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BEHAVIORAL AND ENDOCRINOLOGICAL CHANGES OF SHEEP SUBJECTED TO ISOLATION STRESS

(With 4 Tables and 3 Figures)

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التغيرات السلوكية والهرمونية للأغنام المعرضة للعزل والتسكين الفردى

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تم إجراء هذه التجربة على عدد ٥ من النعاج الغير حوامل أو مرضع والتي كانت من نفس السلالة وفي نفس العمر والوزن تقريبا. تم تسكين هذه الحيوانات جماعيا لمدة ١٤ يوم كفترة تمهيدية ثم ١٤ يوم أخرى كفترة مقارنه ثم تم عزل هذه الحيوانات وتسكينها فرديا في حجرات مستقلة لمدة ١٤ يوم كفترة تجريبية. تم تغذية هذه الحيوانات طوال فترة التجربة على دريس البرسيم الحجازي وتم تحديد متوسط الأكل اليومي لها. تم تسجيل السلوكيات المختلفة لهذه النعاج لمدة ٢٤ ساعة وذلك أثناء اليوم الأول والسابع والرابع عشر من كل من فترتي المقارنة والعزل وكذلك تم أخذ عينات من دم هذه الحيوانات أثناء اليوم الأخير من فترة المقارنة وأثناء نفس الأيام السابقة من فترة العزل وذلك لتحديد نسبة هرمون الكورتيزول بها.أثبتت النتائج التي تم الحصول عليها أن متوسط الأكل اليومي والسلوكيات المختلفة لهذه النعاج وكذلك نسبة هرمون الكورتيزول بدم هذه الحيوانات قد تأثر تأثيرا معنويا بالعزل والتسكين الفردي وأن هذه الحيوانات تحتاج لمدة ١٤ يوم على الأقل حتى تستطيع التأقلم والعودة لحالتها الطبيعية. وقد أوصى الباحث بأخذ هذا في الإعتبار وعدم تقييم النتائج من التجارب التي تتطلب عزل الخراف وتسكينها فرديا قبل مرور أسبوعان من بداية العزل.

SUMMARY

Changes in behavioral pattern as well as plasma cortisol level of sheep subjected to isolation stress have been studied. Five non-pregnant and non-lactating ewes of the same breed, nearly have the same age and weight (50.4 ± 2.1 Kg) were randomly selected from a 25 members sheep flock and stanchioned indoors together for a 14 days preliminary period followed by another 14 days control period. After that, they were

stanchioned individually in separate rooms for a 14 days isolation period. On the 1st, 7th and 14th day of both control and isolation periods, behavioral pattern was recorded for 24 hours using video cameras and video tape recorders. Ewes were *ad libitum* fed on alfalfa hay and the average daily intake was determined. Five blood samples were taken from each ewe during the last day of control period as well as the 1st, 7th and 14th day of isolation period with two hours intervals starting on 9:00 a.m. Sera were separated and assayed for their levels of cortisol. The main behaviours of ewes were significantly different ($P<0.05$) between control and isolation periods. Some other behaviours as bleating and pawing the ground were significantly increased in accompany with isolation. Serum cortisol concentration increased significantly ($P<0.01$) following isolation and remained higher than normal either on the 1st or 7th day of isolation while it was slightly but not significantly higher on the 14th day. Average daily intake as well as body weight gain of the ewes were significantly decreased ($P<0.01$) following isolation. The results suggested that, the animals suffer a lot following their isolation from the flock and may take a long time (not less than two weeks) to adjust to the new environment. This clarifies important welfare issues concerning experimental animals and care is needed when exploratring results from such animals.

Key words: Behaviour, endocrinology, isolation, sheep

INTRODUCTION

In nature, sheep lives in flocks as highly social animals and any changes in their social environment have been shown to cause them diverse distressed conditions as loss of appetite and decreased ability of resistance to pathogens (Adams and Sanders, 1992). The reason of these failures is frequently attributed to stress. The highly variable results of handling, transportation and isolation are likely to be due to different levels of psychological stress. Novelty and novel environment are strong stressors when an animal is suddenly confronted to it (Grandin, 1997).

The response of animals to stress not only limited to alteration of hormonal levels but also involves some behavioral adjustments. Done-currie et al.(1984) As well as Marsden and Wood-Gush (1986) Studied the behaviour of individually penned sheep and they found significant changes in both of their non-active and active behaviours. Moreover, Fordham et al. (1991) found a significant change in both behavioral

patterns and plasma cortisol level of sheep suddenly transferred from pasture to indoor.

As sheep are often kept isolated before surgical operations, before parturition as well as for research purposes and a little is known about how they adjust to the new surroundings, the aim of the present study is to clarify how sheep react with isolation.

MATERIALS and METHODS

I- Animals used:

Five non pregnant and non lactating ewes of the same breed, nearly have the same age and weight (50.4± 2.1 kg) were randomly selected from a 25 members sheep flock and were housed together indoors on a 24 hours lighting for a 14 days preliminary period for group stability and welfare followed by another 14 days as a control period. After that, ewes were isolated and stanchioned individually in separate rooms for a 14 days as isolation period. Allover the experiment, they were *ad libitum* fed on alfalfa hay and the unconsummed portion (feed refusals) was weighed daily before next morning feeding and was deducted from the total weight of the offered hay to calculate the daily intake. Water was freely available allover the experiment. Body weight was measured on the last day of each period to determine the weight gain.

II- Blood sampling:

On the 14th day of control period as well as the 1st, 7th and 14th day of isolation period, five blood samples (10 ml of each) were taken from each ewe with two hours interval starting from 9:00 a.m. The sera of the collected samples were separated by centrifugation at 3000 r.p.m. at 5°C for 30 minutes. Then, the harvested sera were freezed at –80°C until assayed for their levels of cortisol using TDxFLx system with fluorescence polarization and competitive binding techniques according to Dandliker and Feigen, 1970; Dandliker and Saussure, 1973.

III- Behavioral observations:

Behavioral pattern of ewes was recorded for 24 hours starting on the 1st, 7th and 14th day of control as well as isolation period using a video cameras and a video tape recorders. Ewes were fed and cleaned out at about 8:00 a.m. and 16:00 p.m. and during the remainder of the day humans were not normally present. The recorded behaviours were analyzed as follows: -

A- Main behaviours:

1- **Eating behaviour:** Where the total number of eating bouts as well as the total time that each ewe spent eating during 24 hours were calculated. Eating bout was determined similar to Metz, 1975; Morita and Nishino, 1994 as an eating activity which starts by the time that the animal begin to move its jaws to eat and ends when it stop chewing and swallow the very last bolus.

2- **Rumination behaviour:** Where the total number of rumination periods as well as the total time that each ewe spent ruminating during 24 hours were calculated. Rumination period was determined as a series of rumination cycles after at least two minutes of non ruminating activities (Morita and Nishinio, 1994) while rumination cycle was recognized as regurgitation of the ingesta with subsequent remustication, reinsalivation and reswallowing (Stevens and Sellers, 1968).

Resting behaviour: where the total time that each ewe spent lying down and resting was calculated.

Sleeping behaviour: where the total time that each ewe spent sleeping (lying down with resting the head on the floor or the thorax and keeping the eyes closed, Phillips et al., 1997) was calculated.

B- Other behaviours:

The recorded behavioural patterns were also analyzed for other behaviours that may have a relation with fear or frustration (e.g. bleating, pawing, stamping and sniffing the ground) as well as any unidentified behaviour.

IV- Statistical analysis:

Statistical analyses of the collected data were carried out according to GLM (general linear model) procedures of SAS (1995) for completely random design.

RESULTS

Results are obtained at Tables 1, 2, 3, & 4 and Figures 1, 2 & 3.

DISCUSSION

I- Feed intake and body weight gain:

In the present study, voluntary intake of the ewes, either on basis of g/day or g/kg.LBWt was significantly decreased ($P<0.01$) following their isolation and individual housing as shown in Table 1 as well as

Fig. 1 and Fig. 2. Moreover, this decrease in the voluntary intake was accompanied by a significant decrease ($P<0.01$) in their body weight gain (Table 2).

These results agreed with Kiley-Worthington, 1983, Adams and Sanders, 1992 and confirms that isolation and sudden transfer of sheep from a pasture is accompanied by loss of appetite and the animals spend much less time looking for the freely presented food even if it is provided in a form that allows a very rapid consumption.

II- Serum cortisol level:

Cortisol and corticosterone are the principle glucosides of the adrenal cortex. Cortisol predominates in rabbit, mouse and rat. Large ruminants are intermediate case while cortisol is the major corticosteroid secreted by sheep (Linder, 1959 and Paterson, 1964). Studies on the circulating levels of adrenal corticosteroids showed a marked rise of these levels after exposure to any stressful conditions (Johnson and Van Jonack, 1976; Dantzer and Mormede, 1983; Elizabeth and Huda, 1985; Shutt et al., 1988; Minton and Bleacha, 1990 and Parrott et al., 1996).

In the present study, serum cortisol level of ewes at 9:00 a.m.; 11:00 a.m; 1:00 p.m.; 3:00 p.m. and 5:00 p.m. was 0.306, 0.3, 0.322, 0.304 and 0.31 on the 14th day of control period while it was 0.812, 0,808, 0.806, 0,738, 0.678 on the 1st day of isolation, 0.6, 0.61, 0.59, 0.6, 0.58 0n the 7th day of isolation and 0.4, 0.41, 0.39, 0.39, 0.37 on the 14th day of isolation period (Table 4 and Fig.3). This result indicated that, serum cortisol level of ewes was significantly increased ($P<0.01$) following their isolation and individual housing and remained higher than control level during the 1st and 7th day of isolation period while it was slightly but not significantly higher than control level on the 14th day of isolation period.

This finding indicated that, individual housing as a new social environment acts as a stress factor on sheep subjected to it and the animal can not adjust to that new situation and retainted to its normal state before elapsing of a long time which in our experiment was at least 14 days. This is in agreement with (McNatty and Young, 1973; Coppinger et al., 1991; Fordham et al., 1991 and Apple et al., 1993).

III- Behavioral observations:

Exposure to a new environment is indeed a powerful stimulus that has the advantage over other stressors of not inducing physical pain while allowing qualitative and quantitative measurements of behaviour (Dantzer and Mormede, 1983). As a result, it has been used extensively by the behaviorists as an open field test to assess emotions in many

species (Fraser, 1974 for studies with pigs; Jones, 1977 for studies with chicks; Torres-Hernandez and Hohenboken, 1979; Moberg et al., 1980 for studies with sheep). In the present study, isolation and individual housing affected significantly on the main behaviours of sheep. Times spent for eating, ruminating and resting (352, 517, 913 and 258, 388, 819 min./24 hours for control and isolation periods, respectively) as well as number of eating bouts (32 and 22.5 bout/24 hours for control and isolation periods, respectively) were significantly decreased following individual housing ($P<0.05$). Otherwise, time spent for sleeping (114 and 121 min./24 hours for control and isolation periods, respectively) as well as number of ruminating bouts (20.3 and 16.8 period/24 hours for control and isolation periods, respectively) were not significantly affected as shown in table 3. Moreover, other behaviours that may have a relation with fear or frustration (e.g. bleating, pawing, stamping, and sniffing the ground) were significantly increased following isolation and individual housing.

This result is in agreement with Barnett et al., 1985; marsden and Wood-Gush, 1986 and Fordham et al., 1991 indicating that, isolation of sheep acts as a stress factor accompanied by mild frustration and changes in their main behaviours as a result of increased level of their circulating cortisol (Leshner, 1978; Dantzer et al., 1980, Dantzer and Mormede 1983).

CONCLUSION

In conclusion, isolation of sheep was found to act as a stress factor with major changes in their behavioral pattern and circulating cortisol level and the animals may take a long time (not less than 14 days) to adjust to such change in their social environment. As the isolated animal had a differing physiological and behavioral states from those kept in group, then care is needed when managed and extrapolating results from such animals as suggested by Done-Currie et al. (1984) and Fordham et al. (1991).

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Table 1: - Changes in voluntary intake (either on basis of g/day or g/kg.LBWt) of sheep subjected to isolation stress.

Treatment	Control		Isolation	
Day	g/day	g/kg. LBWt	g/day	g/kg. LBWt
1st	1819.32±20.4	34.20±3.1	962.95±13.6	18.80±2.32
2nd	1761.11±18.32	33.10±2.9	981.24±13.87	19.16±2.11
3rd	1784.30±18.88	33.53±2.94	962.76±11.65	18.80±2.13
4th	1908.30±17.9	35.87±3.44	922.26±13.43	18.01±2.24
5th	2043.20±20.6	38.41±4.64	1038.44±13.11	20.28±2.16
6th	2039.40±19.54	38.33±3.98	938.98±12.23	18.34±2.84
7th	2016.34±18.88	37.90±4.1	1031.07±12.87	20.14±3.01
8th	2082.13±18.43	39.13±4.21	997.67±12.43	19.49±2.18
9th	2068.45±20.1	38.88±4.03	999.40±13.22	19.52±2.64
10th	2051.00±18.76	38.55±3.84	999.37±11.89	19.51±2.89
11th	2057.81±18.65	38.68±3.88	968.69±12.54	18.92±3.11
12th	2061.71±20.2	38.75±3.81	1014.13±12.66	19.81±2.88
13th	2053.64±17.98	38.60±4.04	965.30±13.04	18.85±2.64
14th	2058.53±18.76	38.69±3.68	984.64±11.88	18.93±2.61
Mean	1985.71±19.1 **a**	37.33±3.75 **b**	983.35±12.74 **c**	19.13±2.56 **d**

Figures with different superscripts differ significantly ($p < 0.01$).

Table 2:- Effect of isolation and individual housing of ewes on their body weight gain

Experimental period	Initial weight	Final weight	Weight gain
	----------kg----------		
Control	52.3±1.9	54.1±1.7	1.8±0.2[a]
Isolation	54.1±1.7	49.9±1.2	-4.2±0.5[b]

Figures in the same column with different superscripts differ significantly ($p < 0.01$).

Table 3: - Effect of isolation and individual housing of ewes on their behavioral pattern

Items	Control	Isolation	Treatment effect
Main behaviour			
(min./24 hours)			
Eating	352±13	258±18	*
Ruminating	517±15	388±16	*
Resting	913±28	819±11	*
Sleeping	114±17	121±18	
(No./24 hours)			
Eating bouts	32.0±2.1	22.5±1.7	*
Ruminating periods	20.3±1.6	16.8±1.9	

*** $p<0.05$**

Table 4: - Effect of isolation and individual housing on serum cortisol level (Ug/100 ml) of sheep.

Treatment	control	Isolation (1st day)	Isolation (7th day)	Isolation (14th day)
Time				
09:00	0.31±0.02	0.81±0.03	0.6±0.01	0.4±0.01
11:00	0.3±0.01	0.81±0.03	0.61±0.03	0.41±0.01
13:00	0.32±0.02	0.80±0.02	0.59±0.03	0.39±0.03
15:00	0.30±0.03	0.74±0.03	0.6±0.02	0.39±0.02
17:00	0.31±0.02	0.68±0.02	0.58±0.02	0.37±0.01
Mean	0.31±0.02 a	0.77±0.03 b	0.6±0.02 c	0.39±0.02 a

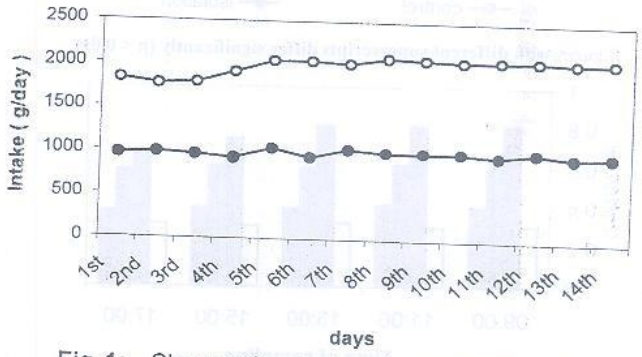

Fig 1:- Changes in voluntary intake (g/day) of sheep subjected to isolation stress.

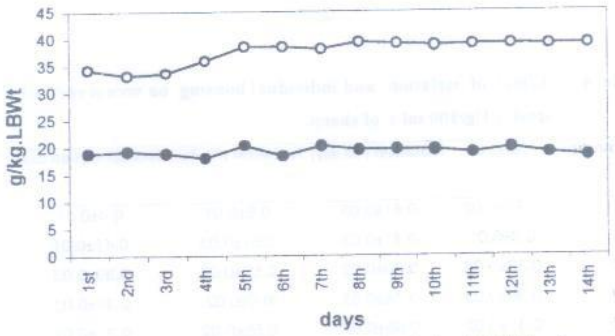

Fig 2:- Changes in voluntary intake (g/kg.LBWt) of sheep subjected to isolation stress.

control isolation

Figures with different superscripts differ significantly ($p < 0.01$).

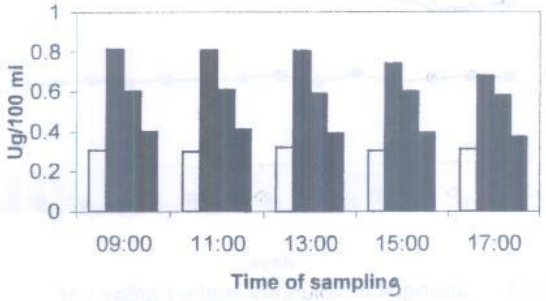

Fig 3 :- Effect of isolation and individual housing on serum cortisol level (Ug/100 ml) of sheep.

□ control ■ Isolation (1st day)
■ Isolation (7th day) ■ Isolation (14th day)