

## HEMATOLOGICAL AND BIOCHEMICAL CHANGES OF BARKI LAMBS FED *Kochia indica* IN SINAI

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### ABSTRACT

The present study was carried out at the experimental farm of Ras Sudr station, South Sinai, belonging to Desert Research Center. A number of 16 growing male Barki lambs at 6 months old and  $18.5 \pm 2.55$  Kg were used to assess the and blood parameters as fed roughage diet of cultivated *Kochia indica* for 75 days. The animals were divided at random into two equal groups; one was fed *ad lib.* manured *Kochia indica* (MKI) while the second group was fed *ad lib.* clover hay (CH). The two groups were supplemented with concentrate feed mixture (CFM) at rate of 100% TDN of their maintenance energy requirements.

The main effects of *Kochia indica* on hemogram showed higher ( $P < 0.01$ ) value of mean corpuscular volume (MCV) compare to the control, while the mean values of hemoglobin (Hb), hematocrit (Ht), erythrocytes count (RBC's) and mean corpuscular hemoglobin concentration (MCHC) were lower in lambs fed *Kochia indica* compared to those fed clover hay. Leukogram picture revealed leukocytosis ( $P < 0.01$ ), the absolute counts of neutrophils and eosinophils were higher ( $P < 0.01$ ) in lambs fed *Kochia indica* compared to their counterparts.

Mean values of total proteins (TP), albumin (AL), globulin (GL), cholesterol (CHO), creatinine (CRE), total lipids (TL) and plasma urea nitrogen (PUN) increased due to feeding lambs on *Kochia indica*. Early changes in plasma enzymes reflected mild hepatocyte damage which is further indicated by significant elevation ( $P < 0.01$ ) of lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). In contrast alkalinephosphatase (ALPh) decreased ( $P < 0.01$ ) in lambs fed *Kochia indica* compared with those fed clover hay.

**Keywords:** sheep, saltbush, blood picture, plasma metabolites

### INTRODUCTION

In arid and semi-arid regions of the world, irrigation, high evaporation and inadequate water management each contributes to increase soil salinity (Choukr-Allah and Harrouni, 1996). The development of halophytes into cultivated plants represents a biological solution to agricultural salinity problems (O'Leary, 1984) and an alternative approach to expand cultivation onto unfavourable land.

Halophytes generally have been considered for their successful growth performance in extremely saline environments and their potential economic use as animal fodder. (Lieth and Al Masoon, 1993 a,b). *Kochia sp.* (halophytic shrubs) is considered one of the most drought resistant, salt tolerant plants (Francois, 1976; Gihad and El Shaer, 1994 and Abou El Nasr *et al.*, 1996), and most valuable species of the chenopodiaceae or goosefoot family produces high quality forages (Davis, 1979). *Kochia (indica or scoparia)*

is an annual bush, both stems and branches are softer in *Kochia scoparia* than in *Kochia indica* (Zahran,1986). *Kochia indica* is a widely adapted plant to many geographical areas. It is grazed by many species of ruminant animals and also used for hay production (Sherrod, 1971).

The aim of this study was to investigate the impact of feeding growing Barki sheep for 75 days on manured *Kochia indica* irrigated with underground brackish water (8000 ppm as TDS) compared with clover hay on their blood parameters under arid conditions in Sinai.

## **MATERIALS AND METHODS**

### **Site description:**

The present study was carried out at the experimental farm of Ras Sudr station, South Sinai, belonging to Desert Research Center. The soil in this farm is characterized as loamy sand texture containing 6100 ppm total salinity.

### **Plant material:**

Clover hay versus and manured *Kochia indica* shrubs were used as basal diet for lambs. *Kochia indica* shrubs were cultivated in agronomic plots in Ras Sudr Experimental Farm. Organic manure was applied during soil preparation at the rate of 40m<sup>3</sup>/fed. *Kochia indica* was irrigated weekly using underground brackish-well water. The daily requirement of green forage was harvested in the morning and mechanically chopped before offering *ad.lib.* to treated animals in two equal meals (at 08:00 and at 15:00 hr.).

### **Experimental design:**

A number of 16 growing male Barki lambs at 6 months old with initial average body weight of 18.5±2.55kg were used in feeding trial for 75 days in summer. They were randomly divided into two equal groups (8 lambs each), They were kept separately in two shaded pens. The first group, as control was fed clover hay *ad lib.* while the second group as treated group was fed manured *Kochia indica ad lib.* as roughage diets. As a feed supplement concentrate feed mixture (CFM), both two groups was given daily to cover 100% of their energy maintenance requirements according to Kearl (1982). Fresh water was available *ad lib.* twice daily. Chemical composition and mineral contents of feed ingredients and tested rations (% on DM basis) are given in Table1.

**Table (1): Chemical composition and mineral contents of feed ingredients and tested rations on dry matter basis (%)**

<b>Item</b>	<b>DM</b>	<b>CP</b>	<b>CF</b>	<b>EE</b>	<b>NFE</b>	<b>Ash</b>	<b>OM</b>	<b>Na<sup>+</sup></b>	<b>K<sup>+</sup></b>
<b>Clover hay</b>	89	13.9	29.6	3.90	38.5	14.1	85.9	2.52	1.96
<b><i>Kochia indica</i></b>	39.9	12.5	27.0	3.53	41.17	15.8	84.2	2.96	1.81
<b>CFM</b>	90	15.5	25.7	3.61	46.99	8.20	91.8	0.80	1.37
<b>C ration</b>	89.5	14.7	27.5	3.74	43.16	10.9	89.0	1.61	1.65
<b>T ration</b>	63.1	13.9	26.4	3.57	43.83	12.3	87.6	1.96	1.60

**C ration:** consists of clover hay plus concentrate feed mixture    **T ration:** Consists of fresh *kochia indica* plus concentrate feed mixture **DM=** dry matter, **CP=** crude protein, **CF=** crude fiber **EE=** ether extract **NEF=** nitrogen free extract **OM=** organic matter **Na<sup>+</sup>=** sodium **K<sup>+</sup>=** potassium

#### **Hematological and biochemical measurements:**

Before offering feedstuffs and water, blood samples were collected biweekly via jugular venipuncture in 10ml test tubes containing lithium heparin as an anticoagulant. About 3ml of whole blood sample was intended for the determinations of complete blood picture.

The largest portion of the blood sample (approximately 7ml) was centrifuged at 3500 rpm for 20 minutes, the plasma obtained was removed into glass vials and frozen at (-20°C) and intended for estimate plasma metabolite profile. Hemoglobin (Hb) concentration (g/dl); hematocrit (Ht%); the erythrocytes (RBC's,  $\times 10^6$  cells/mm<sup>3</sup>) and leukocytes (WBC's,  $\times 10^3$ /mm<sup>3</sup>) were estimated according to Cheryl, *et al.*, (1992). Blood smear slides for differential white cell counts were done using Wright's stain according to (Jain 1993). Proportion of every leukocytic cell type multiplied by the WBC was assumed to give the absolute count of each white cell type. The hematimetric indices were then calculated using the following formulate: mean corpuscular volume (MCV, fl = Ht X 10/RBC's); mean corpuscular hemoglobin (MCH, pg/cell = Hb X 10/RBC's) and mean corpuscular hemoglobin concentration (MCHC, g/dl = Hb X 100/Ht).

For all metabolite assays, total plasma proteins, (TP, g/dl), albumin concentration (A, g/dl), total lipids (TL, mg/dl), plasma urea nitrogen, (PUN, mg/dl), creatinine, (CRE, mg/dl), lactate dehydrogenase (LDH, u/l), alkaline phosphatase (AIPh), alanine aminotransferase (ALT, u/l); aspartate aminotransferase (AST, u/l) activities and total cholesterol (CHO, mg/dl) were analyzed using available kits supplied by bioMe'ricux-France. Total plasma globulins concentration (G, g/dl) was calculated as the difference between total plasma proteins and plasma albumin, then albumin:globulin ratio (A/G, %) was calculated.

#### **Statistical analysis:**

Data were analyzed as split plot repeated measurements using SAS (1998). Differences among means were examined using Multiple Range Test according to Duncan, (1955).

## **RESULTS AND DISCUSSION**

#### **Hematological responses:**

The hematologic responses for control and treated groups are presented in Figure 1. The trend of Hb; Ht and RBC's were slightly lower ( $P < 0.01$ ) in lambs fed *Kochia indica* than those fed clover hay.

With the respect of treatment (T) and experimental period (P), the results showed that, there was ( $P < 0.01$ ) interaction between T x P on Hb ; Ht and RBC's. At the end of experiment the rates of change in Hb ; Ht and RBC's, were -13.5, -3.9 and -16.03% in lambs fed *Kochia indica*. The corresponding values similar results obtained by Rankins *et al* (1991a). On contrast, El-Hassanein and El-Sherif, (1996) reported increase in Hb, Ht, RBC's and MCHC of Barki lambs treated with saline drinking water. Also, Abdelhamid *et al.* (2007) found that Hb, Ht, RBC's and MCV increased in Barki lambs fed *Acacia Saligna* compared with control group.

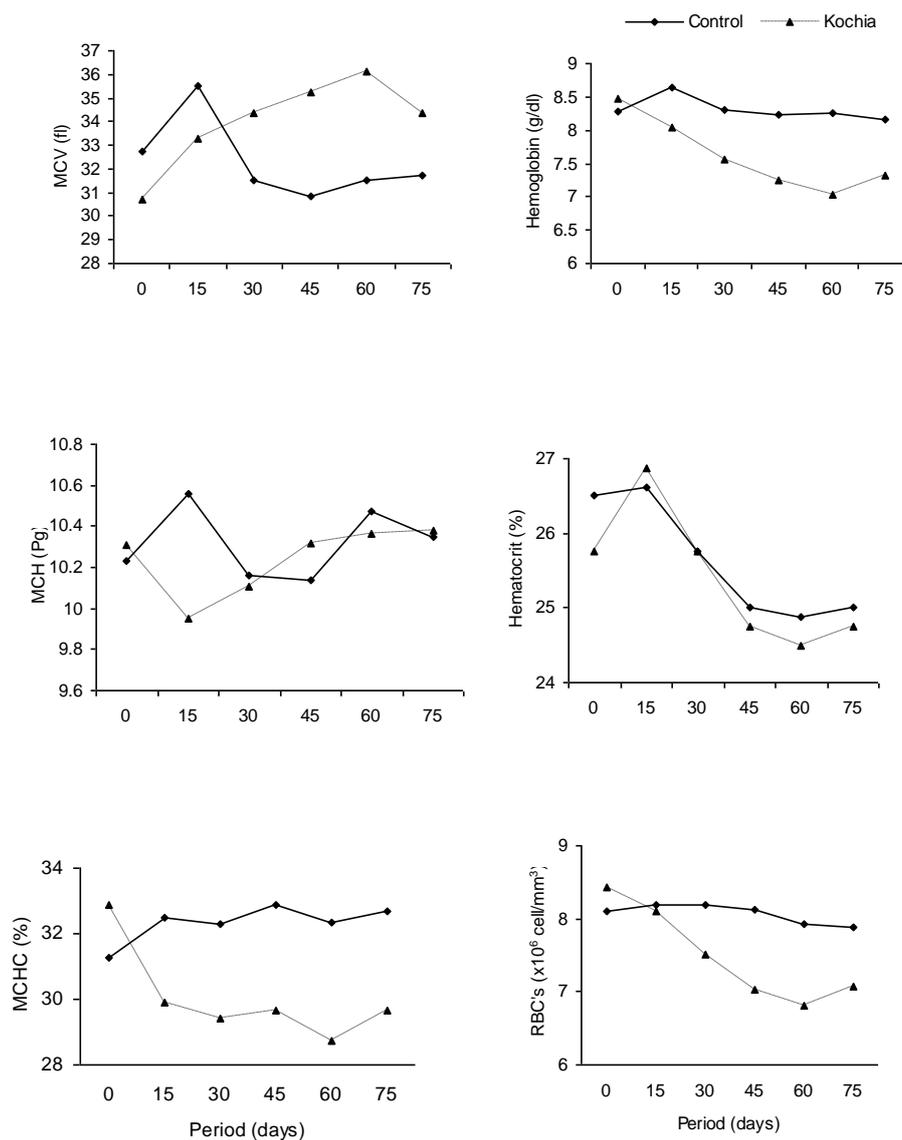
Figure1 indicated that, MCV was slightly higher in lambs fed *Kochia indica*, the rate of change was +11.92% The higher value for MCV as a general finding that can be associated with salinity in *Kochia indica* and subsequent increasing water intake and retention of Na<sup>+</sup> and higher water content in erythrocyte (Upsurge). Therefore, hemolysis in RBC's can be occurred and subsequent decreased RBC's count (Rankins and Smith, 1991).

The present results indicated that, in the erythrocytic series, hematocrit and mean corpuscular volume were most variable, there was inverse relationship between MCV and erythrocyte count (RBC's) values. It should be noted that an index of anemia, the mean corpuscular hemoglobin concentration (MCHC) in lambs fed *Kochia indica* was 29.685% whereas a value of 32.5% or greater recorded in lambs fed alfalfa hay which is considered more normal value. The rate of change in MCHC was +4.5% and -9.76% for control and treated groups respectively. With the respect of treatment (T) and experimental period (P), the obtained data showed interaction (P<0.01) between T x P on MCHC.

Whole blood traits were evaluated at the onset and biweekly intervals (Figure1), although means for most traits were lower in treated group than the control group (P<0.01) at 60<sup>th</sup> day, presumably related to the salinity of *Kochia indica*. The differences between periods as means are small and probably unimportant biologically. Hemodilution can be excluded as the major cause to decrease some determined hemogram traits. Decreased Hb, Ht and RBC's concentrations is likely the result of a shortened half-life of cells or impairment in red blood cell synthesis (Rankins and Smith, 1991).

For cells of the immune system, Figure 2 indicated that, the total number of WBC's increased (leukocytosis) and the peripheral blood smear differential data indicated that, neutrophils and eosinophils elevated in lambs fed *Kochia indica* compared with those fed clover hay. The rate of increase was +5.74, +13.35 and +20.00% for WBC's, neutrophils and eosinophils, respectively. Abdel-Fattah (1994) reported that, 20% salinity in drinking water for Ossimi sheep caused Leukocytosis. El-Hassanein and El-Sherif, (1996) reported leukocytosis and increase in neutrophils, eosinophils and lymphocytes of Barki lambs suffered salinity of drinking water for 6 months. Statistical analysis for the leukocyte picture were not significant on treatment alone whereas, the interaction between treatment and experimental period recorded increase (P<0.01) in leukocyte picture particularly neutrophils and eosinophils during certain weeks which was in agreement with El-Hassanein and El-Sherif, (1996).

The highest count for total WBC's (13.20 X10<sup>3</sup> cell/mm<sup>3</sup>) proved on the 60<sup>th</sup> day. The peripheral blood smear differential data indicated that, the highest count of neutrophils and eosinophils were proved also on the 60<sup>th</sup> day (6.90 and 0.76 X10<sup>3</sup> cell/mm<sup>3</sup>) this increase indicated that, lambs fed *Kochia indica* had neutrophilia and eosinophilia. Nassr *et al.*,(2002) revealed an increase (P<0.01) in neutrophils and eosinophils in goats fed *Atriplex halimus* which may be due to prolonged salt stress and/or irritation of intestine (McLoed,1974).



**Figure 1. Blood hemoglobin, hematocrit, erythrocytes count (RBC's), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) for growing Barki lambs fed manured *Kochia indica* vs. those fed colover hay (control)**

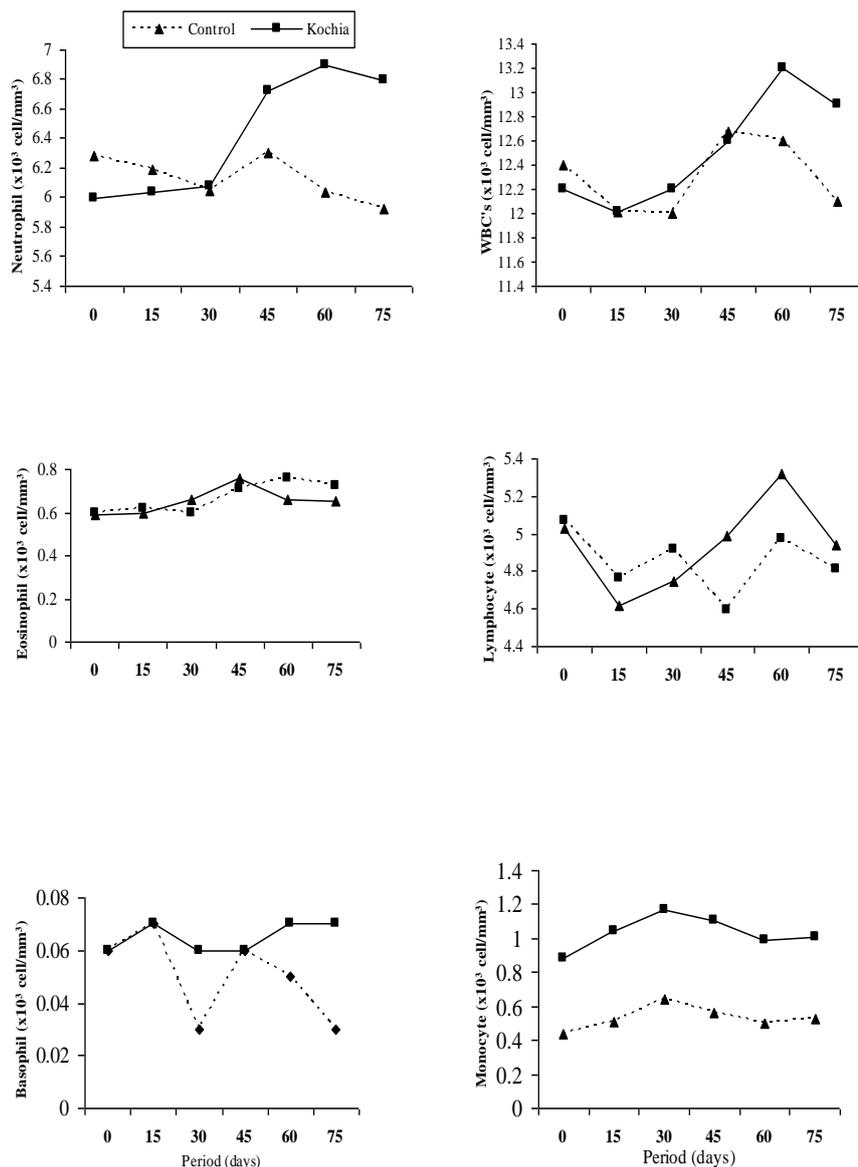


Figure 2. White blood cells (WBC's) , Neutrophils, Lymphocytes, Monocytes eosenophils and basophils counts for growing Barki lambs fed manured Kochia indica vs. those fed colover hay (control)

**Plasma biochemistry responses:**

In respect of the effect of *kochia indica* on plasma proteins, Table 2 revealed that TP, AL, and GL tended to increase in *kochia indica* group than that of the control. This increase was proved to be significant on 15,30 and 45 days till the end of experiment particularly for TP and GL concentrations. The rates of changes were +9.35, +2.92 and +12.75% for TP, AL and GL, respectively.

The elevation in plasma TP and GL for treated group might be attributed to the salt content of *kochia indica* and their effect on kidney, liver and intestine functions. Similar results obtained by Kaneko, (1989); Rankins *et al* (1991b and c) on sheep and cattle; Ibrahim (1995); Nasr *et al.*, (2002) on goats and El-Hassanein *et al* (2002) on Barki lambs and Baladi kids that drank saline water. Increasing plasma total protein may reflect the nutritional status of animals as reported by O'Kelly (1973). Kumar *et al.* (1980) reported a positive correlation between dietary protein and plasma protein. Rankins *et al* (1991b and c) reported that, albumin concentration increased in lambs fed *kochia scoparia* compared with lambs fed clover hay.

Regarding the A/G ratio, the recorded increase in GL concentration in relation to AL concentration due to *kochia indica* affected the A/G ratio ( $P<0.05$ ). The rate of change in A/G ratio recorded -15.1% for lambs fed fresh *kochia indica*. The resultant decrease ( $P<0.01$ ) in A/G ratio probably makes it possible to maintain high colloid osmotic pressure needed for holding more water in blood. Similar results obtained by Assad *et al.* (1989); Ibrahim (1995 and 2001) and El- Hassanein *et al* (2002) recording a reduction in A/G ratio for sheep and goats received drinking saline water or fed Atriplex and Acacia.

**Table (2): Means ( $\pm$ SE) of total protein (TP); albumin (AL); globulin (GL); A/G ratio; cholesterol (CHO) and plasma urea nitrogen (PUN) for growing Barki lambs fed clover hay or manured *kochia indica***

Item	Control group		Treated group		SE ( $\pm$ )	
	Initial	Final	Initial	Final	Treatment	Period
<b>TP, (g/dl):</b>	7.3	8.05	6.95	7.6		0.08
Group mean	7.76 <sup>AA</sup>		7.63 <sup>A</sup>		0.02	
<b>AL, (g/dl):</b>	2.53	2.62	2.4	2.47		0.02
Group mean	2.52 <sup>A</sup>		2.38 <sup>B</sup>		0.02	
<b>GL, (g/dl):</b>	4.77	5.43	4.55	5.13		0.07
Group mean	5.24 <sup>A</sup>		5.25 <sup>AA</sup>		0.001	
<b>A/G, (%):</b>	0.55	0.48	0.53	0.45		0.01
Group mean	0.49 <sup>AA</sup>		0.46 <sup>A</sup>		0.005	
<b>CHO, (mg/dl):</b>	54.87	60.66	55.21	61.45		0.62
Group mean	60.20 <sup>AA</sup>		59.91 <sup>A</sup>		0.05	
<b>PUN, (mg/dl):</b>	28.40	35.75	29.70	38.15		0.83
Group mean	35.80 <sup>AA</sup>		35.37 <sup>A</sup>		0.07	

It is well known that, the level of CHO and PUN is used to reflect the state of glomerular filtration rate and kidney function test (Kaneko, 1989). Concerning the response of CHO and PUN, the results in Table 2 indicated

that, CHO and PUN values were elevated for lambs fed fresh *kochia indica*, the increase in PUN could be attributed to the high level of digestible crude protein in *kochia indica* (Table 1). Nasr *et al* (2002) and Badawy *et al.* (2002) reported that, feeding *Atriplex halimus* resulted in significant increase in serum urea concentration.

Gradually increases were recorded in CHO and PUN concentrations for lambs fed *kochia indica*, the rate of change were +11.3 and +28.45% for CHO and PUN, respectively. However, statistical tests revealed that, overall changes in CHO and PUN concentrations were not significant due to treatment but was significant ( $P < 0.01$ ) on certain experimental weeks. Rankins *et al.* (1991-b) reported that, CHO was elevated in lambs fed *kochia scoparia* than in those fed alfalfa hay. In contrast, Rankins *et al.* (1991-c) reported decrease in PUN in steers fed *kochia scoparia* than in those fed alfalfa hay. This decrease was attributed to low protein intake in *kochia scoparia* which was not low enough to compromise protein synthesis by the liver.

The results in Table 3 show that irrespective of ALPh, there were increases ( $P < 0.01$ ) in plasma CER, TL, ALT, AST and LDH concentrations in lambs fed *Kochia indica* compared with those fed clover hay. The rates of change were +3.3, +12.5, +16.7, and +11.5 and +11.9%, respectively.

**Table (3): Means ( $\pm$ SE) of plasma creatinine (CRE); total lipids(TL); alanine aminotransferase (ALT); aspartate amino transferase (AST) ; alkaline phosphatase (ALPh)and lactate dehydrogenase (LDH) for growing Barki lambs fed clover hat or manured *Kochia indica***

Item	Control group		Treated group		( $\pm$ ) SE	
	Initial	Final	Initial	Final	Treatment	Period
<b>CRE, (mg/dl):</b>	0.95	0.90	0.92	0.95		0.004
Group mean	<b>0.93<sup>A</sup></b>		<b>0.94<sup>AA</sup></b>		0.001	
<b>TL, (mg/dl):</b>	289.6	299.6	283.3	318.8		2.74
Group mean	<b>292.74<sup>A</sup></b>		<b>312.13<sup>AA</sup></b>		3.43	
<b>ALT, (U/L):</b>	5.55	5.40	5.68	6.63		0.09
Group mean	<b>5.46<sup>B</sup></b>		<b>5.98<sup>A</sup></b>		0.09	
<b>AST, (U/L):</b>	30.07	30.55	31.11	34.68		0.37
Group mean	<b>30.69<sup>B</sup></b>		<b>32.37<sup>A</sup></b>		0.29	
<b>ALPh, (U/L):</b>	198.7	198.4	203.7	186.8		1.26
Group mean	<b>199.77<sup>AA</sup></b>		<b>192.41<sup>A</sup></b>		1.3	
<b>LDH, (U/L):</b>	167.7	165.1	172.6	193.1		2.24
Group mean	<b>168.17<sup>B</sup></b>		<b>180.02<sup>A</sup></b>		2.09	

Zhu *et al.* (1992) recorded similar affect on creatinine in sheep; Badawy *et al.* (2002) revealed that, serum creatinine was elevated significantly in both lambs and kids fed *Atriplex halimus* and *Acacia saligna*, respectively. On the contrary, Zhu and Filippich (1995) and El-Bashary (2000) reported no change in serum creatinine in sheep dosed with 1g tannic acid/kg body weight and in camel fed on *Atriplex halimus* for 6 months, respectively.

In contrast, plasma ALPh decreased in lambs fed *kochia indica* at 30-d and continued at the same level till the end of experiment. The rate of change

in plasma ALPh recorded –8.3%. Likewise, Rankins *et al* (1991-b and c) observed decrease in ALPh on sheep and cattle fed *kochia scoparia*.

In essence the detrimental effects of *kochia indica*, which became progressively worse with prolonged the experimental period, were seen as decreased in plasma ALPh (Rankins *et al.*, 1991 c); and increased (greater leakage) of ALT, AST and LDH ( $P<0.01$ ), this results was completely agreement with the previous studies by Badawy *et al.*, (2002) revealed a significant increase in activities of ALT and AST enzymes in lambs and kids fed *Atriplex halimus* and *Acacia saligna*.;El-Bashary (2000) on camels fed *Atriplex halimus* and Rankins *et al.*, (1991b); reported that, elevation of ALT, AST and LDH ( $P<0.01$ ) enzymes in serum of sheep and cattle further establishes that early changes associated with *kochia* toxicosis involve leakage of hepatocellular enzymes.

### Conclusion

Based on the biochemical analysis for *kochia indica* fed-lambs, results from the present study indicated that, most plasma constituents were increased in concentrations. Wherease, certain hematological traits, *i.e.*, Hb,Ht,RBC's and MCHC decreased due to feeding *kochia indica* compared with those fed clover hay. The immunological status would be improved in lambs fed *kochia* plants as indicated by differential leukocytes ( increased the absolute values of neutrophils,basophils, monocytes and lymphocytes) compared with their counterparts of control group. However, *kochia indica* shrubs could be recommended for short-term feeding such as feedlot of growing lambs.

### Acknowledgment

Special thanks are given to Dr. Alsheikh, S.M. Researcher at Department of Animal Breeding for his assistance in statistical analysis.

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## التغيرات الهيماتولوجية والبيوكيميائية للحملان البرقي المغذاة على نبات الكوخيا انديكا المنزرع بجنوب سيناء

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يعتبر الكوخيا انديكا أحد نباتات المراعي الحولية التابعة للعائلة الرمامية التي تتحمل الجفاف والملوحة وتصلح نباتاته لتغذية الحيوانات لإرتفاع قيمتها الغذائية ولذلك تم تنفيذ هذا البحث بمحطة بحوث راس سدر بجنوب سيناء حيث تمت زراعة نبات الكوخيا انديكا وتسميده بالسماد العضوي (بمعدل ٤٠ م<sup>٣</sup> للفدان) تحت ظروف الري بالمياه المالحة (٨٠٠٠ جزء في المليون) واستخدم في هذه الدراسة ١٦ ذكر أغنام برقي نامي (متوسط الوزن ١٨,٥±٢,٥ كجم) وذلك لمدة ٧٥ يوما خلال موسم الصيف وقسمت الحيوانات الى مجموعتين متساويتين حيث غذيت المجموعة الأولى على دريس البرسيم المصري (لحد الشبع) بينما غذيت المجموعة الثانية على شجيرات الكوخيا انديكا (لحد الشبع) الى جانب تغذية تكميلية للمجموعتين بمخلوط العلف المركز بمعدل ١٠٠% احتياجات حافظه من الطاقة وقد اجري هذا البحث بهدف دراسة تأثير التغذية على نبات الكوخيا انديكا المُسدم على الصفات الهيماتولوجية والبيوكيميائية لحملان الأغنام البرقي حيث جمعت عينات الدم مرتين شهريا وقد اوضحت النتائج ما يلي:

- ١- أظهر تركيز كل من الهيموجلوبين والهيماتوكريت وعدد خلايا الدم الحمراء إنخفاضاً معنوياً وعلى النقيض ارتفع متوسط حجم خلايا الدم الحمراء مع نقص في محتواها من الهيموجلوبين وقد يرجع ذلك لزيادة محتوى هذه الخلايا من الماء وذلك في المجموعة المغذاة على الكوخيا انديكا المسددة مقارنة بالمجموعة الكونترول.
- ٢- أظهرت الحملان المغذاة على الكوخيا انديكا إرتفاعاً معنوياً في العدد الكلي لخلايا الدم البيضاء وكان ذلك متمثلاً في زيادة عدد الكريات المتعادلة الصبغة والحامضية والكريات الكبيرة مقارنة بالحملان المغذاة على دريس البرسيم المصري.
- ٣- أظهر النشاط الانزيمي للكبد لكل من الانين امينو ترانسفيراز واسبرتات امينو ترانسفيراز واللاكتات ديهيدروجيناز ارتفاعاً معنوياً مبكراً وعلى النقيض انخفض معنوياً الفوسفاتيز القاعدي في بلازما الحملان المغذاة على الكوخيا انديكا المسددة مقارنة بالمجموعة الكونترول.
- ٤- زادت معنوياً تركيزات البلازما من البروتينات الكلية والالبومين والجلوبولين والكوليستيرول الكلي والليبيدات الكلية والكرياتينين في الحملان المغذاة على الكوخيا انديكا مقارنة بالمجموعة الكونترول.

خلص البحث إلى إمكانية استخدام الكوخيا انديكا في تغذية الحملان البرقي النامية خلال برامج التسمين خاصة في موسم الجفاف في فصل الصيف.