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Effect of *Moringa oleifera* Leaf Extract on Ethogram of Rabbit Does

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ABSTRACT

The present study was conducted to evaluate the effect of *Moringa oleifera* leaves extract (MOLP) on ethogram of common rabbit does. Total (n=32) of *Oryctolagus cuniculus* common rabbit does divided into 4 groups randomly into A, B, C and D. Groups A, B, C and D were treated by MOLP by graded level 150 mg/kg, 300 mg/kg, 450 mg/kg and 0 mg/kg respectively. Water and feed offered to all the groups were *ad libitum.* The ethogram was observed 8 times daily for 4 weeks. Ethogram was manually noted on chart in morning and evening visually. Statistical analysis performed by using Chi-square goodness of fit test ($P<0.05$). Results showed that the behaviors frequency alert, chewing, drinking, eating, foot flickering, grooming, lie recumbent, lie sternal, others, periscope, scratching, sitting and stationery were differed significantly between the groups While jumping, locomotion, nudge, sniffing did not show any significant difference. Major ethograms; group C showed significantly high frequency of inactive (stationary, lie recumbent, lie sternal and sitting) while group D show lesser. Intake (drinking, eating and chewing) was significantly high in group D however significantly low in the group C. Active (locomotion, jumping, alert, foot flickering, periscope, shaking and grooming) was significantly high in group A however significantly low in the group C while the exploratory (sniffing, jumping and nudge) have no statistically significant difference. It was concluded that administration of high doses 450 mg/kg of Moringa rabbit showed abnormal behavior but on low doses of 150 mg/kg show normal behavior, low doses of Moringa are healthier in welfare of rabbit.

Key words: *Moringa oleifera*, leaves extract, Behavior, Rabbit does, *Oryctolagus cuniculus*.

Introduction

Moringa oleifera Lam. (family: Moringaceae) in the Pakistan and its common called as 'Sohanjna' and 'Bata' (Anwar *et al.*, 2005). To overcome malnutrition historical African originally used this herbaceous plant. It seemed that *Moringa*'s clean leaves comprise food plan seven times extra vitamin C if compare with oranges, four times vitamin A greater if compare with carrots, four times calcium greater if compare with milk, three times potassium greater if compare with bananas and two times protein greater if compare with yogurts (Fahey, 2005). Diagnostic studies revealed *M. oleifera* rich in protein, contain desirable amino acids, vitamins, minerals and important source of crucial nutrients with a rather anti-nutrients in little quantity (Falowo *et al.*, 2018). *M. oleifera* genus is *Moringa* concerning to its nature richly incorporate vitamins lipid soluble (A, D, E, K) and water-soluble vitamin C and B (1, 2, 3, 6 and 7). Essential minerals of extract in distinctive components of *Moringa* plant comprise Calcium, Iron, Copper, Potassium, Manganese, Magnesium, and Zinc. It has considerable amount of natural anti-oxidants (Khawaja *al.*, 2010).

The known activities and behaviors list with detail of a specific specie is called ethogram (Martin *et al.*, 1993). The chewing is the worldwide recognized its very characteristic and most common behavior which exhibit by the rabbit. Furthermore, the rabbit showed very complex ethogram. There are many ethogram published till date because the rabbit were

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being used in laboratory from a long time and these article are accessible to the scientific community (Gunn and Morton., 1993; Morton *et al*., 1993; Gunn and Morton., 1995; Krohn *et al*., 1999; Dixon *et al*., 2010; Jordan *et al.*, 2011 and Ferraz *et al*., 2019). In earlier studies (Lidfors, 1997; Harris *et al*., 2001; Johnson *et al*., 2003; Poggiagliolmi, *et al*., 2011). measured the effect of varying objects on behavior of rabbit (Lidfors, 1997; Poggiagliolmi, *et al*., 2011) offered gnawing sticks, grass cubes, hay in a water bottle and a box to rabbits to examine if they would interact with those objects and to assess the influence of the objects on abnormal and normal behaviors.
For the development of an efficient and perfect rabbit production system it is necessary to find out the relationship between the genetic, environmental and nutritional factors (Ferraz *et al.*, 2019). The welfare and behaviour are being influenced by the availability of feed, amount of feed, composition of feed and environmental factors of the rabbits in laboratory (Krohn *et al*., 1999). Evaluation the behaviour of animal which is one of the chief welfare indicators, and determination of possible deviations from the species-specific behaviour, is important in order to evaluate animal welfare (Jordan *et al.*, 2011) like stereotypic behaviors indicative of frustration (Gunn, *et al*.,1995) (DiVincenti, *et al*., 2016) (DiVincenti, *et al*., 2017; Thurston, *et al*., 2018). Animal performance and health is improving by using herbal extracts and leaves as animal feed (Khawaja *et al., 2*010). A feeding system which provides all the nutrients to rabbit and fulfil its nutritional demands this type of housing systems allows the rabbits to present behavior especially species-specific (Wagner, *et al.*, 2008).

The rabbits are most widespread animals and it is third most populated animal after the dogs and cats in the United Kingdom. In 1995 is was kept as companion animal and population was 1.4 million (McBride, 1998; (Dixon, *et al.*, 2010).

The mammalian prey species have abundance and their behaviour was regulated by the food availability they use food for reproduction and maintenance and for protection against predator by reducing the time for outdoor searching of food such as *O. cuniculus* (Lombardi, *et al*., 2007). Rabbits can consume pelleted feeds rapidly (Lidfors, 1997; Prebble *et al.*, 2015) and, whilst they may provide adequate nutrition for the maintenance of the rabbit then foraging behavior become limited. If fed in limited amounts the rapid consumption of the daily ration may leave the rabbit in a state of hunger for a considerable portion of the day (Lidfors, 1997; Prebble *et al.*, 2015).

Animal behaviour is associated with the diet and it shows the positive or negative effects of diet through behavior. If diet fulfills the need of animal then the struggle of animal for feeding becomes low and vice versa. The "Effect of *M. oleifera* leaf extract on the ethogram of common rabbit does" have not yet been studied. Hence, the present work is designed to investigate the "Effect of *M. oleifera* extract on ethogram of common rabbit does".

Materials and Methods

Experimental Animals

Mixed multiparous and nulliparous common domestic healthy rabbit (*O. cuniculus*) does have mix age and mix weight were purchased from the market. Total 32 rabbit does randomly divide into four groups and named A, B, C and D. Each group contained 8 rabbit does. Group D was control group and other three groups A, B and C were the experimental groups. The control group was provided with basal diet and clean water without any supplementation.
The experimental groups rabbit does were treated with the graded levels of *M. oleifera* leaves powder. Group A was given 150mg\kg, group B with 300mg\kg and group C with 450 mg/kg *M. oleifera* leaf extract. Rabbits can survive on all forage diet, but for optimum performance

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feeding mixed with forage and formulates proper diet (Arijeniwa *et al.*, 2000). Feed was given to all groups which was Wheat cracked (*Triticum aestivum*), Millet (*Pennisetum glaucum*), Lucerne (*Medicago sativa*), Sorghum (*Sorghum nitidum*), Lawn grass (*Dactylis glomerata*), and clean water *ad libitum*.

Geographic position

Experimental animals were kept in rabbit house under optimal housing conditions in the Zoology department of Islamia University Bahawalpur. It was high place than the sea approximately118 meters. It was present on 71° 41' 27'' east and 29°.21' 15'' north according to geographical coordinates and present at 29.35 latitude and 71.69.longitude according to geographical position. The animal house is divided into four chambers and each chamber size (1.89m × 3.15m ×3.75m). The light duration was 16L/8D.

Plant extract

Leaves of the plant *M. oleifera* were taken from fully grown trees in village Mubarakpur, district Bahawalpur. The leaves were air-dried at room temperature under shade. The *M. oleifera* leaf which was dried then grinded the leaves with grinder and make powder. Mix the powder in cold water and vortexed energetically for 30 seconds, and placed in the refrigerator for 24 hours and then again vortexed vigorously for 1 min at the room temperature. Centrifuge it twice at 12,000 rpm for 10 minutes and remove the water insoluble fragments of the suspension and the supernatants were filtered and collected and after the drying of extract stored at freezer. For administration weighed amount of the extract mix with water made solution then it was orally administered with the help of pressure drinking bottle to the rabbits in solution form.

Ethogram temperature and humidity

The temperature and humidity were measured two times in morning and two times in evening by the digital LCD temperature humidity meter indoor/outdoor room temperature clock hygrometer with sensor model number HTC-2. The obervstion of ethogram was started after the 3 days treatment of *M. oleifera* leaf extract. Ethogram was manually noted 4 times in morning and evening with interval of 15 minutes through vision. Note the specific behaviour which the rabbit was displaying at that time. Observation for each group in one time was 8 total 4 times in morning total observation 32 as well 32 in evening. Total number of observations for each group in one day is 64 and total reading for 28 days was 1792 for each group. The percentage frequency was analyzed for the specific behavioral activity through following formula:

$$\text{Percentage frequency of } specific\ behaviour = \frac{No.of\ specific\ behaviour\ was\ observed}{No.of\ total\ behaviours\ was\ observed}$$

Ethograms of the behaviours measured based upon (Gunn and Morton., 1993), (Morton *et al*., 1993), (Gunn and Morton., 1995), (Krohn *et al*., 1999), (Dixon *et al*., 2010), (Jordan *et al.*, 2011) and (Ferraz *et al*., 2019)

Alert: Immediate reaction of individual to being disturbed animal momentarily look around

Chewing: Hold food in mouth, above from the floor and crushing from jaws

Drinking: Drinking water from pot
Eating: Eating food from floor

Foot flickering**:** Rabbit give jerk back and forth her hind limb

Grooming: The animal is engaging in Self-directed cleaning behavior or other self-care activity

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Inactive: Animal is relaxing, sleeping, or just sitting still not doing any other activity then this is inactive behavior

Intake: The animal is eating food or drinking water. This includes chewing the food.

Jumping- Moving vertically by animal (rabbit) either into the air or onto an elevated surface.
Lie sternal: Resting with stomach or chest on the floor. Body should be above on tucked hind limb. Fore limbs can be stretched in front of the body or tucked under the body.
Lie recumbent Resting with side on the floor. Outstretched all the four limbs. Chest or stomach partially exposed and head can be resting on the floor
Locomotion: Purposeful movement around the floor pen

Nudge: Rabbit displaced another by physically pushing it out of the way

Others: Infrequent behavior not listed in ethogram

Periscope: Rabbit sit up on their hind feet to get a better view of the surrounding

Scratching: Scratching the own body from hind limbs.

Sitting: Hocks in contact with the floor, forepaws stretched and feet touching the floor.

Sniffing: The rabbit is sniffing environment, inhale the air with its nose the bars of cage
Stationary: Stand up on all four feet with abdomen off the floor and freeze the position

Ethogram Chart									
Time__ /___						Date____/_____/_____			
am/pm	Temperature		Temperature		am/pm	Temperature		Temperature	
Rabbit	Humidity		Humidity		Rabbit	Humidity		Humidity	
A1					C1				
A2					C2				
A3					C3				
A4					C4				
A5					C5				
A6					C6				
A7					C7				
A8					C8				
B1					D1				
B2					D2				
B3					D3				
B4					D4				
B5					D5				
B6					D6				
B7					D7				
B8					D8				

3.4. Experimental length

Experiment last for 31 days. Experiment started on the 14th of September Rabbits were given 3 days to habituate to the environment before the data were collected and observation was started from 17th September and the experiment ends at 14th October.

3.6. Statistical Analysis

The Chi-square goodness of fit test was measured through the minitab-15 for measuring the significance difference between the group for each behavior and the significance level for test was $P<0.05$. Since 17 behaviors were analyzed, a Chi-square test followed by *post-hoc* Benferroni correction resulted in defining a $p \leq 0.05$ as significant through AnalyStat android app. Intake and inactive was measured using One way ANOVA with Duncan's Multiple Range Test as *post-hoc* test through the Statistical Package for Social Sciences (SPSS for Windows Version 12, SPSS Inc., IL. USA. The mean ± SEM of temperature, humidity, five major behaviours measured by descriptive.

Results and discussion

The study was design to evaluate the effect of *M. oleifera* on the ethogram of the healthy rabbit does. The Moringa is given with degraded level and results are observed on the ethogram. Moringa has a nutritional value it fulfills the need of feed and changes the time budget of does and behavior pattern varies due to it. It was also evaluated that there was any hazard effect of Moringa on the behavior. Diet has the effect on behavior and diet can change the behavior pattern of animals.

The diet of lowest nutritional value change the group feeding behavior. The time spend 30-70% of wild rabbit on the ground in eating behaviour (Myers and Mykytowycz, 1958; Mykytowycz, 1958; Devi Prebble *et al* 2015) nevertheless, it increases to 90% when the quality of diet was poor (Myers and Poole, 1961; Prebble *et al* 2015).

The decreases occur in the time consuming in the feeding behavior highly attached upsurges the time consuming in rest, similarly testified in sheep (Ruckebusch and Gaujoux, 1976; Prebble *et al* 2015). The protein diet increases the resting behavior of the sheep (Ruckebusch and Gaujoux, 1976).

The reduction in time spent feeding was matched with corresponding increases in time spent inactive; a similar pattern of behaviour was also reported in sheep (Ruckebusch and Gaujoux, 1976; Prebble *et al* 2015).The protein diet enhance the resting behavior of the sheep (Ruckebusch and Gaujoux, 1976) The increase in inactivity and reduction in active feeding behaviours may have contributed to both the development of abnormal behaviours and high weight gain and increased body condition was observed (Prebble and Meredith, 2014; Prebble *et al* 2015).

The temperature mean ± SEM of overall result was 30.6 ± 0.19 ^{0}C and the average humidity was 61% ± 10% for the overall trail. The rabbits of group A fed extract of *M. oleifera* 150 mg, B fed extract of *M. oleifera* 300 mg, C fed extract of *M. oleifera* 450 mg and D does not fed extract of *Moringa oleifera*, show the behaviors actual frequency and percentage frequency of the specific ethogram was given in table (1).

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Table.1 Rabbit show different behaviours actual frequency in the above row while percentage frequency in lower row in brackets fed graded level of *Moringa oleifera* leaf extract.

Behaviors	**Groups**				Average	*P*-value
	A	B	C	D		
Alertx	76	40	82	36	58.5	<0.001
	(4.24)	(2.23)	(4.57)	(2.00)	(3.27)	
Chewing	110	100	86[a]	194[b]	122.5	<0.001
	(6.13)	(5.58)	(4.80)	(10.82)	(6.83)	
Drinking	4[b]	12	2[b]	52[a]	17.5	<0.001
	(0.22)	(0.67)	(0.11)	(2.90)	(0.97)	
Eating	220	168	116[a]	274[b]	194.5	<0.001
	(12.27)	(9.37)	(6.47)	(15.29)	(10.85)	
Foot flickering	8	10	2[a]	36[b]	14	<0.001
	(0.44)	(0.55)	(0.11)	(2.00)	(0.78)	
Grooming	134[a]	88	46[b]	124	98	<0.001
	(7.47)	(4.9)	(2.55)	(6.91)	(5.45)	
Jumping	2	6	2	10	5	0.228
	(0.11)	(0.33)	(0.11)	(0.59)	(0.28)	
Lie recumbent	288[a]	296	520[b]	316	355	<0.001
	(16.07)	(16.51)	(29.01)	(17.63)	(19.82)	
Lie sternal	394	426	458[b]	230[a]	377	<0.001
	(21.99)	(23.77)	(25.56)	(12.83)	(21.04)	
Locomotion	62	72	56	44	58.5	0.309
	(3.45)	(4.01)	(3.12)	(2.45)	(3.26)	
Nudge	6	16	12	16	12.5	0.071
	(0.33)	(0.89)	(0.67)	(0.89)	(0.70)	
Others	74	64	66	136	85	<0.001
	(4.12)	(3.56)	(3.68)	(7.58)	(4.75)	
Periscope	30	8	10	18	16.5	<0.001
	(1.67)	(0.44)	(0.55)	(1.00)	(0.92)	
Scratching	14	2	2	6	6	<0.001
	(0.78)	(0.11)	(0.11)	(0.33)	(0.33)	
Sitting	302	350	276	252	295	<0.001
	(16.85)	(19.53)	(15.40)	(14.06)	(16.47)	
Sniffing	8	10	8	12	9.5	0.763
	(0.45)	(0.56)	(0.45)	(0.67)	(0.53)	
Stationary	60	124[a]	48	36[b]	67	<0.001
	(3.34)	(6.92)	(2.68)	(2.00)	(3.73)	

[a ,b] superscript show significantly difference (P< 0.05)

Result show that Alert, Chewing, Drinking, Eating, Foot flickering, Grooming, Lie recumbent, Lie sternal, Others, Periscope, Scratching, Sitting and Stationary were differ statistically significant between the groups while Jumping, locomotion, nudge and sniffing show not significant difference between the groups.

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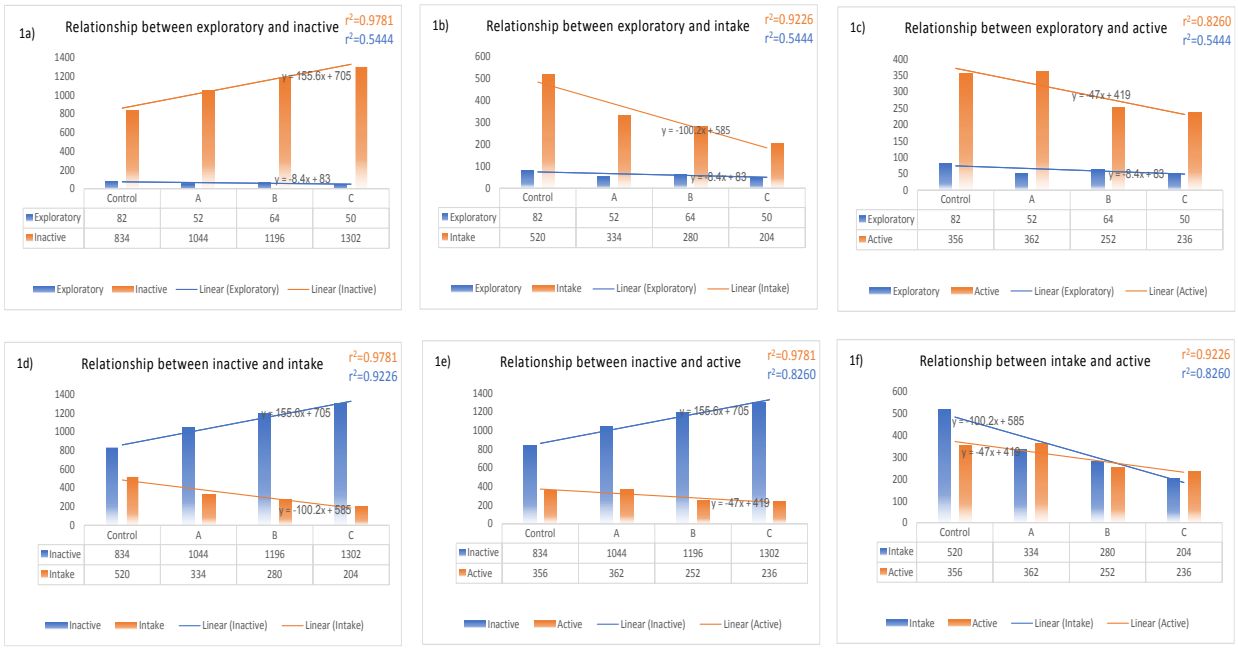

Figure 1. a) The relationship between the exploratory and inactive behaviour. b) Relationship between the exploratory and intake behaviour c) Relationship between the exploratory and active behaviour d) Relationship between the exploratory and intake behaviour e) Relationship between the inactive and active behaviour d) Relationship between the intake and active behaviour of rabbit does treated with graded level of *M. oleifera*.

The chewing behavior significantly high in the group D while low in the C which mean the Moringa effect on the chewing behavior of rabbit therefore the group D has the highest frequency of chewing and chewing gradually decreased in the group when the Moringa was gradually increased and group A, B and C had gradually decrease values of chewing. The Chewing percentage frequency of group A in the agreement with the results of percentage frequency of rabbit which were given with card board rolls experiment conduct by the (Poggiagliolmi, *et al*., 2011).

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Drinking behavior frequency was significantly high in group D while significantly low in the Moringa treated group A and C which indicate that Moringa decrease the thirst in the rabbit. It metabolizes in the body and produce water which fulfill the water requirement of rabbit. Percentage frequency in the control group which was agreement with the percentage frequency of noted by the (Mugnai *et al*., 2009) does kept in single standard cages. While experiment group have slightly low percentage frequency of drinking than the (Poggiagliolmi, *et al*., 2011, 2009) note the behavior form 5 am to 11 pm.

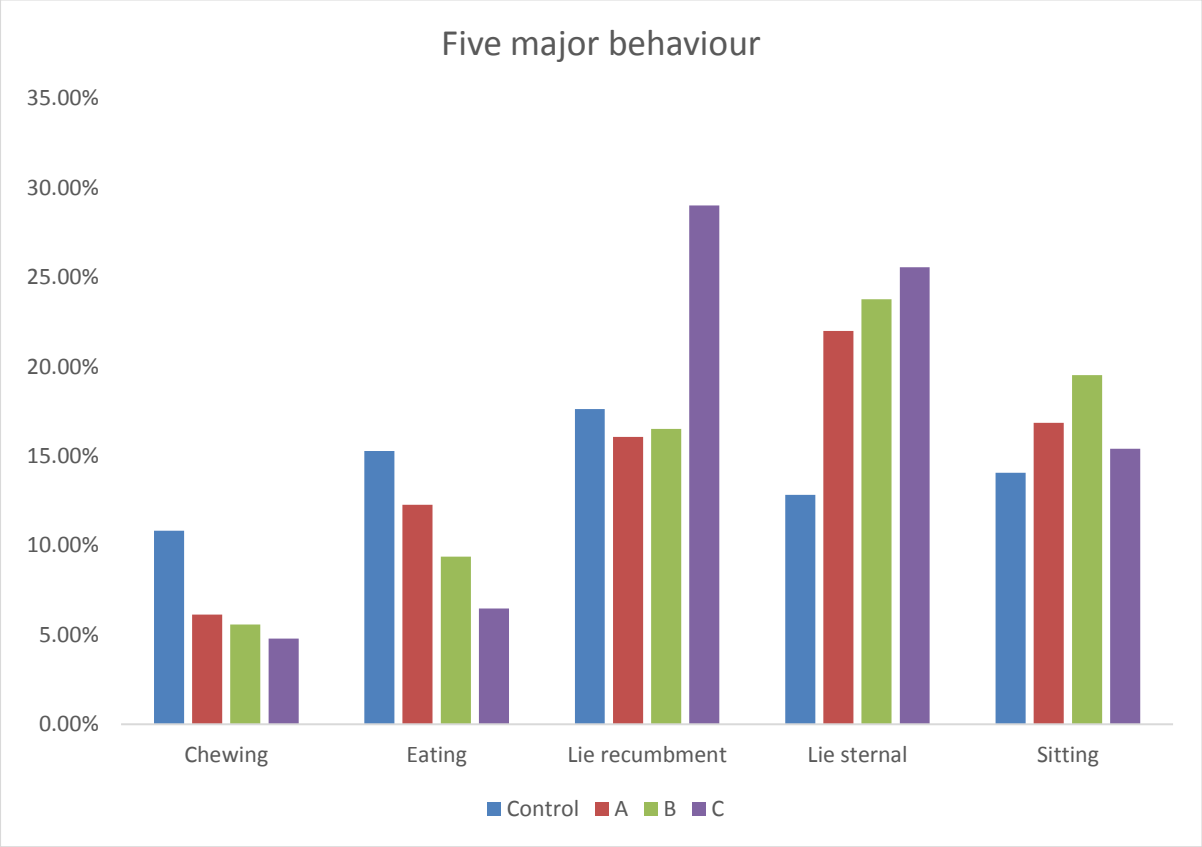

Figure 2:- Percentage frequencies of five major behaviour showed by the group A, B, C, and Control of rabbit does treated with graded level of Moringa.

The eating behavior significantly high in the group D and significantly low in the group C which mean the Moringa effect on the eating behavior of rabbit therefore the group D has the highest frequency of eating it gradually decreased in the group when the Moringa was gradually increased and group A, B and C had gradually decrease values of eating.
Eating percentage frequency of group A group B was agreement with the study of (Princz *et al*., 2008) conducted on the rabbit aged 6.5 weeks and rabbit aged 10.5 week.
Foot flickering was significantly low in the group C while high in the group D.
Grooming was significantly high in the group A while low in the group C it indicates that high dose of Moringa effect the grooming and less care itself while low amount of Moringa has high value of care itself.
Lie recumbent was significantly high was the group D while low in the group A which mean Moringa high values make more sleepy(Inactive) the rabbit while less value make less sleepy (active) the rabbit. The percentage frequency of group D has agreement with percentage frequency recorded by (Mugnai *et al*., 2009) does kept in two colony cages, but not trained to recognize their own nest.

The Lie sternal behavior significantly high in the group C and significantly low in the group D which mean the Moringa effect on the Lie sternal behavior of rabbit therefore the group C has the highest frequency of Lie sternal it gradually increases in the group when the Moringa was gradually increased and group A, B and C had gradually increase values of Lie sternal. It seems that Moringa treated rabbit show more rest. Lie sternal was low in the control group contrary to research conducted by Jordan *et al*., 2011 18.05%. Scratching The group A had the same percentage frequency 0.78 compare than the percentage value of 0.76 noted by Jordan *et al*., 2010. Sitting significantly between the group. And group D had the lowest value of sitting it explore that Moringa treatment increase sitting in all the experimental group.
Stationary significantly high in the group B and significantly low in the group D which mean Moringa increase the rest behavior of rabbit.
The mean ± SEM of intake (eating, chewing and drinking) and Inactive (Lie recumbent, Lie sternal, sitting and stationary) was measured by the one way ANOVA followed by *post-hoc* Duncan's test showed the mean ± SEM in table no. (2) and (3).

Intake mean ± SEM of group D was significantly high in the group D from group B and C. The results of Intake shows that the intake was inversely proportion to the extract treatment of *Moringa* so group D had highest value of intake and intake behavior decrease gradually in the groups A, B and C which shows that Moringa fulfill the requirements of diet so rabbit were less engaged in the feeding habit. Percentage frequency of Intake of group A (18.62) was in the agreement of the result of 18.26% (Poggiagliolmi, *et al*., 2011). And explore that the lowest value of inactive was founded in the group D and it looks like that *Moringa* was directly proportion to this behavior as we increased the quantity of Moringa the rabbit showed more behavior of inactive or resting. It looks that Moringa had any relax effect on the rabbit or Moringa caused laziness in them or Moringa fulfill their requirement of diet so there was less struggle found and rabbit prefer to rest. Inactive percentage frequency of the rabbit reared in Pen value (58.0) and group A 58.25 had the same value while rabbit reared in cage have same value 66.9 which have group B value 66.73 (Princz *et al*., 2008).

Five major behaviours (Chewing, Eating, Lie recumbent, Lie sternal, Sitting) percentage frequency presented in fig (3). It shows that the group C show 81.24% had less diversity in behavior mean showed less other behaviours. The group D has 70.63% of five major behaviors it explore that it was engaged in other diverse activities more than other groups. Result also describe that the Moringa change the behavior of the rabbit as we increase the quantity of Moringa the behavior show less diversity and rabbit showed these five behavior more than other behaviours.

Conclusion

It was concluded less diversity in behavior it was not good in welfare of rabbit and less intake and more inactive can create many health problems in rabbit. Low doses of Moringa does not have significantly effect on rabbit behavior but high doses had significantly risky effects. So, for the sake of welfare of rabbit we should use low quantity of Moringa.

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