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Dept. of Animal Hygiene,
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ACCLIMATIZATION OF SHEEP TO HOUSING STRESS OF METABOLISM CRATES WITH REFERENCE TO THEIR BEHAVIOURAL AND ENDOCRINOLOGICAL ASPECTS

(With 4 Tables and 3 Figures)

By
M.A.ABDEL-RAHMAN; M.M.AHMED and S.A.SOTOHY

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مدى تأقلم الأغنام لضغوط التسكين الفردي في أقفاص التمثيل الغذائي وانعكاسها على السلوكيات والتغيرات الهرمونية

معتز أحمد محمد عبد الرحمن ، مصطفي محمد أحمد ،
سطوحي أحمد سطوحي

لدراسة قدرة الأغنام على التأقلم على ضغوط التسكين الفردي في أقفاص التمثيل الغذائي، تم إجراء هذه الدراسة على عدد ١٠ من النعاج الغير حوامل أو مرضع متماثلة العمر والوزن والسلالة. تم تسكين هذه الحيوانات جماعيا لمدة ٢١ يوم كفترة تمهيدية أتبعت بفترة مماثلـة " ضابطة " ثم تلا ذلك تسكين كل حيوان فرديا في أقفاص التمثيل الغذائي ولنفس الفترة الزمنية مع تقديم كميات كافية من ماء الشرب ودريس البرسيم الحجازي مع وضع قوالب الأمـــلاح المعدنية بصفة دائمة أمام الحيوان. تم تحديد متوسط الإستهلاك اليومي من الدريس وكذلـك حساب معدلات الزيادة في وزن الجسم بالإضافة إلى تسجيل السلوكيات المختلفة لهذه النعاج لمدة ٢٤ ساعة وإجراء بعض القياسات الهرمونية (هرمون الكورتيزول) خلال اليـوم الأول والسابع والرابع عشر والواحد وعشرون لكل من الفترة الضابطة وكذلك الفترة التجريبيـــة. أوضحت النتائج التي تم الحصول عليها أن متوسط الإستهلاك اليومي من الدريس ومعـدلات الزيادة في الوزن وكذلك السلوكيات المختلفة لهذه النعاج ونسبة هرمـــون الكورتـيزول قـد تأثرت تأثرا معنويا بالتسكين الفردي في أقفاص التمثيل الغذائي كما أوضحت النتائج أن نسبة هرمون الكورتيزول عادت تقريبا إلى طبيعتها خلال القياس الأخير مما يدل على عدم مقـدرة هذه الحيوانات على التأقلم على التسكين الفردي في أقفاص التمثيل الغذائي وذلك قبل مرور ثلاثة أسابيع. تم التوصية بأهمية مراعاة هذه الفترة الزمنية أثناء إجراء التجـــارب المختلفـة على الحيوانات تحت ظروف هذا النوع من التسكين.

SUMMARY

Changes in food intake, behavioral pattern as well as plasma cortisol level of sheep housed in metabolism crates have been studied. Ten non-pregnant and non-lactating ewes of the same breed, nearly have the same age (3-3.5 years) and weight (54.3 ± 1.9 kg) were randomly selected from a total 25 members sheep flock and housed indoors together for a 21days preliminary period followed by another 21 days control period. After that, sheep under test were housed individually in metabolism crates for another 21 days. Ewes were *ad libitum* fed on alfalfa hay and the average daily intake was determined. Mineral salt rocks were hanged infront of the animals. On the 1st, 7th, 14th and 21st day of both control and housing in metabolism crates periods, behavioral patterns were recorded for 24 hours using video cameras and video tape recorders. Parallel to that, a 10 ml blood sample was taken from each ewe at 12:00 p.m. Sera were separated and assayed for their levels of cortisol. Obtained results revealed that, non-active behaviors of ewes were significantly different ($p<0.05$) between control and housing in metabolism crates periods. On the other hand, active behaviors were highly increased in accompany with housing in metabolism crates. Serum cortisol concentration increased significantly ($p<0.01$) following housing in metabolism crates and remained higher than control levels till the 14th day. However, cortisol level was not significantly higher than the control period level on the 21st day. Average daily intake as well as body weight gain of the ewes were significantly decreased ($p<0.01$) following isolation. The results indicated that, the animals may take a long time to accommodate to the metabolism crates and the animals does not retained to its normal state before 21 days of housing in metabolism crates. This raises important welfare issues concerning experimental animals and care is needed when exploratring results from such animals.

***Key words:** Acclimatization of sheep to housing stress.*

INTRODUCTION

Housing of domestic animals has through the centuries been linked to the way animal breeding was practiced, so far the history of animal breeding and the housing of animals can't really be separated from each other (Maton et al., 1985). At the time of the first civilization man already possessed herds to produce wool, meat and dairy products. The animals found their pastures in the landscape and as soon as the surroundings of the camp were grazed, the herdsmen drove the herds to new pastures (Maton et al. 1985).

Sheep are essentially a low output enterprise, whose profits do not usually justify sophisticated housing systems. Fortunately the basic needs of a housed sheep are simple. Low level shelters are enough for effective protection of sheep flocks from cold, wind and other adverse weather. 3-6% of ewes in well managed and well-fed flocks that are not housed are lost during winter and are exacerbated by sever weather or disease (Watson, 1992). Housing a flock would not necessarily reduce any losses due to disease but negates adverse effects of bad weather and poached pastures.

Sufficient spare pens should be available to cover periods of bad weather and the intensity of one lambing pen per ten ewes is the recommended guide (ADAS, 1990). Hygiene is paramount during this period but it is often neglected.

If building design is to take into account the comfort of the animals, then clearly one must have some measure of this. An effort to measure 'comfort' in terms of time spent lying down has been made (Kiley-Worthington, 1977). Unless we have detailed knowledge of the normal bahaviour of the animal we can't assess this at any level. For example, there is a suggestion that animals will lie and sleep more, not only when they are comfortable, but in order to switch attention or switch off from an acceptable environment (Kiley-Worthington, 1977).

Sheep are often kept in metabolism crates for research purposes, although little is known about how they adjust to the new surroundings. The length of time taken for sheep to adapt to laboratory conditions when transferred from pasture has been investigated using plasma cortisol levels (McNatty et al., 1972; Pearson and Mellor, 1976). However, studies investigated the behavioral patterns of sheep penned for long periods have found a marked difference in their behavioral pattern from animals kept at pasture with occurrence of abnormal behaviors (Done-Currie et al., 1984; Marsden and Wood-Gush, 1986).

Behavioral observations and changes in serum cortisol level can be used as indicators of stress (Wiepkema, 1987; Ladewig, 1987; Apple et al., 1993 and Grandin, 1997). On the other hand, the independence of behavioral and physiological responses to stress has been illustrated in pigs and calves (Dantzer and Mormede, 1981; Wiepkema et al., 1986). However, little work has been done in this area in sheep.

As sheep are often kept in metabolism crates for research purposes, the aim of the present study is to clarify how they react and adjust to that new situation.

MATERIALS and METHODS

I- Animals used:

Ten non-pregnant and non-lactating ewes of the same breed, nearly have the same age (3-3.5 years) and weight (54.3 ± 2.1 kg) were randomly selected from a total of 25 members sheep flock. The animals were housed together indoors on a 24 hours lighting for a 21days preliminary period (for group stability and welfare) followed by another 21 days as a control period. After that, ewes were isolated and individually housed in metabolism crates (150×70×90 cm) for another 21 days. Each crate had metal bars on the sides, a wire mesh front, a solid back and a wire mesh floor and contained a metal bucket for food and another one for water. Throughout the experiment, animals were *ad libitum* fed on alfalfa hay and mineral salt rocks were hanged in front of them and feed refusal 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 1^{st}, 7^{th}, 14^{th} and 21^{st} days of both control and housing in metabolism crates periods, a blood sample (10 ml of each) was taken from a jugular vein of each ewe at 12:00 p.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 TDxFLx system with fluorescence polarization and competitive binding techniques according to ***Dandliker and Feigen, 1970; Dandliker and Saussure, 1973.***

III- Behavioral observations:

Behavioral patterns of ewes were recorded for 24 hours starting on the 1^{st}, 7^{th}, 14^{th} and 21^{st} days of control as well as housing in metabolism crates periods 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 behaviors were analyzed according to Marsden and Wood-Gush, 1986; Fordham et al., 1991 as follows:

A- Non active behaviors:

1- *Eating behavior*:- 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 behavior*:- 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).

3- *Resting behavior*:- where the total time that each ewe spent lying down and resting was calculated.

4- *Sleeping behavior*:- 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- Active behaviors:- as biting and/or butting the food bucket, biting and/or butting the wall of the crate, bleating, pawing, stamping and sniffing the ground as well as any other activity (e.g. standing on the bars of the crate, biting wool).

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 and DISCUSSION

I- Feed intake and changes in body weight:

In the present study, individual housing of the ewes in metabolism crates was followed by a significant decrease ($p<0.01$) in their voluntary intake, either on basis of g/day or g/kg.LBWt 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 (Table 2). These results agreed with Kiley-Worthington, 1983; Done-Currie et al., 1984; Adams and Sanders, 1992 and confirms that sudden transfer of sheep from a pasture and individual housing of them in metabolism crates is accompanied by a loss of appetite as 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:

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 housed in metabolism crates was significantly higher than control level ($p<0.01$) during the 1st, 7th and 14th days of the experiment (0.29; 0.3; 0.3 and 0.74; 0.65; 0.61 ug/100ml for control and housing in metabolism crates, respectively) while it was higher but not significantly different during the 21st day (0.31 and 0.38 ug/100ml, respectively) as shown in table 4 and fig. 3. This is in agreement with McNatty et al., 1972; Pearson and Mellor, 1976 who found that, cortisol levels of sheep transferred from pasture to laboratory conditions returned to basal levels within 14 to 28 days. This result suggests that, housing of animals in metabolism crates is a mild form of stress to which the animals take a long time to acclimatize. This agrees 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 behavior (Dantzer and Mormede, 1983). Times spent for eating, ruminating and resting (321, 493, 898 and 241, 381, 809 min./24 hours for control and housing in metabolism crates periods, respectively) as well as number of eating bouts (29.6 and 20.7 bout/24 hours for control and housing in metabolism crates periods, respectively) were significantly decreased following individual housing ($p<0.05$). Otherwise, time spent for sleeping (108 and 114 min./24 hours for control and housing in metabolism crates periods, respectively) as well as number of ruminating bouts (19.4 and 15.9 period/24 hours for control and housing in metabolism crates periods, respectively) were not significantly affected (Table 3). Moreover, active behaviors of ewes were highly increased following their housing in metabolism crates. Sometimes ewes physically interacted with each other by either reaching through the bars of the pen or over the top of the pen. Some of these interactions appeared to be friendly (e.g. licking, nudging and leaning on each other) while others appeared to be aggressive (e.g. stamping or butting at other ewe). Moreover, some ewes showed other patterns of active behavior as biting and/or butting the food bucket, biting and/or butting the wall of the crate and bleating.

This result is in agreement with Done-Currie et al., 1984; Barnett et al., 1985; marsden and Wood-Gush, 1986 and Fordham et al., 1991 indicating that individual housing of sheep in metabolism crates acts as a stress factor accompanied by mild frustration and changes in their active and non active behaviors as a result of increased level of their circulating cortisol (Leshner, 1978; Dantzer et al., 1980, Dantzer and Mormede 1983).

CONCLUSION

In conclusion, changes in the level of intake and behavioral pattern as well as elevated level of serum cortisol of ewes housed individually in metabolism crates indicated that these animals were under stress and required at least three weeks to be accommodated to these crates. This raises important welfare issues concerning the way in which the experimental animals are kept. As these animals have different physiological and behavioral states than those kept at pasture, then care is needed when extrapolating results from such animals.

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Table 1: Changes in voluntary intake (either on basis of g/day or g/kg.LBWt) of sheep housed in metabolism crates.

Treat-ment Day	Control		Housing in crates	
	g/day	g/kg LBWt	g/day	g/kg LBWt
1st	1719.32±20.25	30.37±3.25	862.95±15.11	15.49±2.75
2nd	1661.11±18.87	29.34±3.11	881.24±15.25	15.82±2.90
3rd	1684.30±18.66	29.75±2.98	862.76±13.75	15.48±2.25
4th	1808.30±20.26	31.94±3.77	822.26±14.20	14.76±2.70
5th	1943.20±19.75	34.33±3.24	938.44±13.75	16.84±2.72
6th	1939.40±18.64	34.26±3.11	838.98±15.20	15.06±3.05
7th	1916.34±18.74	33.85±2.98	931.07±13.75	16.71±2.95
8th	1982.13±19.22	35.01±3.25	897.67±13.20	16.11±2.25
9th	1968.45±20.02	34.77±2.95	899.40±13.52	16.14±2.25
10th	1951.00±18.88	34.46±2.25	899.37±15.20	16.14±2.55
11th	1957.81±18.81	34.59±2.50	868.69±15.05	15.59±2.90
12th	1961.71±17.98	34.65±3.00	914.13±14.32	16.41±3.10
13th	1953.64±20.12	34.51±2.75	865.30±15.65	15.53±3.00
14th	1958.53±20.16	34.60±2.40	884.64±13.55	15.88±2.75
15th	1954.55±18.82	34.53±3.25	890.55±13.78	15.98±2.75
16th	1956.00±19.02	34.55±2.50	892.75±13.45	16.02±2.55
17th	1953.30±18.25	34.51±3.00	895.65±14.05	16.07±3.00
18th	1952.12±18.35	34.48±2.75	899.18±13.54	16.14±3.05
19th	1955.23±20.00	34.54±2.90	896.23±13.95	16.09±2.75
20th	1957.24±19.25	34.58±2.55	896.65±13.25	16.09±2.90
21th	1955.00±20.02	34.54±2.87	898.76±14.20	16.13±3.00
Mean	1908.99±19.24 a	33.72±2.92 b	887.46±14.18 c	15.92±2.77 d

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

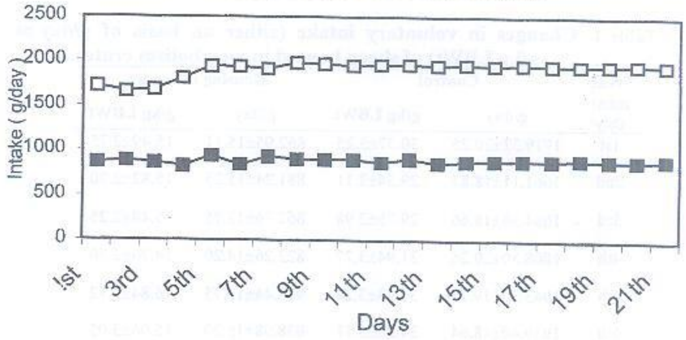

Fig 1:- Changes in voluntary intake (g/day) of sheep housed in metabolism crates.

—□— Control —■— Housing in metabolism crates

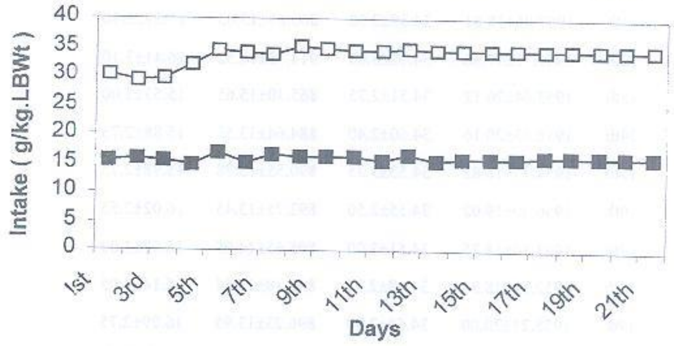

Fig 2:- Changes in voluntary intake (g/kg.LBWt) of sheep housed in metabolism crates.

—□— Control —■— Housing in metabolism crates

Table 2: Effect of individual housing of ewes in metabolism crates on their body weight gain.

Experimental period	Initial weight	Final weight	Weight gain
	----------	kg	----------
Control	55.9±1.6	57.3±1.4	1.4±0.2[a]
Housing in metabolism crates	57.3±1.4	54.1±1.8	-3.2±0.4[b]

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

Table 3: Effect of individual housing of ewes in metabolism crates on their behavioral pattern.

Items	Control	Isolation	Treatment effect
Main behavior (min./24 hours)			
Eating	321±17	241±14	*
Ruminating	493±21	381±13	*
Resting	898±27	809±19	*
Sleeping	108±14	114±16	
(No./24 hours)			
Eating bouts	29.6±1.1	20.7±1.9	*
Ruminating periods	19.4±1.8	15.9±1.3	

* $P<0.05$

Table 4: Effect of individual housing of sheep in metabolism crates on their serum cortisol level (Ug/100 ml).

Treatment Day	Control	Housing in crates	Treatment effect
1st	0.29±0.01	0.74±0.02	**
7th	0.30±0.01	0.65±0.02	**
14th	0.30±0.02	0.61±0.01	**
21st	0.31±0.01	0.38±0.01	

** P<0.01

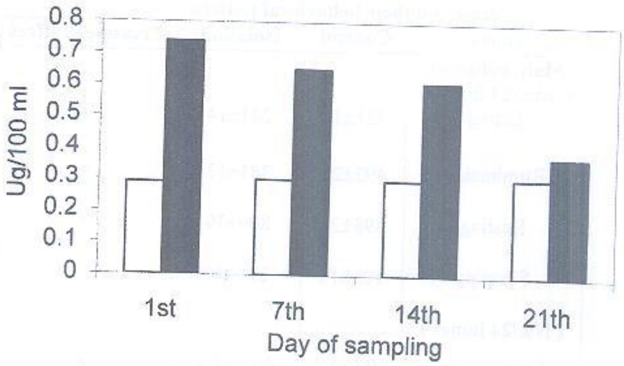

Fig 3 :- Effect of housing in metabolism crates on serum cortisol level (Ug /100 ml) of sheep.

☐ Control ■ Housing in metabolism crates