Journal of Current Veterinary Research

ISSN: 2636-4026

Journal homepage: http://www.jcvr.journals.ekb.eg

Physiology

Consequence of Egyption Green Berseem (*Trifolium alexandrinum*) on Gut Morphology, Performance and Feeding Behavior on Growing Rabbits (*Oryctolagus cunicollus*)

Nazema S. Abdel-Megeid[1*], Enas K. Aziz[2], Hanem El-Gendy[3], Shimaa R. masoud[4], Seham S. Hadad[5]

(1) *Department of Cytology and Histology, Faculty of Veterinary Medicine, University of Sadat City, Sadat City 32897, Egypt.*
(2) *Department of Husbandry and Animal Wealth Development, Animal Behavior and management, Faculty of Veterinary Medicine, University of Sadat City 32897, Egypt.*
(3) *Department of Pharmacology, Faculty of Veterinary Medicine, University of Sadat City 32897, Egypt.*
(4) *Department of Physiology, Faculty of Veterinary Medicine, University of Sadat City, 32897, Egypt.*
(5) *Department of Anatomy & Embryology, Faculty of Veterinary Medicine, University of Sadat City 32897, Egypt.*

**Corresponding author: nazema2009dr@yahoo.com* *Received: 3/11/2022 Accepted: 3/12/2022*

ABSTRACT

Berseem is a conventional and cheapest green forage distributed all over the world. It is a good source of fiber and high nutritive value thus, it used as adlibitum feed for young growing rabbit. Additionally, rabbit is characterized by hind gut fermenter animals through its caecal microbial fermentation as well as rabbit has strong accommodation to high level of fiber diets that increasing the digestive efficiency moreover, it help the rabbits in microbial digestion and production of an energetic substance (volatile fatty acids &high quality proteins). Therefore, this study was applied for examining the outcome of berseem replacement as a cheap green forage by expensive commercial pellets feed in young growing rabbit; by means growth performance, carcass trait, histomorphometric, hematological profiles and behavioural changes. Twenty-four growing male rabbits (white New-Zealand breed) were used and classified into three different groups; two groups of them were supplemented with different two levels of Egyptian green berseem 50% with 50% pellets and berseem 90% with 10% pellets as well as the third group feeds on 100% commercial pellets. The diet was offered to rabbit by rate 4% of their body weight on dry matter basis daily. The study lasted for four weeks. Results showed that, the body weight and weight gain of rabbits were affected by berseem treatments. Moreover, using forage as green Egyptian berseem in rabbit diet with concentrates had a significant effect on feeding and exploration behaviour and a significant increase in total blood protein, globulin, albumin and decrease in A/G ratio. Furthermore, rabbits fed with the mixed diets had lower total cholesterol than those fed with control and they had higher HDL than control group in most blood parameters considered; the values are similar in all groups. Furthermore, there are a significant increase the length of

duodenum and ileum but carcass traits were not affected. As well as increase the height of the intestinal villi, depth of crypt and increase thickness of muscular layer of intestine in rabbits fed on berssem groups.

Key words: Antioxidant, Behaviour, Green Egyptian berseem, Growth performance, Immunity and Rabbit.

INTRODUCTION

Berseem (*Trifolium alexandrinum L*) is described as one of the most important leguminous green forages in Middle East region and it is annual sparsely hairy erect forage (Hannaway et al., 2004 and Hackney et al., 2007). It is valued as winter crop; do not grow well in the summer and in subtropics it grows in a mild winter, besides it recovers shortly after cutting, (Hannaway et al., 2004). Berseem prefers than alfalfa as it grows in wide range of soils as well as it is better in high moisture lands and it tolerant the salinity (Hannaway et al., 2004 and Hackney et al., 2007). Berseem familiarized in Egypt at 6th century (Hannaway et al., 2004); Moreover, it considered as a good source of fiber therefore given to young growing animals adlibitum with few quantities of concentrates (Ghosh et al., 2008); and to lactating does (Hedhly et al., 2011); as well as maintenance for adult rabbit because, it used as sole feed (Deshmukh et al., 1989). Berseem has good nutritive value as alfalfa hay, thus used conventional feeds to rabbit diet usually given at level up to 45-48% dry matter in experimental diets (Asar et al., 2010 and Elamin et al., 2011).

The industry of rabbit plays an important role in overcoming the gap between demand and supply of animal protein. Moreover, the meat of rabbits is more suitable than other for human consumption (Abo Egla et al., 2013). Additionally, the use of locally produced forages can help to overcome the dietary protein gap in rabbit meat production (Lebas, 1983).

Dietery fiber plays a significant role in rabbit feed and it is one of the main components of rabbit diets (Walaasalama et al., 2018); because of its effect on caecal microbial activity (Gidenne et al., 2010). Therefore the rabbit is one of record herbevoiurs animal that ingests large amount of fiber, and reflex it on improvement of their growth performance) (Abou-Ashour et al., 2003; Omara et al., 2005 and Abo Egla et al., 2013). Furthermore, there is strong adaptation of rabbits to high fiber diets due to higher digestible efficiency in volume of intestine especially caecum, colon and small intestine (Gidenne, 2010).

It is necessary to rabbit feeds to contain dietary fiber and roughages to maintain high rate of passage of ingests and avoiding accumulation of it in caecum that reduce feed intake besides, that fiber act as an energetic substrates for energy production by forming volatile fatty acids and high quality microbial proteins (De Balas et al., 1999). Although, Dietary fiber has some adverse effect; as it is a substrate for caecal microorganisms, it's fermentation produce mainly VFA,s which may reduce the incidence of digestive disorders and mortality (Garcia et al., 2000).

Relatively few studies have been made on the comparative study of the nutritional value of forages in rabbits compare to concentrate feeds. Hence, this study was undertaken to evaluate the nutritive values of different percentage of green Egyptian berseem (50%& 90%) with concentrate on growing.

MATERIALS AND METHODS:

All animal handling procedures as well as samples collection and disposal were according to the regulation of institutional Animal Care and Use Committee (IACUC) Faculty of veterinary medicine, University of Sadat city**.**

Twenty-four male New Zealand white rabbits from Badder center farm, approximately 6weeks old with an average weight of 0.85 kg were randomly separated into three groups of eight animals each. The three treatments were consisted of the following:

Group1 (A): Rabbits were fed on pelleted feed(concentrate 100%) (18% protein) as a control group.

Group2 (B): Rabbits were fed on mixture between pellet 50% and green Egyptian berseem 50%.

Group3 (C): Rabbits were fed on pellet 10% and green Egyptian berseem 90%.

Amount of pellet or Egyptian berseem were weighed weekly according to body weight of rabbits. Rabbits were fed on 4% of their body weight according to (Odeyinka et al., 2008). The Egyptian berseem was offered to rabbits on the top of the cage after storing overnight to decrease moisture content and avoid tympani or diarrhea. Rabbit were inspected clinically, weighed at arrival, and housed in galvanized wire cages (45cm×50cm × 30 cm) two rabbits per cage in a ventilated building. Cages were equipped with automatic drinkers, feeding hopper. Animals were confined and allowed to acclimate for two weeks prior to experiment. The house was provided with automatically controlled side exhaustion fans. Housing was cleaned daily and waste product was removed to achieve good hygienic condition. The experiment lasted four weeks till rabbits reach to marketing size.

Data collection:

Performance Data:

Live body weight of each rabbit was measured at 49, 56, 63, and 70 days of rabbit age. Weight gain of rabbits was assessed as difference between the final and initial body weight. Besides, four rabbits of each group were weighted and slaughtered at the end of the experiment for measuring the carcass traits as the following: carcass, liver, spleen and intestine as a percent from rabbit's body weight before slaughter. Additionally, length of each portion of intestine as duodenum, jejunum, ileum and cecum were calculated.

Behavioural observations:

Infrared cameras (SA- 1501s – Sony - Japan recorded behaviours of rabbits during three weeks of research. Behavioural observations were performed for 15 minute at three period morning (6:00- 7:00 am), midmorning (12:00- 1:00 pm) and afternoon (6:00- 7:00 pm). The percentage time and frequency of the following behavioural patterns: feeding (Eating pellets and hay and drinking), inactive (resting), maintenance (grooming), investigative (sniffing and rearing) were recorded according to Prebble et al. (2015) as described in Table (1).

Table (1): Description of rabbit behavior:

Behaviour	Description
Feeding	Eating concentrates: Rabbits stood next to the feeder, taking out pellets and chewing them. Eating hay: ingestion of hay from the top of cage whilst hindquarters were in contact with the floor. Drinking: Ingestion of water from the nipple drinker.
Inactive	Resting with the belly on the ground, hind limbs tucked under the body and forelimbs either tucked beneath the body or stretched forward and the head in an upright position
Maintenance	Grooming as licking the coat with sweeping movements of the head.
Investigative	Sniffing: Rabbits sniffed of items in the environment. Rearing: Rabbits sit up on hind limbs with both forepaws off the ground.

3- Blood samples:

Four blood plasma samples were collected from ear vein of rabbits of each group in vial containing EDTA for hematological studies. In addition, four serum samples were collected for measurement of cholesterol, total proteins, albumin, and globulins.

4-The liver tissues:

Were washed with cold water and sited into petri dishes. After that liver samples stored at –70°C until analysis by using commercial kits for measure MDA (malondialdehyde) and GSH (reduced glutathione) (Turguta et al., 2006).

5- Histomorphological examination:

Tissue samples of jejunum as a part of small intestine, (colon) as part of large intestine and cecal appendix are fixed in 10% neutral buffered formalin for 48h then thoroughly pass through traditional histological technique cited by (Bancroft and Gamble, 2002) .The sections stained with hematoxylin and eosin. The stained sections were examined using bilocular light microscope then photomicrographs were captured using leica digital camera (Swift Imaging 3.0) connected with the microscope. The selected histological images uploaded to Image J program to measure different parameters on the image with unit (um). Villus height (measured from above the crypt to the tip of the villus), crypt depth, and muscularis thickness in jejenum. In large intestine, the height of crypt and thickness of muscle were measured. While in appendix the thickness of epithelium covering the cecal appendix were measured from beginning of the lumen until the apex of lymph nodule while thickness of appendix lymphatic nodules measured from the apex till the base.

6- Statistical analysis:

Values are presented as mean ± standard error. Statistical analysis was carried out using analysis of variance; one-way ANOVA test followed by Duncan multiple comparison tests. All data were statistically analyzed using statistical software program SPSS (Statistical package for Social Sciences) Version 22.

RESULTS

1- Growth performance and carcass traits:

Data summarized in Table (2) indicated that feeding rabbits on mixture between pellet and green Egyptian berseem had a significant effect on rabbit's weight during experimental period. Rabbits that fed on pellet 50% and berseem 50% (B) showed significant ($p<0.05$) or ($p<0.01$) higher weight than others fed on pellet only (A) at 49, 56, 63, and 70 day of rabbit age. Moreover, overall weight and weight gain of rabbits of group B was significant ($p<0.05$) higher than weight and weight gain of rabbits of group A and group C. Beside, Table (3) showed rabbits fed on green berseem not significantly effect on carcass traits as percentage of carcass, heart, liver, stomach and intestine weight. However, groups that fed on berseem either 50% (B) or 90% (C) showed higher length of duodenum and ileum than rabbits fed of pellet only.

Table (2): Weight and weight gain of rabbits (kg) fed on experimental diets during four weeks (Means±SE):

Weight (kg)	Treatments			P value
	A	B	C	
At 49 day	1.02 ± 0.11^{b}	1.21 ± 0.02^{a}	1.06 ± 0.05^{b}	*
At 56 day	1.11 ± 0.11^{b}	1.41 ± 0.05^{a}	1.23 ± 0.06^{ab}	*
At 63 day	1.27 ± 0.14^{b}	1.64 ± 0.04^{a}	1.35 ± 0.06^{ab}	*
At 70 day	1.46 ± 0.13^{b}	1.85 ± 0.04^{a}	1.56 ± 0.03^{ab}	**
Overall weight	1.22 ± 0.09^{b}	1.53 ± 0.07^{a}	1.30 ± 0.06^{b}	*

Weight gain	0.44±0.04[b]	0.63±0.07[a]	0.49±0.03[b]	*

[a, b, c]= Mean values within rows with different superscripts letters are significantly different * ($p<0.05$) or ** ($p<0.01$).

Table (3): Carcass traits (%) and intestine length (cm)fed on experimental diets during four weeks (Means±SE):

Items	Treatments			P value
	Concentrate	Barseem 50%	Barseem 90%	
Carcass %	47.93±0.57	48.04±0.44	45.52±2.63	NS
Liver%	2.15±0.07	2.22±0.14	2.26±0.26	NS
Spleen %	0.06±0.01	0.06±0.01	0.05±0.01	NS
Intestine %	13.60±0.52	13.74±0.50	14.07±0.83	NS
Duodenum length (cm)	37.00±0.96[b]	44.66±1.64[a]	43.00±2.28[a]	*
Jejunum length (cm)	115.00±8.07	121.33±7.45	132.50±7.61	NS
Ileum length (cm)	28.00±0.73[b]	31.66±1.11[a]	30.25±0.16[a]	**
Cecum length (cm)	43.33±1.52	45.00±1.67	43.75±1.37	NS

[a, b, c]= Mean values within rows with different superscripts letters are significantly different * ($p<0.05$) or ** ($p<0.01$) NS ($p>0.05$ or $p>0.01$).

2- Behavioural observation:

Feeding rabbits of diet containing green Egyptian berseem had a significant effect on their behaviour as shown in Table (4). Rabbits of group B and C spent significant ($p<0.05$) long time for feeding compared to rabbits of group A. Moreover, rabbits fed on berseem 50% (B) spent longer time for grooming as maintenance behaviour and sniffing as exploration than other groups. However, feeding rabbits on 90% berseem (C) increase percentage time for rearing than other experimental diets. Additionally, rabbits of group A spent significant long time for drinking than other rabbits of group C.

Table (4): Percentage time of rabbit behaviours (%) fed on experimental diets during four weeks (Means±SE):

Weeks	Treatments			P value
	A	B	C	
Feeding behaviour				
1st	43.19±6.04[b]	45.68±3.72[b]	62.51±8.33[a]	*
2nd	41.10±5.54[b]	51.29±7.54[a]	52.02±5.48[a]	**
3rd	40.66±7.87	45.35±1.56	47.83±2.84	NS
overall	41.66±3.73[b]	52.82±4.38[a]	52.64±3.99[a]	*
Drinking behaviour				
1st	9.95±2.03[a]	5.65±0.60[b]	6.66±1.04[b]	*
2nd	11.50±2.25[a]	7.91±4.61[b]	5.17±1.44[b]	*
3rd	9.38±2.39[a]	4.52±1.46[b]	5.03±2.11[b]	*
Overall	9.96±1.27[a]	7.78±1.48[ab]	4.56±1.38[b]	**
Resting behaviour				
1st	25.33±8.46	24.59±3.43	27.54±4.19	NS
2nd	25.54±7.34	25.97±8.19	27.14±7.67	NS
3rd	30.80±7.76	25.13±5.24	28.37±5.19	NS
Overall	30.58±4.36	27.40±5.51	30.96±5.03	NS
Grooming behaviour				
1st	10.54±3.58	7.28±1.26	10.31±1.84	NS
2nd	18.92±3.72[ab]	24.06±3.67[a]	9.39±1.46[b]	*

3rd	16.14±2.67[ab]	20.67±2.21[a]	9.43±1.21[b]	*
Overall	15.41±1.95[a]	17.56±3.66[a]	7.51±1.05[b]	**
Sniffing behaviour				
1st	9.98±4.60	7.28±2.97	5.59±1.31	NS
2nd	8.45±2.44	7.77±1.80	7.31±4.25	NS
3rd	8.49±1.61[b]	13.86±5.07[a]	7.45±2.01[b]	*
Overall	9.43±1.76[b]	17.31±4.25[a]	9.35±1.89[b]	**
Rearing behaviour				
1st	6.36±1.84[b]	7.91±1.42[b]	28.24±4.31[a]	**
2nd	3.67±0.62[b]	2.96±0.01[b]	6.99±0.22[a]	**
3rd	4.470±1.16[b]	6.07±1.49[b]	14.44±2.79[a]	*
Overall	6.90±1.46[b]	7.64±1.36[b]	18.52±2.64[a]	**

[a, b, c]= Mean values within rows with different superscripts letters are significantly different * ($p<0.05$) or ** ($p<0.01$) NS ($p>0.05$ or $p>0.01$).

Feeding rabbits on different feeding programs containing green Egyptian berseem had a significant effect on their behaviour frequencies as shown in Figure (1). Rabbits fed on concentrate only (A) showed significantly high frequency of drinking (14.33±1.30 %) than other groups (6.66±0.89 %). On the other hand, rabbits of group B showed higher frequency of grooming (12.67±0.87 %) than rabbits of group A (8.98±0.67 %) and group C (7.87±0.71 %). Additionally rabbits of group C showed more rearing (15.14±1.39 %) than others of group A and B (9.10±1.32, 8.74±0.85% respectively).

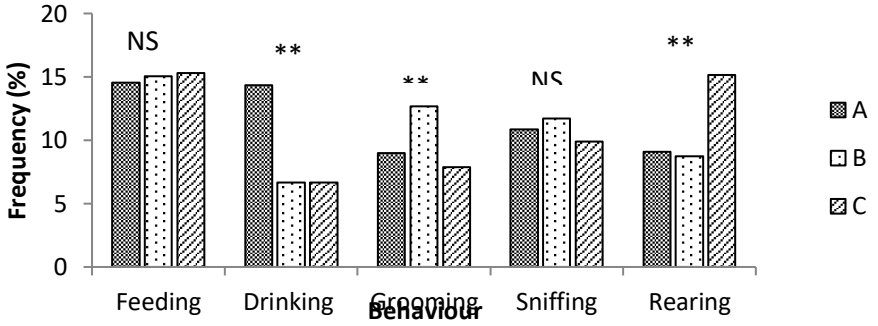

Figure (1): Overall frequency of rabbit behaviours (%) fed on experimental diets during four weeks (Means±SE). Or ** ($p<0.01$) NS ($p>0.05$ or $p>0.01$).

3- Hematological and biochemical parameters:

In most of blood parameters (RBCs, Hb, PCv, MCH and MCHC) considered, the values were similar in all groups; the values were illustrated in Table (5). WBCs and Lymphocyte numbers were significantly higher in berseem treated group than control one. WBCs value in control group, group treated with 50% berseem and group treated with 90% berseem were (5.32, 12.5 and 5.56) respectively. Lymphocyte number in control group, group treated with 50% berseem and group treated with 90% berseem were (1.72, 4.75 and 3.16) respectively.

The results of blood serum constituents as affected by replacement of pellets by green berseem are shown in Table (6). The result in berseem mixture group indicated that there were a significant increase in total proteins, albumin, globulin and HDL. On the other hand, there were a significant decrease in A/G ratio and total cholesterol level. There was a non-significant difference in MDA and GSH than control group.

Table (5): Hematological parameters of rabbits fed on experimental diets during four weeks (Means±SE):

Parameters	Treatments			
	A	B	C	P value
RBCs (10^6/µl)	5.63±0.02[a]	4.86±0.10[ab]	5.09±0.03[a]	**
Hb(g/dl)	11.00±0.03[a]	9.80±0.18[ab]	10.43±0.17[a]	*
PCV (%)	29.12±0.06[ab]	27.65±0.47[b]	31.46±0.16[a]	*
MCV (fl)	51.67±0.10[b]	53.85±0.24[b]	60.90±0.06[a]	*
MCH (pg)	19.70±0.12	20.20±0.03	20.33±0.21	NS
MCHC (%)	38.10±0.19[a]	35.45±0.09[ab]	33.66±0.42[b]	**
WBCs (10^3/µl)	5.32±0.04[b]	12.50±1.54[a]	5.56±0.18[b]	**
Lymphocyte (10^3/µl)	1.72±0.15[b]	4.75±0.58[a]	3.16±0.05[a]	**
Monocyte (10^3/µl)	1.32±0.04[a]	0.75±0.09[b]	0.50±0.03[c]	*
Granulocyte (10^3/µl)	2.27±0.08[b]	7.00±0.86[a]	1.60±0.07[b]	**

[a, b, c]= Mean values within rows with different superscripts letters are significantly different * ($p<0.05$) or ** ($p<0.01$) NS ($p>0.05$ or $p>0.01$).

Table (6): Biochemical parameters of rabbits fed on experimental diets during four weeks (Means±SE):

Parameters	Treatments			
	A	B	C	P value
Total protein (g/dl)	3.92±0.28[b]	6.10±0.23[a]	6.03±0.11[a]	**
Albumin (g/dl)	2.77±0.19[b]	3.72±0.09[a]	3.91±0.02[a]	**
Globulin(g/dl)	1.15±0.10[b]	2.37±0.16[a]	2.12±0.08[a]	**
A/G ratio (%)	2.14 ±0.10[a]	1.57 ±0.10[b]	1.84 ±0.10[b]	
MDA (nM/g pt)	113.33±0.55[b]	117.33±0.63[ab]	117.00±0.96[ab]	*
GSH (µg/mg pt)	22.00±0.36[ab]	16.33±1.28[b]	23.33±3.06[a]	*
Total cholesterol (mg/dl)	113.25±1.85[a]	105.00±7.05[b]	92.66±1.11[b]	**
HDL (mg/dl)	58.00±1.10[b]	62.33±2.76[ab]	71.33±1.28[a]	**

[a, b, c]= Mean values within rows with different superscripts letters are significantly different * ($p<0.05$) or ** ($p<0.01$) NS ($p>0.05$ or $p>0.01$).

4- Histomorphological examination:

The histomorphological characteristics of jejunum, large intestine and appendix on the rabbit in this study are shown in Figures (2), (3) and (4) respectively and Table (7).Data summarized in Table (7) indicated that offering green Egyptian berseem for rabbits improved morphometric measurements of jejunum, large intestine and appendix. Crypt depth of jejunum and colon (large intestine), tunica mucosa thickness of large intestine and epithelium of appendix were significant ($p<0.01$) increase in rabbits fed on mixture between green berseem (50% and 90%) than rabbits fed on concentrate only. However offering 50% green berseem and 50% concentrate had a significant effect on villi length of jejunum and lymphoid follicles length of appendix than other groups. On the other hand offering 90% berseem with 10% concentrate improved muscle thickness of jejunum and thickness of lamina mucosa of large intestine than other treatments.

Table (7): Morphometric measurements of jejunum, large intestine and appendix of rabbits fed on experimental diets during four weeks (Means±SE):

Items	Treatments			
	Control	Group 2	Group 3	P value

Jejunum				
villi height	416.91±8.17^{c}	993.23±4.47^{a}	893.43±3.30^{b}	**
crypts depth	85.01±1.20^{b}	239.43±5.09^{a}	216.47±1.59^{a}	**
Muscle thickness	97.13±3.80^{c}	206.37±2.64^{b}	237.48±8.89^{a}	**
(colon)Large intestine				
crypts depth	314.37±2.27^{b}	464.87±3.06^{a}	543.21±3.06^{a}	**
Lamina mucosa thickness	744.47±3.69^{c}	957.74±8.65^{b}	1761.61±6.22^{a}	**
Tunica mucosa thickness	247.22±6.48^{b}	519.78±52.46^{a}	507.45±30.51^{a}	**
Cecal Appendix				
Epithelium	723.43±1.05^{b}	1503.81±1.64^{a}	1327.50±2.01^{a}	**
Lymphoid follicles length	1646.25±3.77^{c}	2923.99±7.95^{a}	2000.92±5.54^{b}	**

$^{a, b, c}$= Mean values within rows with different superscripts letters are significantly different ** ($p<0.01$)

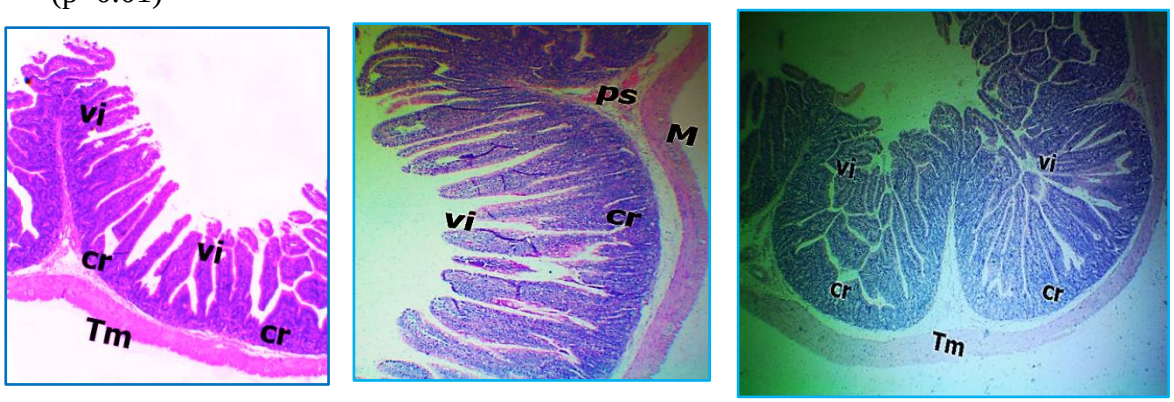

Figure (2): Photomicrograph of Jejunum of male New Zealand white rabbits showing variation in height of villi, crypt and muscle: (a). Rabbits were fed on100% pelleted feed, (b). Rabbits were fed on mixture between pellet 50% and green Egyptian berseem 50%. (C). Rabbits were fed on pellet 10% and green Egyptian berseem 90%. Intestinal villi (Vi) .intestinal crypt (cr). tunica muscularis (Tm). H&E X40.

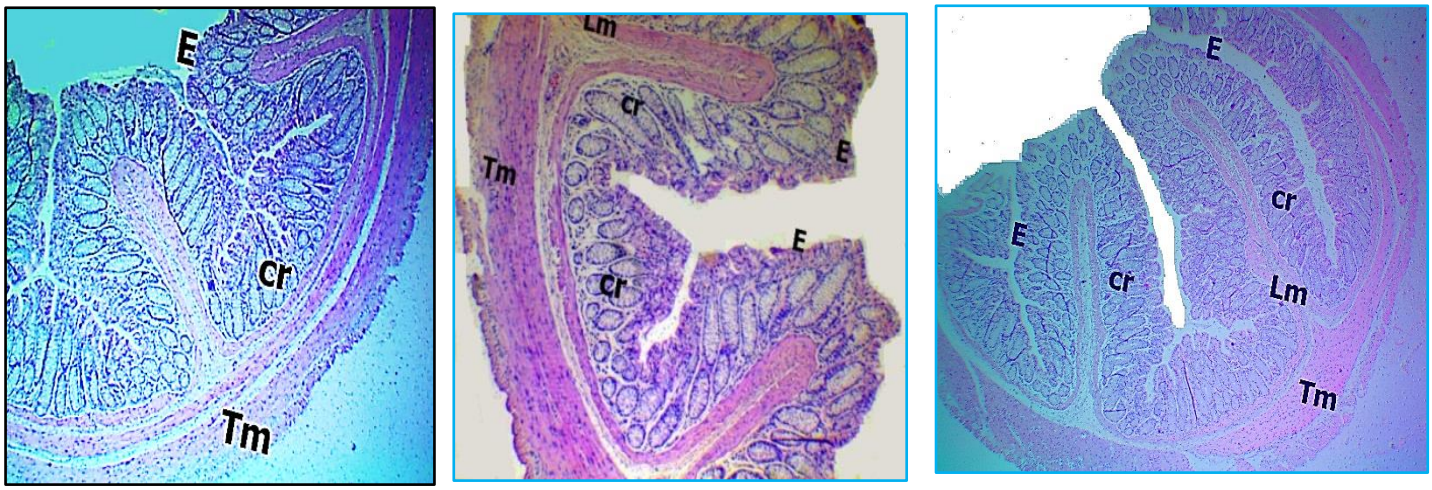

Figure (3): Photomicrograph of large intestine (colon) of male New Zealand white rabbits showing variation in thickness crypt and myscle layer: (a). Rabbits were fed on pelleted feed (as a control group), (b). Rabbits were fed on mixture between pellet 50% and green Egyptian berseem 50%. (C). Rabbits were fed on pellet 10% and green Egyptian berseem 90%. mucosal Epithelium (E). intestinal crypt (cr). tunica muscularis (Tm). H&E X40.

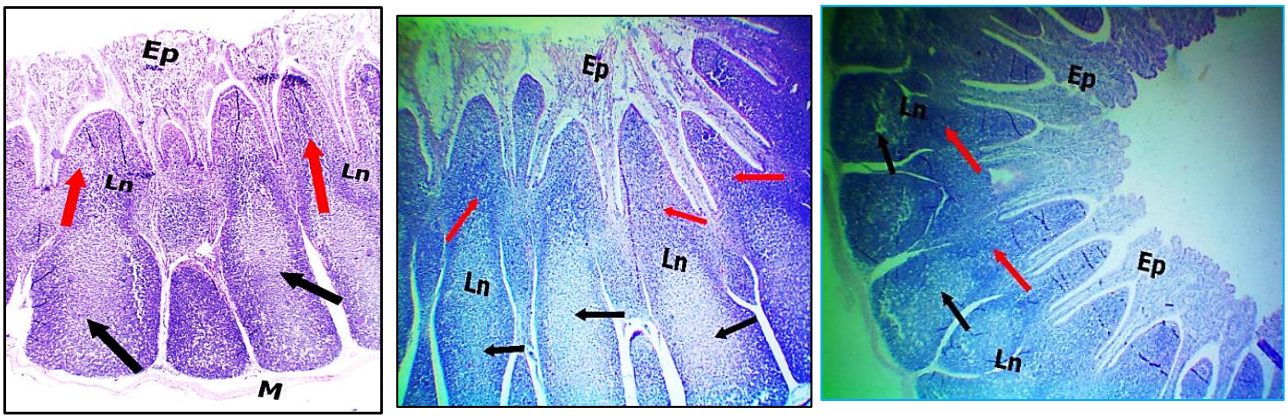

Figure (4): Photomicrograph of cecal appendix of male New Zealand white rabbits showing variation in thickness of epithelium of mucosal appendix and lymph nodules: (a). Rabbits were fed on pelleted feed(as a control group) , (b). Rabbits were fed on mixture between pellet 50% and green Egyptian berseem 50%. (C). Rabbits were fed on pellet 10% and green Egyptian berseem90%. Ep, epithelium of appendix. lymph nodules (Ln) with dark diffuse lymphatic tissue (red arrows) and central germinal center (black arrow) . H&E X40.

DISCUSSION:

Current evolutions in rabbit research had increased the number of nutritional recommendations, especially in the fibrous components (Fortun-Lamothe and Gidenne 2003). More, the basic reason of some recommendations is the highest performance possible in term of production or feed efficiency.

1- Growth performance and carcass traits:

The results of current study revealed that feeding growing rabbits on mixture between green Egyptian berseem 50% and concentrate 50% improved growth performance of rabbits as body weight and weight gain. This might be due to higher fiber content of the forages diet provide similar nutrients like the concentrate that meets the nutrient requirements of the rabbits and promotes the growth of rabbits (Osakwe and Nwose, 2008). Besides, improvement of histomorphological parameters of intestine under feeding Egyptian berseem that increases absorption of nutrient as shown previously in Table (7). In accordance to this study Iyeghe-Erakpotobor and Mohammad (2008) reported usage of forages in the rabbits feed had been recommended as supplement to the basic concentrate diet to meet the fiber and some of the vitamin requirements. Oloruntola et al. (2015) that studied effect of different forage on performance of weaner rabbits reported that total weight gain, weekly and daily weight gain in rabbits were improved under feeding mixture of Tridax and Pueraria (as forage) with 30 g concentrate. Furthermore, Ukpe et al., (2009) recorded higher growth in rabbit fed on forage and recommended the combination of leguminous with non-leguminous forages for better performance in rabbits. Abu Hafsa et al. (2016) reported that final body weight and average daily gain were

reduced with using A. saligna and L. leucocephala instead of Egyptian berseem hey in rabbit's diet. Gaafar et al., (2011) increasing percent of berseem silage in rabbit diet had the best results concerning body weight gain.

On the other hand this study reported carcass traits of rabbits as percent of carcass, heart, liver, gizzard and intestine weight not affected by using green Egyptian berseem in rabbit diet. However, increasing length of duodenum and ileum of rabbits that fed on Egyptian berseem than other groups. Oloruntola et al. (2015) observed decreasing of slaughter weight and non-significant ($p>0.05$) effect in the liver weight and heart weight of rabbits fed on different types of forage. This result is similar to report of Ogunsipe et al. (2014) who observed non-significant effect of internal organ weights in rabbits fed sorghum offal-based diets. However, Gaafar et al., (2011) reported carcass weight increased ($P<0.05$) with increasing the level of berseem silage. This results might attributed to the forages (green Egyptian berseem) might not have anti-nutritive factors or toxins at the levels that change the normal physiological and anatomical functions of liver or heart in growing rabbits.

2- Behavioural observation:

There are limited investigates on application of berseem on rabbits behaviour. Using forage as green Egyptian berseem in rabbit diet had a significant effect on feeding, maintenance and exploratory behaviour of rabbits. Offering green Egyptian berseem (50%, 90%) with concentrate increase percentage time of feeding, and decrease of drinking percentage time and frequency. This results might attributed to differences of feed intake of rabbits to diet that reflect on body weight and weight gain as mentioned before in growth performance results of this study.

Egyptian berseem is palatable for rabbits and contain high level of fiber as major fraction of rabbits diets and moisture content that helps to maintain a high passage rate, avoiding the accumulation of digesta in the caecum so that feeding behaviour increased and regulate the growth performance and the digestive health of rabbits (De Blas et al., 1999 and Gidenne et al., 2010). In accordance to Dehalle, (1981) and Fekete and Bokori, (1985) concluded that feed consumption of rabbits depends basically on nutrient contents and the actual energy need of the animal or protein and fiber level of its ration. Supplying fiber in the feed leads to an energy dilution of the diet so rabbits increased its feed intake to satisfy energetic needs (Gidenne and Lebas, 2002). The opposite effect has been observed by Falcao-e-Cunha et al., (2004) reported the inclusion of soluble and fermentable fiber in the diet increased total retention time and reduced feed intake in accordance to Tschudin et al. (2011) that concluded the majority of water consumed from the diet when the diet has high moisture content (90%) when compared with hay- or concentrate diets so that drinking behavior of rabbit decreased with feeding on green Egyption berseem. Besides, explorations as sniffing and rearing behaviour were increased in rabbits fed on green berseem that indicates the rabbits activities increased.

3-Hematological and biochemical parameters:

RBCs, Hb, PCV, MCH and MCHC showed no significant changes in all treatments that agreed with Ahmad and Zeb (2019) who stated that there was no significant difference among the groups, the aqueous extract of Trifolium repens and other treated

groups. Hemoglobin, mean corpuscular hematocrit (MCHC), eosinophils, and monocytes showed no significant changes in all treatments and also similar to Mahmoud and Ebeid (2014) who mentioned that normal ranges for the blood parameters were observed with no significant differences as the result of feeding all tested rations from them barseem with ration. WBCs and Lymphocyte numbers were significantly higher in barseem treated group than control one, these may be attributed to leaf protein of green barseem had a good and cheap source of protein with immunomodulating properties and increased number of T-lymphocyte (Dewan and Agarwal, 2020). The ranges of Hb values observed in this experiment being within the normal range for rabbits indicates the normal physiological relationship of haemoglobin with oxygen in the transport of gasses to and from the tissues of the body. The MCHC, MCH and MCV values in this experiment were in near similar with the values reported by Oloruntola et al. (2015). This is a signal that the rabbits on all the treatments were not anaemic. There were a significant increase in total proteins, albumin and globulin in barseem treated groups, this agreed with El-Nahas et al. (2004) who stated that concentrations of TP, albumin and globulin significantly increased with rising the level of barseem silage and decreasing the level of orange waste silage in the rations and a significant decrease in A/G ratio. The values of TP in blood were affected by protein intake in ration. This mean the TP in blood may reflect the status of nutrition of rabbits (Abdel-Khalek et al., 2000). Levels of serum cholesterol were significantly decrease and HDL was significantly increase in *Trifolium alexandrium* treated groups than control one, that agreed with Amer et al. (2004) and Khan et al. (2012) who reported that *Trifolium alexandrium* extract in drinking water had greatly improvement to serum lipid profile. There were a non significant difference in MDA and GSH than control group that agreed with Ahmad and Zeb (2019) who reported that there was no significant difference in the amount of GSH among control group (7.01 ± 0.1 µmol/g, mean ± standard deviation) and aqueous extract of *Trifolium* treated group (5.9 ± 0.72 µmol/g). On the other hand, Amer et al. (2004) mentioned that *T. alexandrinum* in drinking water for 4 weeks increase the hepatic GSH content significantly, that difference may due to different animals and different health condition of animals.

4- Histomorphological examination:

There are limited researches on application of berseem on the rabbit diets. The type of diets may affect the gastrointestinal tract size and the morphology of the intestines (Thomas and Ravindran, 2008). Rabbit dietary fiber plays a major role in the maintenance of intestinal mucosa (De Blas et al., 1999). Histomorphological examination of the small intestine (jejenum) ,large intestine (colon) and appendix revealed that there are a significant increase in the height of the intestinal villi, depth of crypt and thickness of muscular layer of intestine, in addition to the epithelium height and aggregated lymphatic nodule showed marked increase in rabbits fed on mixture of pellet 50% and green Egyptian berseem 50% then in group fed on pellet 10% and green Egyptian berseem 90% compared to control group fed on pelleted only. The height of intestinal villi and depth crypt of the absorptive small intestinal epithelium play vital roles in the process of digestion, absorption and

accommodation of nutrients (Wang and Peng, 2008). The crypt depth considered one of the healthiest and functional histomorphometric parameters of the intestinal eminence in chicks (Samanya and Yamauch, 2002; Abou-Elnaga and Selim, 2018) also Crypt depth can be used as a sign of the strength and renewal processes of the intestinal epithelium (Samanya andYamauch, 2002), in addition (Iji, 1999; Yamauchi, 2002 and Xu et al., 2003) mentioned that larger crypts have rapid tissue turnover and a high demand for new epithelium. While (Yegani and Korver, 2008 and Oliveira et al., 2013) indicated that shorter villi and deeper crypts may lead to reduced nutrient absorption, increased digestive secretion and diar-rhea, which in turn diminished growth performance additionally, the increased thickness of tunica muscularis may enhance the contact between the mucosa and intestinal content (De Verdal et al., 2010). Abdel-khalek et al. (2011) reported that increasing the thickness of lamina epithelialis of the tunica mucosa of intestinal cecum has main role in increasing the absorption of microbial products. The dietery fibers may be stimulate multiplication of the epithelial cell of the mucosa therefore increasing the secretion of the mucosa (Jonhsonetal., 1984). In addition the different sources of fibers and high dietery fibers in fleuence the variation thickness of the mucosa and cause distension of all segments of the intestine (Yu and Chiou, 1997). Zitnan et al. (2008) concluded that the higher the duodenum and proximal jejunum of CH bulls adapt to increase the absorptive surface due to the increase in nutrient demand. In addition Kishawy et al. (2018) study the effect of supplementing growing rabbit diets with whey powder and citric acid and reported that these supplements increase in the small intestinal villi, intestinal glands, and amount of goblet cells. Abu afsa et al. (2016) mentioned that Replace 50% of berseem hay in diets of NZW rabbits without any adverse effect on their growth performance instead of tree foliage from *M. oleifera* and *L. leucocephala*

It was concluded that rabbits when fed with diet composed of combination of barseem supplemented with concentrates could give a good performance comparable to rabbits fed only on concentrates.

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