NUTRITIONAL EVALUATION OF A FUNGAL-YEAST PROBIOTIC FORTIFIED WITH NATURAL ANTIOXIDANTS ON PERFORMANCE OF SHEEP. 3- IMPACT ON BODY TEMPERATURE, INTERNAL ANTIOXIDANTS, HEMATOLIGICAL AND HISTOPATHOLOGICAL CHANGES OF LOCAL SHEEP RAISED UNDER DIFFERENT CLIMATIC CONDITIONS.

Azza M. Kamal[1]; Ibtisam M. Azzam[1]; Y.H. Hafez[2]; A.Y. El-Badawi[3] and Faten F. Abou Ammou[2].

[1]Animal Health Research Institute, Agriculture Research Center, Dokki, Giza, Egypt.

[2]Animal Production Research Institute, Agriculture Research Center, Dokki, Giza, Egypt.

[3]Animal Production Dept., National Research Centre, Dokki, Giza, Egypt.

(Received 17/7/ 2012, Accepted 31/8/ 2012)

SUMMARY

This study was carried out on two breeds of local sheep to evaluate the nutritional impact of feeding different supplements of the bio-additive EMMH on body temperature, body weight gain, blood picture, blood antioxidants and histopathological statues of liver and kidney tissues. The tested EMMH is a bio-natural feed additive made of *T. reesei*, A. oryzae and active dry yeast S. cerevisiae in co-culture fortified with mutli-herbal antioxidants. Two feeding experiments were carried out on 32 heads of male Rahmany lambs at El-Serw Station (North Western Egypt) for 168 days and on 24 heads of male Farafra lambs at Mallawy Station (Upper Egypt) for 126 days. Lambs of both breeds were of three months old with an initial average weight of 18.04±0.53 kg and 18.53±0.11 kg, respectively. Lambs of each flock were distributed by weight into four similar groups, where each group was individually fed one of four diets supplemented with EMMH at 0, 0.3, 0.4 and 0.5% of the concentrate feed mixture (CFM). Un supplemented or EMMH-supplemented CFM were offered at 2.5 % of body weight + 1 % of body weight Berseem hay for Rahmany and 3 % of body weight CFM for Farafra lambs, while chopped rice straw was offered adlib to both flocks. Minimum and maximum ambient temperature and relative humidity were recorded. Daily gain and rectal temperature were biweekly recorded while blood samples were individually collected twice during the last month of each feeding experiment. By end of the feeding periods, three representative animals were chosen randomly and slaughtered. Specimen of liver and kidney were collected of each carcass and kept in formalin solution for histopathological examination. The results illustrate that, body temperature of animals in experimental groups were within the normal range with no significant difference among groups. However slight stability of body temperature was noticed in groups fed experimental diets. Average daily gain (ADG) was improved ($p<0.01$) for both breeds with diets contained EMMH and the effect on weight gain was more pronounced with increasing the supplementation level up to 0.5% of CFM. Meanwhile, Farafra sheep exert better response to EMMH supplementation than Rahmany for ADG. No significant differences were attained among groups for blood Hb, g/dl, PVC %, TLC, µL and leukocytic series, µL. However, Hb and PCV tended to increase and neutrophils to decrease with increasing the EMMH supplementation level in diets of both breeds. Significant effect ($p<0.05$) was recorded for EMMH on blood erythrocytes content of glutathione, glutathione peroixidase and superxide dismutase for Rahmany and Farafra sheep. The highest values of blood antioxidants were obtained with the highest EMMH supplementation level (0.5%). The microscopic examination of liver and kidney tissues showed infiltration of inflammatory cells, necrosis, malformation of the liver cord and partial fibrosis of liver and kidney tissues of sheep fed control diets, whereas the case was more acute in Farafra. The tissues of both organs were visually improved and became normal for animals fed supplemented diets, particularly those in group fed 0.5% EMMH level. The results concluded that the feed additive EMMH had potential influence on improving weight gain and withstanding the high environmental temperatures, with advisable EMMH supplementation at 0.5% level of the traditional concentrates mixture.

Keywords: *sheep; growth; probiotic; blood picture; blood antioxidants and histopathological examination.*

INTRODUCTION

Understanding how probiotics exert their beneficial effects is the issue of debate nowadays. The definition of probiotic was formulated simultaneously with the use of living cultures in feed for various animals in order to substitute the application of nutritive antibiotics or chemotherapeutics. The selection of a suitable strain of a microorganism can be regarded as the primary requirement for the use as a probiotic. The composition of the probiotic preparations varies from those containing one strain of microorganism or those containing many strains. The multi-strain probiotic can act in broad spectrum and expected to be active in different species of host animals and against microbial infections (Timmerman *et al.*, 2004). Probiotics have been reported to enhance the growth of many domestic animals i.e.; dairy cows, beef cattle, sheep and goats, neonatal calves and broilers (Musa *et al.*, 2009). The claims for microbial products are: improved performance and feed conversion for the target species, reduced morbidity or mortality and benefits for the consumer through improved product quality. The microorganisms used in probiotics are those derived from *Lactobacillus, Streptococcus, Enterococcus, Bacillus, Clostridum* and *Bifidobacterium* (Kruist *et al.*, 2004). Bacterial probiotics have been effective for chicken, pigs and pre-ruminant calves, whereas yeast and fungal probiotics such as yeast (*Saccharomyces cerevisiae*), *Aspergillus oryzae* and *Tricoderma sp.* have given better results with adult ruminants (Fuller, 1999). It was also noted that the combinations of probiotics strains could increase the beneficial health effects compared with individual strains because of their synergistic adhesion effects (Collado *et al.*, 2007). The probiotic mechanism of action has been summarized by Musa *et al.* (2009) in four mechanisms:

1- Antagonism through the production of antimicrobial substances, 2- Competition with the pathogen for adhesion sites or nutritional sources, 3- Immunomodulation of the host and 4- Inhibition of bacterial toxins. The first three mechanisms are attributed to lactic acid bacteria while the last two are more specifically attributed to yeast. In applied animal nutrition probiotics of multi or separate strains showed significant improvement of feed intake, feed conversion rate, daily weight gain and total body weight in chicken, sheep, goats and cattle (Chiquette, 1995; Samli *et al.*, 2007 and Torres-Rodriguez *et al.*, 2007). Probiotics has positive effects on various digestive processes, specially cellulolysis and synthesis of microbial protein, stabilizers of ruminal pH and lactate, enhancers of the absorption of some nutrients thus display a growth – promoting effect (Mountzouris *et al.*, 2007). It was also noted that the probiotic in organism of a healthy animal stimulate the non-specific immune response and enhance the system of immune protection (Ceslovas *et al.*, 2005). Some medicinal herbs are reached sources of phenolic compounds such as thymol, carvacrol and eugenol. Flavonoids are a large class of polyphenolic of monensin (Yaghoubi *et al.*, 2010). The antioxidant activity to the free radicals and to their ability to scavenge the free radicals and interact with membranes (Tsuchiya., 2010) and/or to induce antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) or catalase (CAT) (Gladine *et al.*, 2007). Based on the pervious review, the objectives of this study were to investigate the effect of a locally made multi-strains of fungal-yeast probiotic with antioxidants (EMMH) on productive and health performance of native growing sheep raised under moderate and hot climatic conditions. The impact of feeding the tested probiotic was monitored through the following parameters: fluctuation of body temperature, weight gain, hematological parameters, blood antioxidants profile and histopathological examination of liver and kidney tissues.

MATERIALS AND METHODS

The nutritional effect of EMMH-feed additive was monitored through body temperature, body weight gain, blood picture, blood antioxidants profile and histopathological examination of liver and kidney of two breeds of local sheep. The tested feed additive abbreviated as EMMH is a co-culture of two strains of fungi (*Trichoderma reesei* and *Aspergillus oryzae*) and live dry yeast (*Saccharomyces cerevisiae*) with mixture of medicinal herbs as a natural source of antioxidants. Each kg of EMMH provides at least 2,200,000 U of fibrolytic, amyliolytic and protiolytic enzymes, 7.5^8 cells of live yeast and 125 mmol of plant antioxidants.

Feeding trials and experimental sites:

Two feeding trials were carried out on two breeds of Egyptian sheep (Rahmany and Farafra) at two stations affiliate the Animal Production Research Institute. The first trial was conducted at El-Serw

Station (Domietta Governorate – 31.28 N and 31.45 E) on 32 heads of Rahmany male lambs and the second trial was at Mallawy Station (El-Minya Governorate – 28.06 N and 30.45 E) on 24 heads of Farafra male lambs. Both breeds of experimental sheep were of three months old with an average initial weight of 18.04 ± 0.53 kg for Rahmany and 18.53 ± 0.11 kg for Farafra lambs.

Table (1): Metrological data of the two experimental sites.

Experimental site	Range of Ambient temperature, °C		Mean	Range of relative humidity,%		Mean
	Min.	Max.		Min.	Max.	
El-Serw	14.9	24.5	22.1	58.1	82.7	61.5
Mallawy	15.0	39.0	31.5	29.0	94.0	72.1

Each value is the mean of two daily records of minimum and maximum ambient temperature and relative humidity for experimental location.

The feeding period lasted 168 days for the first trial on Rahmany and 126 days for the second one on Farafra. Animals in the two feeding experiments were distributed according to their weight in four similar groups where the 1st group fed unsupplemented CFM (Control group), 2nd group 0.3 % EMMH-CFM, 3 rd group 0.4 % EMMH- CFM and the 4th group 0.5 % EMMH-CFM diets. Unsupplemented or EMMH-supplemented CFM were offered at 2.5 % of body weight + 1.0 % of weight Berseem hay with *ad lib* amount of chopped rice straw for Rahmany lambs, while unsupplemented or EMMH-supplemented CFM were fed at 3 % of body weight with *ad lib* amount of rice straw for Farafra lambs. Clean drinking water was available at all times. During the whole feeding periods animals of each group were individually weighed before feeding every two weeks to adjust the daily offered amounts of feeds. Feed refusals (if any) were daily collected, sun dried and weekly weighed to calculate the actual feed intake. Body temperature was recorded biweekly before feeding by inserting individually a clinical thermometer into rectum for one minute and care was taken to keep the thermometer bulb in close contact with rectal mucosa. At the two feeding trials three representative animals of each group were weighed, slaughtered and random samples of liver and kidney were taken for histopathological examinations.

Sampling of blood:

Ten ml of blood were individually collected twice from each experimental group during the last month of each feeding trial. Blood samples were with drawn from the jugular vein before the morning meal and kept in clean heparenized tubes under – 4 °C until analysis.

Blood picture examination:

Hemaglobin concentration (Hb, g/dl) was determined according to Henry (1964), hematocrite (Ht, %) was measured in hematocrite heparinized graduated capillary tubes using a hematocrite centrifuge at 3000 rpm for 15 minutes. White blood cells count (WBCsX10^3/µl) was determined according to Benjamin (1958).

Blood antioxidants examination:

Reduced glutathione (GSH) content was estimated chemically in the whole blood by measuring the optical density (OD) of the yellow colour that developed when 5.5 dithisl-bis (2-nitrobenzoic acid) is added to sulfhydral compounds at wave length µ12 nm according to Beutler *et al.* (1963). Estimation of glutathione peroxidase (GSH-Px) in the whole blood was chemically measured as the amount of residual GSH left after the exposure to enzyme activity for a fixed time and the reaction was measured at wave length 412 nm according to Gross et al.(1967). Superoxide dismutase (SOD) activity was estimated chemically in the whole blood by the method that depends on detecting superoxide anions by nitroblue tetrazoluim formazan and the colour development was measured at 412 nm wave length according to Minami and Yoshikawa (1979).

Histopathological examination:

Specimens of liver and kidney of slaughtered sheep (Rahmany and Farafra) from different experimental groups were collected and kept immediately in 10 % buffered formalin solution. Tissue specimens were processed to obtain live micron thickness paralin sections stained with haematoxylin and eosin according to Bancraft *et al.* (1994) for microscopic examination.

Analytical methods:

Chemical composition of experimental feeds was determined according to the standard methods of A.O.A.C. (1995) for moisture, ash, crude protein (CP, crude fiber (CF) and ether extract (EE), while

nitrogen free extract (NFE) was calculated by difference. Chemical composition of different feed-stuffs is given in Table (2).

Table (2): Chemical composition of experimental feeds.

Item	Moisture	DM composition, %					
	%	OM	CP	CF	EE	NFE	Ash
CFM*	8.70	92.80	14.80	13.80	2.81	61.39	7.20
Berseem hay	10.27	88.15	13.87	29.73	1.35	43.20	11.85
Rice straw	9.37	84.70	4.07	33.74	1.48	45.41	15.30

** Concentrate feed mixture (CFM) consists of: 35% ground yellow corn, 25% undecorticated cotton seed meal, 5% soybean meal, 15% wheat bran, 12% rice bran, 5% cane molasses, 2% lime stone and 1% common salt.*

Statistical analysis:

Data of body temperature, body weight gain and pooled data of blood picture and blood antioxidants of the two collections were subjected to one-way ANOVA according to Snedecor and Cochran (1980) using the following statistical model :

$Y_{ij} = \mu + T_i + e_{ij}$

Where: Y_{ij} is the parameter under analysis, μ is the overall mean, T_i is the effect due to treatment and Eij is the experimental error. The general linear model of SAS (2001) was applied in processing the data. Significant differences among means were tested by Dancan's multiple range test (1955).

RESULTS AND DISCUSSION

Body temperature:

Body temperature changes for Rahmany and Farafra sheep in experimental groups are given in Table (3). Mean values of body temperature were generally within the normal range for sheep (38.9 – 39.2 °C), however the values tended to decrease slightly than control for Rahmany lambs fed EMMH supplemented diets. Farafra lambs body temperature was higher than that of Rahmany lambs, which might suggest the influence of high ambient temperature in Upper Egypt zone on body temperature of Farafra. Among animals exposed to climatic heat stress, groups fed EMMH supplemented diets showed significant ($p<0.05$) decrease in body temperature than control. Moreover, both Rahmany and Farafra lambs showed lower and stable body temperature in groups fed 0.4 and 0.5 % EMMH than other groups (Fig 1). These results are in harmony with the findings of Gomez-Alarcon *et al.*(1988) and Yousef *et al.* (1996) who found that feeding rations supplemented with *Aspergillus oryzae* or yeast culture reduced heat stress which recognized by a decline in rectal temperature of sheep exposed to high ambient temperature during summer season. Similar conclusion was reported by Kobeisy (1997) and Sivakumar *et al.* (2010), who found that rectal temperature was decreased for goats fed diets supplemented with vit. E+ selenium and vit. C under heat stress conditions. They suggested that antioxidants had effective role in ameliorating the heat stress in goats. It seems logic to state that the tested feed additive EMMH had potential role in thermoregulation that allowed sheep under moderate or hot climatic conditions to maintain their body temperature stable. Another explanation might also hold true, that probiotics and natural antioxidants have active compounds against pathogens, inflammatory cells and free radicals, which in turn could help the immune system to stabilize body temperature.

Table (3): Mean body temperature (°C) of sheep in experimental groups.

Breed	Experimental groups				SE	Sig.
	Control	0.3%	0.4%	0.5%		
Rahmany	39.15	39.10	39.11	39.08	0.01	NS
Farafra	39.49^{a}	39.40^{ab}	39.33^{b}	39.33^{b}	0.02	*

a, b: Means within the same row with different superscripts differ at ($P < 0.05$).

NS= Non significant difference.

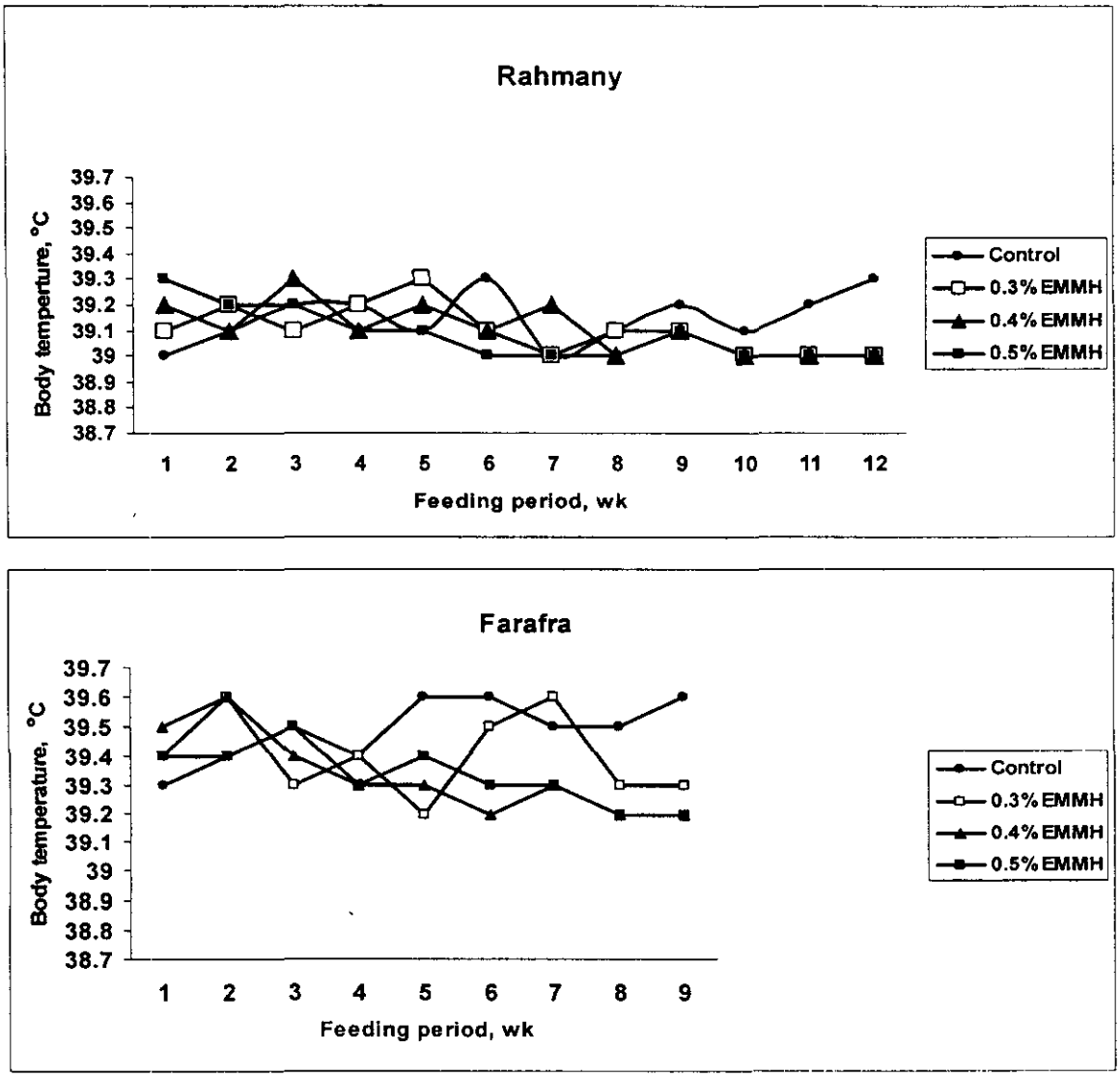

Fig. (1): Body temperature fluctuation of sheep fed experimental diets.

Table (4): Mean body weight gain of Rahmany and Farafra sheep in experimental groups.

Item	Experimental groups				SE	Sig.
	Control	0.3%	0.4%	0.5%		
			Rahmany			
No. of animals, head	8	8	8	8		
Initial weight, kg	17.53	17.97	18.22	18.43	0.18	NS
Final weight, kg	33.75^{b}	38.31^{a}	39.47^{a}	39.66^{a}	0.48	**
Total weight gain, kg	16.22^{b}	20.34^{a}	21.25^{a}	21.23^{a}	0.40	**
Average daily gain$^{!}$, g	96.54^{b}	121.09^{a}	126.49^{a}	126.30^{a}	2.39	**
			Farafra			
No. of animals, head	6	6	6	6		
Initial weight, kg	18.58	18.54	18.33	18.67	0.12	NS
Final weight, kg	32.08^{c}	33.33^{c}	35.75^{b}	38.54^{a}	0.54	**
Total weight gain, kg	13.50^{c}	14.79^{c}	17.42^{b}	19.87^{a}	0.56	**
Average daily gain$^{!!}$, g	107.15^{c}	117.38^{c}	138.25^{b}	157.75^{a}	4.47	**

! Feeding period= 168 days !! Feeding period = 126 days

a, b, c : Means within the same row with different superscripts differ at (P < 0.05).

*NS= Non significant difference ** = significant difference at (P < 0.01).*

Table (5): Blood picture of Rahmany and Farafra sheep in experimental groups.

Item	Experimental groups				SE	Sig.	Normal range
	Control	0.3%	0.4%	0.5%			
			Rahmany				
Hb, g/dl	13.40	13.49	13.92	13.96	1.32	NS	9 – 15
PCV, %	35.00	35.33	37.41	38.60	2.51	NS	27.0 - 45.0
TLC, µl	10517	10233	10017	10000	679	NS	4000 – 12000
Leukocytic series:							
Neutrophil	3868	3950	3670	3555	293	NS	700 – 6000
Lymphocyte	5937	5700	5692	5848	461	NS	2000 – 9000
Monocyte	396	308	391	366	42	NS	0 – 750
Eosinphil	315	275	264	231	58	NS	0 – 1000
Basophil	1	0	0	0	1.0	NS	0 – 300
Percentage distribution:							
N	37	39	37	36	-	-	10 – 50
L	56	56	57	58	-	-	40 – 75
M	4	3	4	4	-	-	0 – 6
E	3	2	2	2	-	-	0- 10
B	0	0	0	0	-	-	0 – 3
			Farafra				
Hb, g/dl	12.70	13.01	13.06	13.55	1.14	NS	9 – 15
PCV, %	34.76	35.01	37.33	38.60	4.05	NS	27.0 - 45.0
TLC, µl	9617	9417	9467	9400	546	NS	4000 – 12000
Leukocytic series:							
Neutrophil	3665	3455	3417	3391	182	NS	700 – 6000
Lymphocyte	5436	5365	5450	5377	321	NS	2000 – 9000
Monocyte	324	346	348	347	49	NS	0 – 750
Eosinphil	192	251	251	285	32	NS	0 – 1000
Basophil	0	0	1	0	1.0	NS	0 – 300
Percentage d' tribution :							
N	38	37	36	36	-	-	10 – 50
L	56	57	58	57	-	-	40 – 75
M	3	4	4	4	-	-	0 – 6
E	3	2	2	3	-	-	0- 10
B	0	0	0	0	-	-	0 – 3

-Each value is the mean of 16 observations (n=64) of Rahmany and 12 observations (n=48) of Farafra.

-Normal range of blood values for sheep was sited from Jain et al., 1993.

NS=non-significant difference between groups. -not statistically tested.

Body weight gain:

Data in Table (4) illustrate average daily gain (ADG) of the two experimental breeds of sheep (Rahmany and Farafra) which were cited from our previous results on the same animals. It was clear that ADG was significantly ($P<0.01$) higher for sheep fed EMMH supplemented diets and the influence of the tested additive on ADG was more marked with increasing the supplementation level. The ADG of Rahmany sheep was 121.09, 126.49 and 126.30g for groups fed 0.3, 0.4 and 0.5 % EMMH, respectively vs. 96.54 g for the control group. Farafra sheep gave better response to the feed additive in terms of ADG than Rahmany sheep. In comparison with the control diet, ADG of Rahmany was increased by nearly 30 % and 47 % for Farafra fed diets supplemented with 0.5 % EMMH. Similar results were previously reported on local sheep. Ali *et al.* (2005) found that average daily gain was improved ($P<0.05$) for animals fed probiotic supplemented diets (207 and 175 g/d) compared with animals fed control diet (115 g/d). It was stated by many investigators that probiotics are linked with a proven efficacy on the gut microflora. Administration of probiotic strains significantly improved feed intake, average daily gain and total weight of sheep, goats and cattle (Torres-Rodriguze *et al.*, 2007, Samli *et al.*, 2007 and Casey *et al.*, 2007). Probiotics were also noted to be stabilizers of ruminal pH and lactate, increased the absorption of some nutrients and displayed a growth-promoting effect.

Hematological changes:

The results of hemoglobin, packed cell volume (PCV) and total leukocyte count (TLC) of experimental groups are presented in Table (5). Hemoglobin and packed cell volume (PCV) were insignificantly increased with increasing the EMMH supplementation level in an ascending order by

either Rahmany or Farafra sheep. Such improvement of hemoglobin and PCV contents could be regarded to the increasing level of dietary antioxidants in groups fed supplemented diets.

This assumption was proposed by Leonart *et al.* (1989) and Kraus *et al.* (1997) whom found increase in Hb and PCV of animals fed diets supplemented with vit. E and C as antioxidants. On the contrary, Srikandakumar *et al.* (2003) on sheep and Sivakumar *et al.* (2010) on goats reported that both Hb and PCV contents were decreased in animals exposed to increased attack of free radicals on the RBCs membrane or to the inadequate nutrients needed for the hemoglobin synthesis as the animals exposed to heat stress consumed less feed due to the decrease of the voluntary feed intake.

Total leukocyte count (TLC) in Table (5) show that total leukocyte of the two breeds (Rahmany and Farafra) was insignificantly decreased in all EMMH treatment groups compared with control group. A typical stress response in ruminants involves the release of glucocorticoids which stimulate some characteristic changes in the pattern of white blood cells (Total leukocyte). This includes increased netriophils, a concurrent drop in total white blood cells count of lymphocytes, eosinophils and basophils which are generally decreased. Richardsson *et al.* (2002) reported that changes in hematological profile are indicative that cattle were mildly stressed while the less efficient steers had more neutrophils, fewer lymphocytes and lower white blood cell count, compared with the more efficient steers. In the study carried out by Scope *et al.* (2002) on racing pigeons, it was noted that stress has an influence on some blood parameters. Although within 3 h, most values did not exceed the reference range, there was a highly significant increase in the number and percentage of neuterophils and decrease in lymphocyte count. An insignificant decrease in the total leucocyte, basophil and monocyte counts were observed.

Blood antioxidants profile:

The blood antioxidant enzymes for Rahmany and Farafra sheep in experimental groups are presented in Table (6). The results revealed that blood concentrations of GSH, GSH-Px and SOD were significantly ($P<0.05$) increased in groups fed diets supplemented with the probiotic EMMH.

Table (6): Blood antioxidant contents of Rahmany and Farafra sheep in experimental groups.

Item	Experimental groups				SE	Sig.
	Control	0.3%	0.4%	0.5%		
			Rahmany			
GSH, mg/dl	19.54^{abc}	20.97^{ab}	21.55^{a}	22.15^{a}	0.65	*
GSH-Px, u/g Hb	51.87^{c}	52.86^{b}	52.98^{b}	53.55^{a}	0.39	*
SOD, mol/ml	21.18^{c}	23.04^{b}	24.17^{a}	23.78^{a}	0.44	*
			Farafra			
GSH, mg/dl	22.02^{c}	24.81^{b}	24.21^{b}	26.35^{a}	0.87	*
GSH-Px, u/g Hb	64.67^{c}	66.03^{b}	66.76^{b}	67.96^{a}	0.76	*
SOD, mol/ml	22.91^{c}	25.75^{b}	26.20^{a}	26.58^{a}	0.45	*

Each value is the mean of 16 observations for Rahmany sheep (n=64) and 12 observations for Farafra sheep (n=48). Mean with difference superscript are significant difference at ≤ 0.05.

The effect of the dietary probiotic on blood antioxidants was highly remarkable with increasing the supplementation level for both breeds of sheep. However, blood antioxidants values were higher for Farafra than Rahmany sheep. The present results might support the idea that the dietary probiotics increased stimulation of immune and internal antioxidants activity. The higher blood antioxidants concentration of Farafra sheep which were raised in hot climatic condition (Upper Egypt) might declare that animals exposed to heat stress produced more internal antioxidants to neutralize the damage effects of the reactive oxygen species. It is worth saying that dietary probiotics or antioxidants are more likely to operate as stimulants for internal antioxidant enzymes rather than scavengers of the free radicals. There are several studies explained the role of probiotics as antioxidants agents. Becker (2004) concluded that oxidative stress resulted in oxidative alteration of biological macromolecules such as lipids, proteins and nucleic acids. It is considerable to play a pivotal role in the pathogenesis of aging and degenerative diseases. In order to cope with an excess of free radicals produced upon oxidative stress, animal bodies have developed sophisticated mechanisms for maintaining redox homeostasis.

These protective mechanisms include scavenging or detoxification of reactive oxygen species (ROS), blocking ROS production, sequestration of transition metals, as well as enzymatic and non enzymatic antioxidant defenses produced in the body. These mechanisms are endogenous, (Hayes and McLellan, 1999 & Mascila, *et al.*, 2005) while others supplied with the diet, namely, exogenous ones. Among them, dietary probiotics have been widely studied for their strong antioxidant capacities and other properties by

which cell functions are regulated. There are several evidences in the literature reported that probiotics have antioxidant properties (Venkatesan *et al.*, 2012). Probiotics had physiologically relevant multivalent antioxidant activity (TAS) to overcome the exogenous and endogenous oxidative stress of the host (Mikelsaar and Zilmer, 2009). Also, In a controlled study involving 2 groups of 12 subjects each, daily supplementation with a mixture of 2 probiotic strains (L. rhamnosus IMC 501 and L. paracasei IMC 502; ~ 10(9) cells/day) for a period of 4 weeks increased plasma antioxidant levels and neutralizing reactive oxygen species. The study concluded that "Athletes and all those exposed to oxidative stress may benefit from the ability of these probiotics to increase antioxidant levels and neutralize the effects of reactive oxygen species (Martarelli *et al.*, 2011 and Shen *et al.*, 2011).

Venkatesan, *et al.*, (2012) revealed that probiotics may enhance phagocytic activity and increase the production of reactive oxygen metabolites by macrophages followed by an increased stimulation of immune and antioxidant activity.

Results of histopathological examination for liver tissue of animals in the experimental groups:

Rahmany sheep:

The liver section of the control group showed formation of fibrous connective tissue, edema dispersed the liver cord, infiltration of inflammatory cells and necrosis of some hepatic (Photo 1). Liver tissue of the second group fed 0.3% EMMH supplemented diet showed edema dispersed organization of the liver cord with few necrotic cells (Photo 2). The liver tissue of the third group fed 0.4% EMMH supplemented diet showed activation of Vankoffer cells, few inflammatory cells, few necrotic cell and less edema dispersion (Photo 3). The liver tissue of the fourth group fed 0.5% EMMH supplemented diet showed slight dilated sinusoid few edema dispersion and normal structure of the hepatic cord (Photo 4).

Farafra Sheep:

The liver section of the control group showed organic thrombus attached the wall of the blood vessel, infiltration of fibrous connective tissue in the portal area, infiltration of mononuclear inflammatory cells and other hepatic are suffering from nucrobiotic changes (Photo 5).Liver of the second group fed 0.3% EMMH supplemented diet showed sever edema dispersed organization of hepatocytes, fibroblast cells and infiltration of inflammatory cells, (Photo 6). The liver tissue of the third group fed 0.4% EMMH supplemented diet showed focal infiltration of mononuclear inflammatory cells, few fibrous connective tissue infiltration in the portal area and few hepatocytes showed necrosis associated with edema (Photo 7). The liver of the fourth group fed 0.5% EMMH supplemented diet is likely to be of normal structure with clear bile ducts lined by cuboidal epithelium, normal hepatic vein and less infiltration of inflammatory cells in the portal area associated with few necrosis of hepatocytes (Photo 8).

Results of histopathological examination for kidney tissue of animals in the experimental groups:

Rahmany sheep:

The kidney section of the control group showed thickening in kidney wall, sever infiltration of inflammatory cells in the interstitial tissue, sever necrosis of cells lining the renal tubules with no nuclear staining while cytoplasm is deeply eosinophilic and some sloughs of necrotic cells are found in the lumen (Photo 9). Kidney tissue of the second group fed 0.3% EMMH supplemented diet showed sever infiltration of mononuclear inflammatory cells, necrosis of cells lining the renal tubules, necrosis in glomuli and densely eosinophilic cast in some renal tubules (Photo 10). The kidney tissue of third group fed 0.4% EMMH supplemented diet showed better structure of kidney tissue than previous groups, however some necrotic cells are shown in glomuli, slight edema and necrosis of cells lining the renal tubules (Photo 11). The kidney tissue of the fourth group fed 0.5% EMMH supplemented diet showed clear and normal glomular structure and normal renal tubules with few mononuclear inflammatory cells (Photo 12).

Farafra Sheep:

The kidney section of the control group showed sever infiltration of fibrous connective tissue, sever infiltration of inflammatory cells, edema and necrosis lining the renal tubules (Photo 13). Kidney tissue of the second group fed 0.3% EMMH supplemented diet showed sever infiltration of inflammatory cells, sever necrosis of renal cells and some sloughs of renal cells in the renal tubules (Photo 14). Kidney tissue of the third group, fed 0.4% EMMH supplemented diet, showed necrosis of some renal tubules, moderate infiltration of inflammatory cells and few densely eosinophilic cast in renal tubules with no clear appearance of glomuli (Photo 15). The kidney tissue of the fourth group fed 0.5% EMMH supplemented diet showed the best and normal kidney tissue, where glomuli and renal tubules are of clear appearance with few inflammatory cells and few necrotic cells lining the renal tubules (Photo 16).

Sections of Rahmany Liver in Experimental Groups

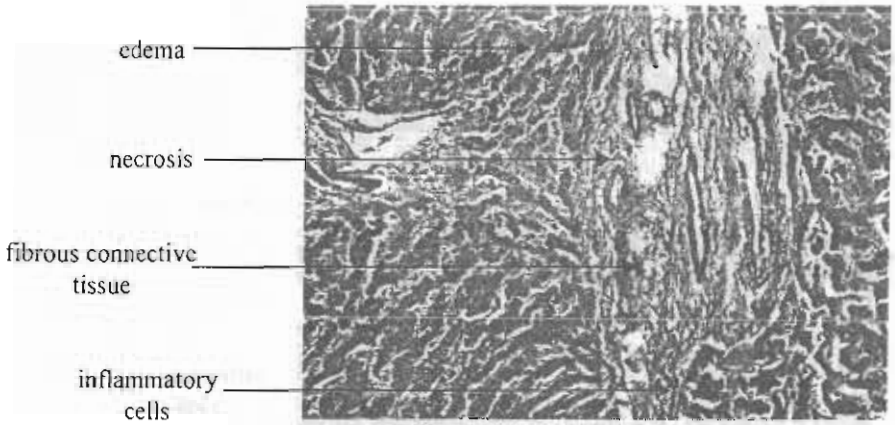

Photo. (1): Control group

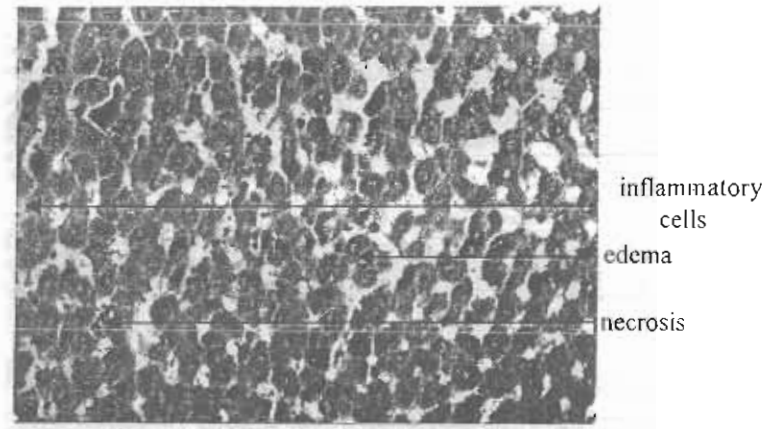

Photo. (2): 0.3% EMMH group

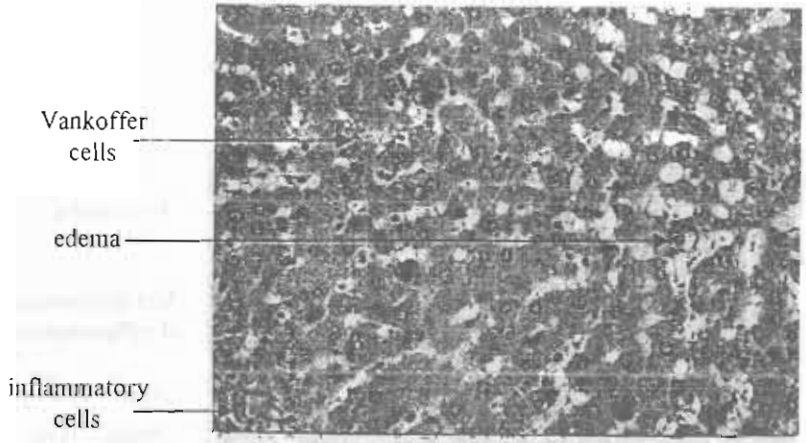

Photo. (3): 0.4% EMMH group

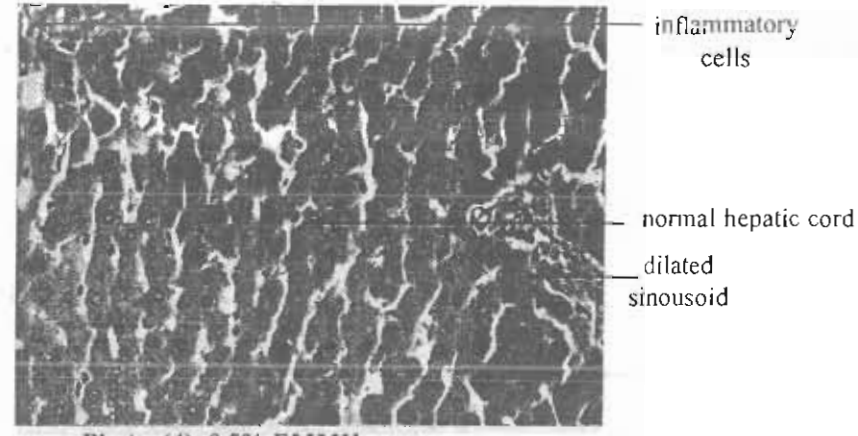

Photo. (4): 0.5% EMMH group

Sections of Farafra Liver in Experimental Groups

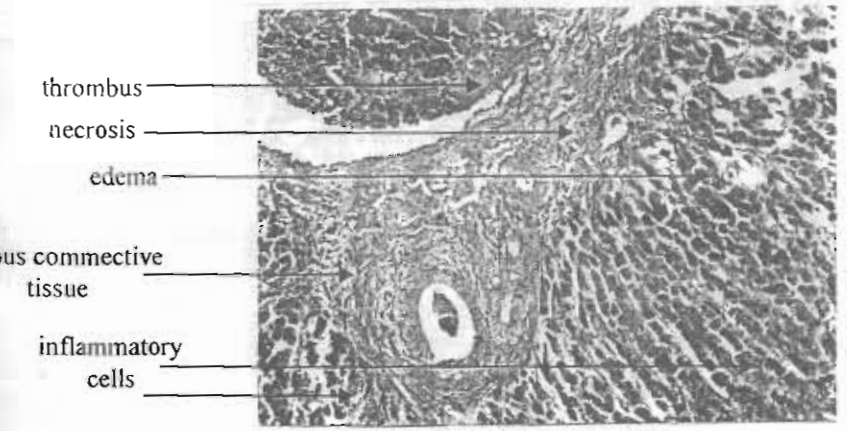

Photo. (5): Control group

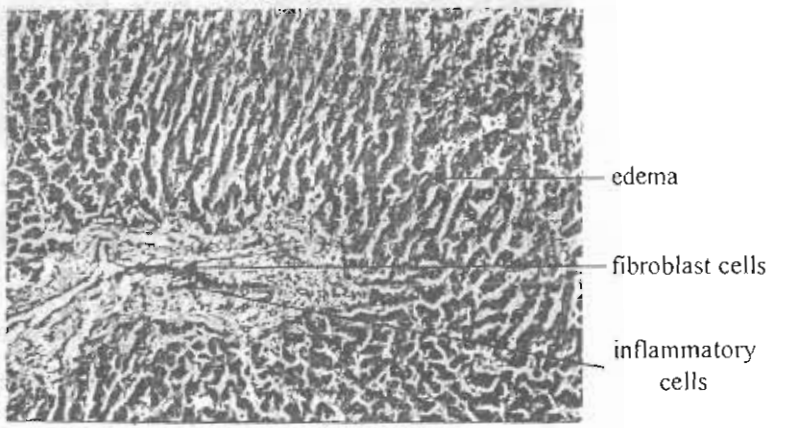

Photo. (6): 0.3% EMMH group

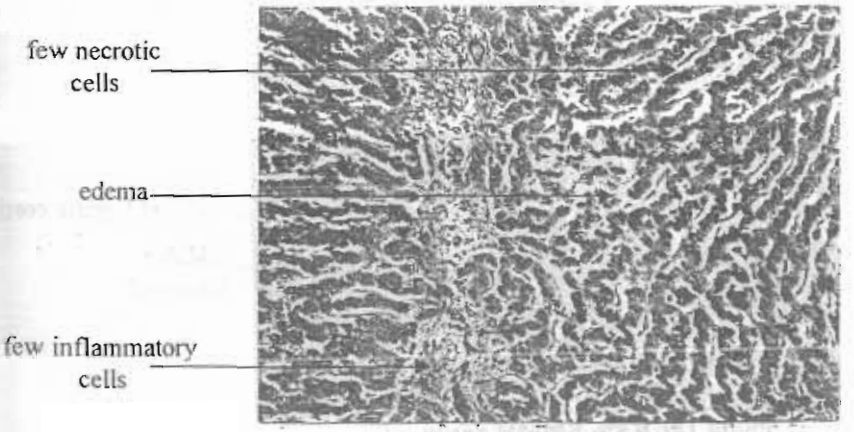

Photo. (7): 0.4% EMMH group

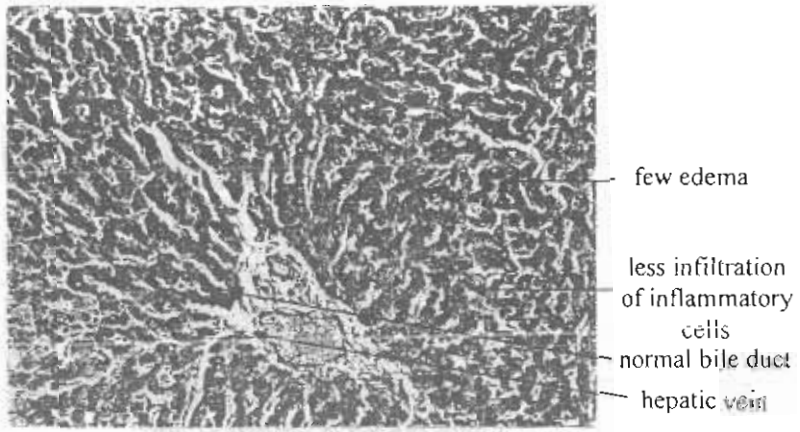

Photo. (8): 0.5% EMMH group

Sections of Rahmany Kidney in Experimental Groups

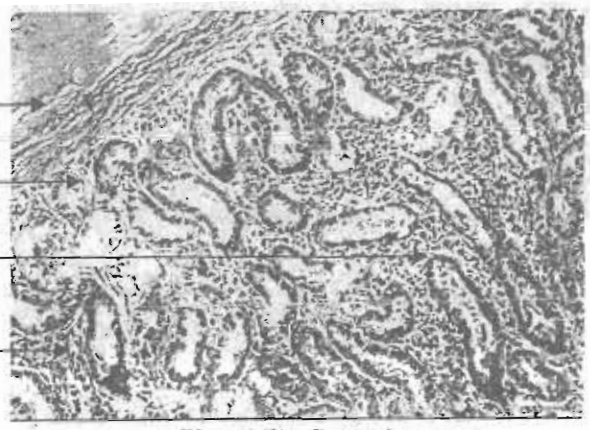

Photo. (9): Control group

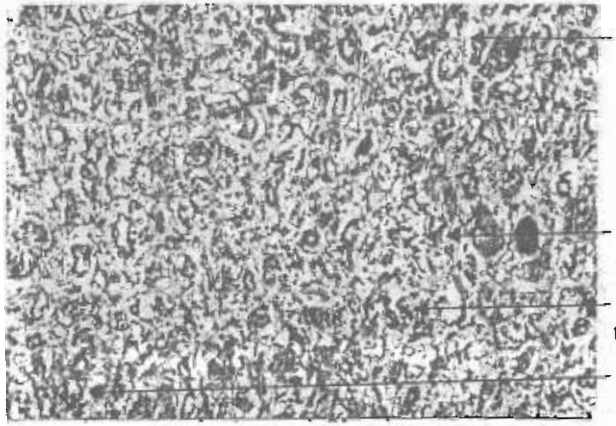

Photo. (10): 0.3% EMMH group

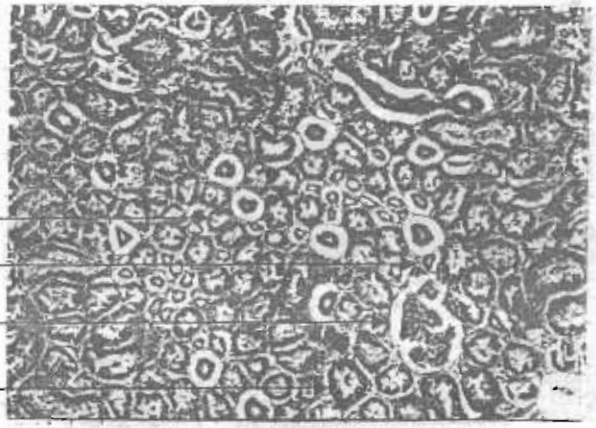

Photo. (11): 0.4% EMMH group

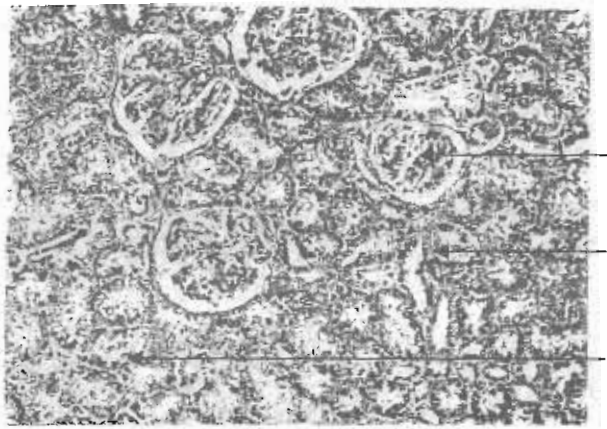

Photo. (12): 0.5% EMMH group

Sections of Farafra Kidney in Experimental Groups

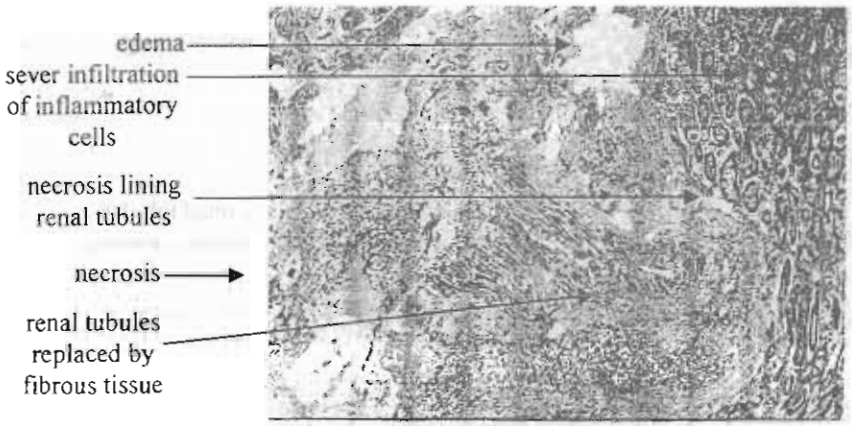

Photo. (13): Control group

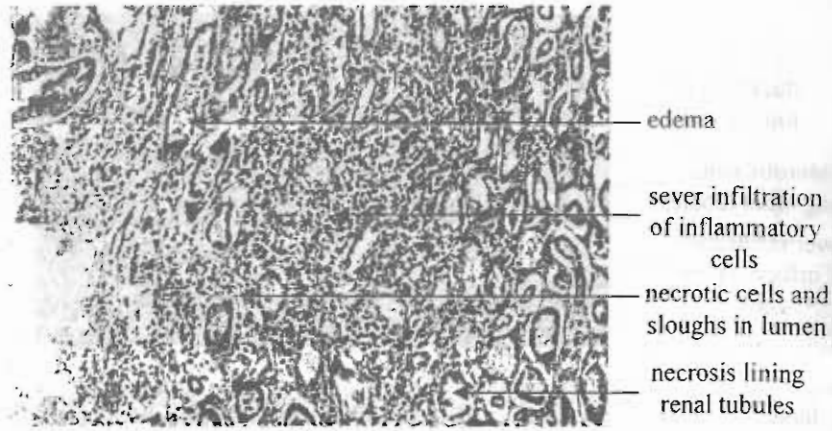

Photo. (14): 0.3% EMMH group

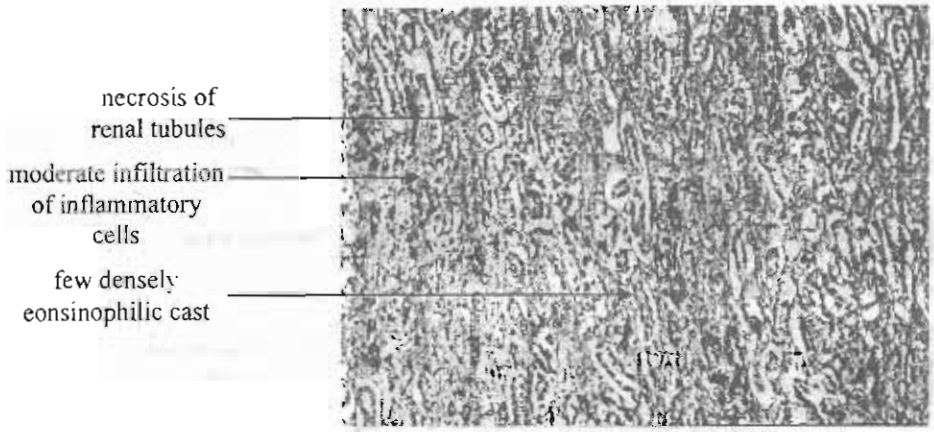

Photo. (15): 0.4% EMMH group

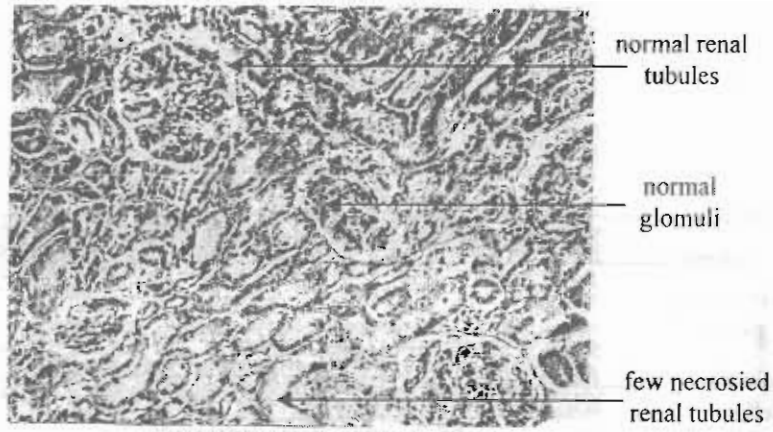

Photo. (16): 0.5% EMMH group

The histopathological results of this study demonstrated that supplemented probiotic EMMH had an effective role in maintaining liver and kidney of native sheep healthy. It was observed clearly that gradually increasing dose of EMMH had been associated with gradually better structural and functional state of cells for both of liver and kidney tissues. This findings are in agreement with previous studies showing that the administration of probiotics diminished the bacterial translocation in models of liver disease (Adawi *et al.*, 2001) and reduced bacterial infection after liver transplantation (Rayes *et al.*, 2005). It is worthy to say that, the bad structure of liver and kidney cells of the control groups (Rahmany and Farafra) might be regarded to disease infection or environmental pollutants in which the supplemental probiotic seems efficient to keep animals in a healthy state. In other words, feeding multi-strains of fungal-yeast probiotic with antioxidants (EMMH) was effective in repairing damaged cells of liver and kidney of growing sheep. The best histological observations were achieved with the supplementation level of 0.5% of the concentrate mixture.

CONCLUSION

The results of this study indicate that, adding the multi-strains of fungal-yeast probiotic with natural antioxidants (EMMH) at 0.3, 0.4 and 0.5% of the traditional concentrate feed mixture for growing native sheep had remarkable effect on improving average daily gain, stabilize body temperature, increase stimulation of immune and internal antioxidants activity and enhance repairing of damaged cells of liver and kidney. The best results were achieved with 0.5% EMMH supplementation level of the traditional concentrates mixture.

REFERENCES

A.O.A.C. (1995). Official Methods of Analysis. Association of Official Analytical Chemists, 16th Ed. Washington, D.C., USA.

Adawi, D., S. Ahrne and G. Molin (2001). Effects of different probiotic strains of Lactobacillus and Bifiodsbacterium on bacterial translocation and liver injury in an cute liver injury model. Int. J. Food Microbial, 70:213-220.

Ali, M.F., M.S. Saleh, N.M. Ewdah and S.A. Mohmoud (2005). Effect of using chamomile (Mtricaria chamomilla) followers as feed additives on performance of growing lambs under desert farming system. Egyptian J. of Nutrition and feeds, 8 (2): 121.

Bancroft, D.J., C.H. Coak, R.W. Stirting and D.K. Turner (1994). Manual of Histolpathological Techniques and their Diagnostic Application. Churcill Livine Stone, Edinburgh, England.

Becker, L.B. (2004). New concepts in reactive oxygen species and cardiovascular reperfusion physiology. *Cardiovasc. Res. 61*, 461-470.

Benjamin, M.M. (1958). Outline of Veterinary Clinical Pathology. 3rd Ed., Iowa State University Press, Ames, Iowa, USA.

Beutler, E., O. Duron and B.M. Kelly (1963). Improved method of the determination of blood glutathione. J. Lab. Clin. Med. 61(5): 882.

Casey, P.G., G.E. Gardiner, G. Casey, B. Bradshaw and P.G. Lawlor (2007). A 5-strain probiotic combination reduces pathogen shedding and alleviates disease signs in pigs challenged with Salmonella enterica serovar Typhimurium. Appled environ.Microb., 73:1858-1863.

Celovas, J., J. Vigilijus and S. Almantas (2005).The effect of probiotic and phtobiotics on meat properities and quality in pigs. Vet. Zootech., 28: 80-84.

Chiquette, J. (1995). *Saccharomyces cerevisiae* and *Aspergillus oryzae* used alone or in combination as a feed supplement for beef and dairy cattle. Can, J. Sci., 75: 405.

Collado, M.C., J. Meriluoto and S. Salminen (2007). Measrement of aggregation properties between probiotics and pathogens: In vitro evaluation of different methods. J. Microbiol Methods., 71:71-74.

Duncan, D.B.(1955). Multiple Range and Multiple F-test. Biometrics, 11:1.

El-Afifi, T.M., M.F. Emara, M.A. El-Sherbiny, H.F.A. Motawe, G.M. El-Moghazy, A.H.M. Gomaa and N.A. Hashem (2010). Effect of photogenic additives on growth performance and intestinal morphology of broilers. Egyptian J. Nutrition and Feeds, 13 (3): 549-562.

Fuller, R. (1999). Probiotics for farm animals. Critical Rev., 8:15-22.

Gladine, C., E. Rock, C. Morand, D. Bauchart and D. Durand (2007). Bioavailability and antioxidant capacity of plant extracts rich in polyenols given as a single acute dose, in sheep made highly susceptible to lipoperoxidation. British J. of Nutrition, 98 (4): 691-701.

Gomez-Alarcon, R.; F. Wiersma and D. Ammon (1988). Effect of feeding Amaferm (*aspergillus oryzae* extract) to cows in early lactation on milk yields and related parameters. J. dairy Sci., 71 (Supp. 1): 302.

Gross, R.T., R. Bracci, N. Rudolph, E. Schroeder and J. A. Kochen (1967). Hydrogen Peroxide Toxicity and Detoxification in The Erythrocytes of Newborn Infants. Blood, 29 (4): 481.

Hayes, J.D., McLellan, L.I. (1999). Glutathione and glutathione-dependent enzymes represent a coordinately regulated defences against oxidative stress. *Free Radic. Res. 31*, 273-300.

Henry, R.J. (1964). Clinical Chemistry-Principals and Techniques. pp.747, Edt. Harper and Row, New York, USA.

Kobeisy (1997). Effect of vitamin C and E on rectal temperature and respiratory rates in heat stressed goats. Assiut. Vet. Med. J. 37 (73):120-132.

Kruis, W., P. Fric, J. Pokrotnieks, M. Lukas and Fixa (2004). Maintaning remission of ulcerative colitis with the probiotic E. coli Nissle 1997 is as effectives as with standard mesalazine. Gut, 53:1617-1623.

Kraus, A., H. P. Roth and M. Kirchgessner (1997). Supplementation with vitamin C, vitamin E or beta-carotene influences osmotic fragility and oxidative damage of erythrocytes of zinc deficient rats. J. Nutr. 127 (7): 1290-1296.

Leonart, M. S., A. M. Weffort-Santos, E. M. Munoz, I. H. Higuti, V. A. Fortes and A. J. Nascimento (1989). Effect of vitamin E on red blood cell preservation. Braz. J. Med. Biol. Res. 22 (1):85-86.

Martarelli D, Verdenelli MC, and Curr Microbiol (2011).Effect of a probiotic intake on oxidant and antioxidant parameters in plasma of athletes during intense exercise training, June; 62 (6): 1689-96.

Masella, R., R. Di Benedetto, R. Vari, C. Filesi and C. Giovannini (2005). Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes. *J. Nutr. Biochem., 16:* 577-586.

Mikelsaar, M. and M. Zilmer (2009). *Lactobacillus fermentum* ME-3 - an antimicrobial and antioxidative probiotic. In *Microbial Ecology in Health and Disease*, vol. 21, 2009, p. 1- 27.

Minami,M. and J. Yoshikawa (1979). A Simplified Assay Method of Superoxide Dismutase. Clin. Chem. Acta., 92:337.

Mountzouris, K.C., P. Tsirtsikos, E. Kalamara, S. Nitsch, G. Schatzmayr and K. Fegeros (2007). Evaluation of the efficacy of a probiotic contaning Lactobacillus, Bifidobacterum, Enterococcus and Pediococcus strains in promoting broiler performances and modulating cecal microflora composition and metabolic activities. Poult. Sci., 86: 309-317.

Musa, H.H., S.L. Wu, C.H. Zhu, H.I. Seri and G.Q. Zhu (2009). The potential benefits of probiotics in animal production and health. J. of Animal and Veterinary Advances, 8, (2): 313-321.

Petrof, E.O., K. Kojima, M.J. Ropeski, M.W. Musch, Y. Tao, C. Desimone and E.B. Chang (2004). Probiotics inhibit nuclear factor kappa-B and induce heat shock proteins in colonic epithelial cells through proteasome inhibition. In astroenterology, Vol. 127, p. 1474-1487.

Rayes, N., D. Sechofer, T. Thenuvath, R.A. Schiller, J.M. Langrcher and S. Jonas (2005). Supply of pre and probiotics reduces bacterial infection rates after liver transplantation, a randomized, double-blind trial. Am. J. Transpl., 5: 125-130.

Richardsson, E.C., R.M. Herd, I.G. Colditz, J.A. Archer and P.F. Arthur (2002). Blood cell profiles of steer progeny from parents selected for and against feed intake. Aust. J. EXP. Agric., 42: 901-908.

Samli, H.E, N. Senkoylu, F. Koc, M. Kanter and A. Agma (2007). Effect of enterococcus faecium and dried whey on broiler performance, gut histomorphology and intestinal microbiota. Arch. Anim. Nutr., 61: 42-49.

S.A.S. (2001). Statistics: User, guide. 6th Ed., Institute, Inc., Cary, N.C., USA.

Scope. A, T. Filip. C. Gabler and F. Resch (2002). The influence of stress from transport and handling on haematologic and clinical chemistry. Blood parameters of racing pigeons. (Columba livia demestica). Avian Dis. 46: 224-229.

Shen, Q., N. Shang and P. Li (2011). *In vitro* and *in vivo* antioxidant activity of Bifidobacterium animalis 01 isolated from centenarians. In *Current Microbiology*, Vol. 62, No. 4, p. 1097-1103.

Sivakumar, A.V.N., G. Singh and V. P. Varshney (2010). Antioxidants Supplementation on Acid Base Balance during Heat Stress in Goats. Center for Advanced Studies on Physiology and Climatology, Indian Veterinary Research Institute, Izatnagar, 243 122, Uttar Pradesh, India. Asian-Aust. J. Anim. Sci. Vol. 23, No. 11: 1462 – 1468.

Snedecor, W. and W. Cochram (1980). Statistical Methods. 7th Edition, Iowa State University Press, Ames, Iowa, USA.

Srikandakumar, A., E.H. Johnson and O. Mahgoub (2003). Effect of heat stress on respiratory rate, rectal temperature and blood chemistry in Omani and Australian Merino Sheep. Small Rum.Res. 49:193-198.

Timmerman, H.M., C.J.M. Koning, L. Mulder, F.M, Rombouts and A.C. Beynen (2004). Monostrain, multistrain and multispecies probiotics: A comparison of functionality and efficacy. Int. J. Food Microbiol., 96: 219-233.

Torres-Rodriguez, A., M. Donghue, D.J. Donoghue, J.T. Barton, G. Tellez and B.M. Hargis, (2007). Performance and condemnationrate analysis of commercial Turkey flocks treated with a Lactobacillus sp-based probiotic Poult. Sci., 86: 444-446.

Tsuchiya, H. (2010). Structure-dependent membrane interaction of flavonoids associated with their bioactivity. Food Chemistry, 120 (4): 1089-1096.

Yaghoubi, S.M.J., G.R. Ghorbani, H.R. Rahmani and A. Nikkhah (2010). Flavonoids to manipulate rumen fermentation: An alternative for monensin.Agri. Seg. Published online.

Yousef, H.M., K.A. El-Masry and A.I. Aboulnaga (1996). Effect of dried yeast supplement on haemobiochemical levels and milk production responses of lactating buffaloes under hot summer conditions in Egypt. Egyptian J. Anim. Prod., 33: 11.

Venkatesan, M. Kirithika, I. Roselin, R. Ganesan and K. Muthuchelian (2012). Comparative *in vitro and in vivo study* of three probiotic organisms, *bifidobacterum* sp., *lactobacillus* sp., *s. cervisiae* and analyzing its improvement with the supplement of prebiotics. International Journal of Plant, Animal and Environmental Sciences, 94-106.

التقييم الغذائى لمعزز حيوي فطري مع الخميرة و مدعم بمضادات الأكسدة الطبيعية على آداء الأغنام. 3- التأثير على درجة حرارة الجسم , مضادات الأكسدة الداخلية , تغيرات مكونات الدم الطبيعية و أنسجة الجسم للأغنام المحلية المرباه تحت ظروف مناخية مختلفة.

عزه محمد كمال[1]، إبتسام محمد عزام[1]، يوسف حسين حافظ[2] ، علاء الدين يحى البدوى[3] و فاتن فهمي أبو عمو[2]

[1]معهد بحوث صحه الحيوان - مركز البحوث الزراعية – الدقى- الجيزة – مصر.

[2] معهد بحوث الإنتاج الحيوانى - مركز البحوث الزراعية – الدقى- الجيزة – مصر.

[3] قسم الإنتاج الحيوانى – المركز القومى للبحوث – الدقى – الجيزة – مصر.

أجريت هذه الدراسة بهدف تقييم تأثير التغذية على مستويات مختلفة من المعزز الحيوي EMMH المكون من فطر تريكوديرما ريزي و اسبرجيلس أوريزي مع خميرة الحية سكاروميسيس سيرفيسيا و المدعم بمخلوط من مضادات الأكسدة الطبيعية و ذلك على درجة حرارة الجسم ، معدل الزيادة الوزنية، صورة الدم، و محتويات الدم من مضادات الأكسدة الداخلية، و مدى تأثر أنسجة الكبد و الكلى بمعدل الإضافة على نوعين من الأغنام المحلية النامية . أجريت تجربتي تغذية الأولى على 32 راس من الحملان الرحماني في محطة السرو بمحافظة دمياط و الثانية على 24 رأس من حملان الفرافرة بمحطة ملوي بمحافظة المينا. أستمرت التجربة الأولى لمدة 168 يوما و الثانية لمدة 126 يوما حيث قسمت الحيوانات في كلا التجربتين إلى أربعة مجموعات متساوية من حيث العدد و الوزن، و كان متوسط أعمار الحيوانات في بداية التجربة ثلاثة أشهر و متوسط أوزانها 18.04±0.53 كجم للرحماني و 18.53± 0.11 كجم للفرافرة. أضيف المعزز الحيوي المختبر بمعدل صفر، 0.3 ،0.4 و 0.5% من مخلوط العلف المركز للمجموعات التجريبية الأولى ، الثانية، الثالثة و الرابعة على التوالي. كان مستوى التغذية اليومية للرأس بمعدل 2.5 % من وزن الجسم مخلوط علف مركز (سواء مدعم أو غير مدعم بالمعزز الحيوي) + 1% من وزن الجسم دريس برسيم للرحماني و بمعدل 3% من وزن الجسم مخلوط العلف المركز للفرافرة و كان قش الأرز المقطع يقدم للشبع لكلا النوعين، و كانت مياه الشرب متاحة لجميع الحيوانات طوال الوقت. تم تسجيل درجة حرارة الجو و الرطوبة النسبية صباحا و مساءا يوميا، و تسجيل درجة حرارة جسم جميع الحيوانات مرة شهريا، و تسجيل الزيادة الوزنية مرة كل أسبوعين، كما تم سحب عينات دم من جميع الحيوانات مرتين خلال الشهر الأخير من تجربتي التغذية. بعد إنتهاء تجارب التغذية تم أختيار ثلاثة حيوانات عشوائيا من كل مجموعة تجريبية لذبحها و أخذ عينات من الكبد و الكلى لفحص أنسجتها إكلينيكيا. و قد أوضحت نتائج الدراسة ما يلي:

- عدم تأثر درجة حرارة الجسم بإضافة المستويات المختلفة من المعزز الحيوي مع ملاحظة أن درجة حرارة الحيوانات المغذاة على العلائق المضاف إليها المعزز كانت ثابتا من تلك المغذاة على العليقة المقارنة للأغنام الرحماني بينما حدث أرتفاع لدرجة حرارة الجسم معنويا ($p<0.05$) للأغنام الفرافرة المغذاة على العليقة المقارنة.

- كان معدل الزيادة الوزنية اليومية مرتفع معنويا ($p<0.01$) لكلا النوعين من الأغنام المغذاة على العلائق المحتوية على المعزز، و كان معدل الزيادة الوزنية يرتفع بزيادة نسبة الإضافة.

- لم تسجل فروق معنوية بين المجموعات التجريبية من حيث تركيز الهيموجلوبين, نسبة حجم كرات الدم، عدد كرات الدم البيضاء و نسب مكوناتها، و إن كان هناك إتجاه لإرتفاع تركيز الهيموجلوبين و نسبة حجم كرات الدم مع إنخفاض عدد كرات الدم البيضاء خصوصا النوتروفيل بزيادة نسبة إضافة المعزز الحيوي في العليقة.

- إرتفاع تركيز مضادات الأكسدة و مضادات الأكسدة الداخلية لكل من الجلوتاثيون بيروكسيديز، سوبر أكسيأكسيد ديسميوتيز معنويا ($p<0.05$) في دم الأغنام المغذاة على المعزز بالنسبة للأغنام المغذاة على عليقة المقارنة، و كان تركيز مضادات الأكسدة و مضادات الأكسدة الداخلية يزداد بزيادة نسبة إضافة المعزز في العليقة.

- أوضحت نتائج الفحص الإكلينيكي لأنسجة الكبد و الكلى أن مجموعات المقارنة كانت تعاني من إنتشار كثيف للخلايا الإلتهابية مع تليف جزئي في الكبد و الكلى مع إنتشار الخلايا الميتة خصوصا لأغنام الفرافرة و كانت حالة الأنسجة تتحسن بشكل ملحوظ عند التغذية على العلائق المحتوية على المعزز، و كانت أفضل النتائج لمجموعات الرحماني و الفرافرة المغذاة على مستوى إضافة المعزز بنسبة 0.5% من مخلوط العلف المركز.

توصي الدراسة بإضافة المعزز الحيوى EMMH للعلف المركز بنسبة إضافة 0.5% لتحسين الحالة الصحية و الزيادة الوزنية للأغنام المحلية النامية.