure of the surgical wound was conducted by the conventional technique to attach the skin with the fascia using absorbable suture (Catgut Chromic® 2/0; Bio SM; Belgium). Those procedures intend to decrease skin tension and space formation that can become the media for both exudate accumulation and bacterial colonisation. The last, simple interrupted suture is performed to link both two sides of the wound edge (Sadhasivan et al., 2017).

**Therapy**

On the wound, the 2% Aloe vera cream is applied topically. The surgical wound was then covered using the antibacterial gauze dressing (Sofra-tulle; Sanofi Aventis; Indonesia). The topical treatment and re-dressing is conducted every 24 hours (once a day). To prevent the secondary infection, the civet was treated twice a day for five days using oral amoxicillin (10 mg/kg of body weight; Amoxicillin IF 500 mg®; Indonesia). The chemotherapy was given using oral cyclophosphamide (50 mg/m$^2$; Cyclophosphamide Tablet Kalbe®; Indonesia) for four days after mastectomy and repeated each week. It combined with vincristine (0.1 mg/m$^2$; Vincristine 2 mg Vial Kalbe®; Indonesia) that injected intravenously and repeated per seven days. The treatment is performed until two months (Karayannopoulou and Lafioniatis, 2016).

**Laboratory test**

The haematological profile is examined on day 0 before surgery, and re-examined on day 7, 30, and 60-post surgery as the monitoring data. Prior histopathology examination, the tissues is fixed in 10% Neutral Buffer Formalin (NBF) for 24 hours. The tissues was processed using the Hematoxylin and Eosin (H&E) staining and Immunohistochemistry (IHC) against CD8+ and CD4+ antibody. All immuno-histopathological slides are analysed by two pathologists.

**RESULTS AND DISCUSSION**

Based on the histopathology examination, the epithelial cells highly differentiated forms the irregular papilla. The lumen covered by the mitotic epithelial cells that indicate the over differentiated (grade III) (Figure 1). On another part showed mineralisation (Figure 2) that is synthesised by the osteoblast in the mammary neoplasm tissue (Figure 3). The nuclear pleomorphism are marked by the variation of the nucleus size, more prominent nucleoli, and loss of basement membrane architecture (Figure 3). These mammary tumour is surrounded by fibrovascular tissue with high angiogenesis (Figure 4). Moreover, the adipocytes are identified in several parts and it is suspected transform into the adipose tissue (Figure 5).

The differentiation of mammary neoplasm depresses and replaces the normal mammary tissue. Immunohistochemistry showed the minimal immune-expression of CD8+ (Figure 6) and surprisingly negative expression of CD4+. Those results indicate an inability of the immune system to inhibit the differentiation of the tumour cells during the tumorigenesis. Based on histopathology and immunohistochemistry the civet is diagnosed suffering mixed mammary carcinosarcoma.
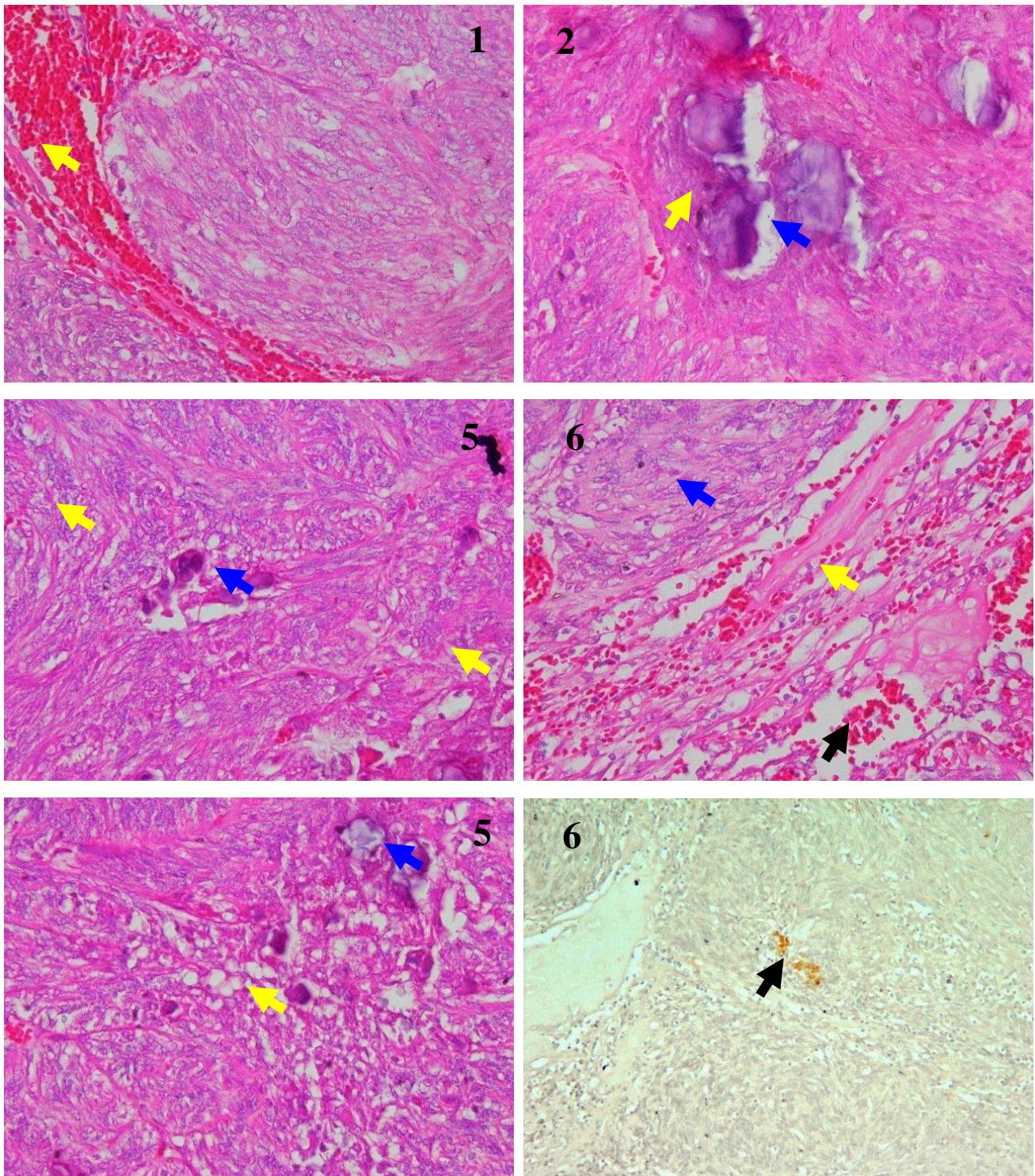


**Figure 1.** High differentiated of epithelial and the lumen not present (blue), predominant of vascularisation around the neoplasm (yellow) in mammary tissue of a female Asian palm civet. H&E, 40×

**Figure 2.** Mineralisation on the central part of mammary tissue (blue) surrounded by the necrosis (yellow) in mammary tissue of a female Asian palm civet. H&E, 40×

**Figure 3.** Osteoblast (blue) identified among the differentiated of epithelial cells, further the nucleus pleomorphism identified as the multiple nuclei (yellow) in mammary tissue of a female Asian palm civet. H&E, 40×

**Figure 4.** High vascularisation (black) in the fibrovascular capsule area (yellow) of a tumour and basement membrane is unidentified (blue) in mammary tissue of a female Asian palm civet. H&E, 40×

**Figure 5.** Adipocytes (yellow) found close to the mineralised area (blue) in mammary tissue of a female Asian palm civet. H&E, 40×

**Figure 6.** Minimal expression of CD8+ (black) in mammary tissue of a female Asian palm civet. IHC anti-CD8+, DAB, 20×

48

Mixed mammary carcinosarcoma is marked by the differentiated of one or both mesenchymal and epithelial cells (Campos et al., 2017). In this case, concluded as the malignant types because of its high index of mitosis, significantly appearance of the nucleoli pleomorphism, and the necrosis on the several parts of tumour tissue (Yoshimura et al., 2015). In human, the carcinosarcoma is growing fast even after surgery and, it is similar to ductal carcinoma (Accurso et al., 2016). The mixed mammary carcinosarcoma is not reported yet in the Viverridae, especially Asian palm civet. This disease suspected due to the utilisation of the progestin as the contraceptive therapy, even, the other factors such as dietary and geographic distribution maybe contributes. As the previous study reported that progesterone administration increase prevalence of the mammary carcinoma in wild animals (Tian et al., 2018). Malignant transformation of a benign tumour not only controlled by gene activity such as cyclin A and p53 but also is played by the cytotoxic T cells (CD8+) via the major histocompatibility complex class I (MHC I).

Cytotoxic T cells potentially control the dysplasia cells. In this case, the minimal expression of CD8+ indicates the failure of the immune system to inhibit tumorigenesis. Moreover, the absence of CD4+ shows the inhibition of healing mechanisms during the disease. CD4+ T cells is a dynamic cell that enhances the antitumor activity of T cell cytotoxic (Borst et al., 2018). Furthermore, the development of the myoepithelial cells in mixed mammary carcinosarcoma has not reported clearly (Singh et al., 2018). It is unclearly understood regarding the origin of carcinosarcoma cells and, further investigations are necessary.

The previous study explained the appropriate therapy against a mammary tumour is a mastectomy and it combines with the chemotherapy agents (Cassali et al., 2017). In this case, the unilateral mastectomy selected because of this procedure indicated for the multiple gland's tumours and easy to perform on skinny animals (Papazoglou et al., 2014). The surgical wound shows positive healing progress and it totally closures on seven days without exudation and secondary infection. It proves that topical combination of 2% aloe vera cream and antibacterial dressing gauze effective to promote wound healing. Moreover, the utilisation of cyclophosphamide and vincristine potentially inhibit the metastases during two months of the observed periods. Cyclophosphamide is the alkylating agent that able to bind the DNA and depress the DNA replication and mitosis (Prajapati et al., 2017), and vincristine binds the tubulin to inhibit cell's metaphase (Dong et al., 2016).

The combination of cyclophosphamide and vincristine generates good prognosis on the mixed mammary carcinosarcoma in civets without creating side effects. It is proved by the haematology results that tend to improve sequentially (Table 1). Further, the leucopenia was unidentified during the utilisation of cyclophosphamide and vincristine, and it is different from the previous study (Huyan et al., 2011). The other haematological results proved that plasma total protein, fibrinogen, and C-reactive protein in a stable condition, following by the total red blood cells and index of erythrocytes that improve concomitantly (Table 1). Those explain the positive response of the civets' physiology and its immune system after mastectomy and during the chemotherapy.

**Table 1.** Routine haematological examination results of Asian palm civet on day 0, 7, 30, and 60 (before and after mastectomy)

| Parameters | Haematology value (day) | | | | Reference value[**] |
|---|---|---|---|---|---|
| | 0 | 7 | 30 | 60 | |
| RBC ($\times10^6$ µL) | 5.44 | 7.78 | 9.81 | 10.14 | 9.16-16.25 |
| Hb (g/ dL) | 7.22 | 7.01 | 7.32 | 9.17 | 8.50-16.70 |
| PCV (%) | 25.11 | 25.8 | 27.41 | 28.02 | 27.00-45.50 |
| MCV (fl) | 46.15 | 33.16 | 27.94 | 27.63 | 24.00-31.00 |
| MCH (pg) | 13.27 | 9.01 | 7.46 | 9.04 | 8.50-19.20 |
| MCHC (%) | 28.75 | 27.17 | 26.70 | 32.72 | 27.60-36.80 |
| WBC ($\times10^3$µL) | 6.83 | 7.25 | 8.44 | 7.99 | 4.40-18.56 |
| L ($\times10^3$ µL) | 3.07 | 3.55 | 4.13 | 4.15 | 1.05-8.26 |
| M ($\times10^3$ µL) | 0.81 | 0.94 | 0.92 | 0.95 | 0.06-1.33 |
| N ($\times10^3$ µL) | 1.63 | 1.81 | 2.27 | 2.07 | 2.30-14.23 |
| B ($\times10^3$ µL) | 0.54 | 0.21 | 0.16 | 0.23 | 0.01-0.22 |
| E ($\times10^3$ µL) | 0.75 | 0.72 | 0.93 | 0.55 | 0.07-1.37 |
| PTP (g/ dL) | 9.15 | 9.03 | 8.71 | 8.44 | 6.40-8.60 |
| Fib (g/ dL) | 1.23 | 1.41 | 1.93 | 1.21 | - |
| CRP (mg/L) | 18.22 | 17.87 | 12.01 | 11.25 | - |

[**]Ahmad et al. (2017), RBC: red blood cells, Hb: haemoglobin, PCV: packed cell volume, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, WBC: white blood cells, L: lymphocytes, M: monocytes, N: neutrophil, B: basophil, E: eosinophil, PTP: plasma total protein, Fib: fibrinogen, CRP: C-reactive protein

## CONCLUSION

In conclusion, it was a unique and rare case of mixed mammary carcinosarcoma in Asian palm civet (*Paradoxurus hermaphroditus*). The mastectomy and the chemotherapy using a combination of cyclophosphamide and vincristine was the appropriate treatment in this case. The periodical observation on the haematological profile of the patients is a good biomarker identify the healing progress before and during the therapy.

## DECLARATIONS

### Acknowledgements

### Competing interests

The authors declare that they have no conflict of interest.

### Consent to publish

The authors guarantee that the contribution to the work has not been previously published elsewhere and that any person named as a co-author of this work is aware of the fact and has agreed to be so named.

### Author's contribution

YAP and K participated during the mastectomy and histopathological analysis. RW, RW, and AK participated in haematology interpretation. All the authors participated in design, writing, revised, and reviewing the manuscript.

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# TABLE OF CONTENTS

# Volume 9 (1); March 25, 2019

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**Research Paper**

## Identification of Locally Isolated *Clostridium difficile* from Rabbits.

Taha MM, El-Helw HA, El-Sergany EF, El Sawy H, Abdella YA and El-Meneisy AA.

*World Vet. J.* 9(1): 01-07, 2019; pii:S232245681900001-9

**ABSTRACT**
*Clostridium difficile* is one of the most important pathogens causing diarrhea and enteritis in rabbits as it causes pseudomembranous colitis that leads to intestinal damage and deaths. In this study, screening of rabbit farms from different localities in Egypt had shown rabbits suffered from diarrhea and enteritis to detect *Clostridium difficile* by ELISA, it revealed that five out of 50 samples (10%) were positive for it. These samples were further identification by cultivation and culture characters, microscopical examination, agglutination test, pathogenicity test and Polymerase Chain Reaction (PCR) by using specific primers for toxins genes (tcdA and tcdB). The results showing that three out of five isolates were confirmed as *Clostridium difficile* and concluded that these isolates causing pseudomembranous enterocolitis in rabbits and this disease unable to be treated by antibiotics, so it used for preparation of vaccine against the disease in rabbits.
**Keywords**: *Clostridiunm difficile*, Rabbits, Enteritis

[Full text-PDF]

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**Research Paper**

## Foodborne Diseases Related to the Consumption of Flesh Foods in Morocco (2010-2016).

Boukili M, Filali FR, Benlarabi S, Hmimou R, Soulaymani-Bencheikh R and Sefiani M.

*World Vet. J.* 9(1): 08-15, 2019; pii:S232245681900002-9

Boukili M, Filali FR, Benlarabi S, Hmimou R, Soulaymani-Bencheikh R and Sefiani M (2019). Foodborne Diseases Related to the Consumption of Flesh Foods in Morocco (2010-2016). *World Vet. J.* 9(1): 08-15. www.wvj.science-line.com

**ABSTRACT**
The current study aimed to determine the epidemiological profile of foodborne diseases associated with flesh foods during 2010-2016 in Morocco. A retrospective study of foodborne diseases caused by flesh foods recorded by the Moroccan anti-poison and pharmacovigilance center during 2010-2016. During this period, 2963 foodborne diseases related to flesh foods were declared to the center, in which 24.83% were registered in 2015, and 20.75% in 2013. Diseases occurred mostly in urban areas (67.06%). The major affected group's ages were adults (33.81%) and children (14.44%). The average patient's age was 25.09 ± 15.37 years. Male were the most vulnerable to infection (54.80%) with a sex ratio (male / female) of 1.72. The most incriminate flesh foods were respectively chicken (47.35%), aquatic products (30.94%) and red meat (16.57%). The high incidence rate was related to chicken skewers (3.55 per 100000 people), while the high fatality rate was associated with giblets (3.33%). Diseases due to the restauration outside home accounted for 58.15%. The majority of cases were collective (84.27%) and occurred significantly in spring (18.49%) and summer (14.51%). clinical symptoms were present in 67.19 % of cases, mostly moderate (81.77%) with four death cases corresponding to fatal condition. The high incidence rates were recorded in the regions of Sahara. Foodborne diseases are spreading progressively in Morocco, especially in summer and hot climates. The majority of these diseases are due to the consumption of contaminated flesh foods. Therefore, the responsible of food safety in Morocco must ensure the quality control of these foodstuffs.
**Keywords:** Epidemiology, Foodborne diseases, Meat, Morocco

[Full text-PDF]

## Effect of Dietary Organic Selenium Supplementation yfon Growth Performance, Carcass Characteristics and Antioxidative Status of Growing Rabbits.
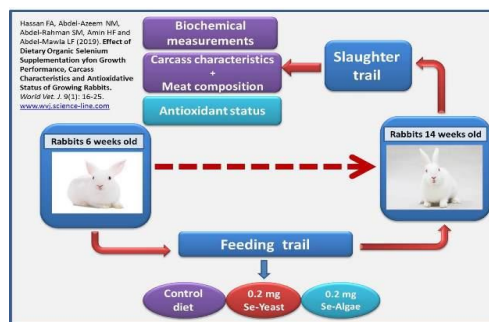
Hassan FA, Abdel-Azeem NM, Abdel-Rahman SM, Amin HF and Abdel-Mawla LF.

Figure 1. Experimental design and feeding trail of New Zealand white rabbit (6-14 weeks old) under Egyptian conditions

**ABSTRACT**
Total of 45 weaned male New Zealand White (NZW) rabbits about six weeks old with an average initial body weight 618.11±10.01g were randomly allotted to three dietary groups; the first group fed the basal diet without organic Se, the second fed basal diet +0.2 mg Se-yeast, the third fed basal diet +0.2 mg Se-algae. The obtained results showed that supplementation rabbit diets with Se-yeast and Se-algae have no impact on final body weight and average daily body weight gain. Se-algae supplementation tended to increase (P < 0.05) average daily feed intake. Rabbits group fed diet supplemented with Se-yeast achieved better (P<0.05) FCR than that group fed Se-algae (5.06 g feed/g gain). Supplementation of Se-algae at 0.2 mg was the highest (P < 0.05) in total protein, albumin, and globulin concentration (7.94, 4.16 and 3.78 g/dl). Diets supplemented with Se-yeast or Se-algae significantly reduced plasma creatinine levels compared to the control group. All recorded values of creatinine and urea concentrations were within the normal ranges. Dietary supplementation with 0.2 mg Se-yeast or Se-algae resulted in a significant (P < 0.05) decrease in the activity of AST enzyme. Plasma total cholesterol and plasma LDL levels were significantly decreased (P < 0.05) with dietary supplementation with Se-yeast or Se-algae. There was a significant (p < 0.05) decrease in plasma MDA level in rabbits fed diets supplemented with Se-yeast or Se-algae. While Catalase activity was significantly (P < 0.05) increased. Rabbits fed diet supplemented with Se-algae was the lowest (P < 0.05) group in ether extract meat content while dietary supplementation of Se-algae significantly increased (P < 0.05) Se content of rabbits meat of hind leg. Conclusively, Se-yeast and Se-algae can be used as selenium sources in growing rabbit diets without causing any adverse effects on growth performance. Besides, their beneficial effects in improving the antioxidative status.
**Keywords:** Anti-oxidative status, Carcass, Growth, Organic selenium, Rabbit

[Full text-PDF]

## Effect of Environmental Heat Stress on Performance and Carcass Yield of Broiler Chicks.

Yousaf A, Jabbar A, Rajput N, Memon A, Shahnawaz R, Mukhtar N, Farooq F, Abbas M and Khalil R.

**ABSTRACT**
Environmental heat stress is one of the most challenging conditions which have adverse effect on the poultry industry. Broiler chickens are sensitive to heat stress mainly due to not having sweat glands. The current study was conducted to observe the effect of heat stress on performance of Ross-308 broiler chickens. 1600 Ross-308 broiler day old chicks were obtained from local hatchery and randomly divided into two groups, the heat stress group A (n = 800) and heat free group B (n = 800). Group A was reared in high temperature (1010F) whereas group B was reared in ideal temperature. To evaluate the physiological stress indicators blood glucose levels and total blood cell count were checked on day 21 and 28. The parameters observed were; feed intake, body weight gain, feed conversion ratio, water intake and carcass yield. The results indicated that feed intake, weight gain, water intake, feed conversion ratio and carcass yield were significantly higher in group B compared to group A. It was concluded that heat stress has deleterious effect over the performance of broiler Ross-308 chicken.
**Keywords:** Broiler Ross-308, Carcass yield, Environmental, Heat stress, Performance
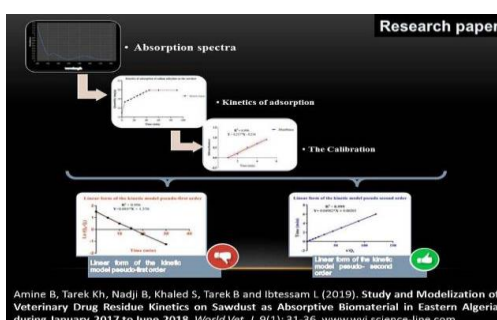
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## Study and Modelization of Veterinary Drug Residue Kinetics on Sawdust as Absorptive Biomaterial in Eastern Algeria during January 2017 to June 2018.

Amine B, Tarek Kh, Nadji B, Khaled S, Tarek B and Ibtessam L.

**ABSTRACT**
Sawdust, an affordable resource, is being investigated as an adsorbent to eliminate residual contaminants from water. Wood processing residues

such as bark and sawdust have been widely studied for some years for their adsorption and removal properties of toxic metals contained in contaminated effluents. The aim of our study is to know the source of chemical contamination of water by using spectrophotometry methods in field ultraviolet, the kinetic study of sodium salicylate adsorption on sawdust was modelized according to two kinetic models (pseudo first and second order), to determine the most optimal pattern to describe this phenomenon, was based in the comparison on the correlation coefficient ($R^2$) between the absorbance and the wavelength for both models and evaluate the optimal time of contact solid/liquid. Present results indicated that the correlation coefficient ($R^2 = 0.999$) for the pseudo-second order, which means that this model is the best for future studies in the kinetics of adsorption of sodium salicylate by sawdust, with a time of contact solid /liquid optimal is 44.5 minutes.

**Keywords:** Residual contaminants, Sodium salicylate, Spectrophotometry, Veterinary drugs

[Full text-PDF]

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## Hormonal Changes in Relation to Productivity of Pregnant Rabbit Does.

Ashour G and Abdel-Rahman SM.

**ABSTRACT**

Pregnancy is a critical period for animals where it undergoes many physiological changes including hormones, which are not received a great attention in rabbit. Therefore, a total number of 25 New Zealand White pregnant rabbit does were used, to assess the changes in concentration of relevant hormones in relation to rabbit productivity. Blood samples were collected on 14, 21 and 28 days of pregnancy and on kindling day to quantify six maternal hormones. Litter size and weight, in addition to average of weekly and total milk yield were determined. Concentrations of the six hormones during the second half of pregnancy ranged between 3.2 to 4.0 ng/ml for progesterone (P4), 47.3 to 89.0 pg/ml for estrogen (E2), 2.0 to 3.7 ng/ml for prolactin (PRL), 114.5 to 136.8 ng/ml for insulin like growth factor-I (IGF-I), 36.0 to 39.2 ng/ml for thyroxine (T4) and 1.9 to 2.2 ng/ml for triiodothyronine (T3). The corresponding values on kindling day were 1.6 ng/ml, 26.8 pg/ml, 4.6 ng/ml, 131.6 ng/ml, 37.4 ng/ml and 0.9 ng/ml, respectively. At day 14, maternal P4, E2, PRL, T4 and T3 were the lowest, whereas IGF-I was the highest compared to the other two days of pregnancy. At day 28, levels of E2, PRL, T4 and T3 were the highest in comparison with days 14 and 21 of pregnancy. On kindling day, P4 and T3 showed the minimal levels, whilst PRL exhibited the maximal level compared to the levels at all the gestational days. The relationship among the different hormones had various trends. The average of total milk yield (129.9 g/d) was negatively correlated with both P4 and E2, and positively associated with the PRL, IGF-I, T4 and T3 hormones. Furthermore, litter size was positively related with P4, E2 and PRL, and negatively with T4, T3 and IGF-I. Whereas, litter weight was negatively correlated with P4, E2 and T4. We recommend giving more attention to the rabbit doe reproduction, particularly close to parturition to achieve good economical return. However, further studies are urgently needed in this area.

**Keywords:** Hormones, Litter size, Litter weight, Milk production, Pregnancy, Rabbits

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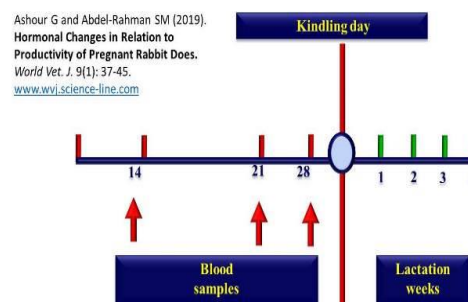
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## Mixed Mammary Carcinosarcoma in Domesticated Asian Palm Civet (*Paradoxurus hermaphroditus*).

Prakoso YA, Widyawati R, Wirjaatmadja R, Kurnianto A and Kurniasih.

**ABSTRACT**

A female Asian palm civet (*Paradoxurus hermaphroditus*), three years old was carried for a medical checkup to Ruddy animal's clinic in Sidoarjo, East Java, Indonesia. The civet suffers enlargement of abdominal mammary glands, painless lump, asymmetric size (4.1 and 8.4 cm in diameter), and lacerated wound on the large one with severe haemorrhage. The unilateral mastectomy was conducted under anaesthesia to handles both haemorrhage and tumour mass. Following the surgery, the tumour mass was stored in 10% neutral buffer formalin for histopathology using Hematoxylin & Eosin (H&E) staining and immunohistochemistry against antibody, anti-CD4+ and CD8+, further, a blood sample collected before and after surgery (on days: 0, 7, 30, and 60) for representing the healing progress. The chemotherapy was given using the combination of oral cyclophosphamide and intravenous injection of vincristine. According to laboratory results, the final diagnosis was mixed mammary carcinosarcoma with minimal expression of CD8+, notwithstanding, it showed the better prognosis after surgery and chemotherapy.

**Keywords:** Asian palm civet, CD4+, CD8+, Mixed mammary carcinosarcoma, Therapy

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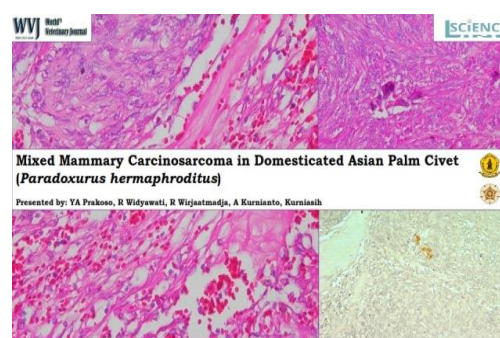
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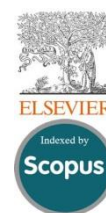
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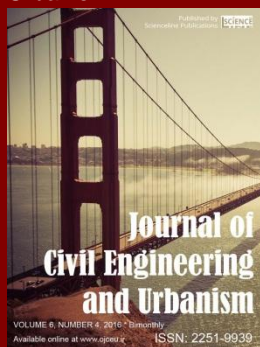
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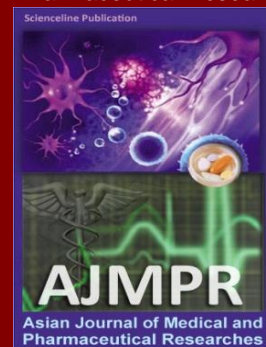
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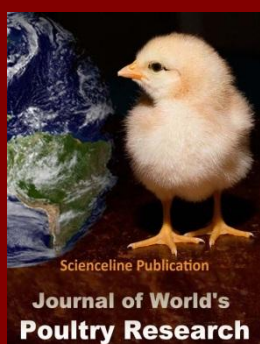
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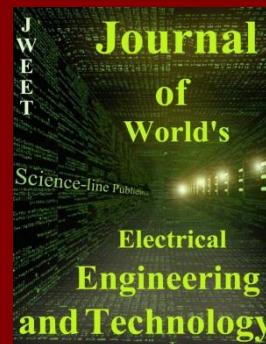
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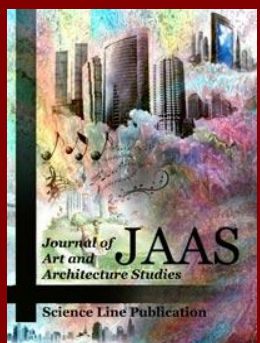
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