Cairo University
Faculty of Veterinary Medicine

Activation of Innate Immune System in Response to Hydrated Nutritional Supplement Treatment

A thesis submitted by

Mai Ramadan Hassan Zaffan

(B.V.Sc, Cairo University, 2013)

For The Master degree in Veterinary Medical Science,
Microbiology
(Bacteriology , Immunology and Mycology)

Under Supervision of

Prof. Dr . Kamelia Mahmoud Osman
Professor of Microbiology
Faculty of Veterinary Medicine
Cairo University

Dr. Ahmed Orabi Hassan
Lecturer of Microbiology
Faculty of Veterinary Medicine
Cairo University

2019

Cairo University

Faculty of Veterinary Medicine

Department of Microbiology

Approval Sheet

This is to certify that the dissertation presented by **Mai Ramadan Hassan Zaffan** to Cairo University for the **Master** degree in Veterinary Science (Bacteriology, Immunology and Mycology) has been approved by the examining committee:

Prof. Dr. Mohamed El Sayed Anany
Professor of Microbiology
Faculty of Veterinary Medicine
Suez Canal University

Prof. Dr. Wagieh Armanius
Professor of Microbiology
Faculty of Veterinary Medicine
Cairo University

Prof. Dr. Kamelia Mahmoud Osman (Supervisor)
Professor of Microbiology
Faculty of Veterinary Medicine
Cairo University

3 / 4 / 2019

Supervision sheet

Supervisors

Prof. Dr . Kamelia Mahmoud Osman
Professor of Microbiology, Faculty of Veterinary Medicine, Cairo University

Dr. Ahmed Orabi Hassan
Lecturer of Microbiology, Faculty of Veterinary Medicine, Cairo University

ABSTRACT

Cairo University

Faculty of Veterinary Medicine

Department of Microbiology

Name: Mai Ramadan Hassan Zaffan

Birth date: 6-3-1992

Nationality: Egyptian

For the degree of: Master (Microbiology)

Title of Thesis: "Activation of Innate Immune System in Response to Hydrated Nutritional Supplement Treatment "

Supervisors:

Prof. Dr. Kamelia Mahmoud Osman

Professor of Microbiology , Faculty of Veterinary Medicine, Cairo University

Dr. Ahmed Orabi Hassan

Lecturer of Microbiology, Faculty of Veterinary Medicine, Cairo University

One hundred and twenty broiler chicks were divided into 6 experimental groups for 6 weeks which received palm date in diet. Ten birds from each group were challenged with bacteria *E.coli* 1st group , *S.* Typhimurium 2nd group , *C. perfringens* 3rd group , *P. multocida* 4th group,*A. paragallinarum* 5th group and ten were control. 5 randomly serum, spleen and intestinal samples were collected from each group for evaluation of innate and acquired immunity of the challenged birds. Serum sample used for evaluation of IL6, IFN-γ and IgA which revealed that the highest level of IgA and IFN-γ was *P. multocida* group, the highest of IL6 was *C. perfringens* group.

Keywords:

Palm date-Immunity- *E.coli* - *S.* Typhimurium - *C. perfringens* - *P. multocida* -,*A. paragallinarum*

Dedication to

My mother

My father

My husband

Acknowledgement

All praise and Glory to ALLAH the Almighty who alone made this small Objective to be accomplished. I feel honored and privileged to glorify his name in the sincerest way through this small accomplishment and ask him to accept my efforts.

I would like to express my gratitude for the encouragement and patience of my major advisor, Prof. Dr. Kamelia Mahmoud Osman, Professor of Microbiology, Department of Microbiology, Faculty of Veterinary Medicine, Cairo University. Her microbiological expertise, steadfast guidance, constant accessibility, unfailing interest, stimulating supervision and constructive criticism are greatly appreciated and are the guidelines which made the completion of this work possible.

I also want to thank Dr. Ahmed Orabi Hassan, Lecturer of Microbiology, Faculty of Veterinary Medicine, Cairo University, for guidance and for valuable direction needed for this research.

My sincere thanks to my mother, my father and my husband for their continuous support and help.

Contents

List of Figures

List of Tables

LIST OF ABBREVIATIONS

AGP	antimicrobial growth promoters
A. paragallinarum	*Avibacterium paragallinarum*
APEC	Avian pathogenic *E. coli*
cDNA	complementary DNA
CE	competitive exclusion
ChIFN-g	Chicken IFN
C. perfringens,	*Clostridium perfringens*
E. coli	*Escherichia coli*
ELISA	enzyme-linked immunosorbent assay
ETEC	Enterotoxigenic *E. coli*
HBSS	Hanks' Balanced Salt Solution
HRP	Avidin-Horseradish Peroxidase
IC	Infectious coryza
IFN-γ	Interferon gamma
IgA	Immunoglobulin A
IL-6	interleukin 6
LPS	Lipopolysaccharide
LT	heat-labile toxin
MHC	major histocompatibility
M. synoviae	*Mycoplasma synoviae*
NE	necrotic enteritis
NK	natural killer
NO	nitric oxide
NOS	Nitric oxide synthase
NPs	Natural products
OD	optical density
OIE	Organization for Animal Health
PAMP	pathogen associated molecular pattern
PBS	phosphate buffered saline
P. dactylifera	*Phoenix dactylifera*
P. multocida	*Pasteurella multocida*

RNA	ribonucleic acid
***S*. enterica**	*Salmonella* enterica
SPF	specific-pathogen-free
spp	species
***S*. Typhimurium**	*Salmonella* Typhimurium
TAE	Tris Acetate EDTA
TJ	tight junctions
TLRs	Toll-like receptors
TS-YE media	Trypticase Soya Broth (**Biolife**) with 0.6% yeast extract (Lamb-fordand, England)
WHO	Whorld Health Organization
XLD	xylose lysine desoxycholate agar

Introduction

Date palm (*Phoenix dactylifera* L.) is a major fruit tree in most of Arabian Peninsula and it is considered one of the most important commercial crops. The beneficial health and nutrition values of date palm, for human and animal consumption, have been claimed for centuries **(Duke, 1992; Vayalil, 2002; Tahraoui *et al*., 2007)**. Phytochemically, the whole plant contains carbohydrates, alkaloids, steroids, flavonoids, vitamins and tannins. The phenolic profile of the plant revealed the presence of mainly cinnamic acids, flavonoid glycosides and flavanols **(Seelig, 1974; Dowson, 1982; Biglari *et al*., 2008)**. Four free phenolic acids and nine bound phenolic acids have been tentatively identified **(Ziouti *et al*., 1996; Eong *et al*., 2006)**. The number of trees in the Kingdom of Saudi Arabia is estimated to be over 23.5 million **(Anonymous., 2009) .** These trees are estimated to yield about 210,000 tons of fronds **(Al Gassim., 2011)**. **Al-Shahib and Marshall (2003)** suggested the potential uses of dates seeds as sources of edible oils and pharmaceuticals. There is a continuous need for the development of new antimicrobial drugs because the increase in number of drug resistant bacteria is no longer matched by discoveries of new drugs to treat infections **(Whitman., 2008)**. According to World Health Organization, medicinal plants can be a good source of variety of drugs. WHO estimated that 80% of the people worldwide rely on plant based medicines for their primary healthcare **(Alagesaboopathi., 2011)**.

Phytochemical show significant antioxidant capacities and antioxidant capability in lowering the prevalence and lower mortality rates of cancer **(Velioglu *et al*.,1998)**. Another finding in the support of dates as antioxidant reported that dates are a good source of antioxidants due to the carotenoids and phenolics with quantity 3942 mg/100 g and antioxidants constituents 80400 μmol/100 g **(Bilgari *et al*., 2008)**.

Phenolic compounds present in dates including p-coumaric, ferulic, and sinapic acids, flavonoids, and procyanidins **(Mansouri *et al*., 2005)**. Other study showed that palm date fruits constitutes thirteen flavonoid glycosides of luteolin, quercetin, and apigenin at different stages of maturity **(Hong *et al*., 2006, Bilgari *et al*., 2008)**.

Ajwa, types of dates that is only cultivated in Saudi Arabia/Al-Madinah Al-Munawara and have significant value in several types of diseases cure and also show protective role in hepatic toxicity **(Abdu SB .,2011)**. An important study based on special type of dates; has shown significant antioxidant activity and caused a significant reversal of the lead induced changes in the oxidative biomarkers in serum and also Ajwa dated extract has a tissue protective effect via a free radical scavenging and antioxidant properties **(Ragab *et al* .,2013)** . Constituents of medicinal plants such as flavanoid and phenol play a significant role in cancer control through the regulation of genetic pathways without any side effect **(Gali-Muhtasib *et al*., 2006- Khan *et al* .,2011)**. Earlier studies reported that beta D-glucan from dates has shown antitumour activity **(Ishurd *et al*., 2002)**. Study on animal model showed that glucans, constituents of date fruits exhibited a dose dependant anticancer

activity with an optimum activity at a dose of 1 mg/kg in tumour **(Ishurda and John .,2005)**. Another important study has also shown that anti-tumor activity for date glucan **(Ishurd *et al*., 2004)**. Study on Ajwa showed a protective effect and ameliorated the lesions of Ochratoxin nephero toxicity which might lead to kidney failure **(Ali and Abdu .,2011)**. An important study showed that the effect of methanol and acetone extracts of leaves and pits *Phoenix dactylifera* inhibited the growth of *F. oxysporum, Fusarium sp., F. solani, A. alternata, Alternaria sp* **(Bokhari and Perveen., 2012)**.Some other important finding showed that methanol and acetone extracts of the *P. dactylifera* pits reasonably inhibited the growth of Gram positive and Gram negative bacteria **(Ammar *et al*., 2009 ,Jassim and Naji., 2010)**. Another recent study in the support of *P. dactilifera* effect as antimicrobial on *Klebsiella pneumonia* and *Escherichia coli* and also showed a role in reducing the side effects due to the use of drugs as methylprednisolone **(Aamir *et al* .,2013)**.

Earlier studies have shown that constituents of plants such as phenolics and flavonoids act as excellent anti-inflammatory agents **(Talhouk *et al*., 2007)**. A study in animal model showed that *Phoenix dactylifera* pollen has potential protective effect via modulation of cytokines expressions **(Elberry *et al*., 2011)**. A study in the support of dates as anti-inflammatory showed that the leaves of dates can be considered as a good source of natural antioxidant and anti-inflammation drugs **(Eddine .,2013)**.

So the aim of work of present study is evaluation of immunological criteria of hydrated palm as following :

1- Detection of IL6.
2- Detection of IFN- gamma.
3- Detection of IgA.
4- Examination of intestinal histopathology .