

ISSN: 2322-4568



World's Veterinary Journal

Scienceline Publication

An international peer-reviewed journal which publishes in electronic format

Volume 8, Issue 4, December 2018

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Volume 8 (4); December 25, 2018

Research Paper

Seroprevalence and Associated Risk Factors of Brucellosis in Sheep and Human in Four Regions in Matrouh Governorate, Egypt.

Diab MS, Elnaker YF, Ibrahim NA, Sedeek EK and Zidan Sh-A-A.

World Vet. J. 8(4): 65-72, 2018; pii:S232245681800007-8

ABSTRACT

Brucellosis is a worldwide zoonosis that has major public health concern in Egypt. The present work was conducted to investigate the seroprevalence of brucellosis in sheep and human in four localities in North Western region of Egypt, on basis of the Rose Bengal plate test (RBPT) and further confirmation by complement fixation test (CFT). A total of 2471 sheep serum samples and 371 human samples were collected. The prevalence of brucellosis in sheep and human by using RBPT were 11% (272/2471) and 24.3% (90/371), respectively while by CFT were 10.56% (261/2471) and 22.91% (85/371). There was significant relationship between age and in infection rate in sheep ($P < 0.01$), with higher percentage of infection was indicated in age group over than 24 months by 14.19% (264/1860) followed by age group less than 24 month and over 12 months by 2.39% (8/335). On studying the relation between locality and infection rate there was no significance in human samples while in sheep it was significant ($P < 0.01$) with higher percentage of infection found in Siwa region by 20.30% (94/463) in sheep and in human by 27.6% (27/98). Concerning season there is highly significant relationship between season and percent of infection with *Brucella*, the high percent of infection found in human and sheep by 43.1% (62/144) and 16.51% (123/745) respectively and lower percent found in spring months by 8% in sheep. From our result, it is concluded that RBPT and CFT used as screening tests for detection the prevalence of species in serum samples, *Brucella* infection is found with high percent in north, west region of Egypt, which need further examination and studying another risk factor associated with infection and isolation of *Brucella* in this area.

Keywords: Brucellosis, Complement fixation test, Human brucellosis, Rose Bengal plate test, Sheep

[Full text-[PDF](#)]



Diab MS, Elnaker YF, Ibrahim NA, Sedeek EK and Zidan Sh-A-A (2018).

Seroprevalence and Associated Risk Factors of Brucellosis in Sheep and Human in Four Regions in Matrouh Governorate, Egypt.

World Vet. J. 8(4): 65-72. www.wvj.science-line.com

Research Paper

Evaluation of Club Foot in Working Donkeys.

Mostafa M, Abdelgalil A, Farhat S.

World Vet. J. 8(4): 73-79, 2018; pii:S232245681800008-8

ABSTRACT

Club foot deformities were studied in 22 donkeys working in brick kilns. Evaluation of hoof capsule was carried out on hard concrete surface. Changes on the hoof capsule were observed from dorsal aspect, lateral aspect, palmar/planter aspect and distal or solar surface. Dorsal hoof wall angle for each hoof (toe angle) was taken using hoof gauge. According to toe angle club foot was classified into three degrees. The first degree the toe angles less than ($< 90^\circ$). The second degree the toe angles less or equal ($\leq 130^\circ$) and the third degree more than ($> 130^\circ$). Club foot in donkeys displayed disparity, increased in heights and lengths associated with contracted heels. The digital cushion appeared atrophied associated with granulomatous inflammations. The third degree club foot revealed horn materials of sole, frog and digital cushion were destroyed and covered by hard keratin materials. The use toe angles for classification of club foot provide a reliable method. Radiography of the 3rd phalanx showed osteophytic and osteolytic changes. Soft tissue reaction and thickening with radio-opaque mass and calcification at palmar/planter structures were observed. Overworking, overloading and pain were considered the predisposing causes in brick kilns working donkeys.

Keywords: Club foot, Deformities, Toe angle, Radiography, Donkey

[Full text-[PDF](#)]



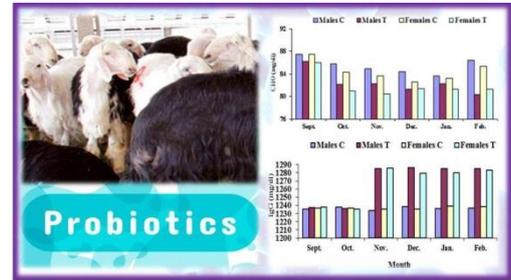
Mostafa M, Abdelgalil A, Farhat S (2018). Evaluation of Club Foot in Working Donkeys. World Vet. J. 8(4): 73-79. www.wvj.science-line.com

Research Paper

Effect of Probiotics on Growth, Some Plasma Biochemical Parameters and Immunoglobulins of Growing Najdi Lambs.

Hussein AF.

World Vet. J. 8(4): 80-89, 2018; pii:S232245681800009-8



Hussein AF (2018). Effect of Probiotics on Growth, Some Plasma Biochemical Parameters and Immunoglobulins of Growing Najdi Lambs. *World Vet. J.* 8(4): 80-89. www.wvj.science-line.com

ABSTRACT

This study was performed at the governorate of Alkhurmah, Kingdom of Saudi Arabia. Twenty growing Najdi lambs aged six months with average body weight 35.22 ± 0.107 and 33.67 ± 0.107 kg for males and females, respectively were allotted based on their gender into two main groups (10 males and 10 females), each gender divided into two subgroups the first subgroup of each gender served as control while the second was supplemented with *Lactobacillus sporogenes* 37.50×10^3 , *Saccharomyces cerevisiae* 625.0×10^3 CFU, 1g Alpha amylase and 20g sea wood powder / kg diet (concentrate feed mixture) for six months. The results indicated that lambs supplemented with probiotics in diet had better improvement ($P < 0.05$) on growth performance indices (Average daily gain, growth rate and total weight gain) compared with control subgroups in both genders. Probiotics increased ($P < 0.05$) the values of plasma total protein, glucose, urea nitrogen and aspartate aminotransferase compared with control group. On the other hand, total cholesterol concentration decreased significantly ($P < 0.05$) in lambs supplemented with probiotics than control groups. The mean values of plasma immunoglobulin A did not differ in both control and treated groups during the study period, while plasma immunoglobulin G increased significantly ($P < 0.05$) in lambs supplemented with probiotics compared with control groups in both genders. Plasma total lipids and aspartate aminotransferase concentrations remained relatively stable throughout the study period in both probiotics and control groups. In conclusion, probiotic supplementations can be used as the important biological additives for enhancing growth indices and immunity status of growing lambs.

Keywords: Body weight, Immunoglobulin, Najdi lambs, Plasma metabolites, Probiotics, Sex

[Full text-[PDF](#)]

Research Paper

Effect of Vitamin E on the Prevention of Peritoneal Adhesions in Sheep.

Borges LPB, Mattos-Junior E-de, Silva MAM, Pereira dos Santos MAA, Garcia DO, Ayer IM, Pereira da Câmara Barros FF and Teixeira PPM.

World Vet. J. 8(4): 90-94, 2018; pii:S232245681800010-8



Borges LPB, Mattos-Junior E-de, Silva MAM, Pereira dos Santos MAA, Garcia DO, Ayer IM, Pereira da Câmara Barros FF and Teixeira PPM (2018). Effect of Vitamin E on the Prevention of Peritoneal Adhesions in Sheep. *World Vet. J.* 8(4): 90-94. www.wvj.science-line.com

ABSTRACT

The objective of this study was to assess vitamin E solution on the prophylaxis of intraperitoneal adhesions in ovine uterine serosal damage model with bipolar diathermy. Therefore, 19 ewes underwent laparotomy for induction of adhesions, using a uterine serosal bipolar electrocauterization model. Cauterizations were performed on the right uterine horn serosa and right ovary. Ewes were randomly divided into three groups: control group (GCT, n=5), with no treatment following electrocoagulation, another group using local rinse of 20 mL of normal saline (GNS, n=8), and the last group using local rinse of 20 mL of vitamin E injection solution (GVE, n=6). On day 21 postoperative, animals underwent laparoscopy for scoring and comparison of intraperitoneal adhesion according to frequency and number. The number of adhesions was compared among groups using the Kruskal-Wallis test and Dunn's post-hoc test. As results, the bipolar uterine serosal coagulation model triggered uterine adhesions in 74% (14/19) of the animals. Frequency of postoperative intraperitoneal adhesions was similar ($P = 0.819$) among groups (80% ewes of GCT, 62.5% of GNS and 83% of GVE). There was no significant difference between treatment groups, however, number of adhesions was lower in GVE and GNS groups than in control group ($P = 0.032$), showing that the addition of these kind of substances are better than not using any type of barrier to prevent the formation of intraperitoneal adhesions.

Keywords: Adhesions, Bipolar diathermy, Laparoscopy, Sheep, Uterus

[Full text-[PDF](#)]

Research Paper

Effect of Dried Rosemary Supplement as Antioxidant Agent on Blood Biochemical Changes in Relation to Growth Performance of Heat-Stressed Crossbred (Brown Swiss × Baladi) Calves.

El-Masry KA, Abdalla EB, Emara SS and Hussein AF.

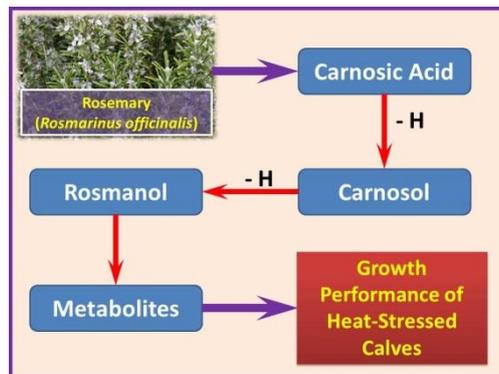
World Vet. J. 8(4): 95-105, 2018; pii:S232245681800011-8

ABSTRACT

Heat exposure is a systemic stressor that adversely influences growth and reproductive performances in cattle. This trial aimed to study the effects of Rosemary (RM) supplementation on reducing the side effect of oxidative stress and its relation with growth performance under heat stress condition. Fifteen male calves were divided into three equal groups, the first was offered the basal diet as a control group, whereas the second and the third groups were fed the same basal diet as in control, in addition to a daily supplement of 3g and 6g dried grinded RM/kg concentrate, respectively, for a period of one month. The results showed that supplement of 3 and 6g dry grinded RM/kg concentrate led to a highly significant ($P < 0.01$) decrease in oxidant status and an increase in total antioxidant capacity, as well as significant ($P < 0.01$) declines were noted in the levels of lipids profile, kidney and liver function indicators, and iron concentration. However, RM supplemented groups showed significant ($P < 0.01$) elevations of feed efficiency and daily weight gain copper and triiodothyronine concentrations. In conclusion, RM improved the calves' growth performance through alleviating oxidative stress side effects under hot summer conditions to improve economic returns.

Keywords: Antioxidant agent, Blood biochemical, Egyptian desert, Growing calves, Oxidative stress

[Full text-[PDF](#)]



Research Paper

Successful Surgical Management of Corneo-conjunctival Dermoid Cyst with Bilateral Nasal Choristoma in a Red Kandhari Calf.

Aher V, Bhadane B, Ramchandra Balage P, Dhage G, Gangane G, Asaramji Mate A and Lokhande Devidas S.

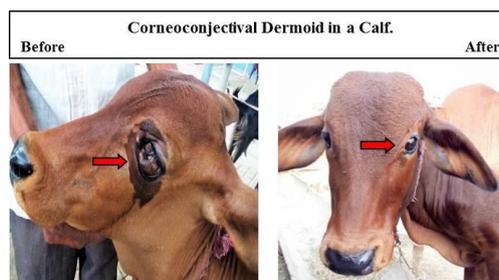
World Vet. J. 8(4): 106-109, 2018; pii:S232245681800012-8

ABSTRACT

An old Red Kandhari calf presented at teaching veterinary clinical complex, veterinary college Parbhani with corneo-conjunctival haired masses on the left eye and bilateral nasal growth at nasolabial planum since birth. As the mass was completely covering on cornea due to which vision was hindered completely after physical examination and considering the health status of the calf the surgery was scheduled. The masses were surgically excised from the cornea and bulbar conjunctiva of eye and the left and right side of the dorsomedial nasolabial planum. Then the eye was flushed with normal saline and the tissue of both corneo-conjunctival and nasal were stored in 10% formalin later histopathology of the excised tissue confirmed as a unilateral corneo-conjunctival dermoid with ectopic lacrimal glands and bilateral nasal choristomas with loose stroma and hair follicle. Two months of follow up was done where there was no reoccurrence of the growth observed. Surgery was curative and healing was uneventful.

Keywords: Calf, Corneoc-onjunctival dermoid, Nasal choristoma

[Full text-[PDF](#)]



Aher V, Bhadane B, Ramchandra Balage P, Dhage G, Gangane G, Asaramji Mate A and Lokhande Devidas S (2018). Successful Surgical Management of Corneo-conjunctival Dermoid Cyst with Bilateral Nasal Choristoma in a Red Kandhari Calf. *World Vet. J.* 8(4): 106-109. www.wvj.science-line.com



World's Veterinary Journal

ISSN: 2322-4568

Frequency: Quarterly

Current Issue: 2018, Vol: 8, Issue: 4 ([December 25](#))

Publisher: [SCIENCELINE](#)

www.wvj.science-line.com

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Seroprevalence and Associated Risk Factors of Brucellosis in Sheep and Human in Four Regions in Matrouh Governorate, Egypt

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ABSTRACT

Brucellosis is a worldwide zoonosis that has major public health concern in Egypt. The present work was conducted to investigate the seroprevalence of brucellosis in sheep and human in four localities in North Western region of Egypt, on basis of the Rose Bengal plate test (RBPT) and further confirmation by complement fixation test (CFT). A total of 2471 sheep serum samples and 371 human samples were collected. The prevalence of brucellosis in sheep and human by using RBPT were 11% (272/2471) and 24.3% (90/371), respectively while by CFT were 10.56% (261/2471) and 22.91% (85/371). There was significant relationship between age and in infection rate in sheep ($P < 0.01$), with higher percentage of infection was indicated in age group over than 24 months by 14.19% (264/1860) followed by age group less than 24 month and over 12 months by 2.39% (8/335). On studying the relation between locality and infection rate there was no significance in human samples while in sheep it was significant ($P < 0.01$) with higher percentage of infection found in Siwa region by 20.30% (94/463) in sheep and in human by 27.6% (27/98). Concerning season there is highly significant relationship between season and percent of infection with *Brucella*, the high percent of infection found in human and sheep by 43.1% (62/144) and 16.51% (123/745) respectively and lower percent found in spring months by 8% in sheep. From our result, it is concluded that RBPT and CFT used as screening tests for detection the prevalence of species in serum samples, *Brucella* infection is found with high percent in north, west region of Egypt, which need further examination and studying another risk factor associated with infection and isolation of *Brucella* in this area.

Key words: Brucellosis, Complement fixation test, Human brucellosis, Rose Bengal plate test, Sheep

INTRODUCTION

Brucellosis is a highly contagious zoonosis caused by genus *Brucella* affecting both humans and animals (Schelling et al., 2003). Sheep brucellosis divided into typical zoonotic brucellosis that caused by *Brucella melitensis* and non-zoonotic ram epididymitis that caused by agent *B. ovis* (Acha and Szyfres 2003). Sheep and goats are primary hosts for *Brucella melitensis* (*B. melitensis*) which is common *Brucella* species in humans (Godfroid et al., 2011). Direct contact with fluids from infected animals in birthing products and other bodily fluids such as urine is the major rout of infections between animals and humans. Symptoms of *B. melitensis* in animals include abortions, stillbirths, infertility and decreased production (Corbel, 2006). While Major symptoms of human brucellosis are undulant fever, headache, muscle pain, lumbar pain and arthritis (Acha and Szyfres 2003; Pal et al., 2017).

Despite of continuous effort for zoonotic brucellosis control, that represent a major public health threats, it remains endemic in the vast majority of middle eastern countries, accused of tens of thousands of new cases yearly (Pappas and Memish 2007; Patel et al., 2017). There are about half a million new human cases of brucellosis occur every year worldwide making it the most common zoonosis (Seleem et al., 2010). Transmission of brucellosis to human occurs through ingestion of the infected product, direct contact with infected animals and its materials and through inhalation of the infected particles (Dieckhaus and Kyebambe 2017). The causative agent has a very low infectious dose, only 10 organisms of *Brucella melitensis* initiate infection (Lopes et al., 2010). Rose Bengal plate test (RBPT) is simple, good, rapid and easy to implement and can be used as herd screening test at remote places (Gul and Khan 2007; Teng et al., 2017). Moreover, Complement Fixation Test (CFT) used as a confirmatory test for diagnosis of brucellosis (Ashraf et al., 2014).

Therefore, this study was intended to study the seroprevalence of brucellosis in sheep and humans in four localities in Matrouh Governorate, Egypt by using Rose Bengal Plate Test and confirmed by complement fixation test.

MATERIALS AND METHODS

Study area

This study completed in four regions (Matrouh, Elhamam, El dabaa, Siwa) in Matrouh Governorate, Egypt, to study seroprevalence of brucellosis, with history of non-vaccination.

Animals

This study was performed during the period between April 2016 to February 2017, a total of 2471 serum samples from Barki and Rahmani free grazing sheep in addition to 271 serum samples from humans at fever hospital in the same area of sheep rearing, the distribution of sheep and human illustrated in table 1 the data of sex and age and previous illness were recorded and there is no previous vaccination against brucellosis.

Sampling

Serum samples. In human blood samples were collected aseptically by vein puncture from each patient at the initiation of therapy and serum separated in two sterile Eppendorf that frozen for serological examination. In sheep, blood was collected (5 ml) from each sheep using plain vacutainer tube, the blood was allowed to clot at room temperature for 1-2 h, stored horizontally overnight at 4° C, then the serum was separated from the clot by centrifugation at 2000-3000 rpm for 10-15 minutes, the serum was labeled and stored at -20° C till tested.

Table 1. Number of serum samples examined from Barki and Rahmani sheep and human in four areas of Matrouh governorate between April 2016 to February 2017.

Locality	Humans	sheep
Matrouh	89	526
El Hamam	90	881
El dabaa	94	601
Siwa	98	463
Total	371	2471

Serological test

Rose Bengal test on human serum samples. The RBPT is a valuable screening test for diagnosis of *Brucella* (Agasthya et al., 2007) spectrum diagnostics *Brucella* Rose Bengal reagent obtained from (MDSS GmbH Schiffgraben 41 30175 Hannover, Germany) it contains ready to use standardized, killed, stained, smooth specific antigen suspensions of *Brucella* having specific reactivity towards antibodies to *Brucella* antigens which planned for quick recognition of *Brucella* (*Melitensis*, *suis*, and *abortus*) specific agglutinins (Alton et al., 1988).

Principle of the test. The smooth, killed stained *Brucella* antigen suspensions are mixed with the patient's serum. Specific antibodies to *Brucella* antigens if present in the patient serum will react with the antigen suspension to produce an agglutination reaction. No agglutination indicates the absence of specific antibodies to *Brucella* antigens.

Rose Bengal plate-agglutination test on sheep serum samples. Prepared from heat-killed *Brucella abortus* cells (strain 99) stained with rose Bengal stain and kept in a stable acidic suspension, the antigen is produced by veterinary serum and vaccine research institute Abbasia, Cairo, Egypt, used for rapid detection of brucellosis in animals (as cattle, buffalo sheep and goat) by rapid agglutination test. The test procedure recommended by Alton et al. (1988) was followed. Briefly, 25 µl of RBPT antigen and 25 µL of the test serum were placed alongside on the plate, and then mixed thoroughly by a tooth pick or glass rod, the plate was shaken for four minutes by electric rocker and the degree of agglutination reactions was recorded. The sample was classified positive if any agglutination was observed and negative if no agglutination was noted.

Complement fixation test. The positive serum samples by RBT were retested using CFT. *B. abortus* S99 antigen for CFT was used to detect the presence of anti-brucella antibodies in the sera. The test antigen obtained from veterinary serum and vaccine research institute Abbasia, Egypt, and the CFT was done at *Brucella* unit in central laboratory evaluation for veterinary biologics, Abbasia, Cairo, Egypt according to Alton et al. (1988). The technique is usually performed using standard 96-well microtitre plates with round (U) bottoms firstly volumes of 25 µl of diluted (1/5) inactivated test serum placed in the well of the first, second and third rows. The first row is an anti-complementary control for each serum. Volumes of 25 µl of CFT buffer added to the wells of the first row to compensate for lack of antigen. Then 25 µl of CFT buffer added to third row. Serial dilutions are made by transferring 25 µl of serum from the third row onwards, 25µl of the resulting mixture in the last row are discarded. Volumes of 25 µl of antigen, added to each well except in the first row. Finally, 25 µl of complement, are added to each well. The plates are incubated at 37°C for 30 minutes, and a volume of 25 µl of sensitized sheep Red Blood Cells is added to each well. The plates are re-incubated at 37°C for 30 minutes. The results are read after the plates have been left to stand at 4°C for 2–3 hours to allow unlysed cells to settle. The degree of haemolysis is compared with standards corresponding to 0, 25, 50, 75 and 100% lysis.

Statistical analysis

Chi square test was used in statistical studies. The P value is the probability of the event occurring by chance if the null hypothesis is true, P-values 0.0001 (<0.01)

Ethical approval

All procedures performed in this study including collection of human serum samples and animals were in accordance with the Egyptian ethical standards of the national research committee. All human subjects gave their consent for the collection of the serum samples, with the agreement that any identifying details of the individuals should not be published.

RESULTS

In table 2 and figure 1, the overall seroprevalence of brucellosis were 11% (272/2471) and 10.56% (261/2471) in sheep serum samples using RBPT and CFT respectively while in human samples were 24.3% (90/371) and 22.91% (85/371). In table 3 and graph 1, studying age as risk factors in infection with brucellosis theirs is highly significant between age of sheep and infection with *Brucella* $P < 0.01$, the high percent of infection found in age group over than 24 months by 14.19% (264/1860) followed by age group less than 24 months and over 12 months by 2.39% (8/335). In table 4 and graph 2, studying locality as other risk factors, theirs is highly significance between locality and percent of infection with brucellosis in sheep $P < 0.01$ while in human samples there is no significance. The high percent of infection found in Siwa region by 20.30% (94/463) in sheep and in human by 27.6% (27/98).

In table 5 obtained the high percent of infection found in human females by 13.6% (21/154) and in sheep by 11.35% (259/2282). In table 6 in studying human the high percent of infection found in contact animals with sheep by 31.5% 68 (216) compared with non-contact human in which the percent of infection were 14.2% (22/155) while table 7, graph 3, studying a season as a risk factors explain that theirs highly significance between season and percent of infection with *Brucella*, the highest percent of infection found in human and sheep in winter by 43.1% (62/144) and 16.51% (123/745) respectively and lower percent found in spring months by 8% in sheep and no examination to human in spring months.

Table 2. Overall prevalence of *Brucella melitensis* by rose Bengal plate test and complement fixation test in Barki and Rahmani sheep at four areas of Matrouh governorate between April 2016 to February 2017

Test	RBPT			CFT		
	Species	Total No	Positive No	%	Positive No	%
Sheep		2471	272	11	261	10.56
Human		371	90	24.3	85	22.91

RBPT; Rose Bengal plate test, CFT; Complement fixation test

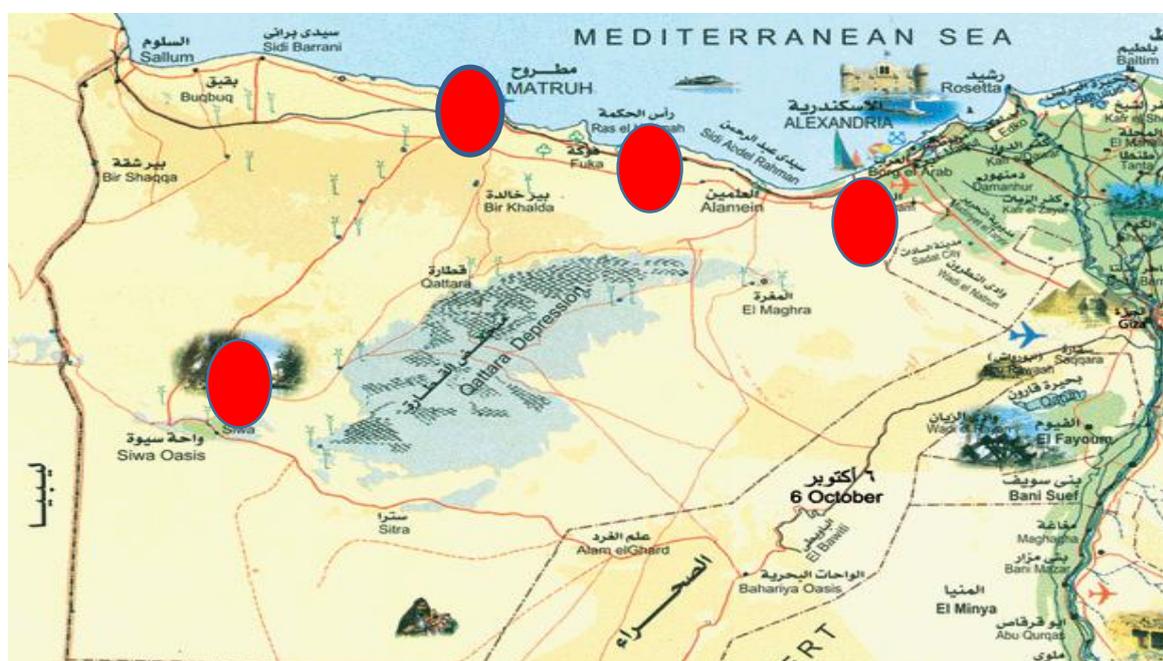
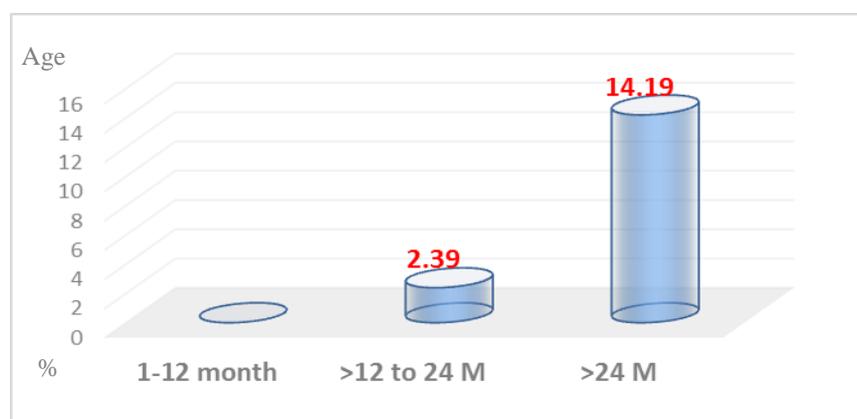


Figure 1. Map for Matrouh governorate, Egypt showing four areas of study between April 2016 to February 2017

Table 3. Prevalence of *Brucella melitensis* by rose Bengal plate test in relation to age in Barki and Rahmani sheep at four areas of Matrouh governorate, Egypt between April 2016 to February 2017

Age (months)	Total	Positive	%
1-12	276	0	0
>12 to 24	335	8	2.39
>24	1860	264	14.19
Total	2471	272	11
Chi-square		78.82**	
P value		0.0001 (<0.01)	

** Highly significant

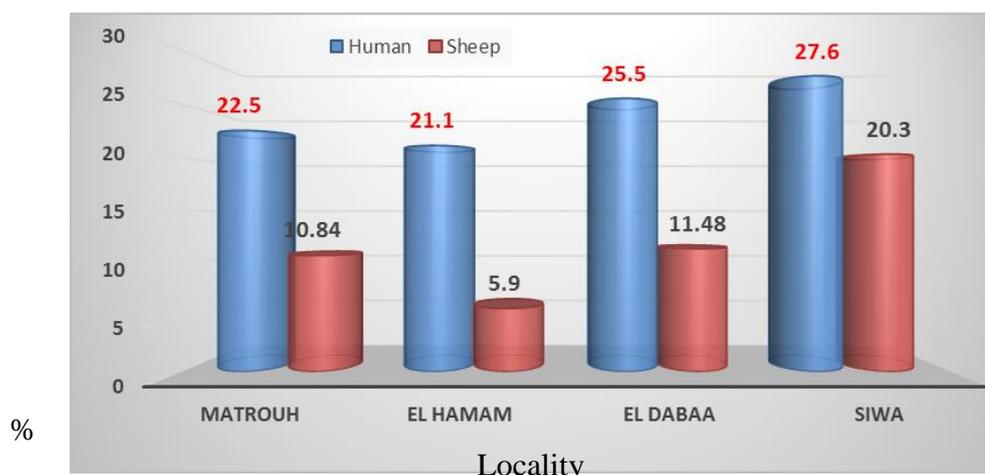


Graph 1. Prevalence of *Brucella melitensis* by rose Bengal plate test in relation to age in Barki and Rahmani sheep at four areas of Matrouh governorate, Egypt between April 2016 to February 2017

Table 4. Prevalence of *Brucella melitensis* by rose Bengal plate test in relation to age in serum samples of Barki and Rahmani sheep and human at four areas of Matrouh governorate, Egypt between April 2016 to February 2017

Locality	Human serum samples			Sheep serum samples		
	Total	Positive	%	Total	Positive	%
Matrouh	89	20	22.5	526	57	10.84
El Hamam	90	19	21.1	881	52	5.90
El dabaa	94	24	25.5	601	69	11.48
Siwa	98	27	27.6	463	94	20.30
Total	371	90	24.3	2471	272	11
Chi-square		1.3 ^{NS}			64.43**	
P-value		0.73			0.0001 (<0.01)	

NS: Non-Significant; **: Highly significant



Graph 2. Prevalence of *Brucella melitensis* by rose Bengal plate test in relation to age in serum samples of Barki and Rahmani sheep and human at four areas of Matrouh governorate, Egypt between April 2016 to February 2017

Table 5. Prevalence of *Brucella melitensis* in serum samples of Barki and Rahmani sheep and human at four areas of Matrouh governorate, Egypt between April 2016 to February 2017 by rose Bengal plate test in relation to sex

Sex	Samples	Human serum samples			Sheep serum samples		
		Total	Positive	%	Total	Positive	%
Male		217	69	31.8	189	13	6.88
Female		154	21	13.6	2282	259	11.35
Total		371	90	24.3	2471	272	11
<i>Chi- square</i>		16.17**			3.56 ^{NS}		
<i>P-value</i>		0.0001 (<0.01)			0.06		

NS: Non-Significant; **: Highly significant

Table 6. Prevalence of *Brucella melitensis* by rose Bengal plate test in relation to history of human contact with sheep in serum samples of Barki and Rahmani sheep and human at four areas of Matrouh governorate, Egypt between April 2016 to February 2017

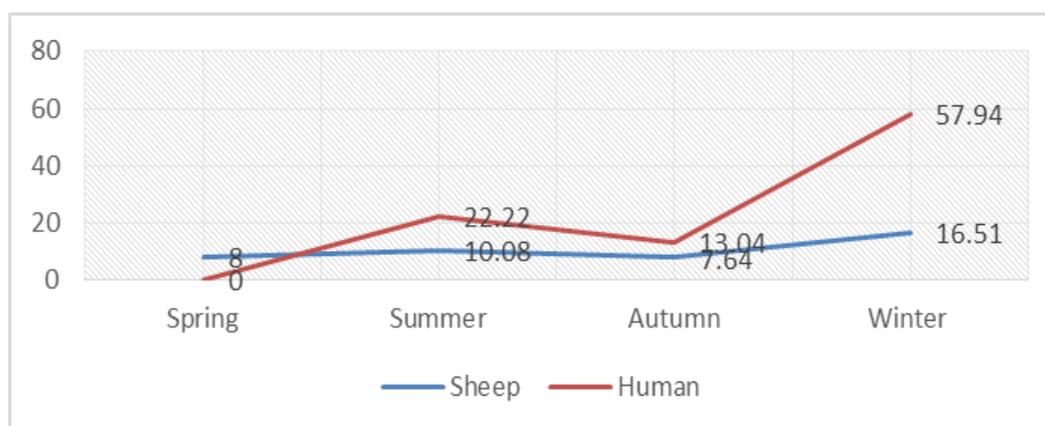
Item	Total	Positive <i>Brucella melitensis</i>	%
Contact	216	68	31.5
Non -contact	155	22	14.2
<i>Chi- square</i>		14.68**	
<i>P value</i>		0.0001 (<0.01)	

** Highly significant

Table 7. Prevalence of *Brucella melitensis* by rose Bengal plate test according to season in serum samples of Barki and Rahmani sheep and human from four areas of Matrouh governorate, Egypt between April 2016 to February 2017

Season	Species	Sheep			Human		
		Total	Positive	%	Total	Positive	%
Spring		400	32	8	-	-	-
Summer		645	65	10.08	107	16	15
Autumn		681	52	7.64	120	12	10
Winter		745	123	16.51	144	62	43.1
Total		2471	272	11	371	90	24.3
<i>Chi- square</i>		35.19**			46.01**		
<i>P-value</i>		0.0001 (<0.01)			0.0001 (<0.01)		

** Highly significant



Graph 3. Prevalence of *Brucella melitensis* by rose Bengal plate test in serum samples of Barki and Rahmani sheep and human at four areas of Matrouh governorate, Egypt between April 2016 to February 2017

DISCUSSION

Brucellosis is a zoonosis of veterinary, major public health and economic importance in most unindustrialized countries including Egypt (Afifi et al., 2005; Asiimwe et al., 2015). The diagnosis is based mainly on the serologic testing, as it is fast and simple in addition to the fact that culture techniques are not available in laboratories in endemic countries (Young, 1995). RBPT and CFT have been used for many decades, confirming to be successful for eradicating brucellosis in some countries (Garin-Bastuji et al., 1998). Therefore, in the present work RBPT were used for determination of seroprevalence of brucellosis in both sheep and human in North West region of Egypt. Results in table 2 indicated that the overall prevalence of brucellosis by RBPT in sheep were 11% (272/2471). Our results were nearly similar to that found by Hegazy et al. (2011) who confirmed that seroprevalence among sheep were 12.2%, Hussain et al. (2014) and who concluded that the overall seroprevalence of ovine brucellosis was 10.0%. The differences in prevalence of brucellosis may be attributed to time and place of sampling in addition to people habits in reporting cases.

On the contrary, higher prevalence were recorded by Al-Majali et al. (2007) 33.1% Ahmed et al. (2010), Kaoud et al. (2010), Mahboub et al. (2013) and Nagati and Hassan (2016) by rates of 24%, 26.6%, 18.09% and 16.4% respectively. On the other hand, lower results were documented by Ferede et al. (2011), Rahman et al. (2011), Horton et al. (2014), Tsehay et al. (2014) and Patel et al. (2017) by rates of 0.74%, 3.08%, 4% , 7% and 8.70 respectively.

RBPT is the ideal screening test for human brucellosis. This is for the reason that it is highly sensitive, simple and rapid technique (Smits and Kadri 2005; Teng et al., 2017). In our study, the overall prevalence of human brucellosis by RBPT as illustrated in table 2 was 24.3% (90/ 371). Nearly similar results were obtained by Fouad et al. (1996) (26%), Kumar et al. (1997) (28.57%) and De Massis et al. (2005) who concluded that Human brucellosis is more widespread in areas where *Brucella* is prevalent in sheep. Hussain et al. (2014) confirmed that nomadic life is characterized by some factors that favor brucellosis infection such as regular migration, inadequate health services, close animal contact, poor hygienic procedures and ingestion of raw animal products.

Lower results were obtained by Afifi et al. (2007) (11%), Hassanain and Ahmed (2012) (6.26%), Hussain et al. (2014) (6%), Ali et al. (2015) (8.6%) , Nagati and Hassan (2016) who declared that the seroprevalence of brucellosis among human was 15.2% and Tsegay et al. (2017) (4.7%). However, higher results were recorded by Ahmed et al. (2010) who founded that the overall seroprevalence of human brucellosis in Libya were 40%.

Data presented in table 3 showed that there is a highly significant difference between age groups, with higher percent of infection in age group more than 24 Month (14.19%) than other groups (2.39%) the obtained data were agreed with Abdallah et al. (2015) and Alhamada et al. (2017) who confirmed that seropositivity were significantly higher older animal ages. On the other hand, the infection rate in human were highly significant in male than female. Our results were agreed with results obtained by Afifi et al. (2005), Khan et al. (2009), Shahid et al. (2014).

A total of 2471 and 371 sheep and human samples were collected from four different localities. Data presented in table 4 showed that however, there a difference in the infection rates in four localities, statistical analysis showed that significant differences found only in sheep samples. Ecological dissimilarity has been reported to influence the seroprevalence of brucellosis (Rahman et al., 2011). Prevalence of brucellosis in relation to sex were illustrated in table 5 and showed that the infection rate in sheep were higher in female (11.35%) than male (6.88%). However statistical analysis showed no significant difference. These results were agreed with that obtained by Ferede et al. (2011), Hussain et al. (2014), Tsehay et al. (2014) and Ali et al. (2015). Rams could be lower than ewes as they may be culled or sold faster. In addition to erythritol and sex hormone in ewes (Rahman et al., 2011).

On the other hand, the infection rate in human were highly significant in male than female. Our results were agreed with results obtained by Afifi et al. (2005), Khan et al. (2009), Shahid et al. (2014). Analysis of the seasonal variation of brucellosis illustrated in table 7 and showed a highly significant differences with the highest infection rate during winter season in both human and sheep. Our results disagreed with Al-Ballaa et al. (1994) who founded that the predominance of cases occurring during spring, summer and early fall and De Massis et al. (2005) who confirmed that the seasonal occurrence of human cases of brucellosis showed a peak in summer.

CONCLUSION

High prevalence of brucellosis in sheep and human in North West region of Egypt, representing major public health threats. We need further investigation including isolation and molecular identification and further analysis of other possible risk factors associated with *Brucella* infection.

DECLARATIONS

Competing interests

All authors have no conflict of interest.

Author's contributions

Mohamed S. Diab, Yasser Elnaker, Nermin Awade, Eman Khalifa and Sherif Zidan conceived and designed the experiments. Yasser Elnaker, Nermin Awade, Eman Khalifa performed the experiments. Sherif Zidan and Yasser Elnaker analyzed the data. Mohamed S. Diab, Sherif Zidan, Nermin Awade and Eman Khalifa contributed reagents/materials/ analysis tools. Yasser Elnaker, Mohamed S. Diab and Nermin Awade wrote the paper.

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Evaluation of Club Foot in Working Donkeys

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ABSTRACT

Club foot deformities were studied in 22 donkeys working in brick kilns. Evaluation of hoof capsule was carried out on hard concrete surface. Changes on the hoof capsule were observed from dorsal aspect, lateral aspect, palmar/planter aspect and distal or solar surface. Dorsal hoof wall angle for each hoof (toe angle) was taken using hoof gauge. According to toe angle club foot was classified into three degrees. The first degree the toe angles less than ($<90^\circ$). The second degree the toe angles less or equal ($\leq 130^\circ$) and the third degree more than ($>130^\circ$). Club foot in donkeys displayed disparity, increased in heights and lengths associated with contracted heels. The digital cushion appeared atrophied associated with granulomatous inflammations. The third degree club foot revealed horn materials of sole, frog and digital cushion were destroyed and covered by hard keratin materials. The use toe angles for classification of club foot provide a reliable method. Radiography of the 3rd phalanx showed osteophytic and osteolytic changes. Soft tissue reaction and thickening with radio-opaque mass and calcification at palmar/planter structures were observed. Overworking, overloading and pain were considered the predisposing causes in brick kilns working donkeys.

Key words: Club foot, Deformities, Toe angle, Radiography, Donkey

INTRODUCTION

Egypt has about three millions of working donkeys and mules. They are carrying goods and people as well as work in brick kilns (Al-Salihi and Farhat, 2014). Club foot conformation in horses have a boxy hoof, thin flat soles, decrease in sole depth, concavity of the dorsal hoof wall, poor hoof wall consistency, toe cracks, hoof wall separation, white line disease and the musculotendinous shortening (Floyd and Mansmann, 2007; O'Grady, 2012). Club foot predisposed the horses to toe first landing, toe and quarter cracks, and dorsal wall flare, hoof wall separation (O'Grady and Dryden, 2012).

Several studies have been conducted on club foot etiology, diagnosis and treatment in horses (Stone and Merritt, 2009; O'Grady, 2014). However, to our knowledge, club foot in donkeys has not yet been studied. There is limited information about the orientation of the club foot in donkeys and radiographic effects on hoof structures. Therefore, the aim of the present study was to carry evaluation of club foot deformity in working donkeys to enable assessment of hoof management.

MATERIALS AND METHODS

Twenty-two donkeys were collected from El Saf brick kilns area from February 2017 to March 2018. A total of 29 hind hooves and three fore hooves showed club foot hoof deformities were examined during this study. This study was approved by the institutional animal care and use committee, Cairo University. These animals were selected on the basis of verbal informed owner consent.

Careful visual inspection and palpation of all hooves were performed according to Ross and Dyson (2011). Evaluation of donkey hoof capsule was carried out on hard concrete surface according to O'Grady (2013). Changes on the hoof capsule were observed from dorsal aspect, lateral aspect, palmar/planter aspect and distal or solar surface especial attentions were observed to the coronet and digital cushion. Dorsal hoof wall angle for each hoof (toe angle) was taken using hoof gauge (Souza et al., 2016).

Lateral radiographic views were taken. The foot was positioned on wooden block perpendicular to the ground and with equal weight bearing according to the method described by Kummer et al. (2004). Radiographic exposure factors were 50 to 55 kV, 10 mAs and 90 cm focal film distance.

ORIGINAL ARTICLE
doi: S232245681800008-8
Received: 20 Oct 2018
Accepted: 29 Nov 2018

RESULTS

Club foot in donkeys were classified in the present study into three degrees according to toe angle (mean± SE), the first degree the toe angles less than ($<90^\circ$) the mean was $77.6 \pm 1.8^\circ$. The second degree the toe angles less or equal ($\leq 130^\circ$) the mean was $106.3 \pm 3.5^\circ$ and the third degree more than ($> 130^\circ$) the mean was $150.3 \pm 7.00^\circ$. Bilateral hind club foot was diagnosed in 9 donkeys and unilateral in 8 hind limbs and 3 forelimbs.

The first degree of toe angle

The dorsal hoof wall appeared straight, convex or even segmented into two parts (Figure 1). The coronary band slope from dorsal hoof wall to heel appeared normal or migrated or concave in shape at the quarters or heel areas (Figure 1). The dorsal coronary band slightly bulged with marked wearing of the toe due to dragging. The loaded side of the hoof wall appeared rolled under and nearly straighter. The unloaded side appeared curved and flared side showed quarter cracks (Figure 2). Unsymmetrical ground surfaces. Atrophied frog migrated backward, out of bearing weight, contracted heels and the center groove of the frog is deep and extended to the coronary band hair line (Figure 3). Disparity in the two heels (Figure 4) and increased in lengths and heights with deep fissure at the base of the frog.



Figure 1. First degree club foot 14 years donkey showing dorsal hoof wall segmented into two parts, contracted heels and frog and the frog out of bearing weight (black arrow).

Figure 2. First degree club foot 9 years, the coronary band is not parallel to the ground surface (red arrow), the loaded side is straight (black arrow), the unloaded side is curved (yellow arrow).

Figure 3. First degree club foot 17 years, unsymmetrical ground surface, contracted, backward migrated and deep fissure at the base of frog (blue arrow), loaded heel is rolled under (yellow arrow) and the unloaded is fared (red arrow).

Figure 4. First degree club foot 14 years donkey, disparity heels with deep fissure at the base of the frog (yellow arrow).

The second degree of toe angle

The dorsal hoof wall was increased in lengths with wearing, damaged, cracks, hoof wall separation and quarter cracks. The overloaded toe appeared damaged (Figure 5). The dorsal coronary band is bulged, lost the slope, appeared parallel to the ground surface, concave in shape, with proximal migration at the medial and lateral quarters, with sever tension and pain on the common digital flexor tendon. The heels contracted and increased in heights and lengths. Fissures in the bulb of the heels with granulomatous inflammation involved the frog and skin of the pastern. Atrophied frog, the lateral and medial sulci of the frog extended backward associated with deep fissure in the central sulcus of the frog extended to the hair line of the coronary band (Figure 6). The solar surface appeared deformed, square in shape and lost its normal shape. The frog deformed, lost the normal wedge shape with widening, deep lateral and medial sulci and migrated backward (Figure 7).

The third degree of toe angle

The entire ground surface of the foot is out of the weight bearing surface, landing and loading mainly on the dorsal wall surface or pastern during walking. The hoof wall showed wearing fragmented and separated. The coronary band appeared bulged, concave and contact with the ground surface during walking associated with proximal migration above the medial and lateral quarters and heels (Figure 8). There was marked increased of the heel height and lengths. The frog lost wedge shape and deformed. The central sulcus of the frog appeared as long narrow fissure and extended up to mid palmar/planter pastern above the base of the frog (Figure 9). The solar surface appeared square or irregular in shape. The horn materials of sole, frog and digital cushion were completely disappeared or destroyed and covered by hard keratin materials (Figure 10).



Figure 5. Second degree club foot 16 years, damaged and cracks of overloaded toe (yellow arrow), granulomatous inflammation at the base of frog, heels and the pastern (red arrow).

Figure 6. Second degree club foot 13 years, the digital cushion is atrophied (red arrow), contracted heels (yellow arrow), the lateral grooves is wider (blue arrow), the central sulcus extended to the hair line (black arrow).

Figure 7. Second degree club foot 13 years, the solar surface is deformed, square in shape (red line), the frog lost shape (yellow arrow) and widening deep lateral sulci (green arrow).

Figure 8. Third degree club foot 19 years, the coronary band concave in shape (red line), dorsal coronary band contact with the ground (yellow arrow), frog migrated rear (blue arrow).

Figure 9. Third degree club foot 19 years, frog and digital cushion out of bearing surface. The central sulcus appeared long narrow and extended to mid planter pastern (red arrow).

Figure 10. Third degree club foot 19 years; solar surface became deformed, square and lost wedge shape (red arrow).

Radiographic evaluation of club foot

The 1st degree club foot showed osteophyte reactions at the proximo-dorsal aspect of the first phalanx. Osteolytic changes were appeared at navicular bone and third phalanx associated with palmar/planter soft tissues reactions (Figure 11). The 2nd degree club foot, radiographic changes displayed osteophyte reactions at dorso-distal aspect of the third metacarpal/metatarsal aspects and at the capsule of fetlock joint with narrowing the joint space. Palmar/ planter soft tissues thickening associated with osteophyte reaction of the navicular bone and at dorsal aspect of the second phalanx. The dorsal aspect of the third phalanx revealed osteolytic changes with decreased radio-opacity (Figure 12). The 3rd degree club foot revealed soft tissue reaction and thickening at the palmar / planter aspect of the distal limb with marked radio-opaque masses and calcification of palmar / planter structures. Osteophyte reaction was seen at the navicular bone, the dorsal aspect of 2nd phalanx and proximo-distal aspect of 3rd phalanx. The distal dorsal aspect of the 1st phalanx had osteophytic reactions with calcification of pastern joint. The 3rd phalanx showed osteolytic reaction at the dorsal, distal and at the tip of 3rd phalanx (Figure 13).



Figure 11. (First degree): Lateromedial radiography revealed palmar radiodense mass (black star).



Figure 12. (Second degree): Lateromedial radiography revealed palmar radiodense mass (black star), osteophyte reaction at the dorsal wall of the Third phalanx (white arrow) with radiolucent zone within the disto dorsal aspect (the tip) of the Third phalanx (black arrow).

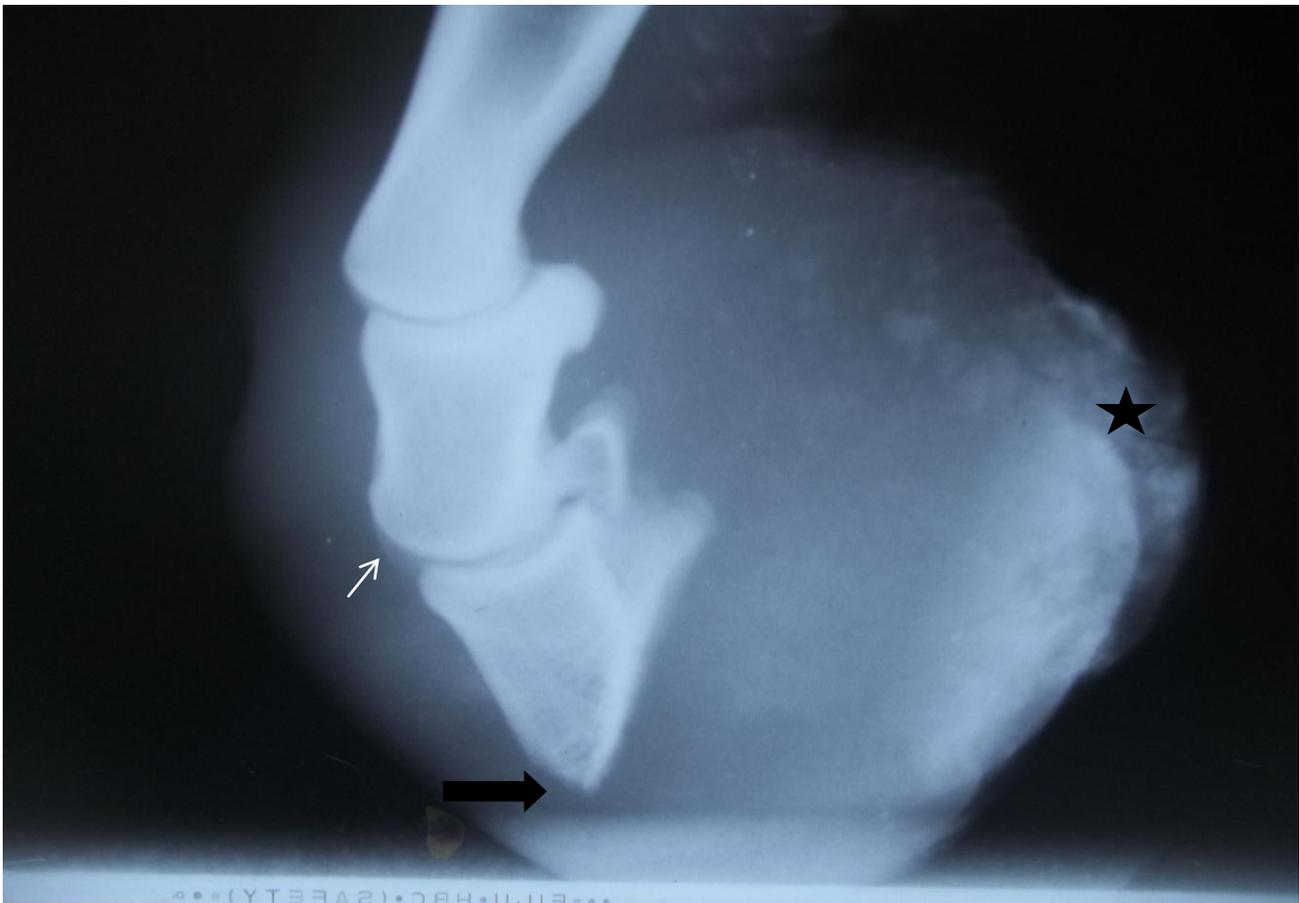


Figure 13. (Third degree): Lateromedial radiography revealed palmar radiodense mass (black star), osteophyte reaction at the distodorsal aspect of the Second phalanx (white arrow) with radiolucent osteolytic change at the disto dorsal aspect of the Third phalanx (bone loss at the tip of Third phalanx) (black arrow).

DISCUSSION

Club foot or Flexural deformity of the distal interphalangeal joint in horses can occur congenitally or acquired later in life due to overload, injury, environmental factors, or farriery practices (Hunt, 2011, Redden, 2014 and Curtis, 2017). Donkey club foot in the present study had three degrees according to measured toe angle. Increased heel heights and lengths, the loaded heels rolled under and the unloaded heels was flared, contracted heels, the digital cushion and frog atrophied and migrated back ward, the coronary band displaced, concave in shape or nearly parallel to the ground surface and dorsal coronary band bulged, hoof wall separations and toe and quarter cracks with wearing were the constant findings. In this respect, similar findings have reported in the horses (O'Grady and Dryden, 2012; O'Grady, 2014; Floyd and Mansmann, 2007; Redden, 2014) in horses. However, club foot in donkeys showed specific findings, the bulb of the heels and base of frog have deep fissures and the central sulcus appeared deep narrow and extended to the hair line. The digital cushion appeared contracted, atrophied associated with granulomatous inflammations. The horny materials of sole, frog and digital cushion were completely disappeared or destroyed and covered by hard keratin materials. These results were contradictory with the findings obtained in horses (O'Grady and Dryden, 2012) in the horses

Several classifications of clubfoot in horses have been reported (Redden, 2003; O'Grady, 2014) they classified club foot or flexural deformity into four grades depending on toe angle opposite to the healthy foot. In the present investigation donkey club foot was classified into three degrees based on the toe angles. The use toe angles for classification of club foot provide a reliable method in donkey due to 9 (41%) donkeys in the present study have bilateral, 8 donkeys unilateral and one had triple club foot deformities. Therefore, the use of opposite limb in the present study is unreliable.

Congenital and acquired club foot were reported and attributed to nutritional imbalance, trauma, improper trimming, persistent musculotendinous contractions of deep digital flexor muscle and pain (Redden, 2014). Club foot in this study could be attributed to stress and pain from hard work during pulling overloaded carts laden with bricks to and from the firing ovens of the brick kilns along the day. Therefore, overworking, stress and pain were considered the predisposing causes. However, acquired club foot in horses may be secondary to chronic lameness or injury (Hunt, 2011).

Club foot in donkeys displayed disparity, increased in heights and lengths associated with contracted heels. The dorsal hoof wall was landing and loading on the ground, wearing fragmented, toe and quarter cracks with hoof wall

separations are common findings. Similar observations have been mentioned previously in horses (Floyd and Mansmann, 2007; O'Grady et al., 2007; Redden, 2014). They reported increased heel growth, raised heels, lead to distal interphalangeal joint flexion altered the distal phalanx alignment, prompts the toe first landing at the dorsum of the foot predisposed to excessive wear, toe and quarter cracks due to lack of adequate horn protection. Moreover, O'Grady et al. (2007) stated that club foot altered biomechanics of the foot result in an increased load being placed on the dorsal section of the foot during landing.

Previous studies proved changes hoof shape in club foot influenced by differing hoof growth rates at various sites around the hoof capsule lead to the hoof wall at the heels growing faster than the toe (Faramarzi et al., 2009), the viscoelastic composite of tubular and inter tubular horn (Dyson et al., 2011), plastic compression (Hood et al., 2001). In addition, the factors influencing changes in hoof capsule shape are thought to be associated with; hoof growth, wear at the bearing border, farriery, plastic deformation and normal weight-bearing and loading (Curtis, 2017). Therefore, the changes in the hoof capsule shapes in the present study could be attributed to multifarious agents mainly overloading, hoof compression, farriery, plastic deformation and hoof growth.

Collins et al. (2011) mentioned that donkey radiography is important for diagnosis of the internal relationships of the osseous structures of the foot and the distal phalanx and subjective assessment of the minor changes, as well as the relationship between the distal phalanx and the hoof capsule (Dyson et al., 2011). Several studies support that radiography foot conformation in horses had lapping and/or bone demineralization of the coffin bone due to the abnormal pressure distribution at the level of the apex of the third phalanx which compresses the blood supply that nourishes it (Redden, 2014; O'Grady and Dryden, 2012). The current findings observed osteophytic and osteolytic in the 3rd phalanx in club foot donkeys. Furthermore, previous guidance on foot conformation in the horses influences the forces acting on the structures in the foot, especially the deep digital flexor tendon, the navicular bone and the distal interphalangeal joint pathology (Wilson and Weller, 2011; Eliashar et al., 2004; Moleman et al., 2006; Holroyd et al., 2013). Similar findings have been seen in deep digital flexor tendon, navicular bones and osteoarthritis in fetlock joints in club foot donkeys supported the same reported in horses.

CONCLUSION

Club foot in donkeys displayed disparity, increased in heights and lengths associated with contracted heels. The central sulcus appeared deep narrow and extended to the hair line. The digital cushion appeared atrophied associated with granulomatous inflammations. The horn materials of sole; frog and digital cushion were destroyed and covered by hard keratin materials in the 3rd degree club foot. The use toe angle for classification of club foot provides a reliable method in donkey. Overworking, stress and pain were considered the predisposing causes in donkeys.

DECLARATIONS

Acknowledgment

We are grateful for the help and assistance received from the staff of Egyptian society for the protection and welfare of the working animals (ESPWWA) and the donkey sanctuary United Kingdom.

Competing interests

The authors have declared that no competing interest exists.

Authors' contribution

M.B. Mostafa was responsible for design, study execution, interpretation and preparation of the manuscript. A.I. Abdelgalil was responsible for data collection, radiographic interpretation and writing the manuscript. S. Farhat was responsible for execution.

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Effect of Probiotics on Growth, Some Plasma Biochemical Parameters and Immunoglobulins of Growing Najdi Lambs

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ABSTRACT

This study was performed at the governorate of Alkhurmah, Kingdom of Saudi Arabia. Twenty growing Najdi lambs aged six months with average body weight 35.22 ± 0.107 and 33.67 ± 0.107 kg for males and females, respectively were allotted based on their gender into two main groups (10 males and 10 females), each gender divided into two subgroups the first subgroup of each gender served as control while the second was supplemented with *Lactobacillus sporogenes* 37.50×10^3 , *Saccharomyces cerevisiae* 625.0×10^3 CFU, 1g Alpha amylase and 20g sea wood powder / kg diet (concentrate feed mixture) for six months. The results indicated that lambs supplemented with probiotics in diet had better improvement ($P < 0.05$) on growth performance indices (Average daily gain, growth rate and total weight gain) compared with control subgroups in both genders. Probiotics increased ($P < 0.05$) the values of plasma total protein, glucose, urea nitrogen and aspartate aminotransferase compared with control group. On the other hand, total cholesterol concentration decreased significantly ($P < 0.05$) in lambs supplemented with probiotics than control groups. The mean values of plasma immunoglobulin A did not differ in both control and treated groups during the study period, while plasma immunoglobulin G increased significantly ($P < 0.05$) in lambs supplemented with probiotics compared with control groups in both genders. Plasma total lipids and aspartate aminotransferase concentrations remained relatively stable throughout the study period in both probiotics and control groups. In conclusion, probiotic supplementations can be used as the important biological additives for enhancing growth indices and immunity status of growing lambs.

Key words: Body weight, Immunoglobulin, Najdi lambs, Plasma metabolites, Probiotics, Sex

INTRODUCTION

Probiotics is defined by the food and agricultural organization of the united nations (FAO/WHO, 2002; Senok et al., 2005) as "live microorganisms used in adequate amounts, which afford a beneficial health effect on the host. Administration of probiotics strains separately and in combination was significantly improved feed intake, feed conversion rate, daily weight gains and total body weight in chicken, sheep, goat and cattle (Chiofalo et al., 2004; Salmi et al., 2007; Saleem et al., 2017), improve absorption of nutrients and thus reduce mortality and accelerated weaning of young animals. A positive effect of probiotics supplementation on intake, growth rate and feed conversion in ruminants has been reported by other workers (Antunovic et al., 2005; Whitley et al., 2009). Probiotics are used in commercial animal production farms to adjust alter gastrointestinal flora, causes improving animal health and productivity. The major outcomes from using probiotics include enhance growth, reduce mortality rate, and increase feed conversion efficiency (Yirga H, 2015)

One of the most important functions of direct-fed probiotics is that they can play a role in the immune system as immunomodulators (Fang et al., 2000; Kaburagi et al., 2007). Immunoglobulin G plays an important role in systemic immune response and is the main antibody in the serum after ingestion of probiotic protein. Immunoglobulin A is a major antibody in the mucosal immunity, and the main function of immunoglobulin A (IgA) is to exert the immune exclusion of pathogenic bacteria by intimate cooperation with innate nonspecific defense mechanisms (Sun et al., 2010). Probiotics increased intestinal IgA secretion both in sows and piglets. Probiotics increase the speed of development of the rumen flora and fauna, enhance immunity (Aattouri et al., 2001), reduced the incidence of intestinal infections, restore an intestinal micro-flora and have a positive effect in cases of diarrhea (Musa et al., 2009). Sohini et al. (2018) reported that probiotic supplementation in rats provide protection against oxidative stress.

Therefore, the objective of this study was the investigating of the effect of *L. sporogenes* (37.50×10^3), *S. cerevisiae* SC-47 (625.0×10^3 CFU), 1g alpha amylase and 20g sea wood powder/kg diet on growth indices, some plasma biochemical parameters and immunoglobulin's levels of growing Najdi lambs.

ORIGINAL ARTICLE
 pii: S232245681800009-8
 Received: 19 Oct 2018
 Accepted: 25 Nov 2018

MATERIALS AND METHODS

Aim of the study

This experiment aimed at studying to evaluate the effect of *Lactobacillus sporogenes* (37.50×10^3), *Saccharomyces cerevisiae* SC-47 (625.0×10^3 CFU), 1g Alpha amylase and 20 g sea wood powder / kg diet on growing Najdi lambs and their growth indices, some plasma biochemical parameters and immunoglobulins levels.

Ethical approval

This experiment was performed according to all ethics and animal rights (Ain Shams University, Egypt).

Animals and experimental design

Twenty growing Najdi lambs (one of the indigenous Saudi Arabia sheep breeds), with average age of six months, average body weight of 35.22 ± 0.107 and 33.67 ± 0.107 kg for males and females, respectively were used. Mineral salt blocks were distributed equally inside each yard. Lambs were allotted based on their sexes into two equal subgroups (10 lambs/each gender). Animals were housed in four shaded pens. The first group of each gender was served as control while the second group was supplemented with *Lactobacillus sporogenes* (37.50×10^3), *Saccharomyces cerevisiae* SC-47 (625.0×10^3 cfu), 1g Alpha amylase and 20g sea wood powder/kg diet [concentrate feed mixture (CFM)] were subjected for six consecutive months. The level of probiotics was listed in table 1.

The experimental diet composed of 60% concentrate feed mixture plus 40 % alfalfa hay. Average daily ration was adjusted according to monthly body weight changes of lambs to achieve adequate growth. Concentrate feed mixture was always provided first. Drinking water was available ad libitum. Chemical analyses of dietary ingredients are reported in table 2.

Table 1. Level of Probiotics added to the experimental diets of Najady lambs

Component	Content / kg diet
<i>Lactobacillus sporogenes</i>	37.50×10^3 CFU
<i>Saccharomyces cerevisiae</i> SC-47	$625. \times 10^3$ CFU
Alpha amylase	1g
Sea wood powder	20g

Table 2. Chemical compositions of concentrate and alfalfa hay (on dry matter basis, %)

Item	DM%	OM	CP	CF	EE	NFE	Ash
Concentrate feed mixture	88.75	91.52	15.31	13.56	2.72	62.23	8.98
Alfalfa hay	92.42	85.35	11.74	31.26	2.39	45.68	11.64

DM= dry matter; OM= organic matter; CP= crude protein; CF= crude fiber; EE= ether extract; NFE= nitrogen free extract

Lambs were weighed monthly by using an aviary weighing-machine to the nearest 100 grams at 07:00 a.m. Furthermore, growth performance indices were calculated as follows: average daily gain (ADG, g/lamb/d) was calculated by subtracting the initial live body weight (ILBW) from final live body weight (FLBW) divided by the time period (180 days). Total weight gain (TWG, kg) was measured as the difference between final LBW and initial LBW. Growth rate (GR%) = (final LBW- initial LBW)/ (initial weight) *100.

Monthly, jugular blood samples were withdrawn from each animal. Plasma total proteins (TP, g/ dl), albumin (A, g/dl), Total Cholesterol (CHO, mg/dl), plasma urea nitrogen (PUN. mg/dl), and plasma alanine transferase (ALT) and aspartate transferase (AST, μ l) concentrations were determined. The concentrations of IgA and immunoglobulin G (IgG) from plasma samples were determined using a sandwich ELISA detection kit for bovine Immunoglobulin A and G against standards, according to the manufacturer's suggested protocol (CUSAbio Biotech Inc., Wuhan, China).

Statistical analysis was conducted using the general linear model (GLM) procedures of SAS (2008). A repeated measurements model was used. Body weight, plasma biochemical and immunoglobulin parameters were compared between the model factors according the following model. Distributed Duncan's test (1955) was used to detect the differences ($P < 0.05$) among different group means. The statistical model was:

$$Y_{ijkl} = \mu + T_i + S_j + P_k (A_l) + e_{ijkl}$$

Where;

μ = the overall mean

T_i = treatment effect, $i = 1: 2$

S_j = sex effect $j = 1$ or 2

$P_k (A_l)$ = animal within period effect $k = 1-6$ and $l = 1-5$

e_{ijkl} = error

RESULTS AND DISCUSSION

Animal growth performance

The initial and final body weight of Najdi lambs are presented in table 3. The results revealed that, lambs supplemented with *Lactobacillus sporogenes* (37.50×10^3), *Saccharomyces cerevisiae* SC-47 (625.0×10^3 CFU), 1g Alpha amylase and 20g sea wood powder/kg diet significantly ($P < 0.05$) improved body weight in both genders compared to the control groups. Similar results were observed by [Abas et al. \(2007\)](#) and [Ismaiel et al. \(2010\)](#) that yeast culture increased average daily gain of lambs. [Hussein \(2014\)](#); [Abdel-Salam et al. \(2014\)](#) and [Mohamed et al. \(2016\)](#) reported that Najdi lambs supplemented with probiotics had better performance ($P < 0.05$) in GR, ADG and TWG than control group. Recently, [Saleem et al. \(2017\)](#) found a positive effect of inclusion probiotics on growth performance by enhancing BW gain, TWG and GR of Saidi lambs during post-weaning period. This is a disagreement with [Baranowski et al. \(2007\)](#), [Titi et al. \(2008\)](#) and [Whitley et al. \(2009\)](#) that yeast supplementation had no effect on growth rate in lambs and goat kids.

Data presented in table 2 indicated that, at the end of experiment, GR, ADG and TWG were higher in male (19.32 %, 35.00 g/h/day and 6.30kg) and female (17.51%, 30.27 g/h/d and 5.45kg) lambs supplemented with *Lactobacillus sporogenes* (37.50×10^3), *Saccharomyces cerevisiae* SC-47 (625.0×10^3 CFU), 1g Alpha amylase and 20 g sea wood powder /kg diet than control groups male (15.29%, 27.77 g/h/day and 5.00 kg) and female (14.53%, 24.94 g/h/d and 4.49 kg), respectively. Similar results observed by [Sarwar et al. \(2010\)](#); [Mukhtar et al. \(2010\)](#); [Khalid et al. \(2011\)](#) and [Adel and EL-Metwaly \(2012\)](#).

With respect to the effect of gender, results in table 3 indicated that gender had a significant effect ($P < 0.05$) where male lambs had the higher mean body weight at the beginning and end of study than females. Similar results were observed by [Abbas et al. \(2010\)](#); [Abdel-Fattah et al. \(2013\)](#) and [Wielgosz-Groth et al. \(2015\)](#). Who demonstrated that gender had significant effect on body weight which may be due to the secretion of sex hormones with advance in age.

Table 3. Means \pm SE of body weight, growth rate, average daily gain and total weight gain of male and female Najdi lambs supplemented with probiotics in diets under Saudi Arabia environment during September 2017 to February 2018

Item	Experimental groups		Females	
	C	T	C	T
No. of animals	5	5	5	5
1st month, kg (Initial weight)	32.70 ^a	32.60 ^a	30.90 ^a	31.13 ^a
2nd month, kg	33.40 ^b	33.20 ^a	31.90 ^b	32.36 ^a
3rd month, kg	34.10 ^b	34.20 ^a	32.96 ^b	33.66 ^a
4th month, kg	35.20 ^b	35.60 ^a	33.82 ^b	34.61 ^a
5th month, kg	36.10 ^b	36.50 ^a	34.64 ^b	35.95 ^a
6th month (Final weight, kg)	37.70 ^b	38.90 ^a	35.39 ^b	36.58 ^a
\pm SE	0.19		0.20	
GR, %	15.29 ^b	19.32 ^a	14.53 ^b	17.51 ^a
ADG (g/h/d)	27.77 ^b	35.00 ^a	24.94 ^b	30.27 ^a
TWG, kg	5.00 ^b	6.30 ^a	4.49 ^b	5.45 ^a

C= control group= *Lactobacillus sporogenes* (37.50×10^3), *Saccharomyces cerevisiae* SC-47 (625.0×10^3 CUF), 1g Alpha amylase and 20g sea wood powder / kg diet; GR= growth rate, ADG= average daily gain; TWG= total weight gain; ^{a,b} within a row indicate a significant difference ($P < 0.05$) between groups. SE= standard error.

Plasma proteins response

Means \pm SE of total plasma protein (TP), albumin (A), globulin (G) concentrations and albumin/ globulin (A/G) ratio for treated and control groups of Najdi lambs are presented in table 4. Results indicated that, probiotics supplementation significantly increase ($P < 0.05$) plasma TP, and, G concentrations, while plasma A and A/G ratio behaved the opposite trend. Figure 1 showed that the increase in plasma TP concentration was more pronounced from the third month till the end of experimental period. The present results may be related to the beneficial effect of probiotics supplementation on increasing protein digestibility through the enzymatic effect of protease and alteration amino acid profile of digesta due to increasing microbial protein synthesis ([Williams, 1989](#); [Abdel-Khalek et al., 2000](#)).

Concerning the rate of change, results in table 4 revealed that, in both genders supplemented with probiotics recorded the higher rate of change in TP and G (12.71, 38.44 % for males and 11.09, 27.44% for females) compared with control group (4.05 and, 9.24 % for males and 5.87, 17.66% for females) at the end of experimental period. The corresponding values for plasma A and A/G ratio recorded (-10.61 and -36.04 % for males; 0.83 and -22.68% for females) of lambs supplemented with probiotics compared with control groups (-1.43 and -10.42 % for males; - 4.74 and -20.72% for females), respectively. These findings are in agreement with [El-Shaer \(2003\)](#) and [El-Ashry et al. \(2003\)](#) in sheep and [Kholif \(2001\)](#), [Abu El-Ella and Kommonna \(2013\)](#) in goats. They found that the Yeast Culture (YC)

supplementation led to increase TP. In contrast, Abdel Rahman et al. (2012) reported that YC supplementation significantly increased albumin concentration, while it was not significantly affected plasma TP or globulin concentrations of sheep. Also, Hossein-Ali et al. (2014) found that lambs supplemented with probiotics decreased significantly ($P<0.05$) the levels of plasma TP and albumin concentrations compared with control group. Recently, Saleem et al. (2017) reported that no significant effects on plasma TP, A and G levels during post-weaning period of Saiidi lambs fed diet supplemented with probiotics.

With respect to the effect of sex, statistical analysis of the obtained results indicated that, there were no significant ($P<0.05$) differences in TP, A, G and A/G ratio due to gender. In accordance, Carlos et al. (2015) found that the values of serum TP, A, G concentrations and A/G ratio were not significantly ($P>0.05$) affected by the gender of Morada Nova sheep. On the other hand, AL-Hadithy and Badawi (2015) found that there were significant ($P<0.05$) differences between males and females of Awassi sheep in serum TP, A and G concentrations where ewe lambs recorded the higher values compared with ram lambs.

Table 4. Means \pm SE of plasma total proteins, albumin, globulin concentrations and A/G ratio of male and female Najdi lambs supplemented with probiotics in diets under Saudi Arabia environment during period from September 2017 to February 2018

Trait	Experimental groups	Males		Females		\pm SE
		C	T	C	T	
TP (g/dl)	Initial	7.16 ^b	7.24 ^b	7.32 ^b	7.39 ^b	0.17
	Final	7.45 ^a	8.16 ^a	7.75 ^a	8.21 ^a	0.15
	Change %	4.05	12.71	5.87	11.09	-
A (g/dl)	Initial	3.49 ^a	3.77 ^a	3.80 ^a	3.60 ^a	0.12
	Final	3.44 ^a	3.37 ^a	3.62 ^a	3.63 ^a	0.12
	Change %	- 1.43	- 10.61	- 4.74	0.83	-
G (g/dl)	Initial	3.68 ^b	3.46 ^b	3.51 ^b	3.79 ^b	0.15
	Final	4.02 ^a	4.79 ^a	4.13 ^a	4.83 ^a	0.13
	Change%	9.24	38.44	17.66	27.44	-
A/G ratio	Initial	0.96 ^a	1.11 ^a	1.11 ^a	0.97 ^a	0.06
	Final	0.86 ^b	0.71 ^b	0.88 ^b	0.75 ^b	0.05
	Change%	- 10.42	- 36.04	- 20.72	- 22.68	-

C= control group; T= *Lactobacillus sporogenes* (37.50×10^3), *Saccharomyces cerevisiae* SC-47 (625.0×10^3 CUF.), 1g Alpha amylase and 20 g sea wood powder / kg diet; TP= total protein; A= albumin; G= globulin; SE= standard error. A/G= Albumin/ globulin ratio, ^{a,b} in the same column are statistically ($P<0.05$) difference between groups

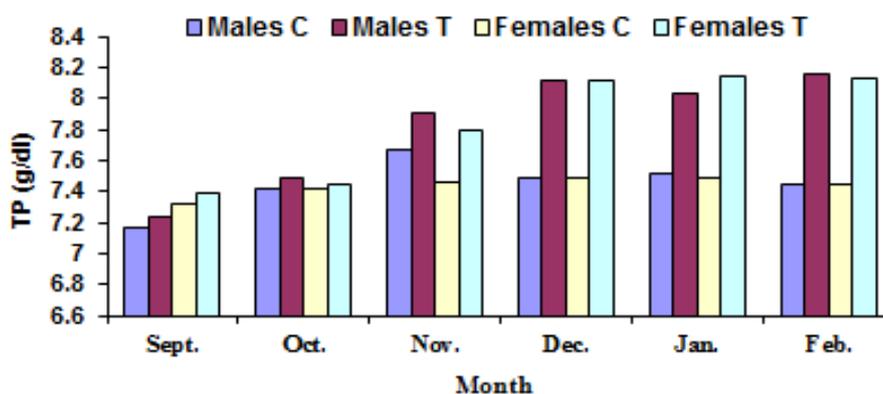


Figure 1. Effect of probiotics supplementation on plasma total proteins concentration of male and female Najdi lambs under Saudi Arabia environment during period September 2017 to February 2018

Plasma total cholesterol response

Means \pm SE of plasma Total Cholesterol (CHO) concentration in Najdi lambs fed diet supplemented with probiotics are presented in table 5. The results indicated that male and female lambs supplemented with probiotics caused significantly ($P<0.05$) low plasma CHO compared with control groups. As shown in figure 2, plasma total CHO began to decrease from the second month of treatment till the end of experimental period. The significant ($P<0.05$) decrease in plasma total CHO concentration in treated groups in comparison to control groups are in agreement with those finding by Abu El-Ella and Kommonna (2013), Hossein-Ali et al. (2014) and Mozghan et al. (2016) who reported that lambs supplemented with probiotics decreased significantly ($P<0.05$) the level of CHO concentration compared with control group. There are two proposed mechanisms for the reduction of plasma cholesterol concentration in animals fed

with probiotics: (1) increase in degradation of cholesterol across the gastrointestinal tract (Zarate et al., 2002) and (2) simultaneous sediment of cholesterol and deconjugation of bile acids (Fernades et al., 1987). In addition, Ooi and Liang (2010) illustrated that cholesterol removed by probiotics by incorporation into the cellular membranes during growth. They added that the cholesterol converted to coprostanol in the intestines which excreted in feces, therefore this reduction in the cholesterol absorbed resulted in decreasing concentration of cholesterol in blood. Hossein-Ali et al. (2014) found that lambs supplemented by probiotics containing *Bacillus subtilis* and *Bacillus licheniformis* with the trade name of Bioplus 2B at two levels (0.5 and 1g Bioplus 2B/kg diets) decreased significantly ($P<0.05$) the level of cholesterol concentration compared with control group.

With respect the effect of gender, results indicated that there was no sex effect ($P<0.05$) on plasma CHO concentration. Similar finding was reported in the study conducted by Carlos et al. (2015). In contrast, Abdel-Fattah et al. (2013) reported that there were significant differences ($P<0.01$) between genders where plasma CHO, concentration was found to be higher in male Barki lambs than that of ewe lambs.

Table 5. Means \pm SE of plasma cholesterol, urea nitrogen, alanine amino transferase and aspartate amino transferase concentrations of male and female Najdi lambs supplemented with probiotics in diets under Saudi Arabia environment from September 2017 to February 2018

Trait	Experimental groups	Males		Females		\pm SE
		C	T	C	T	
CHO (mg/dl)	Initial	87.44 ^a	86.24 ^a	87.52 ^a	85.98 ^a	1.09
	Final	86.92 ^a	76.29 ^b	86.29 ^b	78.22 ^b	1.08
	Change%	- 0.59	- 11.54	- 1.40	- 9.02	-
PUN (mg/dl)	Initial	40.10 ^b	41.68 ^b	42.01 ^b	42.20 ^b	0.77
	Final	43.95 ^a	47.54 ^a	43.81 ^a	48.15 ^a	0.79
	Change%	9.60	14.06	4.28	14.09	
ALT (μ l)	Initial	8.19 ^a	8.07 ^a	7.16 ^a	7.98 ^a	0.38
	Final	8.06 ^a	7.95 ^a	7.17 ^a	7.95 ^a	0.40
	Change%	- 1.58	- 1.49	- 0.28	- 0.37	
AST (μ l)	Initial	14.87 ^b	14.59 ^b	15.93 ^b	15.81 ^b	0.33
	Final	16.28 ^a	16.15 ^a	17.16 ^a	17.46 ^a	0.34
	Change%	9.48	10.69	7.72	10.44	-

C= control group; T= *Lactobacillus sporogenes* (37.50×10^3), *Saccharomyces cerevisiae* SC-47 (625.0×10^3 cuf), 1g Alpha amylase and 20g sea wood powder/kg diet; CHO= total cholesterol; PUN=plasma urea nitrogen; ALT= alanine amino transaminase; AST= aspartate amino transaminase;^{a,b} in the same column are statistically ($P<0.05$) difference between groups; SE= standard error

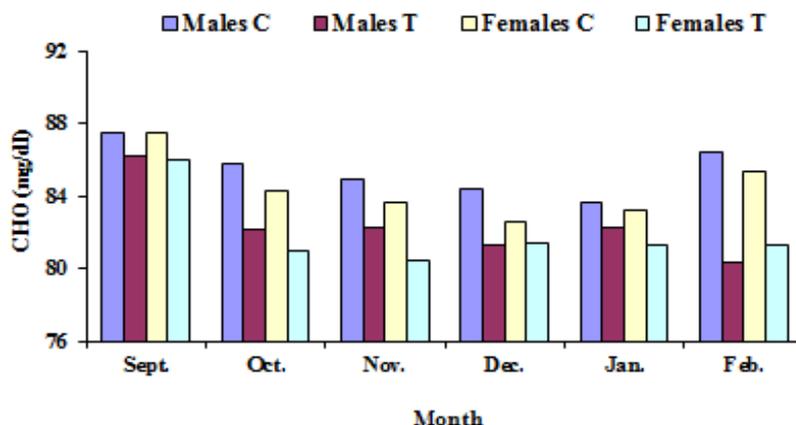


Figure 2. Effect of probiotics supplementation on plasma cholesterol concentration of male and female Najdi lambs under Saudi Arabia environment during period from September 2017 to February 2018

Plasma urea nitrogen (PUN) response

Table 4 summarizes the means \pm SE of plasma urea nitrogen concentrations during the experiment period, results indicated that Plasma Urea Nitrogen (PUN) was increased ($P<0.05$) in male and female lambs supplemented with probiotics compared with control groups. Moreover, the rate of change was slightly higher in treated males (14.06%) and females (14.09%) compared with control males (9.60%) and females (4.28%), respectively. In agreement, Hillal et al. (2011) reported that high concentration of PUN may be due to incapacity of ruminal microflora to detain the ammonia optimally (Butler, 1998). Also, Abdel-Rahman et al. (2012) and Mousa et al. (2012) on sheep found that feeding diets treated with probiotic resulted in an increase of urea concentration. In contrast, no difference ($P<0.05$) was noticed in plasma urea nitrogen concentration of Kajli lambs due to protein sources and probiotics level (Sarwar et al. 2011). Antunovic et al. (2005) in weaned lambs received 0.1% probiotic and Antunovic et al. (2006), where the concentration of urea was lower in the experimental group (5.51:7.97 mmol/l). On the other hand, El-Ashry et al. (2003); Shakweer

(2003) and Petr Dolezal et al. (2011) found lower concentration in serum urea nitrogen of cows in response to probiotic supplementation which suggested as an indicator of better nitrogen metabolism and utilization of protein. With respect the effect of sex, statistical analysis of the obtained results revealed that there was not significant ($P>0.05$) effect in plasma PUN concentrations due to sex. The results of Rabee et al. (2014) and Carlos et al. (2015) were in agreement with the present results. On the contrary, Sitmo (2014) reported that PUN concentration differed significantly between genders ($P<0.05$) where females tended to have higher values than males.

Plasma Alanine transferase (ALT) and Aspartate transferase (AST) response

Means \pm SE of plasma liver enzymes including ALT and AST are presented in table 5. The obtained results revealed that plasma ALT levels decreased significantly ($P<0.05$) in control and treated groups in both genders, this decrease was more pronounced in males (-1.5 and -1.49%) than females (-0.28 and -0.37%) for control and treated groups, respectively. On the other hand, plasma AST behaved the opposite trend where AST concentration increased significantly ($P<0.05$) in lambs supplemented probiotics compared with control groups of both genders. The results of Abdel-Khalek et al. (2012), Adel and El-Metwaly (2012); Abu El-Ella and Kommonna (2013) were in agreement with the present results. On the contrary, Afify et al. (2007) found a significant increase in serum ALT in calves treated with oxytetracycline and erythromycin (as growth promoter). EL-Ashry et al. (2003) found that supplementation of flavomycin (20mg/h/d) and *Saccharomyces cerevisiae* (5mg/h/d) increased ($P<0.01$) plasma ALT and AST than control groups of growing lambs. With respect the effect of sex, statistical analysis of the obtained results indicated that plasma enzymes (ALT and AST) not differed significantly ($P<0.05$) between males and females along the experimental period. In agreement, Kiran et al. (2012), Khan et al. (2013), Abdel-Fattah et al. (2013) and Sitmo (2014). While, Carlos et al. (2015) found that plasma levels of ALT recorded the higher ($P<0.05$) values for male than female of Morada Nova sheep.

Plasma immunoglobulins response

Concerning to the influence of probiotics supplementation on immune response, data in table 6 clearly indicated no significant difference ($P<0.05$) was recorded in plasma IgA concentrations among control and treated groups of both male and females. While the mean IgG concentration of lambs supplemented probiotics showed significantly ($P<0.05$) increase compared with control groups. These findings agree with several studies performed by Al-Saiady (2010), Sarker and Yang (2010), Sun et al., (2010) reported that no difference was observed in serum IgA whereas serum IgG was higher in the probiotic supplemented calves than in the control. Higgins et al. (2007) explained that, Direct Fed Microbial (DFM) have ability to enhance immunity by promoting the production of antibodies and cytokines and colonize the intestines, increasing phagocytosis of pathogens. McBeath et al. (1971) found that serum TP concentration was correlated ($r= 0.72$) with serum Ig concentrations in newborn calves. Also, Zachwieja and Dobicki (1997) found high ($P<0.05$) correlation between TP levels and immunoglobulins (IgA, IgG and IgM) levels in serum of the calves.

As shown in figure 3, on monthly plasma IgA concentration indicated that there was no significant difference ($P<0.05$) at any month along experimental period between treated and control groups and between genders. While figure 4 showed that lambs supplemented probiotics began to increase their plasma IgG from the third month till the end of experimental period in both genders. The rate of change in plasma IgA recorded - 0.10 and 0.00 vs. 0.41 and 0.63% for control and treated groups of both males and females, respectively. The corresponding values for plasma IgG recorded (0.09 and 3.85% vs. 0.16 and 3.62%) for control and treated groups of both males and females, respectively.

The results are disagreed with Patience (2015) found that DFM increased significantly ($P<0.05$) serum IgA levels than the control group, and this increase might be because feeding DFM elicits mucosal immunity and IgA is the dominant antibody of the mucosa. Regarding the effect of sex, statistical analysis of the obtained results indicated that plasma IgA and IgG levels not differed significantly ($P<0.05$) between males and females along the experimental period. Similarly, Kara (2009) found that no significant effect of calf sex on serum Ig concentrations after 24 h of birth.

Table 6. Concentrations of plasma immunoglobulin's (IgA and IgG) in male and female Najdi lambs supplemented with probiotics in diets under Saudi Arabia environment from September 2017 to February 2018

groups Trait	Experimental	Males		Females		±SE
		C	T	C	T	
IgA (mg/dl)	Initial	964 ^a	963 ^a	959 ^a	957 ^a	1.28
	Final	963 ^a	963 ^a	963 ^a	963 ^a	1.30
	Change%	- 0.10	0.00	0.41	0.63	-
IgG (mg/dl)	Initial	1235.6 ^b	1237.2 ^b	1236.6 ^b	1238 ^b	2.46
	Final	1236.8 ^a	1284.8 ^a	1238.6 ^a	1282.8 ^a	2.45
	Change%	0.09	3.85	0.16	3.62	-

C= control group; T= *Lactobacillus sporogenes* (37.50×10^3), *Saccharomyces cerevisiae* SC-47 (625.0×10^3 cfu), 1g Alpha amylase and 20 g sea wood powder / kg diet; IgA=plasma immunoglobulin A; IgG= plasma immunoglobulin G. ^{a,b} in the same column are statistically ($P<0.05$) difference between groups; SE= standard error

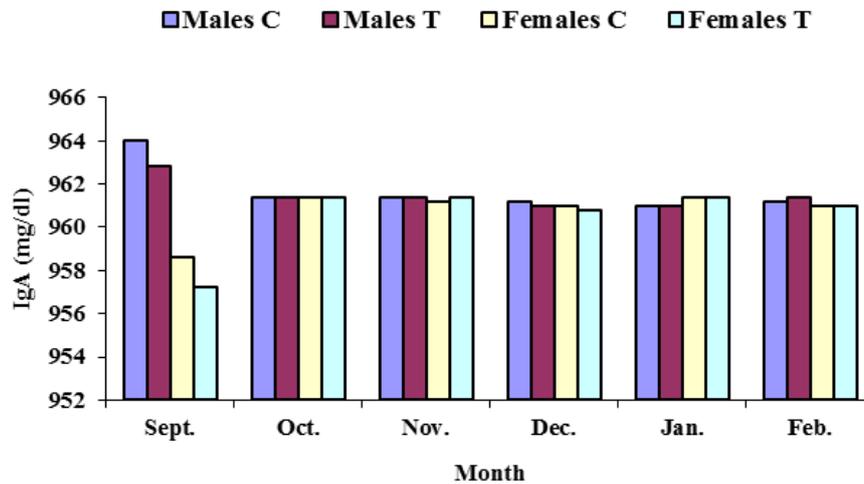


Figure 3. Effect of probiotics supplementation on plasma immunoglobulin A concentration of male and female Najdi lambs under Saudi Arabia environment during period from September 2017 to February 2018

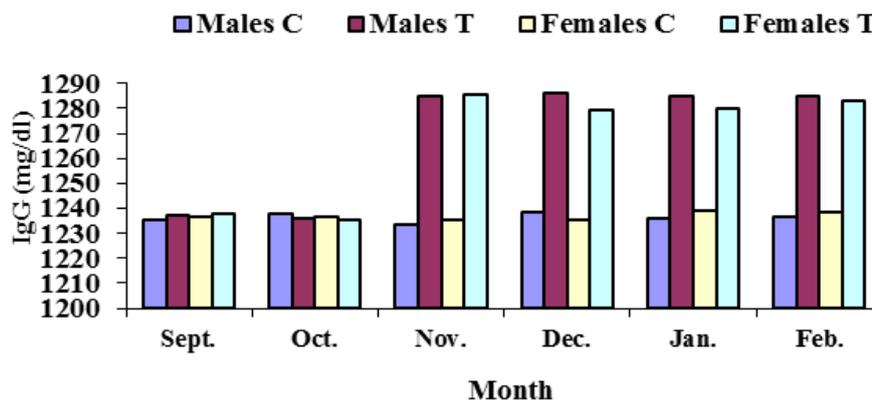


Figure 4. Effect of probiotics supplementation on plasma immunoglobulin G concentration of male and female Najdi lambs under Saudi Arabia environment from September 2017 to February 2018

CONCLUSION

The increase in growth indices (GR, ADG and TWG), plasma TP, G, PUN, GLU, IgG concentrations and the decrease in plasma CHO concentration of lambs supplemented with probiotics are all signs of improved health by potential beneficial effects of probiotics. Accordingly, the results recommended probiotics as one of the important biological additives for enhancing the growth indices and immunity in growing lambs without any side effect on plasma biochemical levels.

DECLARATIONS

Consent to publish

The author agrees to publish this paper in the journal of World's Veterinary Journal.

Competing interests

The author declares that he has no competing interests.

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Effect of Vitamin E on the Prevention of Peritoneal Adhesions in Sheep

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ABSTRACT

The objective of this study was to assess vitamin E solution on the prophylaxis of intraperitoneal adhesions in ovine uterine serosal damage model with bipolar diathermy. Therefore, 19 ewes underwent laparotomy for induction of adhesions, using a uterine serosal bipolar electrocauterization model. Cauterizations were performed on the right uterine horn serosa and right ovary. Ewes were randomly divided into three groups: control group (GCT, n=5), with no treatment following electrocoagulation, another group using local rinse of 20 mL of normal saline (GNS, n=8), and the last group using local rinse of 20 mL of vitamin E injection solution (GVE, n=6). On day 21 postoperative, animals underwent laparoscopy for scoring and comparison of intraperitoneal adhesion according to frequency and number. The number of adhesions was compared among groups using the Kruskal-Wallis test and Dunn's post-hoc test. As results, the bipolar uterine serosal coagulation model triggered uterine adhesions in 74% (14/19) of the animals. Frequency of postoperative intraperitoneal adhesions was similar ($P=0.819$) among groups (80% ewes of GCT, 62.5% of GNS and 83% of GVE). There was no significant difference between treatment groups, however, number of adhesions was lower in GVE and GNS groups than in control group ($P=0.032$), showing that the addition of these kind of substances are better than not using any type of barrier to prevent the formation of intraperitoneal adhesions.

Key words: Adhesions, Bipolar diathermy, Laparoscopy, Sheep, Uterus

INTRODUCTION

Ovine specie has been widely used as an animal model on surgical research and skills training. Moreover, surgical approaches are extensively performed in ovine reproduction science and biotechnology (Teixeira et al., 2013). However, post-operative adhesion formation is one of the main concerns in surgical approach for Artificial Insemination (AI) and Ovum Pick-Up (OPU) in small ruminants (Ward and Panitch, 2011; Menchaca et al., 2016 and Zarkawi and Soukouti, 2018).

Post-operative intraperitoneal adhesions affect both human and veterinary patients, causing chronic abdominal and pelvic pain, bowel obstructions and infertility (Reijnen et al., 2003; Moris et al., 2017). As a result, patients are under risk of repeated surgical approaches for adhesiolysis (Koninckx et al., 2016). There is a growing interest for researches on prophylaxis of adhesions using barrier methods and low molecular weight intraperitoneal solutions (Decherney and Dizerega, 1997; Kamel, 2010; Yildiz et al., 2011).

Pathogenesis of adhesion formation is complex and multifactorial. Peritoneal surgical damage disrupts mesothelial cells and exposes connective tissue layers, triggering inflammation, coagulation, fibrin deposition and fibrinolysis. In a favorable intraperitoneal environment, fibrinolysis overcome fibrin deposition and mesothelial layer heals without permanent adhesion formation (Reijnen et al., 2003). However, intraperitoneal surgical trauma and inflammation may comprise fibrinolysis, resulting in coagulation/fibrinolysis imbalance. In that environment, fibroblasts migrate onto the fibrin matrix and organized collagen fibers, small blood vessels and connective tissue forms. Finally, mesothelial cells cover the connective tissue (Koninckx et al., 2017). At that point, permanent adhesions form and surgery for adhesiolysis is the treatment of choice in cases of adhesion-related complications (Reijnen et al., 2003). Surgical approaches to the

ORIGINAL ARTICLE
 pii: S2322-45681800010-8
 Received: 20 Oct 2018
 Accepted: 22 Nov 2018

uterus are both traumatic and invasive, requiring meticulous and aseptic technique to avoid postoperative adhesiogenesis (Ishwar and Memon, 1996; Wilde et al., 2017). As usual, abdominal cavity should be constantly rinsed with proper solution. The use of an intraperitoneal prophylactic method should also be considered (Decherney and Dizerega, 1997; Moris et al., 2017).

Carboxymethylcellulose, lidocaine chloride, hyaluronic acid, sodium heparin, methylene blue solution and normal saline were assessed as anti-adhesion therapies (Elkelani et al., 2002; Durmus et al., 2011; Mariano et al., 2015). Other management include selection of minimally invasive approaches such as laparoscopy, use of powder free gloves, meticulous hemostasis, use of NSAIDs and antibiotics, cautious visceral handling and less organ exposition (Reijnen et al., 2003 and Kamel, 2010). Vitamin E, a natural cell antioxidant, avoids membrane lipid peroxidation. Vitamin E showed anti-inflammatory, anticoagulant, enzymatic, genic expression and anti-fibroblast properties (Jiang, 2014). Intraperitoneal vitamin E was efficient in preventing intraperitoneal adhesion formation in rat and mouse models (Kokcam and Nairoglu, 2002; Yetkin et al., 2009; Yildiz et al., 2011; Durmus et al., 2011). Moreover, intraperitoneal vitamin E rinse decreased adhesion formation in a mouse uterine horn trauma model (Yildiz et al., 2011).

To the authors' knowledge, there is a gap in the literature concerning the use of vitamin E on the prophylaxis of adhesiogenesis in small ruminants. Thus, the purpose of this study was to assess the efficacy of intraperitoneal vitamin E solution on the prevention of adhesion formation in an ovine uterine and ovarian electrosurgical trauma model.

MATERIALS AND METHODS

Ethical approval

This study was carried out under approval by the committee for ethics in animal use of the university of Franca (protocol no. 2713070316).

Experimental animals

Nineteen healthy adult female Santa Ines ewes, aging two to five years old, weighting thirty to fifty kg and presenting mean corporal score three (scale ranging from one to five), were selected for this study. Inclusion criteria was absence of abnormalities based on clinical, hematological, coproparasitological, gynaecological and abdominal echography assessments. Animals underwent a thirty-day adaptation period, in the veterinary hospital of the university of Franca, São Paulo, Brazil, in 16m² collective stalls, receiving daily diet of Tifton hay, balanced commercial ration for ovine specie, mineral supplementation and water ad libitum.

Experimental design

Ewes were randomly divided into one out of three groups as follows: group GCT (n=5), the control group, without intraperitoneal rinse; group GNS (n=8), receiving intraperitoneal rinse of 20 ml of normal saline (Cloreto de sódio 0,9%, Baxter™, Jurubatuba, São Paulo, Brazil), and group GVE (n=6), undergoing intraperitoneal rinse of 20 ml of vitamin E (Acetato de Vitamina E, 2g, 20 mL, LaboVET™, Bahia, Brazil).

Surgical procedure

Animals were fasted for 48 hours. Prior surgery, ewes were weighted and undergone premedication with acepromazine (0.1 mg/kg, IV - Acepran®, Vetnil, Louveira, São Paulo, Brazil) and methadone (0.5 mg/kg, IM - Mytadon® Cristalia. São Paulo, São Paulo, Brazil). General anesthesia was induced using propofol (6 mg/kg, IV - Propofol® Cristalia. São Paulo, São Paulo, Brazil), 10 minutes following premedication, and maintained with isoflurane (Isoflurano® Cristalia. São Paulo, São Paulo, Brazil) vaporized in 100% oxygen delivered by tracheal tube. Ewes were positioned in dorsal recumbence, followed by aseptic prepare of the ventral abdomen and then positioned in 45° Trendelenburg as described by Teixeira et al. (2013). A 10-cm pre-pubic midline celiotomy was performed, followed by exposition of the uterus and ovaries. A uterine and ovarian serosa damage model was used to trigger adhesiogenesis. Thus, three spots of bipolar electrocoagulation (3-second period each coagulation spot) were performed on the right uterine horn. One spot was carried out on the right ovary using the same technique. A 42 cm laparoscopic bipolar forceps (Lina Tripol Powerblade™, LiNA Medical, Glostrup, Denmark) was used for electrocoagulation. After cauterization, animals were submitted to one of experimental treatments. Group GVE received 20 ml of vitamin E rinsed over the electrocoagulation spots, while group GNS received 20 ml of normal saline in the same fashion and group GCT (control) received no rinse. The reproductive tract was repositioned within the abdominal cavity. Linea alba was closed using cross mattress sutures, followed by subcutaneous space closure and skin synthesis with interrupted horizontal mattress sutures, using nylon 0 thread (Nylon 0 Bionline®, São Paulo, São Paulo, Brazil). A repellent ointment (Unguento Plus®, Pearson, São Paulo, São Paulo, Brazil) was applied on the skin around the surgical site. Skin sutures were removed following 10 days.

Exploratory laparoscopy

Ewes undergone diagnostic laparoscopy to assess uterine and ovarian adhesion formation 21 days after surgery. Anesthetic protocol and positioning for laparoscopy the same as the one employed for adhesion induction approach. A 12 mm trocar cannula was inserted through a small midline celiotomy slightly cranial to the previous surgery's scar. Carbon dioxide pneumoperitoneum was set to eight mmHg intra-abdominal pressure and 5 l/min flow rate. A 10 mm straight angle telescope was used. A 5 mm working port was established under laparoscopic assistance for insertion of an atraumatic Babcock laparoscopic forceps. The right uterine horn and ovary were handled and primarily assessed for presence of adhesion. Adhesions were classified using an adapted scoring system (Farzad et al., 2012 and Mariano et al., 2015). Scores could range from zero to five according to the number of adhesions, with five meaning ≥ 5 adhesion sites. Adhesions were also scored regarding organs involved as follows: (0) no adhesions; (1) on the uterine horn and ovarian bursa; (2) involving the uterus, ovary and uterine tube; (3) among the uterus, ovary and other organs or tissues (bowel, bladder, peritoneum). After laparoscopic adhesion scoring by macroscopic evaluation and manipulation with the forceps to analyze the integrity of viscera, pneumoperitoneum was drained and trocars were withdrawn from the abdominal wall. Skin incisions were closed with simple interrupted nylon 0 USP sutures. Animals were kept in collective stalls following complete anesthesia recovery.

Statistical analysis

All analysis was performed using the Rs (R foundation for statistical computing®, Vienna, Austria). Incidence of adhesion were compared among groups using the Fischer's exact test. Scores of the number of intraperitoneal adhesions were compared among groups using the Kruskal-Wallis test and Dunn's post-hoc test. Significance level was set to 5% ($P < 0.05$) for all tests.

RESULTS

Uterine serosa and ovarian bipolar coagulation induced adhesion formation in 14 out of 19 ewes (74%) regardless of group. Incidence of intraperitoneal adhesions did not differ statistically among groups ($P = 0.819$). Control group presented intraperitoneal adhesions in 80% of ewes (4/5), while group GNS had 62,5% cases (5/8) and GVE presented 83% cases (5/6) of post-op adhesions. Number of adhesions per animal was larger ($P = 0.032$) in control group than in both GNS and GVE (Figure 1). Adhesion scores did not differ statistically among groups regarding adhesion sites or organs ($P = 0.0869$).

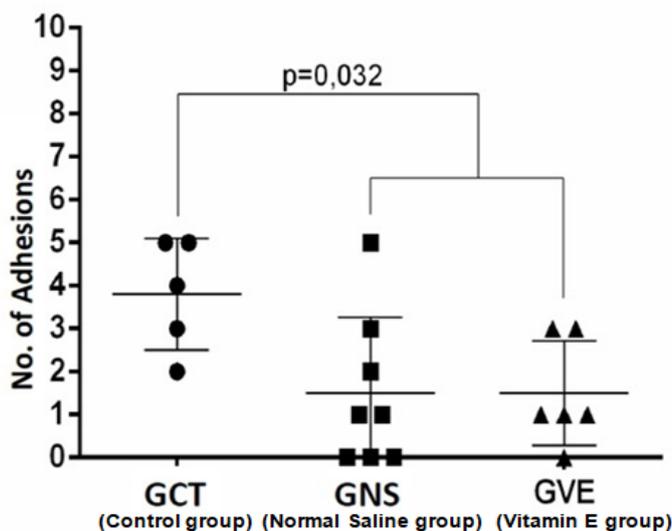


Figure 1. Grades of adhesions evaluated in experimental groups

DISCUSSION

Adhesions formed regardless of group in this study. Bipolar coagulation of uterine horn serosa and ovary was both feasible and effective in inducing intraperitoneal in the ovine experimental model. To the authors' opinion, laparotomy itself inflicts intraperitoneal trauma and desiccation, leading to inflammation, decreased fibrinolysis and adhesion formation (Ewoldt et al., 2004 and Hosseini et al., 2018). Other studies presented lower rates of adhesion induction in animal models, by laparoscopic uterine tract puncture (Mariano et al., 2015) and uterine serosa blunt trauma with

traumatic grasping forceps (Ewoldt et al., 2004). Besides decreased adhesion formation, those experimental models did not provide controlled focal damage to the reproductive tract.

Although adhesions formed in group GVE, intraperitoneal use of vitamin E resulted in fewer adhesion sites in comparison to the control group, as seen in other studies in rats undergone surgical serosa trauma (Corrales et al., 2008 and Yetkin et al., 2009). Another trial reported a decrease of 80% in adhesion formation with intraperitoneal use of vitamin E following laparotomy in rats (De La Portilla et al., 2004). Intraperitoneal vitamin E also decreased substantially adhesion formation following scalpel blade uterine horn scarification in rats (Durmus et al., 2011). Those authors attributed few adhesion formations due to the reactive oxygen specimen (ROS) scavenger and antioxidant properties of vitamin E. Normal saline did not avoid adhesion formation, as well as vitamin E, as reported in other trial (Kappas et al., 1981). That study also reported lower adhesion scores in normal saline group than in control group in a rat laparotomy model, which is in theory less traumatic adhesion induction model than the method applied in this study. Unlike Hosseini et al. (2018), who reported the same amount of adhesions after laparotomy in rats using normal saline and the control group, which no substance was applied. Vitamin E is well known as a natural anti-inflammatory agent, specifically by neutralizing ROS. However, vitamin E did not avoid adhesion formation in this reproductive tract electrocoagulation injury trial.

CONCLUSION

Vitamin E does not affect the incidence of intraperitoneal adhesions following uterine and ovary bipolar electrocoagulation injury in ewes. However, vitamin E reduces the number of adhesion sites and severity, as well as normal saline does when compared to not using any adhesion prevention. We hypothesize that a dose-dependent study should indicate if higher doses of vitamin E given intraperitoneal affect adhesiogenesis, as other studies pointed towards such property. Thus, further research is warranted to fill this gap in the literature. The experimental model of adhesiogenesis was safe and showed to be a controlled method of intraperitoneal adhesion induction since bipolar diathermy provided precise localized spot thermal injury to the ovine reproductive tract.

DECLARATIONS

Acknowledgments

The authors are thankful to the University of Franca and Fapesp (Fundação de Amparo a Pesquisa) for the support and allowing this research work to be performed. We also would like to appreciate all participants who contributed during the performance of the research, especially Dr. Ricardo Uscategui, who helped in the statistical analysis. Also, all the gratitude for Dr Lucas Pereira, who had his particular and unforgettable way of helping the involved ones in the team.

Competing interests

The authors declare that they have no competing interests.

Author's contribution

All authors have revised the work critically for important intellectual content, given their final approval of the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Furthermore, the authors have contributed as follows: substantial contributions to the conception, design and planning of the work, the acquisition, analysis and interpretation of data, and drafting of the work; contributions to the design of the work, and the acquisition and analysis of data. Therefore, all authors contributed equally to this work.

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Effect of Dried Rosemary Supplement as Antioxidant Agent on Blood Biochemical Changes in Relation to Growth Performance of Heat-Stressed Crossbred (Brown Swiss × Baladi) Calves

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ABSTRACT

Heat exposure is a systemic stressor that adversely influences growth and reproductive performances in cattle. This trial aimed to study the effects of Rosemary (RM) supplementation on reducing the side effect of oxidative stress and its relation with growth performance under heat stress condition. Fifteen male calves were divided into three equal groups, the first was offered the basal diet as a control group, whereas the second and the third groups were fed the same basal diet as in control, in addition to a daily supplement of 3g and 6g dried grinded RM/kg concentrate, respectively, for a period of one month. The results showed that supplement of 3 and 6g dry grinded RM/kg concentrate led to a highly significant ($P < 0.01$) decrease in oxidant status and an increase in total antioxidant capacity, as well as significant ($P < 0.01$) declines were noted in the levels of lipids profile, kidney and liver function indicators, and iron concentration. However, RM supplemented groups showed significant ($P < 0.01$) elevations of feed efficiency and daily weight gain copper and triiodothyronine concentrations. In conclusion, RM improved the calves' growth performance through alleviating oxidative stress side effects under hot summer conditions to improve economic returns.

Key words: Antioxidant agent, Blood biochemical, Egyptian desert, Growing calves, Oxidative stress

INTRODUCTION

Rosemary (RM, *Rosmarinus officinalis*) is accepted as one of the medicinal spices with the high antioxidant and anti-carcinogenic activities, as it contains flavonoids, phenols, volatile oil and terpenoids, and natural polyphenols which can lead to a considerable free-radical scavenging activity (Andrade et al., 2018; Petiwala and Johnson, 2015). Heat exposure is a systemic stressor that adversely influences growth and reproductive performances in cattle by disturbing their normal water, minerals and protein metabolism, physiological and blood biochemical processes.

High air temperature, comparative moisture, and radiating energy impair the ability of farm animals, particularly the fattening calves to temperature disposal, resulting in animals subjected to heat stress (Abdalla et al., 2009). A relationship between heat stress and oxidative stress has been established. One of the main reasons for oxidative stress in animals occurs when exposed to heat stress during hot summer conditions in pigs (Montilla et al., 2014), rams (Dehghan et al., 2010), poultry (Abdollah et al., 2016) dairy cows and buffaloes (Kargar et al., 2015 and Maha et al., 2018).

Oxidative stress is a consequence of defect in balance between reactive oxygen species (ROS) generation and efficiency of the antioxidant defense system. While molecular oxygen is needed to continue of normal cellular functions in mammals, excess level of ROS can cause many deleterious effects on organelle, cell and tissue, and disruption of normal metabolism and physiology by oxidative stress (Long et al., 2017). Oxidative stress in animals should be controlled by adding antioxidant nutrients and by minimizing effects of substances that stimulate ROS (Ganaie et al., 2013). The possible role of rosemary as a natural antioxidant was studied on goat's milk (Boutoial et al., 2013), sheep's meat (Vasta et al., 2013), and reproductive performance (Zhong and Zhou, 2013). While the data of the effects of such natural antioxidant especially growing calves under heat stress condition is limited.

Therefore, the objective of the current study was to evaluate the impact of rosemary on antioxidant enzymes, and some biochemical parameters as well as growth performance in heat-stressed calves.

MATERIALS AND METHODS

This study was carried out at a farm belonging to the improving of Cattle production project, in the nuclear research center of the Egyptian atomic energy authority, which was conducted in the desert region of Inshas, Egypt.

Animals and experimental design

The current study involved a two-week adaptation period to the diet followed by four weeks of feeding the experimental diets. The experimental work was carried out during the month of August 2013, where 15 crossbred

ORIGINAL ARTICLE
 pii: S232245681800011-8
 Received: 07 Oct 2018
 Accepted: 12 Nov 2018

(Brown Swiss × Baladi) male calves aging eight to ten months old with average live body weight of 152 kg were used at the beginning of the experiment. The animals were randomly divided into three equal groups (five calves each). The first group was offered the basal diet which was considered as a control group (G1), whereas the second and third treated groups (G2 and G3, treated groups) were fed the same basal diet as in control, in addition to a daily supplement of 3g and 6g dried grinded RM/kg concentrate, respectively.

Ethical approval

This experiment was performed according to all ethics and animal rights in Ain Shams University, Egypt.

Feed and feeding

Feed allowances were offered once a day in the morning at 10 am. The animals were fed in groups. The concentrate feed mixture (CFM) and rice straw were presented on the basis of the average body weight according to NRC (2001). Fresh drinking water was available to all animals at all times in clean basins full of fresh water.

Fresh plant leaves of RM (*Rosmarinus officinalis*) were collected from the Horticulture, Research Institute, agricultural research center, Giza, Egypt. The leaves were dried in a cool dark place at room temperature (25 °C) for 4 days. The average moisture content for the dry plant material was 11%, and then it was grinded very fine using electric grinder (Moulinex-France). Samples of dried RM and concentrate rations were (biweekly) collected. Samples were ground in an hammer mill provided with a 1-mm pore size screen and analysed in triplicate for their content in dry matter (DM) (forced-air oven at 65°C and dried to a constant weight), ash, crude protein (CP) (N × 6.25), crude fibre (CF), ether extract (EE) according to AOAC (2000). Nitrogen Free Extract (NFE) was calculated by differences. The ingredients of CFM are shown in table 1. The chemical compositions and nutritive values of the experimental feedstuffs on dry matter basis are shown in table 2. The chemical compositions of dried rosemary are presented in table 3. Bioactive compound of rosemary was determined using gas chromatography–mass spectrometry (GC–MS).

Table 1. Ingredients of the concentrate feed mixture

Items	CFM
Ingredients (%)	
Crushed yellow maize	50.00
Wheat bran	20.00
Soybean meal	5.00
Uncorticated cotton seed meal	22.50
Lime stone	1.00
Sodium chloride	1.00
Minerals mixture*	0.10
Vitamin mixture**	0.10
Sodium bicarbonate	0.20
Antitoxin	0.10

* Mineral mixture contains: 5g Cu ,30g Fe, 40g Mn , 45g Zn , 0.3g I, 0.1g Se and 881.6g Caco3/ kg mixture. **vitamin mixture contains: 20 million (I.U) vit A, 2 million (I.U) vit D3 and 2g vit E / kg mixture. *** Concentrate Feed Mixture (CFM)

Table 2. The chemical compositions and nutritive values of the experimental feedstuffs on dry matter basis during August 2013

Items	CFM	Rice straw
Chemical composition (%)		
Moisture	12.40	7.50
Dry matter (DM)	100	100
Organic matter (OM)	94.10	81.82
Crude protein (CP)	16.80	3.20
Crude fiber (CF)	8.00	34.05
Ether extract (EE)	2.60	1.94
Ash	5.90	18.18
Nitrogen – free extract (NFE)	66.70	42.63
Nutritive values		
GE (Mcal/kg DM)*	4.30	3.55
NE (Mcal/kg DM)**	1.60	0.94
TDN (%/kg DM)**	70.20	43.24

% NFE = % DM - (% EE + % CP + % ash + % CF) *, GE (Mcal/kg DM) = 0.057 CP % + 0.094 ether extract (EE) % + 0.0415 carbohydrate % (NRC, 2001), **, NE (Mcal/kg DM) = 0.0245 X TDN % - 0.12 (NRC, 2001),***; TDN (%/kg DM) according to the Central Lab for Food and Feed (CLFF), Agriculture Research Center, Egypt (2001). **** Concentrate Feed Mixture (CFM)

Table 3. Chemical analysis of rosemary leaves

Components	Amount
Active components of essential oil %	
Camphor	13.61
Alpha-pinene	17.29
Cineole	34.11
Mineral Elements	
Potassium (%)	1.31
Calcium (%)	2.45
Copper (ppm)	3.4
Zinc (ppm)	31.2
Manganese (ppm)	14.6
Proximate analysis %	
Moisture	8.62
Crude protein	5.08
Ether extract	16
Crude fibre	18.94
Ash	7.52
Nitrogen free extract	43.84
Dried Rosemary obtained from Agricultural Research Center	Giza
Hemicellulose.	6.82
Lignin	6.03
Essential oil	1.33

Meteorological data

The animals were housed in a shaded free stall barn and all experimental groups were kept under the same environmental conditions throughout the experimental period. This experiment was carried out for a period of one month, during August 2013, air temperature (Ta) and the relative humidity (RH) during day and night were recorded using thermo-hygrometer, and the average of each item was calculated, where the Ta and the RH during the days times averaged $38.70^{\circ}\text{C} \pm 0.33$ and $61.55\% \pm 0.74$, [equivalent to temperature humidity index (THI) 92]. While the average of Ta was $29.10^{\circ}\text{C} \pm 0.24$ and in RH was $80.34\% \pm 0.71$, (equivalent to THI 81) during the night times.

THI was calculated using the equation proposed by amundson et al. (2006), where,

$$\text{THI} = (0.8 \times \text{Ta } ^{\circ}\text{C}) + [(\text{RH } \%) \times (\text{Ta } ^{\circ}\text{C} - 14.4) / 100] + 46.4.$$

Blood sampling and analysis

Blood samples were collected before feeding at 10 am from the jugular vein at the beginning and end of the experiment. Serum was separated from clotted blood by centrifugation (20 min, $3000 \times g$) and clear serum collected and stored at -20°C until the biochemical and hormonal determinations. All the following parameters were determined using commercial kits manufactured by bio-diagnostic company, Egypt, unless otherwise indicated.

These parameters were glutathione reductase (GR) and catalase (CAT), uric acid, total antioxidant capacity (TAC), serum Malondialdehyde (MDA). The estimated serum lipids were total cholesterol, high density lipoprotein cholesterol (HDL-cholesterol), low density lipoprotein cholesterol (LDL-cholesterol), and triglycerides. Serum concentration of urea-N and creatinine were determined to indicate kidney functions. For liver function evaluation we evaluated serum concentration of aspartate amino transferase (AST) and alanine amino transferase (ALT). Serum concentrations of iron and copper were determined.

Values of serum very low Density Lipoprotein Cholesterol (vLDL-cholesterol) and phospholipids were determined according to Ellefson and Caraway (1976) using the following equations, vLDL cholesterol = Triglycerides/5, and Phospholipids = $68 + (0.89 \times \text{Total cholesterol})$. Serum concentration of Triiodothyronine (T_3) was determined by using ^{125}I -RIA and antibody-coated tubes kit purchased from Immunotech Beckman Coulter, Inc., Incorporation Prague, Czech Republic, Europe.

Growth performance

Body weight of calves was recorded by digital balance before daily feeding and drinking at the beginning and the end of experimental period. Total and daily body weight gains were calculated for each calf. Dry matter intake (DMI) was determined as kg of calf^{-1} . Feed / gain ratio per day was calculated as kg gain/kg DMI .

The economic gain for rosemary supplementation

The economic gain was calculated as the market values of the total income from body weight gain after subtracting feed cost, and cost of rosemary during the experimental period (one month). Exchange value for Egyptian pounds (LE) to United State Dollar (USD) was equivalent to 8.90 LE at the time of the experiment. The following criteria were calculated in USD: Feed cost per kilogram, total cost of feedstuffs, feed cost per kilogram of gained weight, and net income (total income– expenditure) as presented in table 4.

Table 4. Economic gain per animal during the experimental period (one month) with rosemary supplementation to crossbred calves under Egyptian summer condition during August 2013

Economic indicator	Experimental group		
	Control	3g RM	6g RM
Total gain (kg)	21.04	31.12	33.40
Total income from gain(\$)	70.92	104.90	112.58
Concentrate feed mixture (kg)	120.00	120.00	120.00
Rice Straw (kg)	60.00	60.00	60.00
Cost of feedstuffs (\$)	38.15	38.15	38.15
Cost of rosemary in one month (\$)	--	0.51	1.01
Total expenditure	38.15	38.66	39.16
Net income =Total income –Expenditure	32.76	66.23	73.42
Percentage of gain over control	--	102.15	124.07

Price of kg live body weight = \$3.37; Price of rosemary = \$5.62; Price of feedstuffs (CFM and BH) = \$0.30 and \$0.04, respectively, quoted in Egypt in 2013. RM means rosemary.

Statistical analysis

Data were statistically analyzed using the general linear model procedure of GLM ANOVA procedure of SAS (2000).

The statistical model used was: $Y_{ij} = \mu + T_i + e_{ij}$

Y = the dependent variable,

μ = the overall mean,

T_i = the fixed effect of treatment (1= control, 2= treatment dose 1, 3 = treatment dose 2), e_{ij} = random error.

Significant differences between the means were verified by Duncan (1955).

RESULTS AND DISCUSSION

Chemical analysis of the experimental rosemary leaves

The results of chemical analysis are shown in table 3. The experimental rosemary leaves contained 8.62% moisture, 5.08% crude protein, 16.0% ether extract, 7.52% ash, 18.94% crude fiber and 43.84% nitrogen free extract. The cell walls of rosemary leaves contained high level of cellulose (16.08%), hemicellulose (6.82%) and lignin (6.03%). Furthermore, there are moderate amounts of some macro (calcium, 2.45% and potassium, 1.31%) and micronutrients (Zinc 31.20 mg/kg, Manganese 14.60 mg/kg, Copper 3.40 mg/kg and Zinc 31.2 mg/kg). The percentage of volatile components in the experimental rosemary leaves was 39.31 % for 1,8 cineole, 14.69 % for camphor, 13.85 % for α -pinene, 9.87 % for β - pinene, 6.17 % for camphene, 3.17 % for limonene, 2.58 % for p-cymene, 2.33 % for borneol, 2.02 % for myrcene, for α -terpineol, 1.46 % for bornyl acetate and was 2.27 % for others.

Oxidant and antioxidant status

Both GR and CAT are indicators for serum antioxidant enzymes activities (Sheweita et al., 2016) as well as TAC value is an antioxidant biomarker (Rubio et al., 2016). Serum MDA level was determined as lipid peroxidation marker (Sauriasari et al., 2015).

Data of oxidant and antioxidant parameters are shown in figure 1. Heat stressed calves supplemented with G2 exhibited significant ($P < 0.01$) increase in GR activity by 2.21% and in CAT enzyme activity by 12.75%, while non-significant elevation in the activity of these enzymes was found in G3 (1-A, B). This result indicated that 3g RM/kg concentrate enhanced the activities of oxidant related enzymes. The result in figure (1-C) showed that MDA level was significantly ($P < 0.01$) decreased by 29.74 %, in G3, while insignificant difference was in G2 in compare with the control. The difference between treated groups was insignificant. A significant ($P < 0.01$) increase in TAC concentration was found in RM the treated groups, values were increased by 22.89 and 19.23% in G2 and G3 respectively in compare with the control group, while the difference between RM treated groups was insignificant (Figure 1-D).

Different enzymatic and non-enzymatic mechanisms of the functional ROS as antioxidant have been reported. Since oxidation of reduced glutathione (GSH), acts as the primary non-enzymatic endogenous antioxidant in the cells. Antioxidant enzymes as CAT could promote the reduction of peroxides to alcohols, H_2O or both. GR lowers glutathione disulfide (GSSG), produced through the reduction of peroxides, to the sulfhydryl form of GSH (Zhu et al., 2012).

Oxidation of reduced GSH occurs either directly or through enzymatic means, as free radical (FR) and other reactive oxygen species are scavenged, and GSH; GSSG in heifer was less under hot summer condition than mild condition. A lower ratio is commonly used as an indicator of cellular redox, which indicates an increased oxidative load on the cells or decreased antioxidant defenses, through increasing oxidized glutathione GSSG. Thus one of the main reasons for the occurrence of oxidative stress in animals was the exposure to heat stress during hot summer (Ganaie et al., 2013).

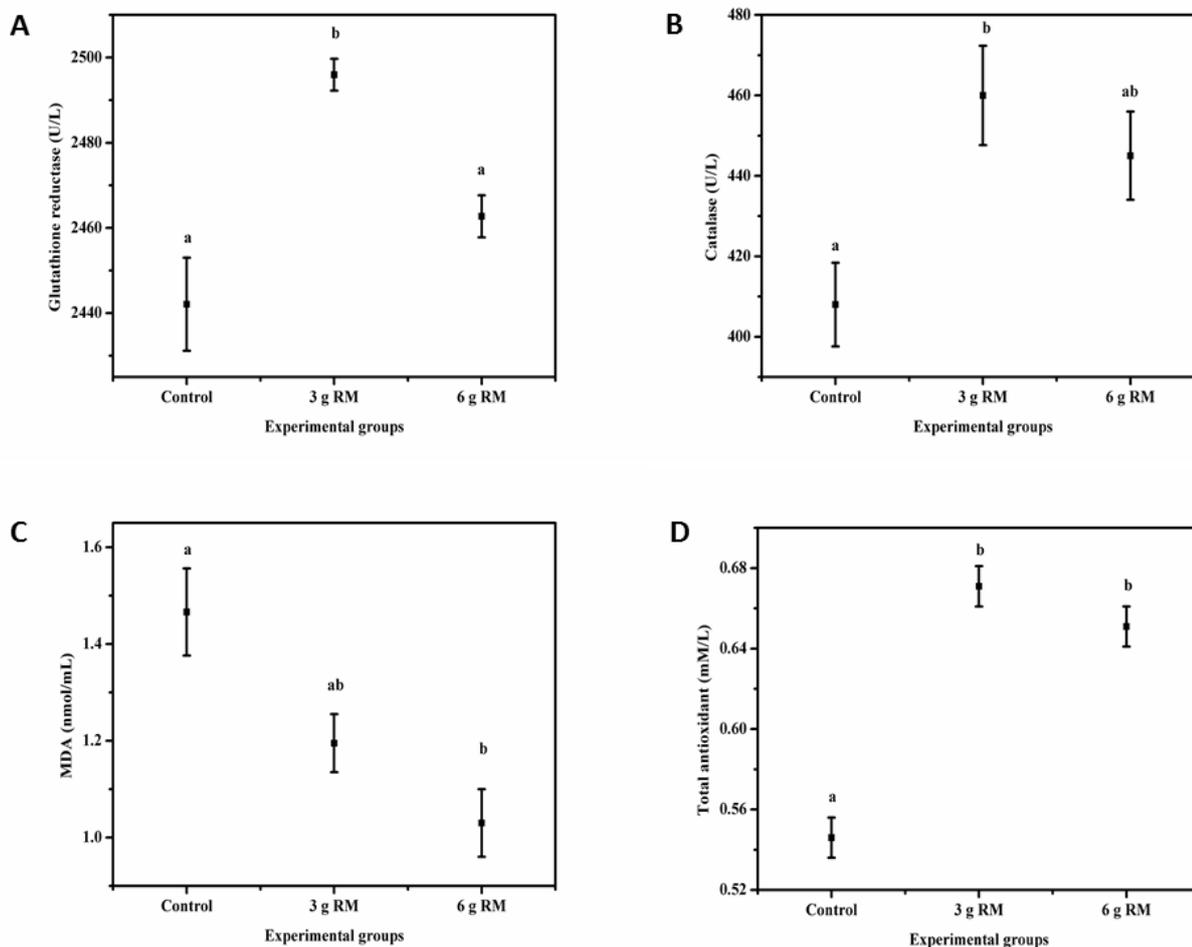


Figure 1. Effect of rosemary supplementation on some oxidant and antioxidant parameters in growing crossbred (Brown Swiss × Baladi) calves under Egyptian summer condition. The means in the same square with a similar superscript are not significantly different ($P < 0.05$)

Trout et al. (1998) found that cattle exposed to heat stress via controlled chambers exhibited no evidence of increased MDA concentration in muscle tissue. While, Bernabucci et al. (2002) noted that the cows exposed to a hot environment display greater MDA than cows exposed to a thermo-neutral environment. Concerning the relationship between hot summer effects and oxidative stress, the use of RM which, has been widely accepted as one of the highest antioxidant agents (Peng et al., 2005), where used as a feed additives because it can alleviate the negative effects of oxidative stress, which occur when the animals were exposed to heat stress during hot summer conditions.

The bioactivities of the RM leaves are comparable with antioxidants components, such as carnosic acid, carnosol, rosmarinic acid, ursolic acid, butylated hydroxyl anisole and butylated hydroxyl toluene (Petiwala and Johnson, 2015). Moreover, RM as an antioxidant has some unique capabilities because most antioxidants need to go through a recycling to maintain antioxidant capability once it is used, while, RM as an antioxidant has a cascade effect. When, the molecule, carnosic acid starts the cascade, after carnosic acid neutralizes aFR it is transformed into carnosol which can neutralize another FR and this cascade continues thus neutralizing several FR without recycling (Masuda et al., 2001). The current findings are comparable with Németh et al. (2004) who found that RM as antioxidant material could lower the oxygen FR formation, increases the antioxidant enzyme activity.

Lipids profile

Data presented in figure (2A and B) shows that G2 and G3 were significantly ($P < 0.01$) lowered the levels of total cholesterol by 7.77%, and phospholipids by 5.28% in compare with control, while the low levels was insignificant in G2. Furthermore, G2 and G3 led to significant ($P < 0.01$) low levels of triglycerides by 18.88 and 21.38%, respectively in compare with control (Figure 2C). The same comparison trend was in LDL-cholesterol levels ($P < 0.01$) by 14.52 and 22.8 %, respectively (Figure 2D), and vLDL-cholesterol ($P < 0.01$) by 18.82 and 21.38 %, respectively (Figure 2E). However insignificant increase in HDL-cholesterol was found (Figure 2F). The means in the same square with a similar superscript are not significantly different ($P < 0.05$). Several investigators reported a significant increases in the levels of serum lipid profile under hot summer conditions in cattle (Kalmath et al., 2015), and in Beetal goats (Kumar et al., 2012). The antioxidant activities of RM could reveal their contents, have been found to inhibit lipid peroxidation. Thus, RM treated animals showed a significant decrease in lipid peroxidation concentration and a rise in GSH content (Sancheti and Goyal, 2007). In general, herbs as RM have high antioxidant concentrations that pose the possibility to prevent the oxidation of LDL, so that, the antioxidant properties of RM are of particular interest in alleviating LDL oxidation (Tapsell et al., 2006).

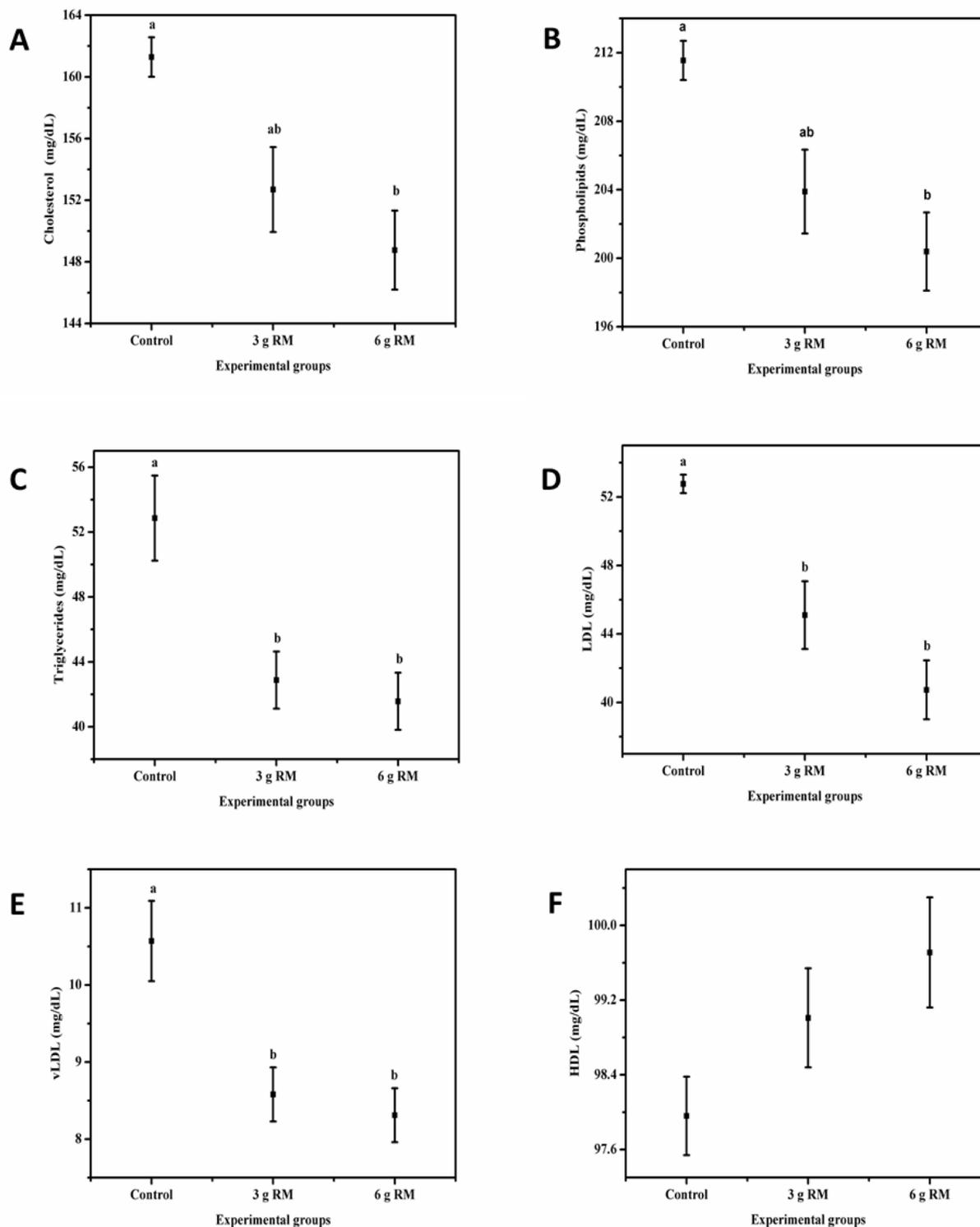


Figure 2. Effect of different rosemary supplementation level g/kg concentrate on lipids profile in growing crossbred (Brown Swiss × Baladi) calves maintained under Egyptian summer condition conditions

Moreover, our present results are consistent with those of Hanafy et al. (2009) who reported that using RM up to 200 mg/kg live body weight as feed additives in rations of Barki growing lambs could decrease serum cholesterol concentration which is positively reflected on lamb's performance. Also, AL-Blooni (2010) found that treating groups of diabetic rats with different levels of dried mixture of chicory and RM induced a significant reduction in serum total cholesterol, triglycerides, LDL and vLDL as compared to control group. On the other hand, Mehmet et al. (2013) reported that total cholesterol, HDL, LDL and triglyceride levels were not significantly different in Rosemary essential oil (REO) groups compared to control groups in Japanese quail under heat stress environmental conditions. This line needs further investigation to identify the possible different actions of RM in both heat stressed birds and animals.

Kidney and liver functions

The influence of RM supplementation on kidney and liver functions are presented in table 5. The results showed G2 and G3 exhibited a significant ($P < 0.01$) low levels in concentration of serum creatinine by 33.90 and 32.58%,

respectively in compare with the control. The same comparison trend in urea -N ($P<0.01$) lowered by 32.13 and 29.82 %, respectively, in AST ($P<0.01$), by 20.94 and 27.75 %, respectively, and in ALT ($P<0.01$) by 16.69 and 14.71 %, respectively.

Uric acid was considered as non-enzymatic antioxidant parameter, because uric acid is a major contributor to total radical trapping capacity (TRAP), which counting approximately 38-47% of the entire TRAP (Kharb, 2000), and uric acid is a better criteria than ascorbate because urate radicals (UH₂) unlike ascorbate do not react with oxygen to give another peroxy radical which make it a good antioxidant (Rodionov, 2003).

Data presented in table 5 showed that uric acid as non-enzymatic antioxidant was significantly ($P<0.01$) increased by 17.29 % in G2, while insignificant difference was noted between G3 and the control group. Generally, the uric acid levels for treated groups were still in the normal range, indicating that used levels of RM improved antioxidant status of animals without the side effect on either the kidney or liver functions.

Ozcan et al. (2004) found lower levels of serum uric acid under different stressors periods, while, it is well known that the stressors is associated with increased FR production and decreased levels of antioxidant defenses. Also, Chaudhari et al. (2010) reported that lower levels of serum uric acid under stress could be related to the high utilization of uric acid for scavenging FR. The higher uric acid concentration found in this study as a result of RM dietary addition could be attributed to the action of bioactive content of flavonoids, phenols, especially the two phenolic compounds, carnosol and carnosic acid (Almela et al., 2006; Petiwala and Johnson, 2015). These natural phenols could exhibit a considerable FR scavenging activity, and decrease the FR formation (Németh et al., 2004).

Terzano et al. (1997) pointed that urea-N is the main indicator of protein degradation for energetic rumen ammonia level and then blood urea release. In hot condition, several investigations have showed that urea-N values were significantly higher in growing heat stressed calves (Atta et al., 2014) and during summer than in winter in lactating buffaloes (Gudev et al., 2007). Montmurro et al. (1995) suggested that the high level of urea-N was related to the low energy/protein ratio and to gluconeogenesis by protein degradation in conditions of insufficient energy for growth.

Creatinine is a chemical waste molecule that is generated from muscle metabolism, and the decrease in its levels means that no muscular wastage which might have been possibly caused by inadequacy of protein intake to animals under stress. In the present trial, the negative effects of hot condition on kidney functions were lower in RM treated groups than the normal group. The current results are in accordance with lower concentrations of serum urea-N nitrogen and creatinine in RM treated rats (AL-Blooni, 2010), and broiler chickens (Ghazalah and Ali, 2008; AL-Blooni, 2010).

ALT and AST are hepatocellular damage biomarkers. The concentrations of ALT and AST activities in the herein study were significantly lower in G2 and G3 in compare with the control group (table 5). A significant high activities in AST and ALT were reported under hot conditions in cows (El-Masry et al., 2010), while these activities were significantly higher for AST concentration and slightly higher in sheep (Okab et al., 1993). The increase in activities of serum AST and ALT in heat stressed animals may be related to the increase in stimulation of gluconeogenesis or gluconeogenesis by corticoids (El-Masry et al., 2010).

In agreement with results reported, Hanafy et al. (2009) showed that AST and ALT were significantly decreased at all RM levels, compared to control groups in Barki growing lambs. Several investigators attributed the decrease in transaminase enzymes in heat stressed calves supplemented with different doses of RM to the physiological effect of phenolic compounds in RM that has a hepatoprotective role (Abdel-Hamid et al., 2011; Rašković et al., 2014). In addition to antilipoperoxidant activity, REO was also found to efficiently reduce the levels of hydrogen peroxide (H₂O₂)- and 2,3-dimethoxy- 1,4-naphthoquinone (DMNQ)-induced oxidative damage of DNA in isolated rat hepatocytes, and testicular cells (Horváthová et al., 2010; Slameňová et al., 2011).

Table 5. Effect of dried rosemary supplementation on kidney and liver functions in growing crossbred (Brown Swiss X Baladi) calves maintained under Egyptian summer conditions during August 2013

Items	Control	3gRM	6gRM	Significance
Uric acid (mg/dL)	0.399 ^a ± 0.01	0.468 ^b ± 0.01	0.435 ^{ab} ± 0.02	P<0.01
Creatinine (mg/dL)	1.277 ^a ± 0.05	0.844 ^b ± 0.03	0.861 ^b ± 0.03	P<0.01
Urea (mg/dL)	10.83 ^a ± 0.01	7.35 ^b ± 0.02	7.60 ^b ± 0.03	P<0.01
AST (U/mL)	76.40 ^a ± 0.93	60.40 ^b ± 0.93	55.20 ^c ± 0.86	P<0.01
ALT (U/mL)	29.71 ^a ± 0.66	24.75 ^b ± 0.58	25.34 ^b ± 0.68	P<0.01

The means in a row with a similar superscript are not significantly different ($P<0.05$). RM means rosemary; AST means Aspartate Amino Transferase; ALT means Alanine Amino Transferase.

Serum minerals

Supplementing diets of heat stressed calves with either 3 or 6g RM/kg concentrate significantly ($P<0.01$) increased mean values of serum Cu concentrations by 24.55 and 20.76 %, respectively (Table 6). However, serum Fe concentration showed a decrease ($P<0.01$) by 2.67 and 4.19 %, respectively as compared with non-supplemented heat stressed calves. Many types of macromolecules are affected by oxidative stress especially if accompanied with environmental stresses. Moreover, elevated concentration of iron that acts as pro-oxidant was reported to be correlated with increased protein carbonyl concentrations that results in oxidative damage in animals (Perucchiatti and Litjens, 2010). The same authors attributed the depression in Cu and the increase in Fe concentration to RBCs destruction in heat stressed animals and thus one of the main reasons for oxidative stress in animals and the changes in these elements was considered as indicator of oxidative stress.

Also, these macromolecules can act as specific antioxidant protecting macromolecules against the negative effects of oxidative stress (Perucchiatti and Litjens, 2010), and the same author attribute the decrease of Cu under oxidative

stress to relatively high level of Fe, which lead to depressed or lowered absorption of zinc, manganese and Cu. The current result about the increase in Cu concentration and decrease in Fe concentration in heat stressed calves supplemented with two levels of RM can explain the role of RM as antioxidants which decrease the ROS formation therefore, increases the antioxidant enzyme activity (Németh et al., 2004), in alleviation of oxidative stress occurring under heat stress conditions.

Triiodothyronine (T₃) hormone

Data presented in table 6 showed that serum T₃ concentrations increased (P<0.01) by 39.43 and 52.87 %, respectively as a function of 3 or 6g RM supplementation per kg concentrate in comparison with the control group. These results confirm that supplementation of heat stressed calves with different doses of RM reduces the negative effect of hot conditions on thyroid activity. Other reports show that, exposure to elevate AT is associated with decrease plasma T₃ and T₄ levels in cattle (El-Masry et al., 2010). Moreover, Sarandöl et al. (2005) showed that this hypothyroidism was accompanied with increased oxidative stress.

The increase in serum T₃ concentration in heat stressed calves fed diets supplemented with RM is related to highest antioxidant activity for RM. The bioactivities of the RM leaves are comparable with known antioxidants constituents, such as carnosic acid, carnosol, rosmarinic acid, ursolic acid, butylated hydroxyl anisole and butylated hydroxyl toluene (Almela et al., 2006; Peng et al., 2005).

Table 6. Effect of dried rosemary supplementation (3 or 6 g/kg concentrate) on some minerals and thyroid hormone in growing crossbred (Brown Swiss X Baladi) calves maintained under Egyptian summer conditions during August 2013

Items	Control	3gRM	6gRM	Significance
Copper (Cu) (µg /dL)	323.42 ^a ± 12.69	402.81 ^b ± 9.93	390.56 ^b ± 11.14	P<0.01
Iron (Fe) (µg/dL)	108.56 ^a ± 0.51	105.66 ^b ± 0.50	104.01 ^b ± 0.46	P<0.01
T ₃ (nmol/L)	0.870 ^a ± 0.02	1.213 ^b ± 0.06	1.330 ^b ± 0.04	P<0.01

The means in a row with a similar superscript are not significantly different (P> 0.05)

Growth performance

The changes in growth performance induced by dried RM additions to the ration of heat stressed calves are presented in table 7. From this table it can be noted before the experimental treatments that the differences in body weight between control and treated groups were insignificant. Addition of 3 or 6g RM/kg concentrate to heat stressed calves showed a significant effect on growth performance, since the mean values of daily gain recorded 47.93 and 58.77 %, respectively, and 47.90 and 58.75 % respectively for total gain as well as 5.00 and 7.12 %, respectively for final body weight, all over the control group.

As a function of supplementation of 3 or 6g RM/kg concentrate to the diet of heat stressed calves, the percentage increase in the mean values of feed efficiency recorded 52.17 and 60.87%, respectively, in comparison with the control group. Moreover, it can be noted that the increases in growth rate of calves were associated with the significant (P<0.01) improvement in feed efficiency as shown in table 7. These results clearly show that supplementation of 3g RM/kg concentrate to heat stressed calves had a significant amelioration in each of daily gain, total gain, final body weight and feed efficiency, while addition 6g RM/kg concentrate to the diet induced higher and better effect on growth performance than those observed by using 3g RM/kg concentrate. Owing the depression in growth performance specially, daily gain in heat stressed animals was observed in growing heat stressed calves. Moreover, the negative changes in protein metabolism, tissues anabolism, most blood constituents, minerals and water metabolism and hormonal levels disturbances, may contribute to such decrease in growth performance in heat stressed cattle (Atta et al., 2014).

Thus, adding RM to the basal diets showed significant alleviation in growth performance of heat stressed calves. These results are in accordance with previous studies which showed good effects of RM plants additive on Seadi lambs performance (Mohamed et al., 2005).

Table 7. Effect of dried rosemary supplementation on growth performance of growing crossbred (Brown Swiss X Baladi) calves maintained under Egyptian summer conditions during August 2013

Items	Control	3g RM*	6gRM	Significance
Initial body weight (kg)	152.60 ± 2.50	151.20 ± 2.33	152.60 ± 1.78	N.S
Daily gain (kg/day)	0.701 ^a ± 0.01	1.037 ^b ± 0.01	1.113 ^c ± 0.01	P<0.01
Total gain (kg)	21.04 ^a ± 0.22	31.12 ^b ± 0.22	33.40 ^c ± 0.22	P<0.01
Final body weight (kg)	173.64 ^a ± 2.37	182.32 ^b ± 2.33	186.00 ^b ± 1.62	P<0.01
Feed / gain ratio (kg/ kg)	0.23 ^a ± 0.01	0.35 ^b ± 0.01	0.37 ^c ± 0.01	P<0.01

The means in a row with a similar superscript are not significantly different (P<0.05). *: RM means rosemary.

Feed / Gain ratio

Also, RM used as natural additive to animal feed may be a good alternative to artificial antioxidants since they showed beneficial effects also on animal welfare and other physiological functions (Tedesco, 2001). Also, Hanafy et al. (2009) concluded that using RM as feed additives in rations of Barki growing lambs up to 200 mg/kg live body weight could be improve the nutrient digestibility, nutritive value, daily gain, feed conversion, which are positively reflected on lambs performance and economic efficiency. The beneficial effects of REO in reducing the negative effects of heat stress

in Japanese quail, was reported by Mehmet et al. (2013) that found a significant increase in feed conversion ratio in RM oil groups than control groups under heat stress environmental conditions. The improved performance of REO groups could be related to these positive effects of REO on digestive system. In sheep, Sahraei et al. (2014) found that REO decreased the ruminal total volatile fatty acids, acetate, butyrate and ammonia-N concentration, these changes makes more adaptable to environmental stress, there more efficient digestion.

CONCLUSION

RM contains antioxidant compounds can be used in alleviation of the negative effect of heat stress, especially oxidative enzymes, some blood constituents and growth performance in heat stressed calves. Further studies on the molecular level should be conducted to show up the possible mechanism of the effects of bioactive component of rosemary on the composition and function of rumen microbiota.

Acknowledgements

The authors would like to offer special thanking for the Nuclear Research Center of the Egyptian Atomic Energy Authority for providing the animals required for the current study

Competing interests

With respect to the research, authorship, and/or publications of this article. The authors declare that they have no competing interests.

Author's contribution

Dr. Kamel Ahmed El-Masry designed the experiment, article writing and revision, Dr. Essmat Bakry Abdalla designed the experiment, manuscript writing, commenting and approval, Dr. Sana Sayed Emara helped in field study, collected data, laboratory analyses, statistical analysis, tabulation of experimental data and article writing; while, Dr. Abdelhady Farouk Hussein helped in statistical analysis, manuscript writing. All authors have read and approved the final manuscript.

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Successful Surgical Management of Corneo-conjunctival Dermoid Cyst with Bilateral Nasal Choristoma in a Red Kandhari Calf

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ABSTRACT

An old Red Kandhari calf presented at teaching veterinary clinical complex, veterinary college Parbhani with corneo-conjunctival haired masses on the left eye and bilateral nasal growth at nasolabial planum since birth. As the mass was completely covering on cornea due to which vision was hindered completely after physical examination and considering the health status of the calf the surgery was scheduled. The masses were surgically excised from the cornea and bulbar conjunctiva of eye and the left and right side of the dorsomedial nasolabial planum. Then the eye was flushed with normal saline and the tissue of both corneo-conjunctival and nasal were stored in 10% formalin later histopathology of the excised tissue confirmed as a unilateral corneoc-onjunctival dermoid with ectopic lacrimal glands and bilateral nasal choristomas with loose stroma and hair follicle. Two months of follow up was done where there was no reoccurrence of the growth observed. Surgery was curative and healing was uneventful.

Key words: Calf, Corneoc-onjunctival dermoid, Nasal choristoma

ORIGINAL ARTICLE
 PII: S232245681800012-8
 Received: 08 Nov 2018
 Accepted: 06 Dec 2018

INTRODUCTION

Ocular dermoid is a congenital defect recognized in animals characterized by skin like appendages present on the eye. These represent histologically normal Island of skin but misplaced to other location usually arising on the limbus, conjunctivae and cornea (Jena et al., 2015). It is believed that these cysts originate from an incarceration and subsequent growth of embryonic epithelial cells during the closure of the neural tube, and therefore, most of these lesions occur along the median line (Tunio et al., 2016). However, there are reports of acquired dermoid cysts, secondary to traumatic epithelial dislocations they are readily diagnosed because they appear as a piece of skin attached to the cornea, sclera, conjunctiva and eyelids (Rashmi et al., 2018) and they usually occurred unilaterally. Most dermoids are quite superficial and involved the epithelium and very superficial stroma. Choristomas are benign, congenital lesions that consist of an over growth of normal tissue in an abnormal location (Rashmi et al., 2018). Dermoids are choristomatous abnormalities that often arise in the ocular region. Bilateral ocular dermoids have been found in animals (Aher et al., 2017). However, ocular dermoids are not common in cattle with their prevalence estimated at 0.002% (Kilic et al., 2016). This paper reports a case of unilateral corneocconjunctival dermoids in a calf in conjunction with bilateral nasal choristomas.

CASE REPORT

History and initial clinical findings

An old Red Kandhari calf (aged one month) was presented with an abnormal appearance of left eye since birth. A large fleshy mass was attached to the dorsal and ventral part of cornea and bulbar conjunctiva spacing on cornea with a large number of superiorly directed hairs arising from the surface of the mass (Figure 1).

The calf exhibited moderate blepharospasm and watery discharge from affected eye. Superficial corneal ulceration was present on the cornea as a result of trichiasis. The corneo-conjunctival dermoid was extending up to medial canthus of the left eye. No other ocular abnormalities were detected in the eye. A nodular skin mass was also present on the left and right dorsomedial aspect of the nasolabial planum of both the nostrils (Figure 2). The clinical diagnosis was unilateral corneal dermoid and bilateral nasal choristomas.



Figure 1. Corneoconjunctival dermoid in one month old Red Kandhari calf before surgery at COVAS Veterinary Hospital



Figure 2. On physical examination observed Bilateral nasal growths on nasolabial planum in calf before surgery at COVAS Veterinary Hospital

Surgical procedure

The calf weighed 30 kg, was in good level of body condition and no further abnormalities were detected on physical examination. Corrective surgery was performed under mild sedation with injection Pentazocine lactate 0.5 mg/kg body weight intravenously followed by supra orbital and Peterson nerves block with 2% lignocain hydrochloride (Figure 3). The dermoids were excised by superficial keratectomy with Bard Parker blade (No. 11) at both dorsal and ventral aspect of cornea and bulbar conjunctiva (Figure 3).

The nasal masses were also excised (Figure 4). All excised tissue was preserved in 10% formalin for histopathological examination. Later cornea was cauterized by 1% silver nitrate and topically applied Tobramycin eye drops two times daily for two weeks (Figure 5). Postoperatively a course of antibiotic gentamycin 3mg/kg body weight, intramuscular, cadistin 0.2mg/kg body weight, intramuscular and Melonex 0.5mg/kg body weight, intramuscular along with this to accelerate the healing injection of vitamin A 10000 IU/kg body weight, intramuscular was given (Figure 5). Ocular and nasal healing was uneventful with prominent corneoconjunctival granulation tissue observed on 6th days postoperatively, which was noticed to be largely resolved by the six-week follow-up examination (Figures 5 and 6).



Figure 3. During surgery performing superficial keratectomy to excise the growth of dermoids in calf at COVAS Veterinary Hospital



Figure 4. Excision of nasal growths from its base in calf at COVAS Veterinary Hospital

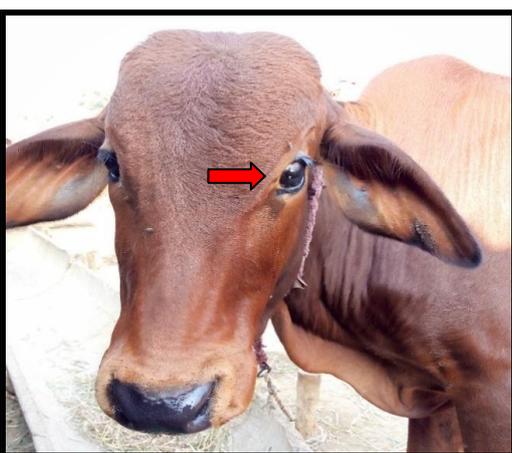


Figure 5. Complete recovery after six weeks of surgery at COVAS Veterinary Hospital

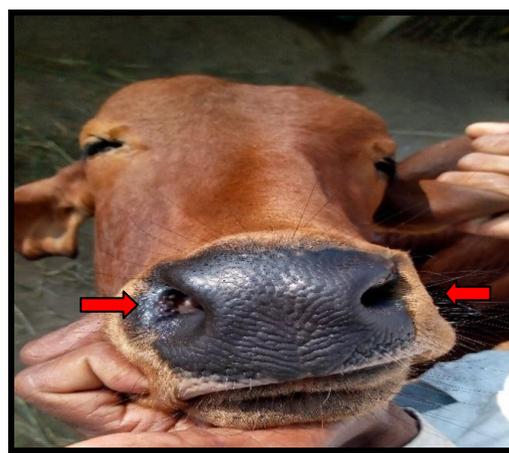


Figure 6. No reoccurrence of the nasal growth after six weeks of surgery at COVAS Veterinary Hospital

Histopathological examination

The corneal lesion exhibited a moderately well delineated but nonencapsulated raised mass comprised of moderately hyperplastic, keratinizing stratified squamous epithelium overlying a thick collagenous stroma, which merged with conjunctival tissue containing submucosal glandular tissue. The corneal mass contained numerous, large, well developed hair follicles and adnexal structures superficially. A band of abortive hair follicles and adnexa was identified as haphazardly arranged clusters of epitheliums, in the absence of hair bulbs, intermingled with tortuous lumen of apocrine glands beneath the productive follicles (Figure 7). This was accompanied by myxomatous stroma and a minimal neutrophilic and eosinophilic inflammatory infiltrate, in addition to this ectopic lacrimal gland was also identified. Histopathology of the excised nasal tissue was similar and characterized by moderately well-delineated but nonencapsulated, raised masses. These were comprised of moderately hyperplastic, keratinizing stratified squamous epithelium over lying large regularly arranged hair follicles (Figure 8) and confirmed as nasal choristoma. Morphologic diagnosis was bilateral corneo-conjunctival dermoids with ectopic lacrimal glands and bilateral nasal choristomas.

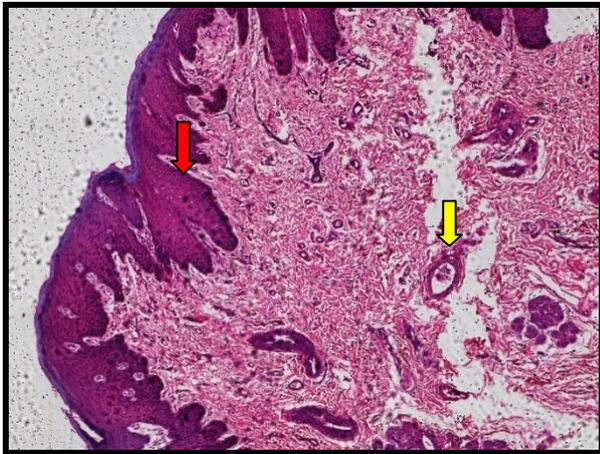


Image 8. Nasal choristoma showing nasal epithelium with loose stroma (red arrow) and having hair follicles (yellow arrow) (H and E stain x100)



Image 7. Corneal conjunctival dermoid cyst with young growing collagen fibres and lacrimal gland (red arrow) characterized by the presence of hair follicles (yellow arrow) and an associated apocrine gland (black arrow) (H and E stain x 100).

DISCUSSION

Metaplasia of mesenchyme (of primarily neural crest origin), resulting in abnormal differentiation of the surface ectoderm, is considered the most likely mechanism. The resulting dermoid consists of ectodermal elements (keratinized epithelium, hairs, sebaceous and apocrine glands. and mesenchymal elements (fibrous tissue, fat and cartilage) combined in different proportions (Rashmi et al., 2018). Our finding in this case was in agreement with Jena et al. (2015) who reported that Holstein Friesians cross bred calf affected with unilateral dermoid on right eye. Dermoid may be located in the third eyelid, cornea, conjunctiva, corneal conjunctival junction and limbus region in this study conjunctivitis these findings were correlated with study of Tunio et al. (2016) who observed that skin tissue and hair attached to the cornea and frequently irritate eye of the animal which leads to conjunctivitis

Dermoid have been reported in many breeds of cattle and can be unilateral or bilateral ocular dermoids in cattle are not common, with an estimated prevalence of 0.002%–0.4% (Rashmi et al., 2018). Ocular dermoids have been reported in cattle of many breeds worldwide, with a similar low prevalence in all breeds other than the Hereford. There are otherwise few reports of unilateral ocular dermoids in calves, each describing single or low numbers of animals and only one reporting a nasal tumor-like growth (Kilic et al., 2016). Of these bilateral cases, inferonasal corneo-conjunctival dermoids were most commonly reported, followed by nasal dermoids (Kilic et al., 2016). Ocular dermoids may be associated with other congenital ocular or multi organ abnormalities (Tunio et al., 2016). Ectopic lacrimal tissue may appear in combination with an ocular dermoid as in this case report and previously reported in cattle (Aher et al., 2017). The ectopic lacrimal tissue in this calf may have represented misplacement of the nictitans gland or additional lacrimal tissue development (Kilic et al., 2016).

The ectopic lacrimal tissue in this calf may have represented misplacement of the nictitans gland or additional lacrimal tissue development. Choristomas are known to grow, with the rate accelerated by trauma, irritation or puberty, in humans. Malignant degeneration is rare in ocular choristomas but transformation of ectopic lacrimal tissue into adenocarcinoma and pleomorphic adenoma has been reported in humans (Rashmi et al., 2018). Cranial meningocele with bilateral nasal choristoma in a cow calf have been reported in calf where the variable staged hyaline and cartilaginous tissue along with young growing fibrous connective tissue seen in the nasal choristoma (Aher et al., 2017). A tumor-like

growth at the entrance of the left nasal passage in association with bilateral corneal and eyelid dermoids in a Rathi calf (Rashmi et al., 2018). Histopathologic examination was not performed on the nasal masses in either of these two reports.

The combination of congenital ocular and nasal abnormalities in this calf is compatible with the intimate early developmental origin of the optic and nasal regions and a common abnormality in neural crest migration whether this abnormality has a genetic basis or not is less clear (Aher et al., 2017). Superficial keratectomy is required to surgically excise a corneal dermoid although the depth of the dermoid within the cornea cannot be ascertained by ophthalmic examination until surgery is undertaken (Rashmi et al., 2018). In the case of large corneal dermoids, surgical excision should be performed early in the life of the patient to achieve optical improvement and allow functional development of the eye.

DECLARATIONS

Acknowledgements

Authors would like to thank Dr. Moregavkar for his contribution in Histopathological examinations.

Author's contribution

P.R. Balage, B. Bhadane and V. Aher performed surgery and written article. G. Dhage, A.A. Mate and D.Sh. Lokhande helped during surgery and writing article. And G. Gangane contributed for histopathological examinations.

Competing interest

The authors declare that they have no conflict of interest with respect to the research, authorship, and/or publication of this article, the author declares that he has no competing interests.

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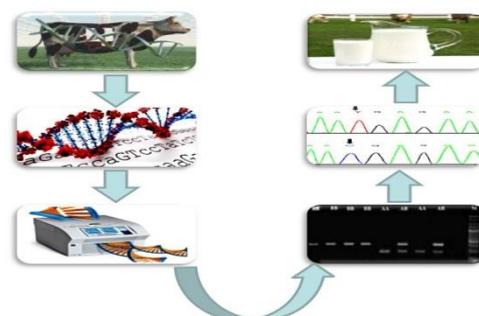
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AOAC (1990). *Association of Official Analytical Chemists. Official Methods of Analysis*, 15th Edition. Washington D.C. pp. 69-88.

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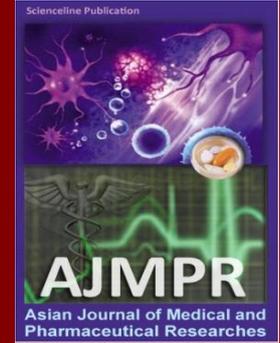
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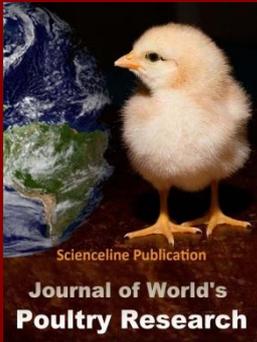
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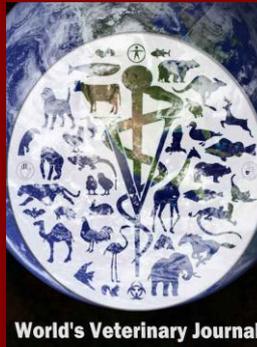
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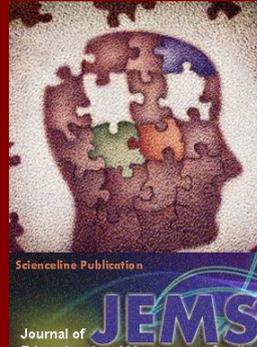
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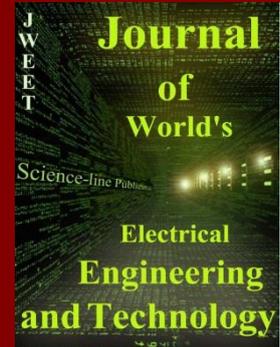
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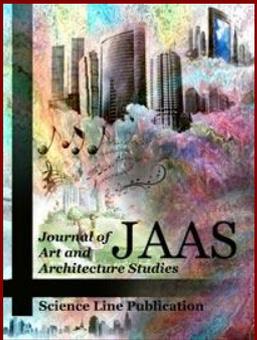
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