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BIOLOGICAL ROLE OF NATURAL ANTIOXIDANT FOR DECREASING THE DAMAGE IMPACT OF FREE RADICALS UNDER AGING AND HEAT STRESS CONDITION IN RABBITS.

By

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LIST OF ABBREVIATIONS

Abbreviations	Descriptions
A.O.A.C	Association of Official Analytical Chemists
ACP	Acid phosphatase
ACTH	Adrenocorticotrophic hormone
ADC	Apparent digestibility coefficient
Alb	Albumin
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BW	Body weight
CAT	Catalase
CF	Crude fiber
CON	Sperm concentration
CP	Crude protein
d	Day
DE	Digestible energy
DM	Dry matter
DNA	deoxyribonucleic acid
EE	Ether extract
EV	Ejaculate volume
FI	Feed intake
g	Gram
g/d	Gram/day
Glb	Globulin
GLM	General Linear Model
Glucose	Glucose
GPx	Glutathione peroxidase
GSH	Glutathione content
GSH-PX	Glutathione peroxidase
GSSG	Glutathione disulfide
GST	Glutathione S-transferase
GSx	Glutathione
H_2O_2	Hydrogen peroxide
HDL	High denisity lipoprotein
HOCl	Hypochlorus acid
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein cholesterol

$LOO^{\bullet}$	Peroxyl radical scavenger
MDA	Malondialdehyde
ME	Metabolizable energy
NAC	N-acetyl-cysteine
NDF	Neutral detergent fiber
NFE	Nitrogen Free Extract
NRC	National Research Council
NZW	New Zealand White rabbit
O^{-2}	Superoxide anion
$^{1}O2$	Singlet oxygen
OH.	Hydroxyl radical
OM	Organic matter
$OONO^{-}$	Peroxynitrite
PCV	Packed cell volume
pH	Initial hydrogen ion concentration
PSV	Packed sperm volume
PUFA	Polyunsaturated fatty acids
RH	Relative humidity
ROS	Reactive oxygen species
RT	Rectal temperature
SC	Sperm concentration
Se-GSH-Px	Se-dependent glutathione peroxidase
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SM	Sperm motility
SOD	Superoxide dismutase
Ta	Indoor ambient temperature
TBARS	Thiobarbituric acid-reactive substances
TFSF	Total functional sperm fraction
THI	Temperature-humidity index
TL	Total lipid
TMS	Total number of motile sperm
TN	Thermoneutral
TP	Tomato powder
TG	Triglyceride
TSO	Total sperm output
VLDL	Very Low-density lipoprotein
WI	Water intake
%	Percent
°C	Air temperature

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CHAPTER 1

INTRODUCTION

Heat stress causes alterations in the biological processes and functions (**Hansen 2009**).

Heat stress thus alters several aspects of reproductive physiology, such as blood flow and steroidogenesis (**Rivera and Hansen 2001**), which manifests in fertility alterations. The harmful effect of summer season on semen quality was reported by **Marai *et al.* (2002**) who found that a rise in testicular temperature in rabbits leads to reduced spermatogenesis; temporary sterility; decreased sexual desire, ejaculate volume, motility, sperm concentration, and total number of spermatozoa in an ejaculate; and increased sperm abnormalities and dead sperm.

Aging is a developmental process and causes physiological, psychological and social changes in humans (**World Health Organization, 2002**). These changes affect the functioning of all body systems and health status. In mammals, deterioration in reproductive performance at the end of reproductive season partly results from a decrease in male fertility. **Alexaki *et al.* (1991)** resulted that age appeared to influence on spermatozoa, and found gradually decreasing in concentrations as the animals advanced in age.Changes in motility, viability and morphology of spermatozoa have been reported in the latter part of the reproductive period in animals (**Wilson, 1995**). Aging causes oxidative stress (**Liu, 2002**) and decreases in levels of antioxidants and antioxidant enzymes in the heart (**Somani *et al.*, 1995**) and blood (Ji, 1993) and accumulation of oxidative damage to deoxyribonucleic acid (DNA), lipids, and protein (**Beckman and Ames 1998**). Also, age factor known to affect semen quality of a male. A significant effect of rabbit's age on its libido, semen volume and pH, and sperm concentration and motility was reported by **Minelli *et al*. (1999**). Studies on turkey showed that the age of the toms affected the sperm quality of both fresh and stored semen. Ageing was accompanied by a reduction in the number of spermatozoa in the ejaculate and in semen volume and by a decrease in motility, viability and membrane integrity of spermatozoa. Consequently, these changes led to a progressive decline in the fertilizing ability of turkey semen and may also affect its preservability during storage **(Laffaldano *et al*., 2007)**.

Reactive oxygen species (ROS; free radicals) are molecules that contain one or more unpaired electrons and are, consequently, very reactive, particularly with respect to lipids. They are produced at different sites in the mammalian body. In the mitochondria, the production of superoxide is a by-product of the respiratory chain (**Balaban *et al*., 2005**). The sources of ROS produced by the sperm, especially the damaged ones, include radicals like hydroxyl ions, superoxide, nitric oxide, peroxyls and others (**Makker *et al*., 2009**). A certain, still low, concentration of ROS is necessary for the sperm function like capacitation, hyperactivation, acrosome integrity and sperm–oocyte fusion (**Awda *et al*., 2009**), but ROS become detrimental at excessive amounts. In the testes ROS are produced during the normal testicular spermatogenesis and steroidogenesis (**Mathur and D'Cruz, 2011**).

Sperm function is undisturbed when the levels of ROS and antioxidants are balanced, as this ensures that no significant damage will occur. In the case of metabolic oxidative stress, however, provoked by excessive ROS production or low antioxidant status or both, impaired sperm function may occur (**Agarwal *et al*., 2003; Aitken and Baker, 2006; Makker *et al*., 2009**). In addition, DNA fragmentation in both nuclear and

mitochondrial genomes is a consequence of oxidative stress (**Agarwal *et al*., 2003; Aitken and Baker, 2006**). The defense mechanism of the sperm consists of three different and interdependent antioxidant protection systems (**Makker *et al*., 2009**), which are dietary (external), enzymatic or non-enzymatic (metabolic).Supplying of antioxidants to sperm samples can protect against the damaging effects of ROS on sperm movement and may be of clinical value in assisted conception procedure **(Baker *et al*. 1996**). It is known that improvement of semen characteristics quality depends on antioxidant capacity to limit the damaging effects of lipid.

Tomato and tomato products are excellent source of vitamins A, C, and E. They also contain many other bioactive compounds, such as carotenoids (α-, β-, γ-carotene, and lutein), and other carotenes including phytoene and phytoflune. Moreover, tomato and tomato products also contain flavonoids. Many of these nutrients and phytochemicals have antioxidant properties and, in combination with lycopene, may contribute to the protection against peroxidation **(Alshatwi *et al*., 2010).**

Castellini *et al*. (2002) demonstrated that supra-nutritional vitamin E administration to fertile rabbits improves the oxidative stability of the sperm but not motion characteristics and fertilising ability of the sperm, in spite of the higher vitamin E concentrations occurring in the semen. Also, lycopene has been shown to have strong antioxidant activity; it exhibits the highest physical quenching rate constant with singlet oxygen; it induces cell-to-cell communication; and it modulates hormones, immune systems, and other metabolic pathways **(Rao and Agarwal, 1999)**. Phenolic compounds exhibit a wide range of physiological properties, such as anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, antithrombotic, cardioprotective, and vasodilator effects **(Balasundram, *et al*., 2006)**. Whereas tocopherol and ascorbic acid are recognized as antioxidant vitamins and heat-labile compounds, lycopene and phenolic compounds are more resistant to thermal processing, being the main antioxidants in processed products.

The present work aimed to study the effect of dietary supplementation with 1% tomato powder on semen quality and blood and seminal plasma biochemical and antioxidant and digestibility coefficient of young and old adults V- line rabbit during winter and summer seasons.

CHAPTER 2

REVIEW OF LITERATURE

2.1. Oxidative stress:

The term oxidative stress; is a state of unbalanced tissue oxidation refers to a condition in which cells are subjected to excessive levels of molecular oxygen or its chemical derivatives called reactive oxygen species (ROS).Under physiological conditions, the molecular oxygen undergoes a series of reactions that ultimately lead to the generation of superoxide anion (O^{-2}), hydrogen peroxide (H_2O_2) and H_2O. Peroxynitrite ($OONO^-$), hypochlorus acid (HOCl), the hydroxyl radical (OH.), reactive aldehydes, lipid peroxides and nitrogen oxides are considered among the other oxidants that have relevance to vascular biology. Oxygen is the primary oxidant in metabolic reactions designed to obtain energy from the oxidation of a variety of organic molecules. Oxidative stress results from the metabolic reactions that use oxygen, and it has been defined as a disturbance in the equilibrium status of pro-oxidant/anti-oxidant systems in intact cells. This definition of oxidative stress implies that cells have intact pro-oxidant/anti-oxidant systems that continuously generate and detoxify oxidants during normal aerobic metabolism. When additional oxidative events occur, the pro-oxidant systems outbalance the anti-oxidant, potentially producing oxidative damage to lipids, proteins, carbohydrates, and nucleic acids, ultimately leading to cell death in severe oxidative stress. Mild, chronic oxidative stress may alter the anti-oxidant systems by inducing or repressing proteins that participate in these systems, and by depleting cellular stores of anti-oxidant materials such as glutathione and vitamin E **(Laval, 1996).** Free radicals and other reactive species are thought to play an important role oxidative stress resulting in many human diseases. Establishing their precise role requires the ability to measure them and the oxidative damage that they cause (**Halliwell and Whiteman, 2004**).

Oxidative stress is involved in the process of aging (**Kregel and Zhang 2007**). Aging is a developmental process and causes physiological, psychological and social changes in humans (**World Health Organization, 2002**). These changes affect the functioning of all body systems and health status. Aging causes oxidative stress (**Liu, 2002**) and decreases in levels of antioxidants and antioxidant enzymes in the heart (**Somani *et al.*, 1995**) and blood (**Ji, 1993**). The levels of reactive oxygen species, notably hydrogen peroxide (H_2O_2), increase with age, resulting in accumulation of oxidative damage to deoxyribonucleic acid (DNA), lipids, and protein (**Beckman and Ames 1998**).

Heat stress stimulates the release of corticosterone, catecholamines and initiates lipid peroxidation in cell membranes (**Freeman and Crapo, 1982**), including membranes of T and B lymphocytes. Heat stress is one of the main causes of economic losses in livestock production in the United States, caused by decreased performance (production, efficiency, and reproduction), health, and well-being, and increased mortality of animals. **St-Pierre *et al.* (2003**) estimated losses of 2.4 billion dollar per year for the livestock industry, with the dairy industry contributing 1.5 billion dollar.

2.2. Antioxidants:

The body produces several enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSHPX), that neutralize many types of free radicals. Supplements of these enzymes are available for oral administration. However, their

absorption is probably minimal at best. Supplementing with the "building blocks" the body requires to make SOD, catalase, and glutathione peroxidase may be more effective. These building block nutrients include the minerals manganese, zinc, and copper for SOD and selenium for GSHPX. In addition to enzymes, many vitamins, minerals and hormones act as antioxidants in their own right, such as vitamin C, vitamin E, beta-carotene, lutein, lycopene, vitamin B2, coenzyme Q10, and cysteine (an amino acid). Herbs, such as bilberry, turmeric (curcumin), grape seed or pine bark extracts, and ginkgo can also provide powerful antioxidant protection for the body. Melatonin is a hormone secreted by pineal gland and proves to be powerful antioxidant and free radical scavenger (**Koc *et al*., 2003**).

Some measurements of quality for foods of animal origin such as colour, oxidative stability, tenderness, storage properties, etc. have been shown to be improved by antioxidant supplementation (**Flachowsky *et al*., 2002**).

Another dietary antioxidant thought to be important in the defence against oxidation is lycopene, of which tomatoes are an important dietary source (**Rao and Agarwal, 1999**). Lycopene is a natural pigment synthesized by plants and microorganisms but not by animals (lycopene is found to concentrate in the adrenal gland, testes, liver and prostate gland, where it is the most prominent carotenoid, (**Stahl, *et al*., 1992**). Lycopene is a highly unsaturated hydrocarbon containing 11 conjugated and 2 unconjugated double bonds. Lycopene from natural plant sources exists predominantly in an all-*trans* configuration, the most thermodynamically stable form (**Nguyen and Schwartz, 1999**). Lycopene, a member of the carotenoid family and mostly found in tomato, is a highly potent antioxidant that provides protection against cellular damage caused by reactive oxygen species (**Rao and Shen, 2002**).

2.3. Oxidative stress, related nutritional factors, and sperm quality:

Reactive oxygen species (ROS; free radicals) are molecules that contain one or more unpaired electrons and are, consequently, very reactive, particularly with respect to lipids. They are produced at different sites in the mammalian body. In the mitochondria, the production of superoxide is a by-product of the respiratory chain (**Balaban *et al*., 2005**).

In the testes ROS are produced during the normal testicular spermatogenesis and steroidogenesis (**Mathur and D'Cruz, 2011**). The sources of ROS produced by the sperm, especially the damaged ones, include radicals like hydroxyl ions, superoxide, nitric oxide, peroxyls and others (**Makker *et al*., 2009**). A certain, still low, concentration of ROS is necessary for the sperm function like capacitation, hyperactivation, acrosome integrity and sperm–oocyte fusion (**Awda *et al*., 2009**), but ROS become detrimental at excessive amounts. The sperm is very sensitive to lipid peroxidation because its plasma membrane is rich in polyunsaturated fatty acids (PUFA), especially in the long-chain PUFA docosahexaenoic acid and docosapentaenoic acid (**Brinsko *et al*., 2005**). The concentration of these PUFA decreases with age in bulls (**Robinson *et al*., 2006**) and the effect of PUFA supplementation are often positive, especially in older animals. However, the variability of its effects on semen characteristics of different mammals is astonishingly high (**Rooke *et al*., 2001; Brinsko *et al*., 2005; Aitken *et al*., 2006; Robinson *et al*., 2006; Samadian *et al*., 2010**). When applied in ruminants, PUFA may be efficient only when provided either in rumen-protected form or at very high levels in order to counteract their extensive ruminal biohydrogenation.

Generally, the ideal supplementation levels are within a narrow range because PUFA at the same time stimulate the formation of ROS and are precursors of them (**Aitken *et al.*, 2006**). Further factors increasing the concentration of ROS in the sperm are various mechanisms present in the sperm itself which are particularly active in defective sperms, and the presence of leukocytospermia (**Aitken, 1995; Agarwal *et al.*, 2003**). Environmental toxins were shown to provoke an imbalance between antioxidants and ROS (**Mathur and D'Cruz, 2011**). Sperm function is undisturbed when the levels of ROS and antioxidants are balanced, as this ensures that no significant damage will occur. In the case of metabolic oxidative stress, however, provoked by excessive ROS production or low antioxidant status or both, impaired sperm function may occur (**Agarwal *et al.*, 2003; Aitken and Baker, 2006; Makker *et al.*, 2009**). In addition, DNA fragmentation in both nuclear and mitochondrial genomes is a consequence of oxidative stress (**Agarwal *et al.*, 2003; Aitken and Baker, 2006**). The defence mechanism of the sperm consists of three different and interdependent antioxidant protection systems (**Makker *et al.*, 2009**), which are dietary (external), enzymatic or non-enzymatic (metabolic). Dietary antioxidants include ascorbic acid (vitamin C), α-tocopherol (the major form of vitamin E), β-carotenes, carotenoids, flavonoids, and retinol (vitamin A). Various studies have investigated their respective roles in determining semen characteristics, as well as the effects of their respective deficiencies or supplementations or both (**Smith and Akinbamijo, 2000; Rao and Sharma, 2001; Castellini *et al.*, 2002; El-Demerdash *et al.*, 2004**). Feeding recommendations, therefore, include vitamins, and currently there is a tendency to use supranutritional doses – supplementation clearly beyond known requirements – in order to provoke a pharmacology type of effect in male reproducing livestock. Not with standing, **Castellini *et al.* (2002**) demonstrated that supra-nutritional vitamin E administration to fertile rabbits improves the oxidative stability of the sperm but not motion characteristics and fertilising ability of the sperm, in spite of the higher vitamin E concentrations occurring in the semen. Superoxide dismutase (SOD), catalase, and the glutathion-peroxidase-reductase system form the complex of the enzymatic antioxidants. The SOD converts superoxide to hydrogen peroxide that catalase subsequently converts to oxygen and water (**Aitken and Baker, 2006**). Zinc, copper, and manganese are components of SOD. Selenium is a cofactor of glutathione peroxidase (GP × 1–8), the enzyme that catalyses the degradation of peroxides, and part of the selenoprotein P. Their respective roles were reviewed by **Flohé (2007)**. The effects of deficiency or supplementation or both of these minerals and trace elements on sperm characteristics have been intensively investigated (**Kendall *et al.*, 2000; Smith and Akinbamijo, 2000; Martino-Andrade *et al.*, 2010**). This knowledge of the importance of distinct minerals and trace elements for semen quality has increased their inclusion in feeding recommendations and their adoption in designing tailor-made mineral supplements for breeding males. Still, there may be many unknown.

2.4. Tomato General Background:

2.4.1. Chemical composition of tomato:

Tomato (Solanum lycopersicum L.), is possesses an array of important micronutrients such as carotenoids, phenolic compounds, vitamin C and folic acid (**Pedro and Ferreira, 2005**). Like in many other plants, carotenoids are the most vital coloured phytochemicals that accumulate as secondary metabolites in the chromoplasts, providing distinct red, pink, orange and yellow colour of tomato (**Georgé *et al.*, 2011**). Previous

studies have suggested that the carotenoids (lycopene, β-carotene and lutein) in tomato can act as antioxidants in the body thus slowing down ageing, and preventing tissue damage, heart disease and certain cancers (**Palozza *et al.*, 2011**).

Tomatoe, also contain higher levels of fructose and glucose than sucrose **(Garvey and Hewitt, 1991)**. **Loiudice *et al.* (1995)** observed that fructose was the most abundant sugar in tomatos but with a similar value to glucose, 1.2 % for glucose and 1.4% for fructose. **Lavelli *et al.* (1999)** showed that the content of ascorbic acid decreased from 3300 mg/kg DM (dry matter) in fresh tomatoes to 400 mg/kg DM in dried tomatoes at temperature of 80^{o}C. Compared to fresh tomatoes, processed tomato products decreased antioxidant capacity in the hydrophilic fraction due mostly to vitamin C losses; however they possess a higher antioxidant capacity in their lipophylic fraction, which contains carotenoids and tocopherols **(Lavelli *et al.*, 2000)**.

Sahin *et al.* (2007) recorded that tomato powder was a sun-dried tomato product and contained 11% crude protein, 4.5% fat, 0.8 mg of lycopene, 0.13 mg of *β*-carotene, 1.73 mg of vitamin C, and 0.07 mg of α- tocopherol per gram of powder.

The results of the chemical analysis of the tomato pulp powder, Protein, fat, total sugar, fiber and ash content were 23.07, 10.46, 13.47, 38.2 and 5.41%, respectively. These data showed that tomato pulp powder is a rich source of fiber and protein; therefore, it can be considered as a key ingredient in the so-called "functional foods" **(Farahnaky *et al.*, 2008)**.

2.4.2. Lycopene-rich by-products from food processing:

Lycopene; a member of carotenoid family; is a lipid soluble antioxidant synthesized by many plants and microorganisms but not by animals and humans, where it serves as an accessory light-gathering pigment and protects them against the toxic effects of oxygen and light. It is a red pigment without provitamin - A activity that imparts colour to many fruits and vegetables (**Paiva, and Russell, 1999**). Tomatoes and processed tomato products (juice, sauce, soup, pizza and spaghetti sauce) constitute the major sources and accounts for more than 85% of all the dietary sources of lycopene. The content differs with the varieties of tomatoes and increases as the fruit ripens2. It varies from 0.85mg to 13.6 mg/ 100g (**Tapiero *et al.*, 2004).**

2.4.3. Antioxidant activity of tomato components:

Jain *et al.* (1999) reported that dietary lycopene decreased serum thiobarbituric acid reactive substances (TBARS) concentration in rat by14%. Also, **Leal *et al.* (1999)** showed that the broilers fed lycopene showed a reduction in MDA production. An opposite correlation between MDA, vitamin E, and lycopene is related **(Halliwell and Gutteridge, 1999)**. **Guo *et al.* (2001)** found that there is a significant inverse relationship between thiobarbituric acid reactive substances (TBARS) value in the thigh meat and egg and the dietary antioxidants.

Consumption of tomato and/or its products is associated with increased lycopene blood level and reduced oxidative damages of lipids, proteins, and DNA (**Rao and Shen, 2002)**. **Sahin *et al.* (2006)** showed that serum and liver MDA levels were decreased in supplemented lycopene and vitamin E groups compared with the control separately, or as a combination. Also, **Alshatwi *et al.* (2010)** reported that nutrients and phytochemicals in

tomato have antioxidant properties and, in combination with lycopene, may contribute to the protection against peroxidation.

2.4.4. Physical properties of lycopene as antioxidant

The Table (1) showed the physical properties of lycopene. The physical quenching rate of lycopene was two times higher than β-carotene and 10 times higher than α-tocopherol. Animals do not have capability to synthesize lycopene, and consequently it must be supplied by the diet **(Di Mascio *et al.*, 1989)**. **Young and Lowe, (2001)** found that the reactivity of carotenoids, especially lycopene, in biological systems depends on their molecular and physical structure, location or site of action within the cells, ability to interact with other antioxidants, concentration and the partial pressure of oxygen. Also, the highly conjugated double bonds of lycopene play the most important role in energy transfer reactions.

Table 1: Physical properties of lycopene:

Molecular Formul	**$C_{40}H_{56}$**
Molecular Weight	**536.85Da**
Melting Point	**172-175^{o}C**
Crystal Form	**Long red needles separatefrom a mixture of carbon disulfide and ethanol**
Powder Form	**Dark reddish-brown**
Solubility	**Soluble in chloroform, hexane, benzene, carbon disulfide, acetone, petroleum ether and oil.** **Insoluble in water, ethanol and methanol.**
Stability	**Sensitive to light, oxygen, high temperature, acids, catalyst and metal inos.**

Source: Shi *et al.* (2002)

Biologically, lycopene tends to act as singlet oxygen ($^1O_2$) and peroxyl radical scavenger ($LOO^{\bullet}$) **(Stahl and Sies, 2003)**. Basically, chain lipid autoxidation reactions can be interrupted by antioxidants such as phenols, vitamin E and flavonoids, which eliminate the lipid peroxyl radicals by donating the hydrogen atom to form lipid peroxide and a resonance-stabilized antioxidant radical **(El-Agamey *et al.*, 2004)**.

However, as a carotenoid compound, lycopene may scavenge the radicals by other ways. The mechanism of action for lycopene towards the reactive species can be predicted through three possible mechanisms: (1) adduct formation, (2) electron transfer to the radical and (3) allylic hydrogen abstraction **(El-Agamey *et al.*, 2004; Krinsky and Johnson, 2005)** as shown in diagram 1.

1. Adduct formation : Lycopene + R* ⟶ R-Lycopene*

2. Electronic transfer : Lycopene + R* ⟶ R-Lycopene** $+R^-$

3. Allylic H abstraction: Lycopene + R* ⟶ Lycopene*+RH

Diagram1**:** Three possible reactions of carotenoids with radical species (R*).

(Source: Krinsky and Johnson (2005).

2.4.5. Antioxidant activity of tomato components against oxidative stress:

In addition, a high protection of lymphocytes from oxidative damage due to singlet oxygen and nitrogen dioxide was found in human subjects with the higher intake of lycopene-rich tomato juice **(Böhm *et al*, 2001)**.

Earlier studies have reported lycopene-rich diet and lycopene supplementation provided protective effects against DNA damage in both normal and cancerous human cells **(Astley *et al*., 2004; Liu *et al*., 2007; Scolastici *et al*., 2008)**. In animals, reduction of lipid peroxidation products (thiobarbituric acid reactive substances, TBARS) and DNA damage markers were found in monkey kidney fibroblast and rat hepatocytes supplemented with lycopene 20 pmol/106 cells and 1.86–18.62 μM, respectively **(Srinivasan *et al*., 2007).**

2.4.6. Protective Effect of Tomato as Antioxidant Agent Against Aged and Heat Stress Factors on:

2.4.6.1. Reproductive performance:

The most obvious limitation to rabbit production in a hot climate area, is their susceptibility to heat stress that produces a series of drastic changes in their biological functions which in turn ends with impairment of reproduction (**Marai *et al*., 1999**).

Kasa, (1991) studied the thermoregulation in rabbits with particular reference to semen production and quality and found significant differences in semen volume for bucks exposed to 32^{o}C than the control**. El-Sherbiny, (1987)** observed that motility of NZW and Bouscat rabbits spermatozoa was significantly reduced during summer, but the differences among values obtained during autumn, winter and spring were not significant. **Tharwat *et al*. (2004)** indicated that the greatest ejaculate volume (0.5 ml) of domesticated Sina Gabali rabbit bucks was obtained during spring and the smallest during summer.

A significant effect of rabbit's age on its libido, semen volume and pH, and sperm concentration and motility was reported by **Luzi *et al*. (1996)** and **Minelli *et al*. (1999).**

Supplying of antioxidants to sperm samples can protect against the damaging effects of ROS on sperm movement and may be of clinical value in assisted conception procedure **(Baker *et al*. 1996**). It is known that improvement of semen characteristics quality depends on antioxidant capacity to limit the damaging effects of lipid peroxidation (**Donoghue and Donoghue, 1997**). Addition of antioxidants significantly decreased the amount of spermatozoa DNA damage induced by ROS in vitro **(Lopes *et al*., 1998)**.

Tomato and its by-products are well established for their antioxidizing effects, mainly attributed to a lipid-soluble carotenoid, lycopene (**Alshatwi *et al*., 2010**). There are

reports on beneficial effects of lycopene on reproductive performance in male mammals (**Uysal and Bucak, 2007**).

Lycopene is a major carotenoid present in tomatoes, and it has been shown to be a powerful antioxidant (**Giovannucci, 1999**). In adult animals, the effects of under-nutrition, with one or more nutrients or energy, malnutrition and nutrient imbalances, include reduced androgen secretion and low semen quality (**Petherick, 2005**). Dietary supplementation of 250 or 500 mg/kg of L-carnitine (one of the antioxidants factors) increased the viability of the mature male Japanese quail breeder spermatozoa **(Sarica *et al*., 2007)**.

Mangiagalli *et al*., (2007) used the lycopene added to the extender for semen storage, and found a significant positive effect on semen viability. Data *in vivo* and *in vitro* showed that lycopene positively affects the semen viability. These results are in accordance with different studies on other animals and humans reporting a potential positive effect of lycopene on sperm characteristics and on structural and functional damage in the testicular tissue **(Goyal *et al*., 2007; Turk *et al*., 2007)**. Also**, Mangiagalli *et al*. (2010)** studied the effect of drinking water supplementation with lycopene (0.5 g/l) on the semen quality in broiler breeder males. The volume and concentration of spermatozoa were affected by lycopene ($P<0.01$). Motility and forward progressive motility were not affected by treatment. Viability was different ($P<0.01$) between birds treated with lycopene and control group, and sperm morphological abnormalities were not different in either group. **Saemi *et al*. (2012)** indicated that dietary inclusion of dried tomato pomace up to 30% increased sperm concentration, accompanied by a decreased seminal volume ($P \leq 0.05$) and percentage of abnormal sperm, while the percentage of live sperm in ejaculate was increased within 4 to 5 weeks in roosters.

Seminal plasma constituents exhibited positive significant responses to tomato powder supplementation, especially during summer season and with old rabbit bucks, showing a constant effect of tomato powder on seminal plasma. The changes in environmental conditions have been reported to significant effect on sexual activity and seminal attributes activities **(Thatcher and Hansen, 1993)**. **Dave and Rindani (1988)** observed an inverse correlation between ACP activity and semen concentration. Statistical analysis revealed that ACP activity was maximal in the azoospermic group, and decreased as the sperm concentration increased. **Chauban *et al*., 1993** found a positive correlation between enzyme release and sperm acrosomal damage. The cyclic changes in pituitary and testicular activity in seasonally breeding mammals in temperate climates are prompter by changes in photoperiod, nutrition, social interactions and temperature **(Bronson and Heideman, 1994).**

Zakrzewska *et al*. (2002) reported that seminal plasma exhibits high activities of acid and alkaline phosphatase, the acid form being predominant. Acid phosphatase (ACP) in stallion is especially localized in corpus epididymidis, ductus epididymidis and vas deferens.

Marai *et al.* (2002) reported that heat stress were not significant on rabbit seminal plasma total protein, globulin, total lipid, cholesterol, creatinine and alkaline phosphatase. Mean while, seminal plasma albumin, acid phosphatase, T_3 and cortisol were significantly lower while GPT and GOT were significantly higher in heat stress than mild condition. **Pesch *et al*. (2006)** reported the correlation between LDH and motility, progressive

motility and living sperm, which may indicates that extracellular LDH ensures metabolism of spermatozoa.

Al-Daraji *et al.* (2010) used n-3 and n-6 fatty acid supplementation to improved semen quality and its seminal plasma constituents in Japanese quail. The authors showed that the decrease in AST and ALT activities indicating a decrease in sperm damage while increasing spermatozoa livability and concentration. The increase in the AlP and the ACP observed in seminal plasma were in agreement with the result of **Al-Daraji *et al.* (2001)** reported that both AlP and ACP are involved in the metabolism of spermatozoa via the hydrolysis of carbohydrates. **Saemi *et al.* (2012)** indicated that dietary inclusion of dried tomato pomace up to 30% lowered concentration of thiobarbituric acid reactive substances (as an index of sperm membrane lipid peroxidation) in the roosters.

2.4.6.2. Blood plasma biochemical status:

Blood biochemical parameters have been shown to be subject to change with increasing age in many animal species (**Mohri *et al.*, 2007**).

Matsuzawa *et al.* (1993) reported that the biochemical changes were related with age in a large number of animals (monkeys, dogs and rats). They supported that as a general trend, the hepatic enzymes increased with age, which however does not necessarily reflect a corresponding liver pathology. **Daramola *et al.* (2005)** resulted that serum alkaline phosphatase (ALP) levels were higher in adult African Dwarf goats animals compared to young animals ($P<0.05$). Serum urea, total protein, albumin, triglyceride, glutamate pyruvate transaminase (ALT) and serum glutamate oxaloacetate transaminase (AST) levels were comparable in both age groups.

Heat stress is a significant financial burden to animal agriculture in most areas of the world. Acclimation to heat stress imposes behavioral, physiological and metabolic adjustments to reduce the strain and enhances the likelihood of surviving the stress (**Bernabucci *et al.*, 2010).**

Serum transaminase activities differ with the change in environmental temperatures. Serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) activitiesare increased during the hot summer in rabbits **(El-Masry *et al.*, 1994)**. Also, **Salem *et al.* (1998)** stated that the serum SGPT concentration was significantly higher in summer than in winter, while serum SGOT was not significantly affected by season on sheep. The increase of SGPT and SGOT levels with exposure to hot temperature may be due to an increased stimulation of gluconeogenesis by corticoids (increase in cortisol, cortisone or adrenocorticotrophic hormones**.**

Alkaline phosphatase in dairy cattle decreased significantly due to heat stress. This may be attributed to a reduction in thyroid hormones which takes place under heat stress conditions (**Shaffer *et al.*, 1981)**. While using rabbits, **Ibrahim (1994**) found that ASTand ALT activities were significantly lower during the summer than during the winter season due to the increase of water intake and, consequently, the increase in enzyme dilution during the hot conditions. With regard to acid phosphatase level, **Aboul-Naga (1987)** reported that it was not affected by heat stress.

Ayyat *et al.* (2002) observed that blood cholesterol of rabbits significantly increased by 9.37% in summer compared to winter. **Meshreky *et al.* (2005)** found that plasma protein in rabbit was higher in summer months than in winter months, which help

the animals to withstand heat stress by aiding in body water retention to provide water for intense evaporative heat loss.

Abdel-Azeem *et al.* (2007) conducted that plasma triglycerides recorded higher values in litter through January and decreased with the rise of temperature.

Averos *et al.* (2007) found that albumin concentration was the lowest during summer season, whereas, higher concentration was found in rainy season as compared to others season. This finding is compatible with the role of albumin in maintaining plasma osmotic pressure and transportation of protein in the blood. **Suntorn *et al.* (2009)** reported reduction in albumin level in goats during summer. The difference in the globulin may be due to the various physiological adaptation and genetic factors. **Abdalla *et al.* (2009)** reported that higher plasma globulin level was observed during winter season. It may suggest that the goats are more adapted to the arid environment so that their immunity is potentiated. **Maria *et al.* (2008)** found that plasma total lipids and cholesterol of NZW male (6 months) increased during summer compared with spring. This might be due to an increased activity of HMG-CoA reductase (or 3-hydroxy-3-methyl-glutaryl-CoA reductase) – the limiting enzyme in cholesterol synthesis.

Moreover, **Ondruska *et al.* (2011)** studied the effect of heat stress on blood parameters of NZW rabbit and showed that plasma total lipids and cholesterol were significantly higher in high temperature compared to natural temperature.

Paran and Engelhard (2001) reported that lycopene supplementation reduced blood lipids, lipoproteins and oxidative stress markers in hypertensive patients. **Rao and Shen (2002)** reported that plasma level of total cholesterol is reduced by lycopene supplementation. Dietary lycopene supplementation (60 mg/d) to 6 men for a 3-month period resulted in a significant reduction (14%) in their plasma LDL-C levels **(Rao, 2002)**. These results suggest that dietary supplementation of carotenoids may act as moderate hypocholesterolemic agent secondary to their suppressor influence on macrophage 3-methyl glutaryl coenzyme A reductase. Furthermore, lycopene augmented the activity of the macrophage LDL receptor **(Fuhrman *et al.*, 1997)**.

Elkomy and Hassan (2005) also, observed decrease in the activity of AST and ALT of thioacetamide-treated male rat fed tomato-juice as supplemented diet. They added also, that lycopene may reduce lipids by inhibiting the enzyme macrophage-3- hydroxy-3-methyl glutaryl coenzyme A (HMG-Co A) reductase (an important step in cholesterol synthesis) and by enhancing low density lipoprotein LDL degradation **(Sesso *et al.* 2003)**. Other previous studies also confirmed that lycopene or tomato products reduced triglycerides, total cholesterol, and LDL-C in blood serum and in liver **(Silaste *et al.*, 2007)**.

Sahin *et al.* (2006) showed that the supplementation of lycopene in Japanese quail increased HDL concentration whereas VLDL and LDL concentrations were reduced by lycopene supplementation ($P \leq 0.01$), particularly at a dietary concentration of 200 mg lycopene/kg. **Blum *et al.* (2006)** also found that tomato-rich diet (300 g daily for one month) increased the HDL-cholesterol level significantly by 15.2%.. On the contrary, **Frederiksen *et al.* (2007)** stated that dietary supplementation with an extract of lycopene-rich tomatoes had no effect on cholesterol and triacylglycerol levels measured in total plasma in rabbits. **Sahin *et al.* (2006b)** reported that supplementing with a combination of dietary lycopene and vitamin E reduced serum and yolk cholesterol concentrations ($P \leq 0.05$).

Napolitano *et al.* (2007) investigated the effects of lycopene on the induction of foam cell formation by modified LDL. They suggested that lycopene may reduce the macrophage foam cell formation induced by modified LDL by decreasing lipid synthesis and down regulating the activity and expression of scavenger receptor activity.

Hsu *et al.* (2008) found that tomato paste was effective in lowering total cholesterol and LDL-C in cholesterol-fed hamsters. Lycopene has been observed to enhance LDL degradation and alterations in lipoprotein composition and particle size. **Ševčikova *et al.*, (2008)** found that supplemented with 50 mg of lycopene had a higher content of HDL cholesterol, followed by groups receiving 100 mg of lycopene, and the lowest values were recorded in groups without lycopene. A significant difference ($P \leq 0.05$) in the content of HDL cholesterol was only in group with 50 mg lycopene in comparison with group without lycopene.

Rahmatnejad *et al.* (2009) reported that inclusion of 24% dried tomato pomace into the broilers diet significantly decreased concentration of total protein, cholesterol. **Abdulazeez and Thiruvengadam (2012)** investigated the effect of lycopene on basic biochemical parameters during D-galactosamine /lipopolysaccharide (D-GalN/LPS) induced hepatitis in experimental rats. They concluded that administration lycopene helps to maintain the normal values of biochemical parameters during experimental hepatitis in rats and restore the normal liver function through its protective effect due to its antioxidant defense mechanism.

2.4.6.3. Blood plasma antioxidant status:

Antioxidants, both enzymatic (viz. superoxide dismutase, glutathione peroxidase & catalase) and nonenzymatic (vitamins C, E and A, glutathione, pyruvate etc) provide necessary defence against oxidative stress generated due to high ambient temperature. Catalase detoxifies H_2O_2 produced during different metabolic processes and also in stressful conditions by reducing it to H_2O and O_2 **(Fridovich, 1978)**. **Kihlström *et al.* (1986)** reported that long-term daily intake of 0.5% H_2O_2 (as environmental stress factor) decreased Se-dependent glutathione peroxidase (Se-GSH-Px) activity in rat skeletal muscle, kidney, and liver, as well as decreased the activity of catalase in rat skeletal muscle. The increase in MDA associated with feeding of H_2O_2 was inhibited by feeding tomato powder or lycopene beadlet. **Aboul-Naga (1987)** reported that the latter component was not affected by heat stress.

Cand and Verdetti (1989) have reported a 20 % loss in catalase activity in 24 months old rat brain in comparison to that of the 4 months old young ones. As in same way, **Rao *et al.* (1990)** reported a 40% decrease in SOD activity in brain of the 26 months old male Fisher rat when compared with the 6 months old animal brain. External factors such as heat, trauma, ultrasound, infections, radiations, toxins etc. can lead to increased free radicals and other ROS and may lead to oxidative stress **(Halliwell *et.al.*, 1992)**. Total SOD activity has been observed to decline in aged skeletal muscle, MnSOD activity was observed to increase, presumably to defend against the age-associated increase in ROS generation (**Pansarasa *et al.*, 1999)**.

Gul *et al.* (2000) reported a decrease in GPx activity with age in the cerebral hemisphere, cerebellum and the brain stem. There is evidence that aging has little effect on liver GSH whereas glutathione disulfide (GSSG), the product of GSH oxidation, is elevated (**Leichtweis *et al.*, 2001**), reflecting an age-associated increase in oxidative stress.

Finally, it has also been reported that both myocardial (**Leichtweis *et al.*, 2001**) and hepatic (**Bejma *et al.*, 2000**) GSH content is augmented with aging; however, there also appears to be an age-related increase in GSSG in these tissues

High environmental temperature not only has adverse effects on rabbits performance but also cause an increase in oxidative stress (**Lee *et al*, 2000**) and reactive oxygen species (ROS) production (**Bouchama and Nochel, 2002)** which can impede disease resistance and impairs antioxidant status (**Sahin *et al.*, 2001**) because of the positive correlation between oxidative stress and illnesses (**Chirase *et al.*, 2004**). The biochemical and enzymatic markers malondialdehyde (MDA), glutathione (GSx), glutathione peroxidase (GPx), superoxide dismutase (SOD) and glutathione-S-transferase (GST) were significantly affected by the time of sampling, indicating that a perturbation of homeostasis has occurred, probably as a result of animal handling during the time course of the experiment (**Cook *et al.*, 2001**).

Altan *et al.* (2003) reported that heat stress increases lipid peroxidation as a consequence of increased free radical generation. It can enhance the formation of reactive oxygen species (ROS) and induce oxidative stress in cells. Oxidative damage may be minimized by antioxidant defence mechanisms that protect the cell against cellular oxidants and repair systems that prevent the accumulation of oxidatively damaged molecules. Antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) play a vital role in protecting cellular damage from harmful effects of ROS.

Agarwal and Prabhakaran (2005) found that heat stress may lead to increased production of transition metal ions (TMI), which can make electron donations to oxygen forming superoxide or H_2O_2 which is further reduced to an extremely reactive OH radical causing oxidative stress **(**Superoxide dismutase (SOD) in conjugation with catalase and glutathione peroxidase (GPx) scavenges both intracellular and extracellular superoxide radicals and prevents lipid peroxidation**.**

Rao and Agarwal (1999) showed that dietary lycopene protected lipid, protein and DNA from oxidation. An inverse association between MDA and antioxidant vitamins has been mentioned by others **(Halliwell and Gutteridge, 1989)**. **Leal *et al.* (1999)** reported that the broilers exposed to lycopene showed a reduction in MDA production. The author attributed such depression to the decrease of endogenous heat production by the animals as a result of the thyroid hormone stimulation which decreases the heat production. In chicks, lycopene showed protection against depletion of glutathione, an endogenous antioxidant, during viral-induced acute oxidant stress **(Leal *et al.*, 1999)**.

Jain *et al.* (1999) reported that dietary lycopene decreased serum TBARS concentration by 14% in rats. **Paran and Engelhard (2001)** reported that lycopene supplementation reduced oxidative stress markers such as homocysteine in hypertensive patients.

Decreasing MDA may be attributed to lycopene because of its ability to protect cells against oxidative damage **(Heber and Lu, 2002)**.

Kim *et al.* (2004) observed that tomato pomace, but not lycopene, partially protected against acute injury due to chemically induced oxidant stress. Therefore, an important question raised by this study is why was tomato pomace more effective than lycopene beadlet in reducing liver MDA? One possibility is that tomato and tomato

products are excellent source of vitamins A, C, and E; they also contain carotenoids (α-, β-, γ-carotene, and lutein) and other carotenes including phytoene, phytoflune, and the provitamin A, β-carotene. In addition, tomato and tomato products also contain flavonoids (**Campbell *et al.*, 2004**). Many of these nutrients and phytochemicals have antioxidant properties and in combination with lycopene may contribute to the protection against oxidation. Another possibility is the action of lycopene from Tomato pomace may be enhanced by other components of the tomato **(Kim *et al.*, 2004)**.

Lycopene may elevate the levels of superoxide dismutase (SOD), GSH-Px, and glutathione reductase, which are the most important enzymes involved in antioxidant activity, thereby, decreasing oxidative stress **(Bose and Agrawal, 2007)** .

Alshatwi *et al.* (2010) showed that the concentration of hepatic lycopene in rats fed lycopene was higher than in rats fed tomato powder. They showed that the tomato powder treatment was more effective than lycopene in decreasing liver MDA and in preventing the enhanced MDA production when challenged with H_2O_2.

2.4.6.4. Testosterone concentration level:

Particularly, accessory gland secretion and spermatogenesis are controlled by testosterone hormone concentration which is lowest in the summer **(Bone, 1979; Hammond *et al.*, 1983)**. A significant rise in blood and seminal plasma testosterone levels by about 22.9% was reported in male rabbit treatment with Se+Vit E (as antioxidant factors) under hot summer conditions **(El-Masry *et al.*, 1994**). Exposure to hyperthermia is harmful for spermatogenesis and also decreases testosterone levels **(Murray, 1997)**. This transient decrease in serum testosterone levels with short-term carotenoid intake can be considered beneficial, as excessive androgen status can ultimately give rise to prostate cancer (**Taplin and Ho, 2001**). The decrease in serum testosterone levels in sham-operated rats with short-term carotenoid intake is likely a transient effect, as we have previously reported that longer carotenoid feeding did not significantly alter serum testosterone concentrations **(Canene-Adams *et al.*, 2007**). Also, **Yousef *et al.* (2006)** reported that folic acid significantly increased plasma testosterone levels in male rabbits. **El-Hanoun *et al.* (2007)** observed that the testosterone hormone was higher significantly during winter than summer seasons. Testosterone is required for maturation of male germ cells and sperm production and quality **(Walker, 2009)**. **Kamel (2012)** reported that supplemented male rabbits with organic selenium and folic acid or their combinations significantly improved blood plasma testosterone concentration under summer condition compared with control group.

CHAPTER 3
MATERIALS AND METHODS

The present study was carried out at the Rabbit Research Laboratory, in the Animal and Fish Production Department, Faculty of Agriculture (Saba Basha) During the period from January to march 2010 (winter) and from july to September 2010 (summer). The analytical part of this study was performed at the laboratories of Animal and Fish production Department, Faculty of Agriculture (Saba Basha), Alexandria University.

The present study was designed to evaluate the effect of tomato powder (TP) (1%) of complete diets as a non-conventional ingredient on reproductive performance, digestibility, semen quality,digestibility, some blood and seminal plasma components, and antioxidant status under aging and heat stress conditions in bucksV-line rabbits.

3.1. Environmental Condition:

The rabbitry was open air sided with electric exhausted fans. Indoor ambient temperature (Ta, ^{o}C) and relative humidity (RH, %) were daily recorded inside the rabbitry using electronic digital thermo-hygrometer. Overall means of maximum and minimum temperatures (°C), relative humidity (RH %) and temperature-humidity index (THI) during the experimental period are shown in Table (4). Temperature-humidity index (THI) was calculated according to **Marai *et al.* (2001)**:

$$THI = db\ ^{o}C - [(0.31 - 0.31 \times RH) \times (db\ ^{o}C - 14.4)]$$

Where, db ^{o}C = dry bulb temperature and RH = relative humidity %. The THI values were classified as absence of heat stress (<27.8), moderate heat stress (27.8-28.8), severe heat stress (28.9-29.9) and very severe heat stress (>30.0) (**Marai *et al.*, 2005).**

Table (2): Overall means of air temperature, relative humidity, temperature-humidity index (THI) and day light length during the experimental period.

Month	Air temperature (°C)			Relative humidity (%)			THI*	Day light length (hrs)
	Max.	Min.	Mean	Max.	Min.	Mean		
January	20.6	11.3	15.2	80.8	47.7	64.4	16.02	10.20
February	25.0	12.1	17.2	82.3	40.9	64.4	18.47	11.05
March	31.1	16.2	23.3	74.6	36.8	55.0	22.81	11.56
Average	**25.6**	**13.2**	**16.6.3**	**79.2**	**41.4**	**61.3**	**19.1**	**10.9**
July	40.5	26.4	33.7	75.0	27.1	49.0	31.08	14.09
August	37.8	27.3	32.5	85.6	35.4	60.0	30.74	13.18
September	38.2	25.1	32.4	76.4	34.1	54.1	29.68	12.23
Average	**38.8**	**26.3**	**32.9**	**79**	**32.2**	**54.4**	**30.5**	**13.20**

*the temperature-humidity index (THI) was calculated using the equation reported by **Marai *et al.*, (2005).**

3.2. Experimental Diets:

Tomato powder was purchased from Fathalla super market. Pellets of the experimental diet were made as follow, pelleting was initiated by molasses addition as binding material. Feed and water were offered *ad libitum* throughout the whole experiment which lasted for 8 weeks in both two seasons. The composition and chemical analysis

(**AOAC, 1995**) of the experimental diets are presented in Table 4. All rabbits were kept under similar managerial and environmental condition. Rabbits were housed in galvanized batteries provided with feeders and automatic drinkers in a windowed rabbitry.

Table (3): The ingredient and chemical composition of the basal diet (control) and the experimental diets (1% Tomato Powder) (AOAC, 1995).

Ingredients	Control	1% Tomato Powder
Berseem hay	40.0	40.0
Yellow corn	10.0	8.2
Barley	13.0	14.0
Wheat bran	15.0	16.3
Soybean meal	17.5	17.0
Tomato powder	---	1.0
Molasses	3.0	2.0
Di-calcium phosphate	0.8	0.8
Sodium chloride	0.3	0.3
Vit+Min Premix[1]	0.3	0.3
DL-Methionine	0.1	0.1
Total	**100**	**100**
Determined and Calculated composition		
Dry matter (DM)[2], %	89.67	89.83
Crude protein (CP)[2], %	17.18	17.19
Crude fiber (CF)[2], %	13.05	13.14
Ether extract (EE)[2], %	3.41	3.91
Nitrogen free extract (NFE)[3], %	56.03	55.31
Ash[2], %	10.33	10.17
Digestible energy (DE)[3] Kcal/Kg	2519	2516
Calcium [3], %	0.83	0.83
Available phosphorus[3], %	0.31	0.31
Methionine[3], %	0.36	0.36
Total sulphur amino acid [3], %	0.68	0.68
Lysine[3], %	0.98	0.98

[1]Vit+Min mixture provides per kilogram contains: Vit A 6000 IU; Vit D_3 450 IU; Vit E 40 mg; Vit K_3 1 mg; Vit B_1 1 mg; Vit B_2 3 mg; Vit B_3 180 mg; Vit B_6 39 mg; Vit B_{12} 2.5 mg; Pantothenic acid 10 mg; biotin 10 mg; folic acid 2.5 mg; choline chloride 1200 mg; Manganese 15 mg; Zinc 35 mg; Iron 38 mg; Copper 5 mg; Selenium 0.1 mg; Iodine 0.2 mg; Selenium 0.05 mg. [2]Analyzed values according to **AOAC (1995).** [3]Calculated values according to **NRC (1977).** DE calculated according to **Cheeke *et al* (1987)**. DE=4.36-0.0491*NDF%, NDF%=28.92+0.657*CF%.

3.3. Animals and Treatment:

Twenty mature V line (VL) rabbit bucks were randomly taken and classified individually into four groups each of 5 bucks. The first group included bucks at 7-9 months without TP supplementation, the second group included bucks at 7-9 months with supplementation of 1% TP/kg diet, while third group included aged bucks at 22-24 months-old without TP supplementation, and fourth group included aged bucks at 22-24 months-old with supplementation of 1% of TP/kg diet, respectively.

3.4. Digestion Trial:

At the end of experimental period, three rabbits were randomly taken from each group to digestion trial evaluation. Rabbits within each treatment were randomly housed individually in metabolic cages that allowed separation of feces and urine. A preliminary period of 7 days was followed by five days for measurements of actual consumed feed. The animals were fed twice daily at 8 a.m. and 4 p.m. Water was available all time. Feces of each rabbit was collected quantitatively once a day before offering the morning meal at 8 a.m. Daily feces of each rabbit were stored at -20^{0}C. The five days combined collection fecal samples were kept for routine analysis. Fecal samples were oven dried at 60^{0}C for 48 h (partial drying) then ground through a 1 mm screen on a Wiley mill grinder. Samples were composite per treatment per animal for analysis. Representative samples of feed offered and feces of each rabbit were chemically analyzed for determinations of (DM), crude protein (CP), and ether extract (EE), crude fiber (CF) and (ash) which were carried out according to **AOAC (1995)** methods. Nitrogen free extract (NFE) was determined by difference.

Apparent digestibility coefficient (ADC) was calculated as follows:

ADC= [total nutrient intake – total nutrient in feces] / (Total nutrient intake) x 100.

3.5. Data Collected:

3.5.1. Body Weight and Feed and Water Intakes:

Rabbits were individually weighed weekly in the morning before offering feed. Daily feed and water intakes of each animal were recorded weekly throughout the experiment. The feed intake was calculated weekly by subtracting the unconsumed feed from the total amount offered during this period and recorded as g/kg BW.

3.5.2. Semen Quality:

Semen collection occurred weekly over the 8 weeks; in both seasons. Ejaculates were collected using an artificial vagina maintained at 45°C to 46°C and a teaser doe. The volume of each ejaculate was recorded after removal of the gel mass. Initial hydrogen ion concentration (pH) of semen samples was determined immediately after collection using a pH paper (Universalindikator pH 0–14 Merck, Merck KgaA, 64271 Darmstadt, Germany). Immediately after collection, semen was maintained at 35°C in a water bath for evaluation. Fresh semen (two drops) was placed on a warm slide and covered with a cover slip to determine sperm motility. sperm motility from at least three fields was examined at 37°C under a microscope with phase-contrast optics, at 403, and assessed from 0 to 100%.

A weak eosin solution was used at a rate of 1 : 99 before counting the cells for the evaluation of sperm concentration, SC (x10^6/ml) according to **Smith and Mayer (1955)**

using the improved Neubauer hemocytometer slide (GmbH1Co., Brandstwiete 4, 2000 Hamburg 11, Germany). The total sperm output (TSO) was calculated by multiplying semen ejaculate volume and semen concentration. Total number of motile sperm (TMS) was calculated by multiplying percentage of motile sperm and total sperm outputs. Assessment of live and abnormal spermatozoa was performed using an eosin–nigrosin blue-staining mixture **(Blom, 1950)**. The percentage of live, dead and abnormal spermatozoa was determined using stains that penetrated cells with damaged membranes. Normal live sperm exclude the eosin stain and appear white in color, whereas dead sperm take up eosin and appear pinkish in color because of loss of membrane integrity. Normal sperm have an oval head with a long tail. Abnormal sperm have head, midpiece or tail defects, such as a large or a misshapen head or a crooked or a double tail. The total number of motile sperm was calculated by multiplying the percentage of motile sperm and the total sperm outputs. The total functional sperm fraction (TFSF) was also calculated by multiplying the total sperm output and sperm motility and normal-morphology sperm (**Correa and Zavos, 1996**). Determination of initial fructose (IF) concentration in semen was carried out immediately after collection according to **Mann (1948)**. Packed sperm volume (PSV) was recorded.

3.5.3. Seminal and Blood Plasma Constituents:

Seminal plasma samples were analyzed weekly for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AlP), acid phosphatase (ACP) and lactate dehydrogenase (LDH) using commercial kits of bio-diagnostic company (Recycling Crusher-SBM®).

Seminal plasma samples were analyzed weekly for thiobarbituric acid-reactive substances (TBARS) using the method of **Tappel and Zalkin (1959)**. Seminal plasma glutathione S-transferase (GST) activity was determined according to **Habig et al. (1974)** using P-nitrobenzylchloride as a substrate. Seminal plasma Superoxide dismutase (SOD) activity was assayed according to **Misra and Fridovich (1972)**.

Blood samples were collected from the ear vein of each buck every other week and placed immediately on ice in heparinized tubes. Blood plasma was collected from blood by centrifugation at 860xg for 20 min at 4°C and stored at -20°C. Blood plasma total protein, albumin (Alb), glucose, total lipid (TL), triglycerides, and urea ere determined by colorimetric enzymatic methods using commercial kits purchased from bio-diagnostic company (Recycling Crusher-SBM_R). Also, the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), alkaline phosphatase (AlP) and acid phosphatase (ACP), lactate dehydrogenase (LDH), in blood plasma were determined by colorimetric enzymatic methods using commercial kits purchased from bio-diagnostic company (Recycling Crusher-SBM_R). Thiobarbituric acid-reactive substances (TBARS) were assayed in the blood plasma using the method of **Tappel and Zalkin (1959).** Blood plasma glutathione S-transferase (GST) activity was determined according to **Habig et al. (1974)** using p-nitrobenzylchloride as a substrate. SOD activity was assayed according to **Misra and Fridovich (1972)**.

Blood plasma testosterone concentration was measured using immunoassay commercial kits (Biosource-Europe S.A. 8, rue de L'lndustrie.B-1400 Nivelles, Belgium).

3.6. Statistical analysis:

Statistical analysis of the Data was done by using the general linear model (GLM) procedure of the Statistical analysis system (**SAS, 2000)**. differences between means were detected using Duncan test **(Duncan, 1955).**

Data were analysis by using the following model:

$$Y_{ijk} = U + S_i + A_j + T_k + (S\times A)_{ij} + (S\times T)_{ik} + (A\times T)_{jk} + (S\times A\times T)_{ijk} + E_{ijk}$$

Where:

Y= any observation.

U= over all mean.

Si= effect due to season of the year.

A_j= effect due to age.

T_k= effect due to treatment.

$(S\times A)_{ij}$ = first order interaction between season of the year and age.

$(S\times T)_{ik}$ = first order interaction between season of the year and treatment.

$(A\times T)_{jk}$= first order interaction between age and treatment.

$(S\times A\times T)_{ijk}$ = first order interaction between season of the year, age and treatment.

E_{ijk} = Random error.

CHAPTER 4
RESULT AND DISCUSSION

4.1. Effect of Tomato Powder Supplementation on Aged Rabbit under Winter and Summer Conditions:

4.1.1. changes in body weight, feed,water intake and rectal temperature:

Table (4) shows the overall means and the interaction of body weight (BW), feed intake (FI), water intake (WI) and rectal temperature (RT) in young and old VL rabbit bucks as affected by 1% tomato powder dietary supplementation during winter and summer seasons. The data in Table 4 showed that BW and FI were significant decrease, while WI and RT were significant increase in rabbits during summer compared to winter season regardless of age or treatment effect. Significant increased in BW, WI and RT were detected in older rabbits compared with young rabbits, regardless of season or treatment effects, while the young rabbits significantly consumed more diet than older rabbits. Dietary supplementation with 1% TP caused significant increase in BW, FI and WI while RT was significant increased compared to unsupplemented group (Table 4), regardless of season or age effects.

The results in Table (4) indicated highly significant interaction between season (S) and age (A) on body weight, feed intake and water intake. The highest significant weight was shown in old rabbit during winter season. Young rabbit significantly consumed more feed during winter season. On the other hand, the young rabbit was significantly drank more water during summer season compared to other groups. No significant different between S*A on rectal temperature.

The significant interaction between season (S) and treatment (T) was detected only in body weight and water intake. No significant different was found between S*T on feed intake and rectal temperature. The highest significant body weight was obtained in the rabbit that fed TP during winter season. During summer season, the rabbit in both treatment (0 and 1% TP) drank significant more water compared to winter season (Table 4). The interaction between A and T was shown in body weight and rectal temperature. The young rabbit that treated with 0% TP had the significant lowest BW than other groups. The old rabbit that fed 1% TP showed significant improvement in rectal temperature compared to the old group fed diet free of tomato powder.

The results in Table 4 showed that there are significant interaction between S, A and T on BW, WI and RT, while FI was not significant. Old rabbits had the heavier BW during winter season regardless of TP treatment. Tomato powder supplementation caused significant increase in BW especially during winter season for young rabbits compared with unsupplemented group.

The significant increased in water intake was detected during summer season for young rabbits that supplemented with TP or old rabbit that unsupplemented with TP compared to other groups. The significant highest increased in RT was found in old rabbits regardless TP treatments especially during summer season.

Table (4): Overall means (Mean ±SE) and the interaction of live body weight (BW), feed intake (FI), water intake (WI) and rectal temperature(RT) of young and old V-line bucks rabbit as affected by tomato powder dietary supplementation during winter and summer seasons.

Item	BW (Kg)	FI (gm/kg/day)	WI (ml/kg/day)	RT (°C)
Effect of season (S)				
Winter (W)	3.33 ± 0.02^{a}	48.17 ± 0.51^{a}	57.15 ± 0.52^{b}	39.27 ± 0.02^{b}
Summer (Su)	3.14 ± 0.02^{b}	45.96 ± 0.43^{b}	64.03 ± 0.48^{a}	39.45 ± 0.02^{a}
P value	<0.0001	<0.0001	<0.0001	<0.0001
Effect of age (A)				
Young (Y)	3.17 ± 0.02^{b}	49.08 ± 0.43^{a}	59.58 ± 0.62^{b}	39.24± 0.03^{b}
Old (O)	3.30 ± 0.02^{a}	43.53 ± 0.46^{b}	61.61 ± 0.49^{a}	39.48 ±0.01^{a}
P value	<0.0001	<0.0001	<0.0006	<0.0001
Effect of treatment (T)				
Without TP (NTP)	3.19± 0.02^{b}	44.48 ± 0.48^{b}	59.21 ± 0.55^{b}	39.24 ± 0.02^{b}
With TP (WTP)	3.28 ±0.02^{a}	49.56 ± 0.40^{a}	61.97 ± 0.56^{a}	39.48± 0.03^{a}
P value	<0.0001	<0.0001	<0.0001	<0.0001
Interaction (S*A)				
W*Y	3.22 ± 0.02^{b}	51.37 ± 0.59^{a}	54.23 ± 0.64^{d}	39.14 ± 0.03
Su*Y	3.12 ± 0.02^{c}	46.79 ± 0.42^{b}	64.93 ± 0.65^{a}	39.34 ± 0.04
W*O	3.44 ± 0.03^{a}	44.98 ± 0.43^{c}	60.08 ± 0.64^{c}	39.41 ± 0.03
Su*O	3.16 ±0.02^{bc}	44.96 ± 0.74^{c}	63.13 ± 0.71^{b}	39.55 ± 0.02
P value	0.0001	<0.0001	<0.0001	0.2376
Interaction (S*T)				
W*NTP	3.26 ± 0.02^{b}	45.69 ± 0.65	54.57 ± 0.54^{c}	39.39 ± 0.03
Su*NTP	3.11 ± 0.02^{c}	43.27 ± 0.37	63.85 ± 0.62^{a}	39.51 ± 0.02
W*TP	3.40 ± 0.02^{a}	50.66 ± 0.64	59.74 ± 0.79^{b}	39.16 ± 0.04
Su*TP	3.16 ± 0.02^{c}	48.47 ± 0.35	64.21 ± 0.71^{a}	39.38 ± 0.04
P value	0.0272	0.7742	<0.0001	0.0809
Interaction (A*T)				
Y*NTP	3.07 ± 0.02^{b}	46.62 ± 0.47	57.22 ± 0.76^{b}	39.37 ± 0.03^{b}
Y*TP	3.27 ± 0.02^{a}	51.53 ± 0.67	61.44 ± 0.93^{a}	39.12 ± 0.04^{c}
O*NTP	3.30 ± 0.02^{a}	42.33 ± 0.34	60.70 ± 0.75^{a}	39.54 ± 0.04^{a}
O*TP	3.30 ± 0.03^{a}	47.59 ± 0.35	62.51 ± 0.62^{a}	39.42 ± 0.03^{b}
P value	0.0001	<0.6550	0.1001	0.0144
Interaction (S*A*T)				
W*Y*NTP	3.12 ± 0.02^{d}	48.96 ± 0.59	52.65± 0.62^{e}	39.24 ± 0.04^{b}
W*Y*TP	3.32 ± 0.04^{b}	53.79 ± 0.85	55.80 ± 1.10^{d}	39.05 ± 0.05^{c}
W*O*NTP	3.40 ± 0.03^{a}	42.42 ± 0.64	56.48 ± 0.70^{d}	39.55 ± 0.03^{a}
W*O*TP	3.48 ± 0.03^{a}	47.52 ± 0.50	63.68 ± 0.71^{b}	39.28 ± 0.05^{b}
Su*Y*NTP	3.02 ± 0.03^{e}	44.29 ± 0.46	62.77 ± 0.66^{bc}	39.50 ± 0.03^{a}
Su *Y*TP	3.22 ± 0.03^{c}	49.27 ± 0.44	67.09 ± 0.78^{a}	39.19 ± 0.06^{b}
Su *O*NTP	3.21 ± 0.02^{c}	42.25 ± 0.90	64.93 ± 0.93^{ab}	39.53 ± 0.03^{a}
Su *O*TP	3.11 ± 0.03^{d}	47.66 ± 0.51	61.33 ± 0.99^{c}	39.57 ± 0.03^{a}
P value	0.0272	0.9127	<0.0001	<0.0001

^{a,b,c,d,e} Means within a column not sharing similar superscripts are significantly different (P<0.05).

Marai *et al.* (2001) found that exposure rabbits to a high ambient temperature decreased embryonic weight and length and growing live body weight, where average daily body gain of rabbits was found to be lower in summer than in winter**. Also, Marai *et al*. (1996)** reported that weekly feed intake was decreased during summer compared with winter in NZW rabbits. **Kamel, (2012)** reported that dietary supplementation of folic acid and selenium (as antioxidant potent) caused significant improve in live body weight and feed intake of rabbit bucks and decreased the harmful effects of heat stress during summer season on rectal temperature. In the same way, **Khattak *et al.*, (2012)** showed that appropriate feed supplements attenuate the decline in performance caused by heat stress, and diet supplements with feed additives (betaine and $NaHCO_3$) offered better protection against heat–stress related depression in performance of broilers.

The decline in feed intake of heat stressed rabbits may be due to the decrease of T_3 hormone level under high ambient temperature **(Habeeb *et al.*, 1997)**. Also, **Marai, *et al.*, (2002)** reported that thermoregulatory parameters (respiration rate and temperatures of ear, rectum and skin) and water consumption were significantly ($P < 0{\cdot}01$) higher, while consumption of food was significantly ($P < 0{\cdot}01$) lower in heat stress than in mild conditions.

Yasmeen *et al.* (2008) reported that there was no significant difference in the feed consumption values of pullets (24 weeks old) and spent layers (76 weeks old), indicating that increase in age did not exert any effect on feed intake of birds. **Applegate *et al.* (1999)** and **Schafer *et al.* (2005)** also observed that feed intake did not differ significantly with increase in age of birds.

The present study showed that tomato powder supplementation caused significant improve in rabbits body weight and feed intake, and this is agreement with **Sahin *et al.*, (2011)** who found that tomato powder supplementation improved feed intake and egg production in quail reared under heat stress (34 ◦C) condition.

4.1.2. Reproductive performance:

Table (5) shows the overall means and the interaction of ejaculate volume (EV), initial hydrogen ion concentration (pH), sperm motility (SM) and packed sperm volume (PSV). Ejaculate volume, sperm motility and packed sperm volume of rabbit bucks showed significant ($P < 0.01$) decrease during summer compared with winter season, regardless of age or treatment effect, except rabbit semen pH that significantly decreased through winter as compared with summer season. The effect of rabbit age on EV, pH, SM and PSV values were observed. The old rabbits were showed significant ($p \leq 0.01$) lower improvement in pervious parameters than young one regardless of season or treatment effects. Dietary supplementation with 1% TP caused significant increase in EV, SM and PSV and significant decrease in pH values compared to unsupplemented group (Table 5) regardless of season or age effects.

Table (5) showed significant interaction between S*A on EV and SM. On the other hand, no significant different was obtained on pH and PSV. The highest significant increased in EV and SM was detected in young rabbits during winter season compard other groups. Only significant interaction between S*T was found on SM, while EV, pH and PSV were not significant.

Table (5): Overall means (Mean ±SE) and the interaction of ejaculate value (EV), initial hydrogen ion concentration (pH), sperm motility (SM) and packed sperm volume (PSV) of young and old V-line bucks rabbit as affected by tomato powder dietary supplementation during winter and summer seasons.

Item	EV (ml)	pH	SM (%)	PSV (%)
Effect of season (S)				
Winter (W)	0.73±0.012^{a}	6.95 ± 0.04^{b}	68.66±0.88^{a}	15.61 ± 0.18^{a}
Summer (Su)	0.65 ± 0.01^{b}	7.03 ± 0.04^{a}	64.5 ± 0.87^{b}	14.54 ± 0.16^{b}
P value	<0.0001	0.0129	<0.0001	<0.0001
Effect of age (A)				
Young (Y)	0.73 ± 0.01^{a}	6.63 ± 0.02^{b}	71.58 ± 0.89^{a}	16.30 ± 0.18^{a}
Old (O)	0.65 ± 0.01^{b}	7.35 ± 0.03^{a}	61.59 ± 0.69^{b}	13.85 ± 0.10^{b}
P value	<0.0001	<0.0001	<0.0001	<0.0001
Effect of treatment (T)				
Without TP (NTP)	0.62 ± 0.01^{b}	7.21 ± 0.04^{a}	60.48 ± 0.75^{b}	14.20 ± 0.13^{b}
With TP (WTP)	0.76 ± 0.01^{a}	6.77 ± 0.03^{b}	72.69 ± 0.75^{a}	15.94 ± 0.19^{a}
P value	<0.0001	<0.0001	<0.0001	<0.0001
Interaction (S*A)				
W*Y	0.79 ± 0.02^{a}	6.59 ± 0.04	75.50 ± 1.05^{a}	16.91 ± 0.28
Su*Y	0.67 ± 0.01^{b}	6.66 ± 0.03	67.66 ± 1.31^{b}	15.69 ± 0.20
W*O	0.67 ± 0.01^{b}	7.31 ± 0.04	61.81 ± 0.91^{c}	14.31 ± 0.11
Su*O	0.62 ± 0.01^{c}	7.40 ± 0.05	61.38 ± 1.05^{c}	13.38 ± 0.16
P value	0.0049	0.6266	<0.0001	0.4246
Interaction (S*T)				
W*NTP	0.66 ± 0.01	7.19 ± 0.05	63.75 ± 1.12^{b}	14.65 ± 0.16
Su*NTP	0.57 ± 0.01	7.24 ± 0.06	57.22 ± 0.87^{c}	13.75 ± 0.18
W*TP	0.81 ± 0.02	6.71 ± 0.04	73.56 ± 1.13^{a}	16.56 ± 0.29
Su*TP	0.72 ± 0.01	6.82 ± 0.04	71.8 ± 0.98^{a}	15.32 ± 0.23
P value	0.6484	0.2793	0.0038	0.3273
Interaction (A*T)				
Y*NTP	0.65 ± 0.01	6.82 ± 0.02	64.47 ± 0.98^{b}	15.14 ±0.14^{b}
Y*TP	0.82 ± 0.02	6.43 ± 0.03	78.69 ± 0.98^{a}	17.46 ±0.28^{a}
O*NTP	0.58 ± 0.01	7.60 ± 0.05	56.50 ± 0.95^{c}	13.27± 0.15^{d}
O*TP	0.71 ± 0.01	7.11 ± 0.02	66.69 ± 0.61^{b}	14.42 ±0.11^{c}
P value	0.0519	0.1084	0.0146	0.0008
Interaction (S*A*T)				
W*Y*NTP	0.69 ± 0.01	6.83 ± 0.03	70.38 ± 1.09	15.63 ± 0.20
W*Y*TP	0.89 ± 0.03	6.36 ± 0.04	80.63 ± 1.40	18.18 ± 0.45
W*O*NTP	0.62 ± 0.02	7.56 ± 0.06	57.13 ± 1.26	13.68 ± 0.14
W*O*TP	0.73 ± 0.01	7.06 ± 0.03	66.50 ± 0.80	14.95 ±0.10
Su*Y*NTP	0.59 ± 0.01	6.82 ± 0.03	58.56 ± 0.95	14.64 ± 0.15
Su *Y*TP	0.74 ± 0.02	6.49 ± 0.03	76.75 ± 1.35	16.74 ± 0.29
Su *O*NTP	0.55 ± 0.02	7.65 ± 0.08	55.88 ± 1.43	12.86 ± 0.26
Su *O*TP	0.69 ± 0.02	7.15 ± 0.04	66.88 ± 0.92	13.89 ± 0.16
P value	0.1222	0.2916	0.0554	0.7631

^{a,b,c} Means within a column not sharing similar superscripts are significantly different (P<0.05).

Tomato powder supplementation in both seasons causd the highest significant increased in SM as compared to unsupplemented groups. The significant interaction between A*T was shown on SM and PSV, while EV and pH were not significant. The old rabbit that fed diet contained 0% TP showed the lowest significant values in SM and PSV as compared with the other groups. The interaction effect between S*A*T on previous parameters in Table (5) was not significant. In a general, fed male rabbits diet supplemented with 1% tomato powder significantly decreased the harmful effects of age and heat stress factors on above mentioned parameters.

The data in Table (6) shows the overall means and the interaction of sperm concentration (CON), total sperm output (TSO), total motile sperm (TMS) and the total functional sperm fraction (TFSF). Significant ($P < 0.05$) decrease of CON, TSO, TMS and TFSF were shown during summer compared to winter season, regardless of age or treatment effect.

The old rabbits had significantly lower CON, TSO, TMS and TFSF values than young rabbits, regardless of season or treatment effects. Significant increased for CON, TSO, TMS and TFSF were observed due to fed buck rabbits on diet supplemented with 1% TP, regardless of season or aged effects.

The significant interaction between S*A and A*T only detected on TSO, TMS and TFSF, while the CON was not significant. The old rabbits had significantly lower values in TSO, TMS and TFSF than young one during summer season, and this trend was continuous during winter season. The highest ($P \geq 0.05$) values in TSO, TMS and TFSF were shown in young rabbits that fed diet supplemented with 1% tomato powder compared with other groups in both winter and summer seasons. Fed old rabbits with 1% TP decreased significantly the harmful effects of the progress of age on rabbits. The interaction between S*T and between S*A*T was not significant different on CON, TSO, TMS and TFSF in Table (6).

The data in Table (7) represents the overall means and the interaction of live and abnormal sperm and initial fructose in semen rabbits. Live sperm and initial fructose were significantly ($P < 0.05$) decreased and abnormal sperm was significantly increased during summer compared to winter season, regardless of age or treatment effect. Also, the old rabbits had significant lower live sperm and initial fructose values and significant higher abnormal sperm than young rabbits, regardless of season or treatment effects. Significant increased for live sperm and initial fructose while, significant decreased for abnormal sperm were observed due to fed buck rabbits on diet supplemented with 1% TP as compared to rabbits fed diet free of TP, regardless of season or aged effects.

Table (6): Overall means (Mean ±SE) and the interaction of sperm concentration (CON), total sperm output (TSO) total motile sperm (TMS) and the total functional sperm fraction (TFSF) of young and old V-line bucks rabbit as affected by tomato powder dietary supplementation during winter and summer seasons.

Item	CON ($\times 10^6$)	TSO ($\times 10^6$)	TMS ($\times 10^6$)	TFSF ($\times 10^6$)
Effect of season (S)				
Winter (W)	277.48 ± 3.95[a]	208.77 ± 6.06[a]	150.39 ± 6.19[a]	123.16 ± 5.62[a]
Summer (Su)	252.31 ± 3.91[b]	166.77 ± 4.55[b]	111.90 ± 4.13[b]	89.09 ± 3.63[b]
P value	<0.0001	<0.0001	<0.0001	<0.0001
Effect of age (A)				
Young (Y)	290.48 ± 3.51[a]	217.35 ± 5.93[a]	161.79 ± 6.13[a]	134.93 ± 5.56[a]
Old (O)	239.30 ± 3.51[b]	158.19 ± 4.10[b]	100.49 ±3.26[b]	77.32 ± 2.64[b]
P value	<0.0001	<0.0001	<0.0001	<0.0001
Effect of treatment (T)				
Without TP (NTP)	237.66 ± 3.59[b]	149.02 ± 3.85[b]	93.27 ± 3.27[b]	72.74 ± 2.73[b]
With TP (WTP)	292.13 ± 3.26[a]	226.52 ± 5.41[a]	169.02 ± 5.60[a]	139.51 ± 5.18[a]
P value	<0.0001	<0.0001	<0.0001	<0.0001
Interaction (S*A)				
W*Y	306.22 ± 4.85	247.60 ± 9.02[a]	192.96 ± 9.42[a]	162.71± 8.62[a]
Su*Y	274.75 ± 4.46	187.10 ± 6.10[b]	130.63 ± 6.18[b]	107.15± 5.55[b]
W*O	248.73 ± 4.28	169.95 ± 5.33[b]	107.81 ± 4.46[c]	83.62 ± 3.60[c]
Su*O	229.88 ± 5.39	146.43 ± 5.98[c]	93.17 ± 4.64[c]	71.02 ± 3.75[c]
P value	0.0835	0.0003	<0.0001	<0.0001
Interaction (S*T)				
W*NTP	252.51 ± 4.75	167.89 ± 5.25	110.64 ± 4.91	87.70 ± 4.11
Su*NTP	222.81 ± 4.89	130.14 ± 4.82	75.88 ± 3.35	57.79 ± 2.75
W*TP	302.44 ± 4.94	249.65 ± 8.83	190.13 ± 9.51	158.63 ± 8.84
Su*TP	281.81 ± 3.96	203.39 ± 5.13	147.91 ± 4.96	120.39 ± 4.56
P value	0.2125	0.4000	0.4249	0.3055
Interaction (A*T)				
Y*NTP	264.84 ± 3.79	172.19 ± 4.52[c]	112.93 ± 4.23[b]	90.04 ± 3.47[b]
Y*TP	316.13 ± 4.32	262.52 ± 8.35[a]	210.67 ± 8.55[a]	179.82 ± 7.85[a]
O*NTP	210.48 ± 4.36	125.85 ± 5.08[d]	73.61 ± 3.91[c]	55.45 ± 3.24[c]
O*TP	268.12 ± 3.09	190.53 ± 3.93[b]	127.37 ± 3.04[b]	99.120 ± 2.34[b]
P value	0.3818	0.0115	0.0115	<0.0001
Interaction (S*A*T)				
W*Y*NTP	281.94 ± 4.84	196.11 ± 5.44	139.39 ± 5.27	112.69 ± 4.13
W*Y*TP	330.50 ± 6.44	299.09 ± 12.81	246.54 ±13.57	212.72±12.49
W*O*NTP	223.09 ± 4.84	139.68 ± 6.43	81.90 ± 5.24	62.71 ± 4.39
W*O*TP	274.38 ± 4.13	200.21 ± 5.19	133.72 ± 4.31	104.54 ± 3.31
Su*Y*NTP	4.88 ± 4.46	148.27 ± 4.88	86.47 ± 2.96	67.39 ± 2.31
Su *Y*TP	301.75 ± 4.83	225.94 ± 7.06	174.79 ± 6.79	146.92± 6.18
Su *O*NTP	197.88 ± 6.73	112.02 ± 7.30	65.31 ± 5.56	48.19 ± 4.54
Su *O*TP	261.88 ± 4.44	180.84 ± 5.56	121.03 ± 4.08	93.86 ± 3.12
P value	0.6169	0.0970	0.2249	0.1347

[a,b,c] Means within a column not sharing similar superscripts are significantly different (P<0.05).

Table (7): Overall means (Mean ±SE) and the interaction of live and abnormal sperm and initial fructose of young and old V-line rabbit bucks as affected by tomato powder dietary supplementation during winter and summer seasons.

Item	Live Sperm (%)	Abnormal sperm (%)	Initial Fructose (mg/100ml)
Effect of season (S)			
Winter (W)	70.9 ± 0.79[a]	20.0 ± 0.38[b]	232.7 ± 3.16[a]
Summer (Su)	68.9 ± 0.68[b]	22.3 ± 0.42[a]	213.9 ± 2.99[b]
P value	0.0070	<0.0001	<0.0001
Effect of age (A)			
Young (Y)	74.3 ± 0.64[a]	18.0 ± 0.31[b]	242.5 ± 2.82[a]
Old (O)	65.5 ± 0.67[b]	24.2 ± 0.34[a]	204.1 ± 2.72[b]
P value	<0.0001	<0.0001	<0.0001
Effect of treatment (T)			
Without TP (NTP)	65.1 ± 0.68[b]	23.5 ± 0.38[a]	207.8 ± 3.04[b]
With TP (TP)	74.8 ± 0.59[a]	18.8 ± 0.35[b]	238.8 ± 2.78[a]
P value	<0.0001	<0.0001	<0.0001
Interaction (S*A)			
W*Y	77.05 ± 0.93[a]	16.77 ± 0.39	254.49 ± 4.16
Su*Y	71.64 ± 0.77[b]	19.21 ± 0.44	230.46 ± 3.33
W*O	64.73 ± 0.84[c]	23.13 ± 0.41	210.90 ± 3.28
Su*O	66.23 ± 1.05[c]	25.28 ± 0.52	197.29 ±4.23
P value	<0.0001	0.6883	0.1167
Interaction (S*T)			
W*NTP	66.81 ± 0.98[b]	21.78 ± 0.49[b]	219.62 ± 3.99
Su*NTP	63.33 ± 0.89[c]	25.13 ± 0.52[a]	195.97 ± 4.22
W*TP	74.96 ± 1.07[a]	18.13 ± 0.51[c]	245.78 ± 4.46
Su*TP	74.54 ± 0.53[a]	19.37 ± 0.47[c]	231.77 ± 3.16
P value	0.0343	0.0032	0.1463
Interaction (A*T)			
Y*NTP	69.58 ± 0.59	20.52 ± 0.28	225.06 ± 3.18
Y*TP	79.11 ± 0.85	15.47 ± 0.39	259.90 ± 3.77
O*NTP	60.56 ± 0.98	26.39 ± 0.53	190.54 ± 4.43
O*TP	70.39 ± 0.48	22.03 ± 0.26	217.65 ± 2.36
P value	0.8315	0.3387	0.2434
Interaction (S*A*T)			
W*Y*NTP	73.04 ± 0.64	19.02 ± 0.31	237.51 ± 4.25
W*Y*TP	81.06 ± 1.52	14.53 ± 0.53	271.48 ± 6.12
W*O*NTP	60.59 ± 1.22	24.54 ± 0.69	201.73 ± 5.49
W*O*TP	68.86 ± 0.68	21.73 ± 0.34	220.08 ± 3.03
Su*Y*NTP	66.13 ± 0.65	22.02 ± 0.32	212.60 ± 3.88
Su *Y*TP	77.15 ± 0.66	16.41± 0.54	248.33 ± 3.66
Su *O*NTP	60.54 ± 1.56	28.25 ± 0.71	179.35 ± 6.56
Su *O*TP	71.93 ± 0.59	22.33 ± 0.39	215.23 ± 3.61
P value	0.9688	0.1596	0.2344

[a,b,c] Means within a column not sharing similar superscripts are significantly different (P<0.05).

The significant interaction between S*A only showed in live sperm while, no significant different was detected on abnormal sperm and initial fructose. The significant lowest value of live sperm was shown in the old rabbits in both seasons than young one. Significant interaction between S*T was obtained in live and abnormal sperm, while no significant different was found for intial fructose. The significant highest value of live sperm and the significant lowest value of abnormal sperm were shown due to supplemented rabbit with tomato powder during winter season. The interaction between A*T and between S*A*T was not significant on live, abnormal sperm and initial fructose.

Heat stress causes alterations in the biological processes and functions **(Hansen, 2009)**. Heat stress thus alters several aspects of reproductive physiology, such as blood flow and steroidogenesis **(Rivera and Hansen, 2001)**, which manifests in fertility alterations. The harmful effect of summer season on semen quality in our study is compatible with **Marai *et al.* (2002)** who reported that a rise in testicular temperature in rabbits leads to reduced spermatogenesis; temporary sterility; decreased sexual desire, ejaculate volume, motility, sperm concentration, and total number of spermatozoa in an ejaculate; and increased sperm abnormalities and dead sperm. **El-Sherbiny (1987)** who observed that motility of NZW and Bouscat rabbit spermatozoa was significantly reduced during summer, but the differences among values obtained during autumn, winter and spring were not significant. **Kasa (1991)** found significant differences in semen motility for bucks exposed to 32 ^{o}C compared to their control. **Kasa, (1991)** studied the thermoregulation in rabbits with particular reference to semen production and quality and found significant differences in semen volume for bucks exposed to 32 ^{o}C than the control. Also, **Tharwat *et al.* (2004)** indicated that the greatest ejaculate volume of domesticated Sina Gabali rabbit bucks was obtained during spring and the smallest during summer. **Gao *et al.* (2007)** indicated that sperm concentration, sperm count per ejection, sperm progressive motility (%), and sperm viability (%) of healthy men were lowest in summer.

In all species increasing age negatively influences reproductive performance. In mammals, deterioration in reproductive performance at the end of reproductive season partly results from a decrease in male fertility. Changes in motility, viability and morphology of spermatozoa have been reported in the latter part of the reproductive period in animals (**Wilson, 1995**). **Alexaki *et al.* (1991)** showed that age appeared to influence on spermatozoa, and found gradually decreasing in concentrations as the animals advanced in age.

Our results are in accordance with recent studies on sperm characteristics and a potential positive effect of lycopene on it **(Goyal *et al.*, 2007; Turk *et al.*, 2007)**.
These provided evidence suggesting a more potent protective role for tomato powder, in decreasing the circulatory and hepatic lipid peroxidation, in rats (**Alshatwi *et al.*, 2010)**. There are also reports on detrimental effects of higher lycopene levels on the quality of ram spermatozoa (**Uysal and Bucak, 2007**) and of higher inclusion levels of tomato pulp (>10%) on reproductive performance in layers (**Jafari *et al.*, 2006**), mainly due to its high fiber content **(Lira *et al.*, 2010). Saemi *et al.* (2012)** indicated that dietary inclusion of dried tomato pomace up to 30% increased sperm concentration, accompanied by a decreased seminal volume ($P \leq 0.05$) and percentage of abnormal sperm, while the percentage of live sperm in ejaculate was increased within 4 to 5 weeks in roosters.

4.1.3. Seminal plasma Biochemistry:

Tables (8 and 9) show the overall means and the interaction of seminal plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AlP), acid phosphatase (AcP) and lactic dihydrogenase (LDH) in young and old VL rabbit bucks as affected by 1% tomato powder dietary supplementation during winter and summer seasons. The results showed that regardless of age and treatment effects, seminal plasma ALT and LDH were significant increased, while seminal plasma AcP significant decreased during summer compared to winter season, while seminal plasma AST and AlP were not significantly different.

Significant increase in seminal plasma AlP and AcP, while significant decreased in seminal plasma ALT, AST and LDH in young rabbits was observed compared with older one, regardless of season or treatment effects. The same trend was shown in the previous parameters when rabbit bucks were fed diet supplementation with 1% TP compared to unsupplemented group (Tables 8 and 9) regardless of season or age effects.

The significant interaction between S*A was shown in seminal plasma ALT, AST and AcP, while seminal plasma AlP and LDH were not significant different (Tables 8 and 9). Seminal plasma ALT and AST significantly increased and AcP significantly decreased in old rabbits compared to young rabbits in both winter and summer seasons. No significant interaction between S*T was shown in seminal plasma ALT and AlP, while seminal plasma AST, AcP and LDH were significantly different, where the lowest significant values in AST and LDH and the highest significant value in AcP were obtained in TP treatment in both seasons. Seminal plasma ALT and LDH were not significant, while seminal plasma AST, AlP and AcP showed significant interacted between A*T, where the young rabbit that fed diets contained 1% of TP showed the lowest level of seminal plasma AST and the highest level of AlP and AcP compared to the other groups. Old rabbits that fed 1% TP showed significant improved in the previous parameters compared unsupplemented groups. On the other hand, the significant interaction between S*A*T was obtained for seminal plasma ALT, AST and AcP, while seminal plasma Alp and LDH were not significant. The lowest significant levels of seminal plasma ALT and AST and the highest significant level of seminal plasma AcP were shown in young rabbits that fed 1% TP during winter season compared to the other groups (Tables 8 and 9), while the highest significant levels of seminal plasma ALT and AST and the lowest significant level of seminal plasma AcP were shown in old rabbits that fed 0% TP, especially during summer season. Seminal plasma constituents exhibited positive significant responses to TP supplementation, especially during summer season and with old rabbit bucks, showing a constant effect of TP on seminal plsma.

The improvements found herein are in general agreement with those reported by **Al-Daraji et al. (2010)** who used n-3 and n-6 fatty acid supplementation to improved semen quality and its seminal plasma constituents in Japanese quail. The author showed that the decrease in AST and ALT activities indicating a decrease in sperm damage while increasing spermatozoa livability and concentration. The increase in the AlP and the ACP observed in seminal plasma is in agreement with the result of **Al-Daraji et al. (2001)**, who reported that both AlP and ACP are involved in the metabolism of spermatozoa via the hydrolysis of carbohydrates.

Table (8): Overall means (Mean ±SE) and the interaction of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) of young and old V-line bucks rabbit as affected by tomato powder dietary supplementation during winter and summer seasons.

Item	ALT (IU)	AST (IU)	AlP (U/L)
Effect of season (S)			
Winter(W)	22.22 ± 0.27^{b}	34.21 ±0.36	58.06 ± 0.76
Summer (Su)	23.48 ± 0.36^{a}	35.05 ± 0.56	57.92 ± 0.87
P value	0.0001	0.0515	0.8470
Effect of age (A)			
young(Y)	20.75 ±0.26^{b}	31.65 ± 0.39^{b}	64.07 ± 0.47^{a}
old (O)	24.95 ± 0.29^{a}	37.60 ± 0.43^{a}	51.91 ± 0.80^{b}
P value	<0.0001	<0.0001	<0.0001
Effect of treatment (T)			
Without TP (NTP)	25.03±0.28^{a}	38.24 ± 0.40^{a}	52.92 ± 0.85^{b}
With TP (WTP)	20.68 ± 0.27^{b}	31.02 ± 0.36^{b}	63.06 ±0.53^{a}
P value	<0.0001	<0.0001	<0.0001
Interaction (S*A)			
W*Y	20.64 ± 0.41^{c}	32.10 ±0.53^{c}	63.49± 0.66^{a}
Su*Y	20.86 ± 0.33^{c}	31.21± 0.57^{c}	64.64 ±0.66^{a}
W*O	23.81± 0.25^{b}	36.31±0.37^{b}	52.62 ±1.06^{b}
Su*O	26.10 ± 0.50^{a}	38.90±0 .76^{a}	51.21 ±1.20^{b}
P value	0.0004	0.0001	0.0673
Interaction (S*T)			
W*NTP	24.26 ± 0.22	37.03 ±0.32^{b}	53.26 ±1.09
Su*NTP	25.80 ± 0.49	39.44± 0.70^{a}	52.57±1.31
W*TP	20.19 ± 0.37	31.38 ±0.48^{c}	62.85± 0.73
Su*TP	21.16 ± 0.39	30.66± 0.54^{c}	63.28 ±0.76
P value	0.3249	0.0001	0.4222
Interaction (A*T)			
Y*NTP	23.06± 0.12	34.89± 0.19^{b}	60.38± 0.34^{b}
Y*TP	18.44 ± 0.36	28.42± 0.55^{d}	67.76± 0.66^{a}
O*NTP	27.00 ± 0.44	41.58 ±0.56^{a}	45.46 ±1.18^{d}
O*TP	22.91 ± 0.21	33.62± 0.21^{c}	58.37± 0.36^{c}
P value	0.3612	0.0435	<0.0001
Interaction (S*A*T)			
W*Y*NTP	23.23 ± 0.22^{c}	35.39 ±0.27^{c}	59.81± 0.46
W*Y*TP	18.04 ± 0.38^{d}	28.81 ± 0.81^{e}	67.18± 0.94
W*O*NTP	25.28 ± 0.30^{b}	38.67 ± 0.30^{b}	46.71±1.5
W*O*TP	22.34 ± 0.18^{c}	33.94± 0.30^{cd}	58.53± 0.57
Su*Y*NTP	22.88 ± 0.19^{c}	34.39± 0.20^{cd}	60.94± 0.47
Su *Y*TP	18.85 ± 0.41^{d}	28.02 ± 0.71^{e}	68.34± 0.92
Su *O*NTP	28.72 ± 0.28^{a}	44.50 ± 0.23^{a}	44.20 ±1.76
Su *O*TP	23.48 ± 0.15^{C}	33.30 ± 0.29^{d}	58.21± 0.45
P value	<0.0029	0.0001	0.4379

^{a,b,c,d,e} Means within a column not sharing similar superscripts are significantly different (P<0.05).

Table (9): Overall means (Mean ±SE) and the interaction of seminal plasma acid phosphatase (ACP) and lactic dihydrogenase (LDH) of young and old V-line bucks rabbit as affected by tomato powder dietary supplementation during winter and summer seasons.

Item	ACP (U/L)	LDH (IU)
Effect of season (S)		
Winter(W)	30.90±0.30[a]	1137.3±11.3[b]
Summer (Su)	28.46±0.35[b]	1218.4±16.3[a]
P value	<0.0001	<0.0001
Effect of age (A)		
young(Y)	31.55±0.29[a]	1101.5±10.5[b]
old (O)	27.80±0.32[b]	1254.2±15.1[a]
P value	<0.0001	<0.0001
Effect of treatment (T)		
Without TP (NTP)	27.51±0.36[b]	1263.5±15.2[a]
With TP (WTP)	31.85 ±0.21[a]	1092.2 ± 9.5[b]
P value	<0.0001	<0.0001
Interaction (S*A)		
W*Y	33.40 ± 0.27[a]	1072.3 ±13.3
Su*Y	29.71 ± 0.44[b]	1130.6 ±15.7
W*O	28.40 ± 0.37[c]	1202.4 ±15.1
Su*O	27.20 ± 0.51[d]	1306.1 ±25.0
P value	<0.0001	0.1107
Interaction (S*T)		
W*NTP	29.06 ± 0.41[c]	1199.1 ±15.2[b]
Su*NTP	25.95 ± 0.54[d]	1327.8 ±24.3[a]
W*TP	32.74 ± 0.33[a]	1075.6 ±13.5[c]
Su*TP	30.96 ± 0.21[b]	1108.9 ±13.2[c]
P value	0.0331	0.0009
Interaction (A*T)		
Y*NTP	30.03 ± 0.42[b]	1176.40 ±12.8
Y*TP	33.07 ± 0.34[a]	1026.54 ±11.72
O*NTP	24.98 ± 0.42[c]	1350.54 ±23.89
O*TP	30.62 ± 0.15[b]	1157.92 ±10.82
P value	<0.0001	0.1318
Interaction (S*A*T)		
W*Y*NTP	31.79 ± 0.18[b]	1129.33 ±14.97
W*Y*TP	34.99 ± 0.37[a]	1015.19 ±17.87
W*O*NTP	26.33 ± 0.52[e]	1268.79 ±21.46
W*O*TP	30.48 ± 0.24[c]	1135.96 ±15.35
Su*Y*NTP	28.27 ± 0.73[d]	1223.39 ±18.07
Su *Y*TP	31.15 ± 0.36[bc]	1037.90 ±15.17
Su *O*NTP	23.63 ± 0.60[f]	1432.30 ±38.84
Su *O*TP	30.77 ± 0.20[bc]	1179.88 ±14.64
P value	0.0081	0.3952

[a,b,c,d,e,f] Means within a column not sharing similar superscripts are significantly different (P<0.05).

The changes in environmental conditions have been reported to significant effect on sexual activity and seminal attributes activities **(Thatcher and Hansen, 1993)**. The cyclic changes in pituitary and testicular activity in seasonally breeding mammals in temperate climates are prompter by changes in photoperiod, nutrition, social interactions and temperature **(Bronson and Heideman, 1994).** These results also agree with **Hussain (1995)** who reported an increase concentration and activity of AST in local goats in winter.

Juma and Al-Kassab (2009) showed a significant marked increase ($P<0.01$) in the activity of AlP during summer. Highest ($P<0.01$) monthly was recorded in August. The increase in ALP activity in summer may be due to increase secretion adrenocorticotrophic hormone (ACTH) due to environmental stress **(Litwack, 1972**). Whereas, seminal acid phosphatase (ACP) activity showed a significant increase in winter.

Roussal and Stallcup, (1965) found negative correlation between AST activity of seminal plasma and with each of ejaculate volume, sperm motility, concentration and percent live cells. Also, **Chauban *et al.* (1993**) found a positive correlation between enzyme release and sperm acrosomal damage.

Table (10) shows the overall means and the interaction of seminal plasma thiobarbituric acid-reactive substances (TBARS), superoxide dismutase (SOD) and Glutathione S-transferase (GST) of rabbit bucks. Significant increased of seminal plasma TBARS and significant decreased of seminal plasma SOD and GST were shown during summer as a compared with winter season, regardless of age or treatment effect. The same trend was shown in the previous parameters as affected by rabbit age regardless of season or treatment effects. Dietary supplementation with 1% TP caused significant decreased in seminal plasma TBARS and significant increased in seminal plasma SOD and GST compared to unsupplemented group (Table 10) regardless of season or age effects.

The present study in Table (10) showed no significant interaction effect between S*A, S*T, A*T, and S*A*T for seminal plasma TBARS and GST, except the interaction between A*T on GST.

Significant interaction between S*A was shown on seminal plasma SOD, where the highest significant value was found for young rabbits during winter season, while the lowest significant value was detected for old rabbits during summer season. Also, the significant interaction between S*T showed highly significant value of SOD during winter season for rabbits that fed 1% TP. Seminal plasma SOD significantly affected by the interaction between S*A*T, where the highest significant level was obtained in young rabbit that fed diet contained TP during winter season. Fed old rabbits with 1% TP caused significant increased in seminal plasma SOD as compared with unsupplemented group especially during summer season

Table (10): Overall means (Mean ±SE) and the interaction of seminal plasma thiobarbituric acid-reactive substances (TBARS), superoxide dismutase (SOD) and Glutathione S-transferase (GST) of young and old V-line bucks rabbit as affected by tomato powder dietary supplementation during winter and summer seasons.

Item	TBARS (nmol/ml)	SOD (IU)	GST (IU)
Effect of season (S)			
Winter(W)	1.110 ± 0.016^{b}	7.13±0.034^{a}	1.22 ±0.02^{a}
Summer (Su)	1.182 ± 0.017^{a}	6.74±0.037^{b}	1.14 ±0.02^{b}
P value	<0.0001	<0.0001	<0.0001
Effect of age (A)			
young(Y)	1.037 ± 0.015^{b}	7.13 ± 0.037^{a}	1.29 ± 0.01^{a}
old (O)	1.27 ± 0.0141^{a}	6.74 ± 0.034^{b}	1.08 ± 0.01^{b}
P value	<0.0001	<0.0001	<0.0001
Effect of treatment (T)			
Without TP (NTP)	1.263 ± 0.014^{a}	6.72 ±0.041^{b}	1.07 ±0.01^{b}
With TP (WTP)	1.029 ± 0.011^{b}	7.15 ± 0.027^{a}	1.29 ± 0.01^{a}
P value	<0.0001	<0.0001	<0.0001
Interaction (S*A)			
W*Y	1.003 ± 0.022	7.37 ± 0.037^{a}	1.32 ± 0.02
Su*Y	1.054 ± 0.023	6.90 ± 0.051^{b}	1.25 ± 0.02
W*O	1.216 ± 0.024	6.89 ± 0.044^{b}	1.12 ± 0.02
Su*O	1.32 ± 0.022	6.58 ± 0.048^{c}	1.04 ± 0.02
P value	0.0901	0.0285	0.9421
Interaction (S*T)			
W*NTP	1.222 ± 0.017	7.00 ± 0.055^{b}	1.11 ± 0.02
Su*NTP	1.303 ± 0.015	6.44 ± 0.042^{c}	1.03 ± 0.02
W*TP	0.994 ± 0.021	7.26 ± 0.035^{a}	1.32 ± 0.02
Su*TP	1.064 ± 0.023	7.04 ± 0.039^{b}	1.25 ± 0.02
P value	0.6014	0.0001	0.7945
Interaction (A*T)			
Y*NTP	1.154 ± 0.011	6.93 ± 0.058	1.19 ± 0.01^{b}
Y*TP	0.902 ± 0.022	7.34 ± 0.032	1.38 ± 0.02^{a}
O*NTP	1.367 ± 0.017	6.51 ± 0.048	0.96 ± 0.02^{c}
O*TP	1.162 ± 0.011	6.97 ± 0.033	1.20 ± 0.01^{b}
P value	0.3812	0.5043	0.0487
Interaction (S*A*T)			
W*Y*NTP	1.13 ± 0.01	7.30±0.058^{ab}	1.22 ± 0.01
W*Y*TP	0.88 ± 0.03	7.43±0.041^{a}	1.42 ± 0.03
W*O*NTP	1.32 ± 0.03	6.70±0.063^{e}	1.01 ± 0.03
W*O*TP	1.12 ± 0.01	7.08±0.041^{c}	1.23 ± 0.01
Su*Y*NTP	1.17 ± 0.01	6.55±0.052^{e}	1.16 ± 0.01
Su *Y*TP	0.93 ± 0.03	7.24±0.044^{b}	1.33 ± 0.02
Su *O*NTP	1.44 ± 0.03	6.32±0.060^{f}	0.90 ± 0.03
Su *O*TP	1.19 ± 0.01	6.84±0.046^{d}	1.17 ±0.01
P value	0.3341	0.0038	0.2083

^{a,b,c,d,e,f} Means within a column not sharing similar superscripts are significantly different (P<0.05).

In the general, the lowest values in TBARS and the highest values in GST and SOD were detected in seminal plasma of young rabbits that fed diet supplemented with 1% tomato powder compared with other groups in both winter and summer seasons. Fed old rabbits diet supplemented with tomato powder significantly decreased the harmful effects of age factor on the above mentioned parameters.

Under normal condition, the seminal fluid surrounding sperm contains antioxidant factors (such as glutathione, urate, ascorbate, a-tocopherol, taurine, etc) protecting them from oxidative damage (**Kim and Parthasarathy, 1998**). While under heat stress, the seminal fluid may either lack sufficient protective elements or the buck's body may be so overloaded with ROS, so as to overwhelm the normal inherent antioxidative mechanisms. Increased levels of ROS may be generated internally from damaged or defective sperm, as well as from leucocytes in the seminal plasma (**Tamura *et al.*, 1988**). So, Lycopene is the most potent singlet oxygen quencher, in vitro, among the natural carotenoids and other micronutrients, such as VitE and VitC **(Di Mascio *et al.*, 1989)** and therefore delay or prevent oxidative damage (**Willis and Wians, 2003)**.

Pansarasa *et al.* (1999) reported that total SOD activity has been observed to decline in aged skeletal muscle, MnSOD activity was observed to increase, presumably to defend against the age-associated increase in ROS generation.

Also, **Chi *et al.*, (2008**) indicated that *in vitro* and *in vivo* studies the antioxidants supplementation attenuated the negative effects of ROS and improved sperm function, capacity for fertilization and sperm membrane fluidity.

In chicks, lycopene showed protection against depletion of glutathione, an endogenous antioxidant, during viral-induced acute oxidant stress **(Leal *et al.* 1999)**. **Juma and Al-Kassab (2009)** showed a marked significant increase ($P<0.01$) in Glutathione S-transferase (GST) activity in winter season.

Attia and kamel (2012) reported that supplemented rabbit bucks diets with omega 3 and omega 6 as antioxidant factor caused significant increase in the antioxidant status of seminal plasma (GSH, GPx, SOD and GST) and the decrease in TBARS caused higher cell stability.

4.1.4. Blood plasma biochemistry:

Table (11) shows the overall means and the interaction of blood plasma total protein, albumin (Alb), globulin (Glb) and glucose. The present data showed that blood plasma total protein, Alb, and Glb were significantly ($P < 0.05$) decreased and blood glucose was significantly increased of rabbits buck during summer compared with winter season, regardless of age or treatment effect. The old rabbits on previous parameters, except blood plasma glucose were shown significantly lower values than young one, regardless of season or treatment effects. Dietary supplementation with 1% TP caused significant increased the blood plasma total protein, Alb and Glb and significant decreased blood plasma glucose compared to unsupplemented group (Table 11), regardless of season or age effects.

The results of the study indicated insignificant interaction between season (S) and age (A), season (S) and treatment (T), age (A) and treatment (T) and between S*A*T on blood plasma total protein, Alb, Glb and glucose, except the interaction between S*T and between S*A*T on Glb, the interaction between A*T on Alb.

Table 11: Overall means (Mean ±SE) and the interaction of blood plasma total protein, albumin (Alb) globulin (Glb) and glucose of young and old V-line rabbit bucks as affected by tomato powder dietary supplementation during winter and summer seasons.

Item	Total Protein (g/dl)	Alb (g/dl)	Glb (g/dl)	Glucose (mg/dl)
Effect of season (S)				
Winter(W)	7.33± 0.08^{a}	4.16± 0.05^{a}	3.32 ± 0.05^{a}	113.4 ± 1.53^{b}
Summer (Su)	7.09 ± 0.08^{b}	3.98 ± 0.05^{b}	3.12 ± 0.06^{b}	119.8 ±1.57^{a}
P value	0.0003	0.0007	0.0037	0.0001
Effect of age (A)				
young(Y)	7.66 ± 0.06^{a}	4.28 ± 0.05^{a}	3.39 ± 0.05^{a}	108.8 ±1.13^{b}
old (O)	6.76 ± 0.06^{b}	3.86 ± 0.04^{b}	3.04 ± 0.05^{b}	124.4 ± 1.49^{a}
P value	<0.0001	<0.0001	<0.0001	<0.0001
Effect of treatment (T)				
Without TB (NTB)	6.89 ± 0.07^{b}	3.93 ± 0.04^{b}	3.10 ± 0.06^{b}	121.7 ± 1.56^{a}
With TB (WTB)	7.53 ± 0.07^{a}	4.21± 0.05^{a}	3.33 ± 0.05^{a}	111.6 ±1.40^{b}
P value	<0.0001	<0.0001	0.0010	<0.0001
Interaction (S*A)				
W*Y	7.80 ±0.09	4.36±0.07	3.45±0.08	105.3 ±1.47
Su*Y	7.53± 0.07	4.21±0.06	3.33±0.07	112.4 ±1.53
W*O	6.87 ± 0.08	3.96 ± 0.05	3.18 ±0.05	121.6 ±2.05
Su*O	6.65 ±0.10	3.75±0.06	2.91±0.08	127.3 ±2.09
P value	0.6692	0.5663	0.2419	0.6197
Interaction (S*T)				
W*NTB	7.01 ± 0.09	4.02±0.05	3.27 ± 0.07^{a}	117.2 ±2.13
Su*NTB	6.77±0.11	3.84 ±0.06	2.93±0.09^{b}	126.2 ±2.05
W*TB	7.65 ±0.10	4.30 ±0.07	3.36 ±0.07^{a}	109.7 ±2.13
Su*TB	7.40±0.08	4.12± 0.07	3.30 ±0.07^{a}	113.5 ±1.80
P value	0.9260	1.0000	0.0460	0.0768
Interaction (A*T)				
Y*NTB	7.34 ±0.05	4.03 ±0.05^{b}	3.31±0.08	115.0 ±1.22
Y*TB	7.98 ±0.07	4.54 ± 0.06^{a}	3.46±0.07	102.7 ±1.32
O*NTB	6.44 ± 0.09	3.83 ± 0.06^{c}	2.89±0.08	128.4 ±2.46
O*TB	7.07±0.06	3.88±0.04^{bc}	3.20 ±0.06	120.4 ±1.46
P value	0.9260	<0.0001	0.2289	0.1415
Interaction (S*A*T)				
W*Y*NTP	7.44±0.07	4.09±0.07	3.36 ±0.11^{ab}	119.20±1.16
W*Y*TP	8.16 ±0.11	4.63± 0.09	3.54 ± 0.10^{a}	133.25 ±3.28
W*O*NTP	6.59 ± 0.10	3.96 ±0.08	2.64 ± 0.10^{c}	105.65 ±1.86
W*O*TP	7.15±0.07	3.97±0.05	3.18 ± 0.08^{b}	121.25 ±1.84
Su*Y*NTP	7.25 ± 0.08	3.98 ±0.06	3.27 ± 0.11^{ab}	114.29 ±2.03
Su *Y*TP	7.80 ± 0.07	4.44 ± 0.08	3.38 ± 0.09^{ab}	123.50 ±3.40
Su *O*NTP	6.30±0.14	3.71±0.10	2.60 ± 0.10^{c}	99.70 ±1.68
Su *O*TP	7.00±0.09	3.80±0.07	3.21±0.10^{b}	119.60 ±2.31
P value	0.2739	0.4447	0.0119	0.3412

^{a,b,c} Means within a column not sharing similar superscripts are significantly different (P<0.05).

The interaction between S*T and S*A*T on blood plasma globulin showed that the lowest significant concentration in globulin was found in old rabbit that unsupplemented with 1% TP especially during summer season. Fed rabbits diet contained 1% TP caused significant increase in Glb level in both age during both season compared unsupplemented groups. The significant interaction between A*T found only in blood plasma albumin, where the highest significant level of albumin concentration was found in young rabbit that fed diet contained tomato powder compared to unsupplemented group (Table 11).

Fed old rabbits diet supplemented with tomato powder decreased the harmful effects of age and season factors on above mentioned parameters.

The effect of climatic conditions on globulin was not significant, similar to that reported by **Marai *et al.* (1994).** The significant decrease in glucose was similar to the results of **Marai *et al.* (2002)** and **Habeeb *et al.* (1997).** Such changes relate in part to the decrease in concentrations of insulin as a function of heat stress (**Habeeb, 1987)**.

Similar results are in agreement with **(Abdelatif *et al.*, 2009)** resulted that total protein, glucose and albumin parameters in goats were found to be the maximum in winter and were minimum in wet summer. Also, **(Al-Eissa *et al.*, 2008)** reported that biochemical parameters could be affected by many factors including sex, age, reproductive status and seasonal variations.

Table (12) shows the overall means and the interaction of blood plasma of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AlP) and acid phosphatase (ACP). The present data showed that blood plasma ALT, AST and ACP were significantly increased, while blood plasma AlP was significantly ($P < 0.05$) decreased during summer as compared with winter season, regardless of age or treatment effect. The same trend was shown as affected by age factor on the pervious parameters, regardless of effect of season or tomato powder treatment. Dietary supplementation with 1% TP caused significant decreased in the blood plasma ALT, AST and ACP and significant increased in blood plasma and AlP compared to unsupplemented group (Table 12), regardless of season or age effects.

The results in Table (12) indicated insignificant interaction between season (S) and age (A), season (S) and treatment (T), age (A) and treatment (T) and between S*A*T on blood plasma ALT, AST, AlP and ACP, except the interaction between S*T on ALT and the interaction between A*T and the interaction between S*A*T on AlP.

The results in Table (12) indicated that significant interaction between S*T in blood plasma ALT, where the lowest level was found in rabbit that fed diet contained 1% TP during winter season. The interaction between A*T was found in blood plasma ACP, where the young rabbits had the lowest significant concentration of ACP due to fed the 1% TP in its diets. Fed old rabbit with TP significantly closed the blood plasma ACP value to the young rabbit. Interaction between S*A*T on blood plasma AlP showed that the young rabbit that fed 1%TP during winter season had the highest levels compared to the other groups. Old rabbits that fed TP showed significant improved in this parameter near to the young rabbit especially during summer season.

Table 12: Overall means (Mean ±SE) and the interaction of blood plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AlP) and acid phosphatase (ACP) of young and old V-line rabbit bucks as affected by tomato powder (TP) dietary supplementation during winter and summer seasons.

Item	ALT	AST	AlP	ACP
Effect of season (S)				
Winter(W)	23.23 ± 0.48[b]	52.27 ± 0.62[b]	58.45 ± 0.66[a]	10.83 ± 0.13[b]
Summer (Su)	30.94 ± 0.51[a]	59.26 ±0.55[a]	56.48 ± 0.66[b]	11.21 ± 0.13[a]
P value	<0.0001	<0.0001	0.0093	0.0168
Effect of age (A)				
young(Y)	25.31± 0.63[b]	53.36 ± 0.66[b]	58.96 ± 0.64[a]	10.61 ± 0.10[b]
old (O)	28.85 ± 0.62[a]	58.17 ± 0.65[a]	55.98 ± 0.66[b]	11.43 ±0.14[a]
P value	<0.0001	<0.0001	0.0001	<0.0001
Effect of treatment (T)				
Without TP(NTP)	29.20 ± 0.69[a]	57.60 ± 0.72[a]	54.29 ± 0.57[b]	11.47 ±0.14[a]
With TP (WTP)	24.96 ± 0.52[b]	53.93 ± 0.63[b]	60.64 ± 0.56[a]	10.57 ±0.10[b]
P value	<0.0001	<0.0001	<0.0001	<0.0001
Interaction (S*A)				
W*Y	21.18 ± 0.54	49.23 ± 0.68	60.28 ± 0.99	10.42±0.15
Su*Y	29.45 ± 0.65	57.50 ± 0.66	57.64 ± 0.77	10.79±0.14
W*O	25.28 ± 0.64	55.31 ± 0.80	56.63 ± 0.77	11.24 ± 0.18
Su*O	32.43 ± 0.72	61.03 ± 0.80	55.33 ± 1.07	11.62±0.20
P value	0.2980	0.0595	0.3721	0.9532
Interaction (S*T)				
W*NTP	24.65 ± 0.69[c]	53.61 ± 0.87	55.48 ± 0.72	11.30 ± 0.19
Su*NTP	33.76 ± 0.64[a]	61.60 ± 0.72	53.11 ± 0.86	11.63±0.20
W*TP	21.80 ± 0.58[d]	50.93 ± 0.85	61.43 ± 0.87	10.36±0.13
Su*TP	28.13 ± 0.48[b]	56.93 ± 0.65	59.86 ± 0.69	10.78±0.14
P value	0.0106	0.1433	0.5920	0.7522
Interaction (A*T)				
Y*NTP	27.40 ± 0.90	55.39 ± 0.92	55.56 ± 0.61	10.88±0.14[b]
Y*TP	23.23 ± 0.75	51.33 ± 0.85	62.36 ± 0.83	10.33 ±0.14[c]
O*NTP	31.01 ±0.99	59.82 ± 1.00	53.02 ± 0.94	12.05 ± 0.20[a]
O*TP	26.70 ± 0.60	56.53 ± 0.74	58.93 ± 0.65	10.81±0.13[b]
P value	0.9057	0.5688	0.5467	0.0268
Interaction (S*A*T)				
W*Y*NTP	22.55 ± 0.67	50.75 ± 0.85	56.20 ± 0.93[cd]	10.70 ±0.21
W*Y*TP	19.80 ± 0.75	47.70 ± 0.97	64.35 ± 1.19[a]	10.14±0.20
W*O*NTP	26.75 ± 1.02	56.48 ±1.24	54.75 ± 1.12[d]	11.90 ±0.25
W*O*TP	23.80 ± 0.62	54.15 ± 0.96	58.50 ± 0.90[bc]	10.57±0.17
Su*Y*NTP	32.26 ± 0.62	60.03 ± 0.71	54.91± 0.79[d]	11.06±0.18
Su *Y*TP	26.65 ± 0.74	54.96 ± 0.78	60.37±1.01[b]	10.52±0.19
Su *O*NTP	35.26 ± 1.04	63.16 ± 1.17	51.30 ± 1.43[e]	12.20±0.32
Su *O*TP	29.60 ± 0.44	58.90 ± 0.86	59.35± 0.96[b]	11.04±0.17
P value	0.9463	0.9748	0.0206	0.8114

[a,b,c,d,e] Means within a column not sharing similar superscripts are significantly different (P<0.05).

These results are in agreement with those reported by **QingHua and Genlin (2007)** who found that during heat stress, levels of liver enzymes tend to rise suggesting some liver damage in mammals. With regard to acid phosphatase level, **Aboul-Naga (1987)** reported that it was not affected by heat stress.

Other studies indicated that SGOT (**Ibrahim, 1994**) and SGOT and SGPT activities (**Ayyat and Marai, 1997**) were significantly lower during the summer than during the winter season due to the increase of water intake and, consequently, the increase in enzyme dilution during the hot conditions. Alkaline phosphatase decreased significantly (**Peterson and Waldern, 1981; Shaffer *et al*., 1981)** due to heat stress. This may be attributed to a reduction in thyroid hormones which takes place under heat stress conditions (**Habeeb *et al*., 1992)**.

Salem *et al*. (1998) stated that the serum SGPT concentration was significantly higher in summer than in winter, while serum SGOT was not significantly affected by season. The increase of SGPT and SGOT levels with exposure to hot temperature may be due to an increased stimulation of gluconeogenesis by corticoids (increase in cortisol, cortisone or adrenocorticotrophic hormones(**Thompson, 1973).**

Elkomy and Hassan (2005) observed decrease in the activity of AST and ALT of thioacetamide-treated male rat fed tomato-juice as supplemented diet. **Kalender *et al*. (2005) and Ogur *et al*. (2005)** also recorded decrease in activity of AST and ALT after diazinon-induced and nitrate-induced stress in male rats fed vitamin E as supplemented diets.

Table (13) shows the overall means and the interaction of blood plasma total lipid (TL), triglyceride (TG), urea and lactic dihydrogenase (LDH) in young and old VL rabbit bucks during dietary supplementation without or with 1% tomato powder through winter and summer seasons.

Data showed that blood plasma TL, TG, urea and LDH were significantly increased in rabbit bucks due to exposure to heat stress in summer season compared to winter, regardless of age or treatment effect. Significantly increased in blood plasma TL, TG, urea and LDH were detected in older rabbits compared with young rabbits, regardless of season or treatment effects. Dietary supplementation with 1% TP caused significantly decreased blood plasma TL, TG, urea and LDH as compared to unsupplemented group (Table13), regardless of season or age effects.

The results of the study indicated insignificant interaction between season (S) and age (A), season (S) and treatment (T), age (A) and treatment (T) and between S*A*T in the effect on blood plasma TL, TG, urea and LDH.

The young rabbits had insignificant lower values in pervious parameters than older rabbits during winter season, The data showed insignificant improvement in the previous parameters due to fed diet supplemented with 1% tomato powder compared with other groups in both winter and summer seasons. Fed old rabbits diet supplemented with tomato powder decreased the harmful effects of age factor on above mentioned parameters.

Heat stress increases lipid peroxidation and depresses growth in birds **(Sahin et al., 2003; Sahin and Kucuk, 2003)**. These finding coincide with previous findings by **Porrini and Riso, (2000)** who reported that dietary supplementation with tomato products increased serum lycopene levels and reduced endogenous levels of oxidation of lipids, proteins, lipoproteins, and DNA.

Table 13: Overall means (Mean ±SE) and the interaction of blood plasma total lipids (TL), triglyceride (TG), urea and lactic dihydrogenase (LDH) for young and old VL rabbit bucks as affected by tomato powder dietary supplementation during winter and summer seasons.

Item	TL (mg/dl)	TG (mg/dl)	Urea (mg/dl)	LDH (IU)
Effect of season (S)				
Winter(W)	449.50 ± 5.76[b]	52.11 ± 1.57[b]	44.16 ± 0.64[b]	1073.63±12.04[b]
Summer (Su)	467.42 ±5.93[a]	57.52 ±1.70[a]	46.04± 0.64[a]	1133.13±13.40[a]
P value	0.0036	0.0023	0.0026	0.0001
Effect of age (A)				
young(Y)	430.39 ±4.37[b]	48.38 ±1.27[b]	41.85 ± 0.46[b]	1053.13±12.01[b]
old (O)	486.53 ± 5.59[a]	61.25 ±1.71[a]	48.34 ± 0.61[a]	1153.63±11.78[a]
P value	<0.0001	<0.0001	<0.0001	<0.0001
Effect of treatment (T)				
Without TP (NTP)	479.98 ± 5.97[a]	61.49 ±1.68[a]	47.77 ± 0.63[a]	1145.23±11.23[a]
With TP (WTP)	436.94 ± 4.80[b]	48.14 ±1.29[b]	42.42± 0.51[b]	1061.5 ±13.28[b]
P value	<0.0001	<0.0001	<0.0001	<0.0001
Interaction (S*A)				
W*Y	418.58 ±5.70	45.56±1.67	41.31±0.75	1027.25 ±16.28
Su*Y	442.20± 6.15	51.20 ± 1.79	42.40±0.52	1079.00±16.88
W*O	480.43 ± 7.27	58.66 ± 2.26	47.00 ± 0.82	1120.00 ±14.55
Su*O	492.64 ±8.49	63.84 ±2.52	49.67±0.85	1187.25 ±17.11
P value	0.3470	0.8968	0.1983	0.6046
Interaction (S*T)				
W*NTP	469.93 ± 7.93	59.17 ± 2.18	47.10 ± 0.85	1120.13 ±15.15
Su*NTP	490.04 ±8.73	63.81±2.54	48.45 ±0.93	1170.33 ±15.79
W*TP	429.08 ±7.07	45.05 ±1.66	41.21 ±0.69	1027.13 ±15.71
Su*TP	444.79 ±6.33	51.23 ±1.76	43.63 ± 0.71	1095.93 ±20.19
P value	0.7161	0.6592	0.3858	0.5344
Interaction (A*T)				
Y*NTP	449.84 ±4.54	54.48±1.41	43.99±0.50	1101.05±13.83
Y*TP	410.93 ±6.12	42.27 ±1.58	39.72±0.61	1005.20 ±16.60
O*NTP	510.13 ± 8.7	68.49 ±2.64	51.55 ± 0.79	1189.40 ± 14.8
O*TP	462.94±4.59	54.01 ±1.50	45.12 ± 0.57	1117.85±16.64
P value	0.4944	0.5176	0.0812	0.4172
Interaction (S*A*T)				
W*Y*NTP	438.08±4.88	117.37±1.46	44.06±0.77	1078.50±20.34
W*Y*TP	399.08±8.34	103.75 ±2.10	38.56 ±0.96	976.00±19.95
W*O*NTP	501.77 ±11.30	130.97 ±3.54	50.14 ± 1.17	1161.75±18.59
W*O*TP	459.09±6.41	116.35±1.65	43.87±0.59	1078.25±18.44
Su*Y*NTP	461.60±6.81	121.60 ± 2.36	43.93 ±0.65	1123.60±17.82
Su *Y*TP	422.80±8.33	111.29±2.26	40.87±0.67	1034.40±25.36
Su *O*NTP	518.49±13.47	136.01±3.93	52.97±1.00	1217.05 ±21.81
Su *O*TP	466.79±6.63	122.18±2.37	46.38±0.90	1157.45 ±25.14
P value	0.7032	0.7187	0.2642	0.8594

[a,b] Means within a column not sharing similar superscripts are significantly different (P<0.05).

Suganuma and Inakuma (1999) reported that the uptake of tomato inhibited the increase of lipid peroxide in mouse plasma. They add that lycopene might act as an antioxidant in plasma and that it protected plasma lipoprotein from oxidative modification.

Table (14) shows the overall means and the interaction of blood plasma thiobarbituric acid-reactive substances (TBARS), superoxide dismutase (SOD) and Glutathione S-transferase (GST) in young and old VL rabbit bucks during dietary supplementation without or with 1% tomato powder through winter and summer seasons.

Data showed that blood plasma TBARS were significant ($p \leq 0.01$) increased, and blood plasma SOD and GST were significant ($p \leq 0.01$) decrease in rabbit bucks due to exposure to heat stress in summer season compared to winter, regardless of age or treatment effect. Significantly decreased ($p \leq 0.01$) in blood plasma SOD and GST were shown in old rabbits compared with young one, while significant ($p \leq 0.01$) increased was detected in blood plasma TBARS regardless of season or treatment effects. Dietary supplementation with 1% TP caused significant ($p \leq 0.01$) decrease in blood plasma TBARS and significant increase in blood plasma SOD and GST compared to unsupplemented group (Table 14), regardless of season or age effects.

The results in Table (14) indicated no significant between S*A on blood plasma TBARS, while blood plasma SOD and GST were significantly affected, where the old rabbit had the lowest values in both seasons compared to other groups. Significant interaction only between S*T in blood plasma TBARS was found, where the highest concentration was obtained for rabbit that not fed TP during summer season. Significant interaction between A*T was found only in blood plasma GST, while no significant detected on TBARS and SOD. The highest concentration of blood GST was obtained in young rabbits that fed tomato powder.

On the other hand, the highly significant interaction between S*A*T was found only in blood plasma SOD, where the lowest significant concentration was obtained in old rabbit that fed 0%TP during summer season (Table 14), tomato powder supplementation decreased significantly the progress of rabbit age especially during summer season. No significant different was found on blood plasma TBARS and GST.

In general, improvement the blood plasma antioxidant and reduction in blood plasma TBARS was observed due to fed diet supplemented with 1% tomato powder compared with other groups in both winter and summer seasons. The results of the study are in agreement with **Bose and Agrawal (2007)** who showed that lycopene may elevate the levels of superoxide dismutase (SOD), GSH-Px, and glutathione reductase, which are the most important enzymes involved in antioxidant activity, thereby, decreasing oxidative stress. Also, **Sgorlon *et al.* (2006)** showed that superoxide dismutase (SOD) was up-regulated for tomato pomace (TOM, 10 g/head/day) in ewes.

Decreasing TBARS may be attributed to lycopene (tomato powder containing) because of its ability to protect cells against oxidative damage **(Heber and Lu, 2002)**. Because of its high number of conjugated, double-bound lycopene might have quenched superoxide and other free radical anions. In addition, lycopene may elevate the levels of superoxide dismutase (SOD), GSH-Px, and glutathione reductase, metabolite, which plays

a major role in maintaining high concentrations of GSH-Px activity. Glutathione peroxidase is the main enzyme involved in removing H_2O_2 generated from superoxide anion by superoxide dismutase (**Alshatwi *et al.* 2010)**.

Table (15) shows the overall means and the interaction of blood plasma testesterone in young and old VL rabbit bucks during dietary supplementation with 1% tomato powder through winter and summer seasons.

Data showed that blood plasma testosterone did not significant differences in rabbit bucks due to exposure to heat stress in summer season compared to winter, regardless of age or treatment effect. Significant decreased in blood plasma testosterone was shown in old rabbits compared with young one, regardless of season or treatment effects. Dietary supplementation with 1% TP caused significant increase in blood plasma testosterone compared to unsupplemented group (Table 15), regardless of season or age effects.

The results in Table (15) showed significant interaction in blood plasma testosterone only between A*T, where the highest significant concentration in blood plasma testosterone was found in the young rabbit that fed diet contained TP supplementation. Also, the significant interaction between S*A*T showed highly significant level of blood plasma testosterone during winter and summer seasons for young rabbit that fed diet contained 1% TP supplementation. No significant interaction between S*A and S*T on blood plasma testeserone.

The data showed significant improvement in the blood plasma testosterone due to fed diet supplemented with 1% tomato powder compared with other groups in both winter and summer seasons. Also, fed old rabbits diet supplemented with tomato powder significantly decreased the harmful effects of age factor on blood plasma testosterone.

Testosterone is required for maturation of male germ cells and sperm production and quality **(Walker, 2009).** Exposure to hyperthermia is harmful for spermatogenesis and also decreases testosterone levels **(Graves, 1978)**. A significant rise in blood and seminal plasma testosterone levels by about 22.9% was reported in male rabbit treatment with Se+Vit E under hot summer conditions **(El-Masry *et al*., 1994)**. Also, **Yousef *et al.* (2006)** and **Kamel (2012)** reported that folic acid as antioxidant nutrients significantly increased plasma testosterone levels in male rabbits.

Table 14: Overall means (Mean±SE) and the interaction for blood plasma thiobarbituric acid-reactive substances (TBARS), superoxide dismutase (SOD) and Glutathione S-transferase (GST) for young and old VL rabbit bucks as affected by tomato powder dietary supplementation during winter and summer seasons.

Item	TBARS (nmol/ml)	SOD (IU)	GST (IU)
Effect of season (S)			
Winter(W)	1.669 ± 0.025^{b}	2.60 ± 0.04^{a}	3.63 ± 0.05^{a}
Summer (Su)	1.744 ± 0.021^{a}	2.45 ± 0.05^{b}	3.37 ± 0.05^{b}
P value	0.0056	0.0011	<0.0001
Effect of age (A)			
young(Y)	1.607 ± 0.022^{b}	2.61 ± 0.04^{a}	3.64 ± 0.06^{a}
old (O)	1.807 ± 0.020^{a}	2.44 ± 0.05^{b}	3.36 ±0.05^{b}
P value	<0.0001	0.0075	0.0001
Effect of treatment (T)			
Without TP (NTP)	1.813 ±0.020^{a}	2.31 ± 0.04^{b}	3.27 ± 0.04^{b}
With TP (WTP)	1.601 ± 0.020^{b}	2.74 ± 0.04^{a}	3.73 ± 0.05^{a}
P value	<0.0001	<0.0001	<0.0001
Interaction (S*A)			
W*Y	1.545 ± 0.040	2.73 ± 0.06^{a}	3.83 ± 0.09^{a}
Su*Y	1.669 ± 0.030	2.49 ± 0.06^{ab}	3.56 ± 0.07^{b}
W*O	1.794 ± 0.02	2.46 ± 0.06^{b}	3.43 ± 0.06^{b}
Su*O	1.819 ± 0.03	2.42 ± 0.07^{b}	3.30 ± 0.07^{b}
P value	0.0566	0.0159	0.0260
Interaction (S*T)			
W*NTP	1.746 ± 0.034^{b}	2.40 ± 0.05	3.50 ± 0.06
Su*NTP	1.879 ± 0.030^{a}	2.23 ± 0.06	3.17 ± 0.06
W*TP	1.592 ± 0.035^{c}	2.80 ± 0.06	3.89 ± 0.08
Su*TP	1.609 ± 0.027^{c}	2.68 ± 0.05	3.57 ± 0.06
P value	0.0296	0.4709	0.3068
Interaction (A*T)			
Y*NTP	1.732 ± 0.029	2.40 ± 0.05	3.32 ± 0.06^{c}
Y*TP	1.482 ± 0.029	2.83 ± 0.05	3.95 ± 0.08^{a}
O*NTP	1.894 ± 0.033	2.35 ± 0.07	3.34 ± 0.07b^{c}
O*TP	1.719 ±0.020	2.66 ± 0.06	3.51± 0.06^{b}
P value	0.1646	0.9906	0.0064
Interaction (S*A*T)			
W*Y*NTP	1.76 ± 0.02	2.46 ± 0.06^{cd}	3.44 ± 0.06
W*Y*TP	1.56 ± 0.04	3.01 ± 0.07^{a}	4.23 ± 0.10
W*O*NTP	1.84 ± 0.04	2.34 ± 0.08^{d}	3.29 ± 0.10
W*O*TP	1.75 ± 0.03	2.58 ± 0.08^{bc}	3.56 ± 0.06
Su*Y*NTP	1.80 ± 0.03	2.33 ± 0.08^{d}	3.20 ± 0.08
Su *Y*TP	1.64 ± 0.03	2.64 ± 0.06^{bc}	3.67± 0.09
Su *O*NTP	1.88 ± 0.05	2.12± 0.08^{e}	3.13 ± 0.10
Su *O*TP	1.76 ± 0.03	2.73 ± 0.07^{b}	3.47 ± 0.09
P value	0.6702	0.0004	0.0916

^{a,b,c,d,e} Means within a column not sharing similar superscripts are significantly different (P<0.05).

Table 15: Overall means (Mean ±SE) and the interaction for blood plasma testesterone of young and old V-line rabbit bucks as affected by tomato powder (TP) dietary supplementation during winter and summer seasons.

Item	Testosterone
Effect of season (S)	
Winter (W)	5.38 ± 0.13
Summer (Su)	5.12±0.15
P value	0.0568
Effect of age (A)	
young(Y)	6.07 ±0.12[a]
old (O)	4.44 ±0.09[b]
P value	<0.0001
Effect of treatment (T)	
Without TP (NTP)	4.89 ± 0.12[b]
With TP (WTP)	5.61± 0.15[a]
P value	<0.0001
Interaction (S*A)	
W*Y	6.12 ± 0.18
Su*Y	6.01± 0.16
W*O	4.65 ± 0.09
Su*O	4.22 ± 4.21
P value	0.2275
Interaction (S*T)	
W*NTP	5.08 ± 0.11
Su*NTP	4.70 ± 0.20
W*TP	5.69 ± 0.22
Su*TP	5.53 ± 0.21
P value	0.4462
Interaction (A*T)	
Y*NTP	5.56 ± 0.10[b]
Y*TP	6.57± 0.19[a]
O*NTP	4.22 ± 0.15[d]
O*TP	4.65 ± 0.10[c]
P value	0.0372
Interaction (S*A*T)	
W*Y*NTP	5.50 ± 0.14[b]
W*Y*TP	6.73 ±0.28[a]
W*O*NTP	4.30 ± 0.19[cd]
W*O*TP	4.65 ± 0.12[c]
Su*Y*NTP	5.62±0.15[b]
Su *Y*TP	6.42 ±0.26[a]
Su *O*NTP	3.79 ± 0.25[d]
Su *O*TP	4.65 ±0.16[c]
P value	0.0227

[a,b,c,d] Means within a column not sharing similar superscripts are significantly different (P<0.05).

4.1.5. Apparent digestibility coefficients of nutrients:

Tables (16 and 17) show the overall means and the interaction of dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), ether extracts (EE) and nitrogen Free extract (NFE) in VL ageing rabbit during dietary supplementation without or with 1% tomato powder through winter and summer seasons.

Data showed that DM, OM, CP and NFE were significantly decreased, while CF and EE were not significantly differences in rabbits during summer season as compared to winter season, regardless of age or treatment effect. Significantly decreased in DM, OM, CP, CF, EE and NFE were detected in old rabbits compared with young rabbits, regardless of season or treatment effects. Dietary supplementation with 1% TP caused significantly increased in DM, CP and CF, while OM, EE and NFE were not significantly differences compared to unsupplemented group (Tables 16 and 17), regardless season or age effects.

No significant different between S*A on DM, while the significant interaction was found on OM, CP, CF, EE and NFE where the lowest significant values were found for old rabbit in both seasons. Also, all previous parameters were significantly interacted between A*T except EE, where the young rabbit that fed diet contained 1% TP showed highest significant values as compared other groups.

On the other hand, no significant interaction was detected between between S*T and between S*A*T on the previous digestive coefficients parameters (Tables 16 and 17).

In general, tthe data showed improving in the digestibility coefficients as affected by tomato powder supplementation, especially with the young rabbit bucks. Also, fed old rabbits diet supplemented with tomato powder significantly decreased the harmful effects of age factor and heat stress on the digestibility coefficients.

The results of the study are in agreement with **Tůmova *et al*. (2006a, b)** who found that the dry matter and crude protein digestibility was higher in early weaned rabbits and decreased with age.

Studies showed the digestibility of dry matter (DM), crude protein (CP), ether extract (EE) and energy (E) and metabolizable energy (ME) of diets to be depressed when the animals are exposed to high temperatures **(Marai *et al*., 2001).**

Shafie *et al*. (1994) found the digestibilities of DM, OM, CP, CF and NFE to be slightly more in rams maintained at 35 ˚C.

In rabbits **Ahmed *et al.* (1994)** concluded that DM digestibility of 10% tomato pomace diet was significantly ($P < 0.05$) higher than that of 30% tomato pomace one, but it was insignificantly higher as compared to control and 20% tomato pomace ones. The average OM and CF digestibility values were significantly ($P<0.05$) higher in 10% tomato pomace diet than those in 20 and 30% tomato pomace diet and insignificantly higher than those of control diet. The EE digestibility value was significantly ($P <0.05$) higher at 20% dietary level of tomato pomace than in control and 30% level of tomato pomace, meanwhile it was insignificantly higher than that in the diet with 10% tomato pomace. The NFE digestibility of the control diet (control) was significantly ($P < 0.05$) higher than that for dietary level of 30% tomato pomace, while it was insignificantly higher than that in diets with 10 and 20% level of tomato pomace. There were no significant differences in CP digestibility values among all groups. The TDN value of 10%

tomato pomace fed rabbits was significantly ($P < 0.05$) higher than that of 30% TP fed ones, and insignificantly higher than that of control and 20% TP fed rabbits. The DCP value of 10% TP fed group was insignificantly higher than that of other groups, and that may be due to the increase in DM, OM, CF and CP digestibilities. They also concluded that the 10% TP fed group had the highest values of DM, OM, CF and CP digestibility coefficients.

Abd El-Razik (1996) reported that substitution of maize with tomato pomace in rabbit diets did not affect nutrients digestibility.

Lloyd and McCay (1954) resulted that increasing age in Beagles was not accompanied by a decline in digestive efficiency. Conversely, the older dogs demonstrated higher apparent digestibility coefficients for fat and protein.

Table 16: Overall means (Mean ±SE) and the interaction for digestibility coefficients of dry matter (DM), organic matter (OM), crude protein (CP) and crude fiber (CF)of young (Y) and old (O) V-line rabbit bucks as affected by tomato powder (TP) dietary supplementation during winter and summer seasons.

Item	DM (%)	OM (%)	CP (%)	CF (%)
Effect of season (S)				
Winter(W)	60.54 ± 0.99[a]	57.75 ±1.44[a]	60.75 ± 0.84[a]	34.25 ±1.74
Summer (Su)	58.13 ±0.86[b]	54.58 ±0.80[b]	55.83 ±1.55[b]	33.75 ±1.07
P value	<0.0001	0.0002	<0.0001	0.3597
Effect of age (A)				
young(Y)	62.13 ±0.63[a]	59.50 ±0.92[a]	61.83 ±0.72[a]	38.33±0.70[a]
old (O)	56.54±0.41[b]	52.83 ±0.56[b]	54.75±1.19[b]	29.67±0.53[b]
P value	<0.0001	<0.0001	<0.0001	<0.0001
Effect of treatment (T)				
Without TP (NTP)	58.42±0.80[b]	55.92±1.00	57.17±1.27[b]	32.92 ±1.26[b]
With TP (WTP)	60.25±1.09[a]	56.42 ±1.47	59.42 ±1.54[a]	35.08 ±1.54[a]
P value	0.0002	0.4499	0.0011	0.0011
Interaction (S*A)				
W*Y	63.50 ±0.82	62.17 ±0.60[a]	63.00 ±0.97[a]	39.67 ±0.99[a]
Su*Y	60.75 ±0.54	56.83 ±0.70[b]	60.67±0.88[b]	37.00 ±0.68[b]
W*O	57.58±0.37	53.33 ±0.99[c]	58.50±0.43[c]	28.83 ±0.75[d]
Su*O	55.50 ±0.43	52.33 ±0.56[c]	51.00 ±0.63[d]	30.50 ±0.62[c]
P value	0.3739	0.0046	0.0003	0.0011
Interaction (S*T)				
W*NTP	59.50 ±1.10	57.83 ±1.49	59.67 ±0.76	32.83±2.26
Su*NTP	57.33 ±1.09	54.00 ±0.86	54.67 ±1.99	33.00 ±1.37
W*TP	61.58±1.64	57.67 ±2.63	61.83 ±1.45	35.67±2.72
Su*TP	58.92 ±1.34	55.17 ±1.40	57.00 ±2.45	34.50 ±1.73
P value	0.5021	0.3175	0.8814	0.2273
Interaction (A*T)				
Y*NTP	60.75±0.57[b]	58.33 ±1.28[b]	60.00 ±0.58[b]	36.67 ±0.67[b]
Y*TP	63.50±0.80[a]	60.67 ±1.23[a]	63.67 ±0.76[a]	40.00±0.77[a]
O*NTP	56.08±0.58[c]	53.50 ±0.67[c]	54.33 ±1.87[c]	29.16±0.95[c]
O*TP	57.00±0.58[c]	52.17±0.87[c]	55.17 ±1.62[c]	30.17 ±0.48[c]
P value	0.0242	0.0128	0.0216	0.0443
Interaction (S*A*T)				
W*Y*NTP	61.83 ±0.60	61.00±0.58	61.00 ±0.58	37.67 ±0.88
W*Y*TP	65.16 ±0.44	63.33 ±0.33	65.00 ±0.58	41.67 ±0.33
W*O*NTP	57.17 ±0.44	54.67 ±0.88	58.33 ±0.88	28.00 ±1.15
W*O*TP	58.00 ±0.58	52.00 ±1.53	58.67 ±0.33	29.67 ±0.88
Su*Y*NTP	59.67 ±0.33	55.67 ±0.88	59.00 ±0.58	35.67±0.67
Su *Y*TP	61.83 ±0.44	58.00 ±0.58	62.33 ±0.88	38.33±0.33
Su *O*NTP	55.00 ±0.58	52.33 ±0.33	50.33 ±0.88	30.33 ±1.33
Su *O*TP	56.00 ±0.57	52.33 ±1.20	51.67 ±0.88	30.67±0.33
P value	0.3739	0.3175	0.4599	1.0000

[a,b,c] Means within a column not sharing similar superscripts are significantly different (P<0.05).

Table 17: Overall means (Mean ±SE) and the interaction for digestibility coefficients of ether extract (EE) and nitrogen-free extract (NFE) of young (Y) and old (O) V-line rabbit bucks as affected by tomato powder (TP) dietary supplementation during winter and summer seasons.

Item	EE (%)	NFE (%)
Effect of season (S)		
Winter(W)	61.92 ±1.31	57.75 ±1.51[a]
Summer (Su)	60.75 ±0.84	54.42±0.75[b]
P value	0.0642	0.0010
Effect of age (A)		
young(Y)	64.50 ±0.63[a]	58.92 ±1.13[a]
old (O)	58.17 ±0.52[b]	53.25 ±0.79[b]
P value	<0.0001	<0.0001
Effect of treatment (T)		
Without TP (NTP)	60.92±0.94	55.92±0.84
With TP (WTP)	61.75 ±1.26	56.25±1.63
P value	0.1733	0.6868
Interaction (S*A)		
W*Y	66.00 ±0.73[a]	62.00 ±0.97[a]
Su*Y	63.00 ±0.58[b]	55.83 ±0.95[b]
W*O	57.83 ±0.60[c]	53.50 ±1.38[c]
Su*O	58.50 ±0.89[c]	53.00 ±0.89[c]
P value	0.0070	0.0035
Interaction (S*T)		
W*NTP	61.83 ±1.49	58.00 ±1.03
Su*NTP	60.00 ±1.15	53.8 ±0.54
W*TP	62.00 ± 62.01	57.50 ±3.00
Su*TP	57.00 ±2.45	34.50 ±1.73
P value	0.2703	0.3209
Interaction (A*T)		
Y*NTP	63.50 ±0.76	57.16 ±1.35[b]
Y*TP	65.50 ±0.89	60.67 ±1.61[a]
O*NTP	58.33 ±0.80	54.67 ±0.80[c]
O*TP	58.00 ±0.73	51.83 ±1.14[d]
P value	0.0642	0.0016
Interaction (S*A*T)		
W*Y*NTP	65.00 ±0.58	60.00 ±0.58
W*Y*TP	67.00 ±1.15	64.00 ±0.58
W*O*NTP	58.67 ±0.88	56.00 ±1.00
W*O*TP	57.00 ±0.58	51.00 ±1.53
Su*Y*NTP	62.00 ±0.58	54.33 ±0.88
Su *Y*TP	64.00 ±0.58	57.33 ±1.20
Su *O*NTP	58.00 ±1.53	53.33 ±0.67
Su *O*TP	59.00 ±1.15	52.66 ±1.86
P value	0.2703	0.1219

[a,b,c,d] Means within a column not sharing similar superscripts are significantly different (P<0.05).

SUMMARYAND CONCLUSION

The present study was carried out at the Rabbits Research Unit, Department of Animal and Fish Production, Faculty of Agriculture (Saba Basha), Alexandria University, during the period from from January 2010 to March 2010 (winter) and from July 2010 to September 2010 (summer).

A factorial design (2 **X** 2 **X** 2) was applied in this experiment, to study the effect of three ambient heat stress conditions with or without tomato powder supplementation growth performance, digestibility, semen quality, some blood and seminal plasma components, and antioxidant status under age in V-line rabbits.

Twenty mature V line (VL) rabbit bucks were randomly taken and classified into four groups each of 5 bucks. The first group included bucks at 7-9 months without TP supplementation (YNTP), the second group included bucks at 7-9 months with 1% of TP/kg diet supplementation (YTP), while the third group included aged bucks at 22-24 months-old without TP supplementation (ONTP), and fourth group included aged bucks at 22-24 months-old with 1% of TP/kg diet supplementation (OTP), respectively.

The results obtained could be summarized as follows:-

1- Significantly increased in BW, WI and RT were dictated in older rabbits compared with young rabbits, regardless of the season or treatment effects, while the young rabbits significantly consumed more diet than older rabbits. Dietary supplementation with 1% TP caused significantly increased in BW, FI and WI while RT was significantly decreased compared to the unsupplemented group in spite of season or age effects. Highly significant interaction between season (S) and age (A), season and treatment (T), age and treatment and between S*A*T in the effect on BW, FI, WI and RT except the interaction between S*A on RT and the interaction between S*T on FI, RT and the interaction between A*T on WI.

2- Ejaculate volume (EV), sperm motility (SM), sperm concentration (CON), in V-Line were significantly increased in rabbits during winter compared to summer season regardless of age or treatment effect. Significantly increased in EV, SM and CON were dictated in young rabbits compared with older rabbits, regardless of season or treatment effects. Dietary supplementation with 1% TP caused significantly increased in EV, SM and CON compared to unsupplemented group despite season or age effects. Insignificant interaction were found between season (S) and age (A), season and treatment (T), age and treatment and between S*A*T in the effect on EV, SM and CON except the interaction between S*A on EV and SM, the interaction between S*T on SM, the interaction between A*T on EV.

3-Total sperm output (TSO), total number of motile sperm (TMS) and packed sperm volume (PSV %) in V-Line were significantly increased in rabbits during winter compared to summer season regardless of age or treatment effect. Significantly increased in TSO, TMS and PSV % were dictated in young rabbits compared with older rabbits despite the consequences of season or treatment effects. Dietary supplementation with 1% TP caused significantly increased in TSO, TMS and PSV % compared to unsupplemented group regardless season or age effects. Significant interaction had been found between season (S) and age (A), and age and treatment in the effect on TSO, TMS and PSV %, while the

interaction between S*T and the interaction between S*A*T had no any significant effect on TSO, TMS and PSV %.

4- abnormal sperm, , Live sperm and The total functional sperm fraction (TFSF) in V-Line were significantly increased, while abnormal sperm was significantly decreased in rabbits during winter compared to summer season regardless of age or treatment effect. Significantly increased in Live sperm and TFSF were dictated, while abnormal sperm significantly decreased in young rabbits compared with older rabbits, regardless of season or treatment effects. Dietary supplementation with 1% TP caused significantly increased in Live sperm and TFSF, while abnormal sperm decreased significantly compared to unsupplemented group regardless season or age effects. There were significant interactions between season (S) and age (A), season and treatment (T) in the effect on abnormal sperm, Live sperm and TFSF, except the interaction between S*A on abnormal sperm and the interaction between S*T on TFSF, while the interaction between A*T and S*A*T had no any significant effect on abnormal sperm, Live sperm and TFSF except the interaction between A*T on TFSF.

5- Initial hydrogen ion concentration (PH) and initial fructose in V-Line were significantly increased in rabbits during winter compared to the summer season, while PH non significant changes by the season effect regardless of age or treatment effect. Significantly increased in initial fructose was dictated, while PH significantly decreased in young rabbits compared with older rabbits, apart from season or treatment effects. Dietary supplementation with 1% TP caused significantly increased in initial fructose, while PH decreased significantly compared to the unsupplemented group regardless season or age effects.

6- Acid phosphatase (ACP) was significantly increased, while alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were no significant differences in rabbits during winter compared to summer season regardless of age or treatment effect. Significantly increased in ALP and ACP were dictated, while ALT, AST and LDH significantly decreased in young rabbits compared with older rabbits, regardless of season or treatment effects,. Dietary supplementation with 1% TP caused significantly increased in ALP and ACP, while ALT, AST and LDH decreased significantly compared to the unsupplemented group regardless season or age effects. There were insignificant interaction between S*A, S*T and S*A*T in the effect on ALT, AST, ALP, ACP and LDH except the interaction between S*A on ACP, S*T on ACP and LDH, S*A*T on ALT and ACP. The results indicated a significant interaction between A*T in the effect on ALT and LDH.

7- Glutathione S-transferase (GST) was significantly increased, while a thiobarbituric acid-reactive substance (TBARS) was significantly decreased in rabbits during winter compared to summer season regardless of age or treatment effect. Significantly increased in GST was dictated, while TBARS was significantly decreased in young rabbits compared with older rabbits, regardless of season or treatment effects. Dietary supplementation with 1% TP caused significantly increased in GST and significantly decrease in TBARS compared to the unsupplemented group regardless season or age effects.

8- Alkaline phosphatase (ALP) of blood plasma of V-Line rabbits was significantly increased, while alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were significantly

decreased and ACP was no significant differences in rabbits during winter compared to summer season regardless of age or treatment effect. Significantly increased in ALT, AST, ACP and LDH were dictated in older rabbits compared with young rabbits, regardless of the season or treatment effects, while the older rabbits significantly decreased in ALP compared with young rabbits. Dietary supplementation with 1% TP caused significantly increased in ALP, while ALT, AST, ACP and LDH were significantly decreased compared to the unsupplemented group regardless season or age effects.

9- Plasma total protein, albumin (Alb), globulin (Glb) and glucose in V-Line were significantly increased, in rabbits during winter compared to summer season regardless of age or treatment effect. Significantly increased in total protein, Alb, Glb and glucose were dictated in young rabbits compared with older rabbits, regardless of season or treatment effects. Dietary supplementation with 1% TP caused significantly increased in total protein, Alb, Glb and glucose compared to the unsupplemented group despite the consequences season or age effects.

10- Plasma total lipid (TL), triglyceride (TG) and UREA in V-Line were significantly increased in rabbits during summer compared to winter season regardless of age or treatment effect. Significantly increased in TL, TG and UREA were dictated in older rabbits compared with young rabbits, despite the consequences of season or treatment effects. Dietary supplementation with 1% TP caused significantly decreased in TL, TG and UREA compared to unsupplemented group regardless season or age effects.

11- Superoxide dismutase (SOD) and Glutathione S-transferase (GST) of blood plasma V-Line rabbits were significantly increased, while thiobarbituric acid-reactive substances (TBARS) was significantly decreased in rabbits during winter compared to summer season regardless of age or treatment effect. Significantly increased in SOD and GST were dictated, while TBARS was significantly decreased in young rabbits compared with older rabbits, in spite of of season or treatment effects. Dietary supplementation with 1% TP caused significantly increased in SOD and GST, while TBARS was significantly decreased compared to the unsupplemented group despite season or age effects. There were significant interactions between season and treatment (T), age and treatment and between S*A*T in the effect on TBARS, SOD and GST, except the interaction between S*T on GST and TBARS, the interaction between A*T on SOD and the interaction between S*A*T on TBARS.

12- Testosterone concentration was no significant differences in rabbits during winter compared to summer season regardless of age or treatment effect. Significantly increased in testosterone concentration was dictated in young rabbits compared with older rabbits, at any rate of season or treatment effects. Dietary supplementation with 1% TP caused significantly increased in testosterone concentration compared to the unsupplemented group regardless season or age effects. Significant interactions were found between, age and treatment and between S*A*T in the effect on testosterone concentration, while the interaction between S*A and S*T had an insignificant effect.

13- Dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), ether extracts (EE) and nitrogen Free extract (NFE) in V-Line were significantly increased, while CF and EE were no significant differences in rabbits during winter compared to summer season regardless of age or treatment effect.

Significantly increased in DM, OM, CP, CF, EE and NFE were dictated in young rabbits compared with older rabbits, in spite of the season or treatment effects. Dietary supplementation with 1% TP caused significantly increased in DM, CP and CF while OM, EE and NFE were no significant differences compared to the unsupplemented group regardless season or age effects. There were significant interactions between S*A and A*T in the effect on DM, OM, CP, CF, EE and NFE except the interaction between S*A on DM and the interaction between A*T on EE while The results indicated insignificant interaction between S*T and between S*A*Ton all apparent digestibility coefficients studied.

Conclusion:

It could be concluded from the present study that heat stress significantly dramatically affected the productive performance and physiological responses of Buck rabbits. Also, results showed that increasing age of V-Line rabbit bucks had significant bad effects in the most studied parameters. However, dietary supplementation with 1% tomato powder alleviated the harmful and adverse effects to season and age on productive and reproductive performance and biochemical parameters of V-Line rabbit bucks.

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