Department of Animal hygiene,
Faculty of Veterinary Medicine, Assiut University

BEHAVIORAL, PHYSIOLOGICAL AND ADRENAL RESPONSES TO REGROUPING AND RELOCATION IN SHEEP

(With 4 Tables and 3 Figures)

By

M.A. ABDEL-RAHMAN
and MADEHA H.A. DARWISH

(Received at 5/3/2006)

الاستجابات السلوكية والفسيولوجية والهرمونية والناتجة عن إعادة تجميع وتسكين الأغنام

معتز أحمد محمد عبد الرحمن ، مديحه حسنى أحمد درويش

تم إجراء هذه التجربة على عدد ٤٠ من النعاج الغير حوامل أو مرضع من سلالة أغنام محلية (الأوسيمى) والمتماثلة فى العمر والوزن تقريبا وسبق لها الولادة عدة مرات. قسمت هذه الحيوانات إلى أربعة مجموعات متساوية وتم تسكين كل مجموعة على حدة فى حظائر منفصلة تحت الظروف البيئية السائدة لمدة ١٠ أيام كفترة تمهيدية. أستخدمت حيوانات المجموعة الأولى كمجموعة ضابطة حيث لم يتم إضافة أو نقل حيوانات من أو إلى هذه المجموعة بينما أستخدمت حيوانات المجموعة الثانية لاختبار إعادة التجميع مع المجموعة الثالثة أو إعادة التجميع والتسكين مع المجموعة الرابعة. تم تغذية هذه الحيوانات طوال فترة التجربة على قش القمح و مخلوط المركزات مع توافر ماء الشرب بحرية وتم تحديد متوسط الأكل اليومي لها. تم تسجيل السلوكيات المختلفة لهذه النعاج وذلك مع كل إعادة تجميع أو تسكين. بالإضافة إلى هذا، تم فحص الحالة الصحية للحيوانات المستخدمة فى هذه التجربة وذلك لتعيين معدلات النبض والتنفس ودرجة حرارة جسم هذه الحيوانات. كذلك أخذت عينات من دم هذه الحيوانات فى بداية كل مرحلة تجريبية وعوملت هذه العينات المعاملة الخاصة لتعيين نسبة هرمون الكورتيزول بها. أثبتت النتائج التى تم الحصول عليها أن إعادة التجميع أو إعادة التجميع والتسكين بالنسبة للأغنام كانت من العوامل الضاغطة والتى أتت بانعكاسات سلبية مباشره على السلوكيات المختلفة ومتوسط الأكل اليومى لها وكذلك نسبة هرمون الكورتيزول بدم هذه الحيوانات. وقد نوهت هذه الدراسة عن مدى أهمية تجنب إعادة تجميع الأغنام داخل نفس الحظائر أو إعادة التجميع والتسكين داخل حظائر جديدة وذلك للوصول للمستوى الإنتاجي المطلوب.

SUMMARY

Forty non-pregnant and non-lactating multiparus ewes of the local Ossemi breed, about 4 years in age and 50 kg in weight were used in this investigation. They were divided into four groups each of 10 ewes.

Animals in each group were housed together under normal environmental conditions in a separate well-ventilated and well-lighted straw-bedded pen. All groups were subjected to a 10 days preliminary period for group acclimatization, stability and welfare. After that, ewes in the first group were used as control animals where they were never moved from their pen and no more animals were added. However, animals of the second group were used for making regrouping with that of the third group or regrouping and relocation with that of the fourth one for three times during the studying period at weekly intervals. Ewes were ad libitum fed on commercial concentrate mixture and wheat straw and average daily food intake was calculated, however drinking water was freely available allover the experiment. Behavioral pattern of the experimented animals was recorded at each social and pen exchange. Moreover, they were clinically examined to determine their average pulse rate, respiratory rate and body temperature. Blood samples were collected to measure their serum cortisol level. The obtained results indicated that, moving and merging of sheep through regrouping and regrouping with relocation were resulted in aversive environmental conditions and leaded to increased aggression and consequent injuries and reflected prominently on their behavior, food intake and serum cortisol level. Therefore, stability of the herd as well as avoidance of moving and merging as much as possible should be considered during establishing their farms.

Key words: *Behavior, physiology, adrenal, regrouping, relocation, sheep*

INTRODUCTION

Many conventional management practices may be stressful for livestock. This stressful situations annoy the animals and a brief period of it may cause a variety of physiological changes that result in poor production performance and reduced defense mechanisms against diseases. So, it is considered as a managemental stresses (Klastrup *et al.*, 1987 and Fraser & Broom, 1990).

Stress is defined as an external body forces that tend to displace the homeostatic state of the animal (Scott, 1981). In lactating animals, stressful events can hamper milk secretion and ejection reflex and may enhance the risk of mastitis (Bruckmaier *et al.*, 1993). The inhibition of the milk ejection reflex is a consequence of activation of the sympathetic-adrenal system, which reduces the release of oxytocin and its access to the mammary epithelium (Findlay and Grosvenor, 1969; Goodman and Grosvenor, 1983 & Bruckmaier and Blum, 1992).

Moreover, increased level of plasma corticosteriods in stressed animals may impair the immune functions and reduce the synthesis of milk proteins due to amino acid utilization for gluconeogenesis (Hart, 1983; Napolitano *et al.*, 1995 and Rhind *et al.*, 1998).

The disruption of suitable social links may lead to increased aggression and consequent injuries as well as reduced growth rates (Friend *et al.*, 1983 and Rushen, 1987). Moving and merging were found to cause increased cortisol secretion and to have slight and short-term effect on the productive traits (Varner and Johnson, 1983 and Hasegawa *et al.*, 1997).

Indeed, sheep are gregarious and relatively defenseless animals and any husbandry operations may result in the motivational state of fear, anxiety and frustration (Lynch *et al.*, 1992 and Casamassima *et al.*, 2001). So, the present study focused on the stressfulness of regrouping and regrouping with relocation and its effect on behavioral, physiological and adrenal responses in sheep.

MATERIALS and METHODS

I- Animals, experimental design and management: -

Forty non-pregnant and non-lactating multiparus ewes of the local Ossemi breed, nearly of about 4 years in age and 50 kg in weight were used in this investigation. They were divided into four groups each of 10 ewes. Animals in each group were housed together under normal environmental conditions in a separate well-ventilated and well-lighted straw-bedded pen. Each pen was provided with a manger and water trough with a sufficient feeding and watering space per animal. Used pens were selected to be faraway from each other so that, animals in each pen were isolated from tactile, visual and auditory stimuli of animals in other pens.

The experiment was designed according to Sevi *et al.* (2001). All groups were subjected to a 10 days preliminary period for group acclimatization, stability and welfare. After that, ewes in the first group were used as control animals where they were never moved from their pen and no more animals were added. However, animals of the second group were used for making regrouping with that of the third group or regrouping and relocation with that of the fourth one for three times during the studying period at weekly intervals (day 1, 8, 15). Animals of the third and fourth groups were individually marked for further behavioral observations and experimental analyses. At early morning of these initiative days, five ewes of the second group replaced others of

the third group which never moved from their pens (regrouping) while other animals of the second group replaced others of the fourth group and all members were moved to a new pen of the same conditions (regrouping and relocation).

Allover the experiment, ewes were ad libitum fed on commercial concentrate mixture and wheat straw and feed refusal was weighed daily before next morning feeding and was deducted from the total offered weight to calculate the daily intake. Water was freely available allover the experiment.

II- Behavioral observations and measurements: -

Ewes were fed and cleaned out at 8:00 a.m. and during the remainder of the day humans were not normally present. Their behavioral pattern was recorded according to Marten and Bateson (1988); Fordham *et al.* (1991) and Sevi *et al.* (2001) using the scan sampling technique in which a screen was erected at one end of the room so that the observer can study all the animals at the same time without being seen by them. Three behavioral recordings were conducted after each social and pen exchange (day 1, 8 and 15). Experimented ewes were observed for nine hours per day (three hours per group). Each group was observed for one hour in the morning (9:00 to 12); one hour in the afternoon (13:00 to 16:00) and one hour around dusk (17:00 to 20:00). Animals were observed for their postural situation (standing or lying); behavioral activity (walking, eating, ruminating, drinking or not active) as well as social and aggressive interactions (butting, chasing, sniffing each other). Postural situation and behavioral activities were expressed as percentage of the observation / recorded hour. Meanwhile, social and aggressive interactions are short-lasting events so, they were expressed as number of total observations / day.

III- Physiological measurements: -

At the initiative day of each social and pen exchange and just before starting blood sampling and behavioral recording, experimented ewes were clinically examined according to Blood and Radostits (1990) to determine their average pulse rate, respiratory rate and body temperature.

IV- Blood sampling: -

At each social and pen exchange, Blood samples were collected from the jugular vein of the experimented animals at the initiative day just before starting the behavioral recording. Samples were allowed to coagulate at room temperature and serum separated by centrifugation.

The sera were freezed at -20 °C till further analysis to determine its cortisol level using TDxFLx system with fluorescence polarization and competitive binding technique according to Dandliker & Feigen (1970) and Dandliker & Saussure (1973).

V- Statistical analysis: -

Statistical analyses of the collected data were carried out according to procedures of completely random design, SAS (1995).

RESULTS

The results of this study were illustrated in tables 1,2,3,4 as well as Figures 1, 2 and 3.

Table 1: Postural situation and behavioral activities of the experimented ewes

Item	Treatment		
	Control	Regrouping	Regrouping and relocation
	---------- Postural situation (% / hour) ----------		
Standing	60 [a]	70 [b]	100 [c]
Lying	40 [a]	30 [b]	0 [c]
	---------- Behavioral activities (% / hour) ----------		
Walking	0 [a]	30 [b]	80 [c]
Eating	50 [a]	30 [b]	10 [c]
Ruminating	30 [a]	20 [a]	0 [b]
Drinking	10	10	10
No activities	10	10	0
Aggressive interactions (No./day)	4±1 [a]	18±2 [b]	21±2 [b]

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

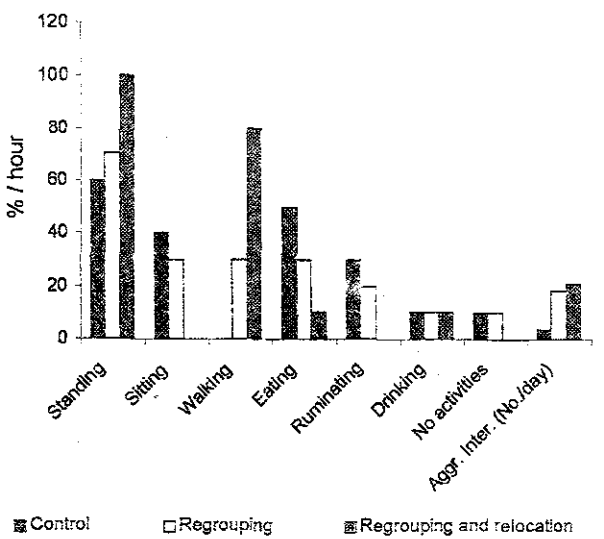

Fig. 1: Postural situation and behavioral activities of the experimented ewes.

Table 2: Changes in voluntary food intake of the experimented ewes.

Day	Treatment		
	Control	Regrouping	Regrouping and relocation
	Average (g / day)		
1st	1730±25	1250±50	930±25
2nd	1710±50	1260±30	980±20
3rd	1690±45	1290±10	1050±20
4th	1700±20	1330±30	1130±35
5th	1720±25	1370±50	1180±50
6th	1680±35	1510±20	1250±35
7th	1700±20	1550±20	1270±50
Average	1704±31[a]	1366±30[b]	1113±34[c]

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

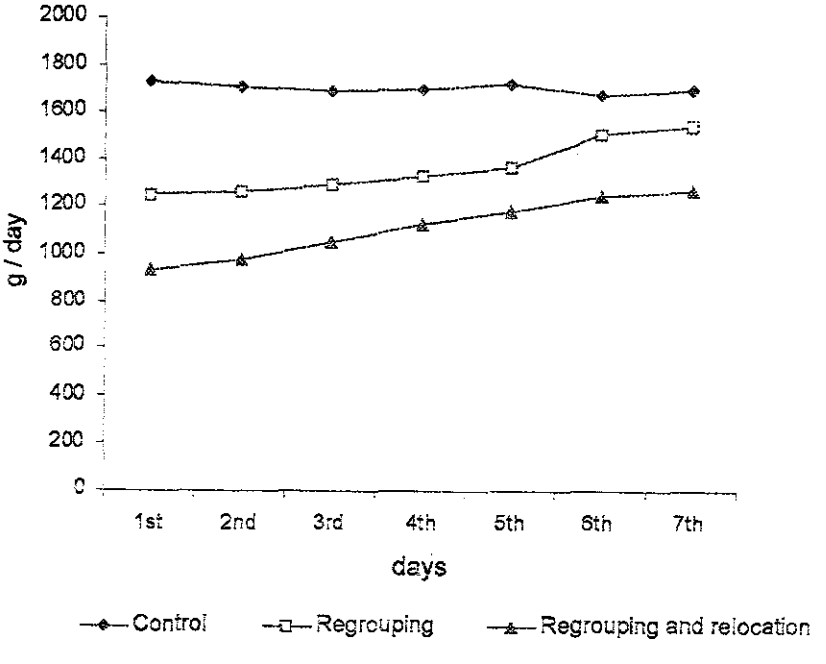

Fig. 2: Changes in voulantry food intake of the experimented ewes.

Table 3: Changes in the physiological status of the experimented ewes

Items	Treatment		
	Control	Regrouping	Regrouping and relocation
Pulse rate (No. / min.)	74±4	82±2	84±4
Respiratory rate (No. / min.)	18±2	22±2	26±2
Body temperature (°C)	39.1±0.1	39.3±0.1	39.1±0.1

Table 4: Changes in serum cortisol level of the experimented ewes

Item	Treatment		
	Control	Regrouping	Regrouping and relocation
	---------- Average (µg / 100 ml) ----------		
Cortisol level	0.47±0.01 [a]	0.71±0.01 [b]	0.94±0.01 [c]

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

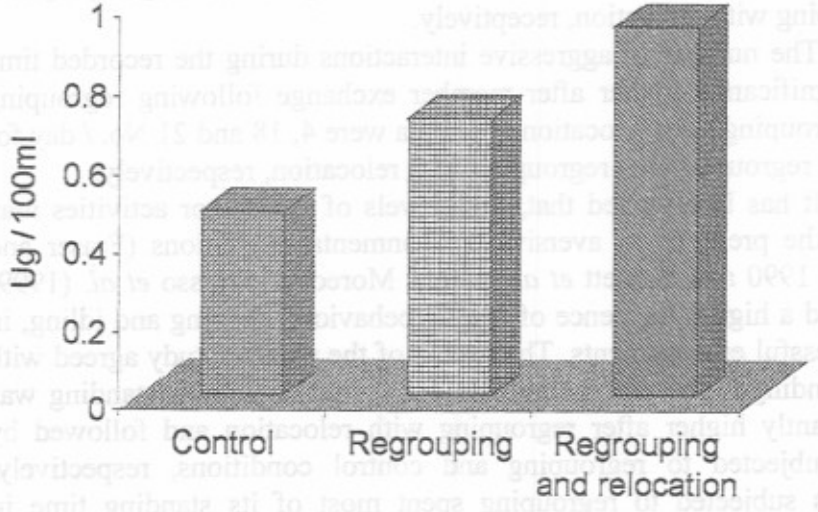

Fig. 3: Changes in serum cortisol level of the experimented ewes

DISCUSSION

I- Behavioral observations of experimented animals: -

The data represented in table (1) and assimilated on figure (1) showed the effect of the studied regrouping as well as regrouping and relocation on the behavior of the experimented ewes. These data revealed that, both of postural situation, behavioral activities and social interactions were significantly affected with the studied conditions ($P<0.01$).

The percentage of ewes that were recorded in different postural situation was 60; 70 and 100 in standing and 40, 30 & 0 in lying or idling following control; regrouping and regrouping with relocation, receptively. Locomotiom activity was higher for sheep subjected to group and pen exchange than for those subjected to regrouping or control conditions. The percentage of ewes that were recorded walking during the observation time were 0, 30 and 80 following control; regrouping and regrouping with relocation, respectively.

Eating and rumination activities were also affected with these treatments. The percentage of ewes that were recorded eating or ruminating was 50, 30, 10 and 30, 20, 0 following control; regrouping and regrouping with relocation, receptively. However, the percentage of ewes that were recorded drinking or not active during the observation time was not significantly affected following these treatments and the values were 10, 10,10 and 10, 10, 0 following control; regrouping and regrouping with relocation, receptively.

The number of aggressive interactions during the recorded time was significantly higher after member exchange following regrouping and regrouping with relocation. The data were 4, 18 and 21 No. / day for control, regrouping and regrouping with relocation, respectively.

It has been stated that, high levels of locomotor activities may reflect the presence of aversive environmental conditions (Fraser and Broom, 1990 and Barnett *et al.*, 1992). Moreover, Grasso *et al.* (1999) observed a higher incidence of resting behaviors, as lying and idling, in less stressful environments. The results of the present study agreed with these findings. The percentage of ewes spent the time in standing was significantly higher after regrouping with relocation and followed by those subjected to regrouping and control conditions, respectively. Animals subjected to regrouping spent most of its standing time in eating and walking, however, regrouped and relocated ewes spent most of their standing time in walking. This finding may be related to the new

aversive environmental conditions of regrouping and relocation where the animals may induced by forced social interactions to fight or escape and active behaviors may be adopted to get readily prepared to react (Hanlon *et al.*, 1994 and Sevi *et al.*, 2001).

In the same time, the obtained results indicated that, aggressive behavioral interactions were significantly increased either with regrouping or regrouping and relocation. This finding agreed with Tennessen *et al.* (1985), Kondo and Hurnic (1990) and Sevi *et al.* (2001) who indicated that, aggression can be employed to gain more food or a higher-quality food or to establish a place in a social hierarchy with new situations and it lasts about 4 days after strange animals are mixed.

II- Food intake of the experimented animals: -

The data represented in table (2) showed the effect of the studied regrouping as well as regrouping and relocation on voluntary food intake of the experimented ewes. These data revealed that, average food intake of the experimented ewes was significantly affected with the studied conditions ($P<0.01$). Average food intake of the experimented ewes was 1704; 1366 and 1113 g / day for control; regrouping and regrouping with relocation, respectively. Moreover, Fig. (2) showed that, daily food intake of the experimented ewes was the lowest allover the experiment for those subjected to regrouping with relocation while it was markedly lower for those subjected to regrouping only as compared to control ones. However, the effect of regrouping and regrouping with relocation on food intake was gradually decreased from the 1st to the 7th day of the experiments. This finding agreed with Varner *et al.* (1983); Hanlon *et al.* (1994) and Sevi *et al.* (2001) and could be attributed to higher aggression and unfamiliarity to the new aversive environmental conditions of regrouping and regrouping with relocation.

III- Physiological status of the experimented animals: -

The data represented in table (3) showed the average pulse rate, respiratory rate (No. / min.) and body temperature (°C) of the experimented ewes. It was 74, 18, 39.1; 82, 22, 39.3 and 84, 26, 39.1 after control, regrouping and regrouping with relocation, respectively. These results indicated that, non-of the studied conditions had a significant effect on physiological and health status of the experimented ewes. However, the slight increase in their pulse and respiratory rates after regrouping and regrouping with relocation was within the normal range and could be attributed to the physiological and biological adjustments and changes in the animal body to meet that new stressful situation (Hafez, 1975; Banerjee, 1982 and Radostits *et al.*, 1994).

IV- Serum cortisol level of the experimented animals: -

Studies on serum levels of adrenocorticoids showed a marked rise in cortisol after exposure to several or any stressor, which are known to cause an increased outpouring of ACTH that induce the adrenal cortex to increase its secretion of glucocorticoids (McDonald, 1969 and Burchfield *et al.,* 1980). The effect of regrouping and regrouping with relocation on serum cortisol level of the experimented ewes was illustrated in table (4) and assimilated on fig. (3). The data of this table showed that, serum cortisol level of the experimented ewes was significantly affected by the studied conditions ($P<0.01$). Serum cortisol level of ewes in control or subjected to regrouping and regrouping with relocation were 0.47; 0.71 and 0.94 μg / 100 ml, respectively. This finding indicated that, social and environmental instability act as emotional stress due to the need to establish a new social hierarchy. This stressful stimuli triggers a nonspecific endocrine response which characterized by activation of the hypothalamic-pituitary-adrenal axis, as demonstrated by increased level of circulating ACTH with subsequent increase of serum cortisol level (Ekkel *et al.,* 1995; Hasegawa *et al.,* 1997 and Hopster *et al.,* 1999).

CONCLUSION

In conclusion, moving and merging of sheep through regrouping and regrouping with relocation were result in aversive environmental conditions and leaded to increased aggression and consequent injuries and reflected prominently on their behavior, food intake and serum cortisol level. Therefore, stability of the herd as well as avoidance of moving and merging as much as possible should be considered during establishing their farms.

REFERENCES

Banerjee (1982): A textbook of animal husbandry. 5th Ed., Oxford and publishing company.

Barnett, J.L.; Hemsworth, P.H.; Cronin, G.M. and Chilton, D. (1992): Effect of pen size, partial stalls and method of feeding on welfare-related behavioral and physiological responses of group housed animals. Appl. Anim. Behav. Sci., 34: 207-220.

Blood, D.C. and Radostitis, O.M. (1990): Veterinary medicine. 7th Ed., UK.

Bruckmaier, R.M. and Blum, J.W. (1992): Disturbed milk removal in dairy cows. J. Dairy Res., 60: 481-486.

Bruckmaier, R.M.; Schams, D. and Blum, J.W. (1993): Milk removal in familiar and unfamiliar surroundings. J. Dairy Res., 60: 449-456.

Burchfield, S.R.; Wood, S.C. and Elich, M.S. (1980): Pituitary adrenocortical response to chronic intermittent stress. Physiol. And Behav., 24; 297-302.

Casamassima, D.; Sevi, A.; Palazzo, M.; Ramacciato. R. and Belliti, A. (2001): Effects of two different housing systems on behavior, physiology and milk yield of Comisana ewes. Small Rum. Res., 41: 151-161.

Dandliker, W.B. and Feigen, G.A. (1970): Quantification of the antigen-antibody reaction by polarization immunochemistry. Immunochemistry, 7: 799-828.

Dandliker, W.B. and Saussure, D.V. (1973): Review article: fluorescent polarization immunoassay. Theory and experimental method. Immunochemistry, 10: 219-227.

Ekkel, E.D; Van Doorn, E.A.; Hessing, C. and Tielen, M. (1995): The specific-stress-free housing system has positive effects on productivity, health and welfare of pigs. J. Anim. Sci., 73: 1544-1551.

Findlay, A.L. and Grosvenor, C.E. (1969): The role of mammary gland innervation in the control of the motor apparatus of tea mammary glands. Dairy Sci. Abstr., 31: 109-116.

Fordham, D.P.; Gahtani, S.A.; Durotoye, L.A. and Rodway, R.G. (1991): Changes in plasma cortisol and B-endorphin concentrations and behaviour in sheep subjected to a change of environment. Anim. Prod., 42: 157-159.

Fraser, A.F. and Broom, D.M. (1990): Farm animal behavior and welfare. Bailliere Tindall, London.

Friend, T.H.; Knabe, D.A. and Tanksley, T.D. (1983): Behavior and performance of pigs grouped by three different methods at weaning. J. Anim. Sci., 57: 1406-1413.

Goodman, G.T. and Grosvenor, C.E. (1983): Neuroendocrine control of milk ejection reflex. J. Dairy Sci., 66: 2226-2235.

Grasso, F.; Napolitano, F.; De Rosa, G.and Bordi, A. (1999): Effect of pen size on behavioral, endocrine and immune responses of water buffalo calves. J. Anim. Sci., 77: 2039-2046.

Hafez, E.S. (1975): The behavior of domestic animals. 3rd Ed., Bailliere-Tindall-London.

Hanlon, A.J.; Rhind, S.M.; Reid, H.W. and McMillen, S.R. (1994): Relationship between immune response, live weight gain, behavior and adrenal function in red deer calves derived from wild and farmed stock. Appl. Anim. Behav. Sci., 41: 243-255.

Hart, I.C. (1983): Endocrine control of nutrient partition in lactating ruminants. Proc. Nutr. Soc., 42: 181-194.

Hasegawa, N.; Nishiwaki, A.; Sugawara, K. and Ito, I. (1997): The effect of social exchange between two groups of lactating primiparous heifers on milk production, dominance order, behavior and adrenocortical response. Appl. Anim. Behav. Sci., 51: 15-27.

Hopster, H.; Van der Werf, J.; Erkens, J. and Blohkius, H. (1999): Effects of repeated jugular puncture on plasma cortisol concentrations in loose-housed dairy cows. J. Anim. Sci., 77: 708-714.

Klastrup, O.J.; Bakken, J.B. and Bushnell, R. (1987): Environmental influences on bovine mastitis. FIL-IDF Bulletin no. 217, Brussels, Belgium.

Kondo, B. and Hurnik, J.F. (1990): Stabilization of social hierarchy in dairy cows. Appl. Anim. Behav. Sci., 27: 287-297.

Lynch, J.J.; Hich, G.N. and Adms, D.B. (1992): The behavior of sheep. Biological principles and implications for production. CAB International, Wallingford, UK.

Marten, P. and Bateson, P. (1988): In Measuring behaviour. Cambridge University Press, Cambridge, pp. 48-69.

McDonalds, L.E. (1969): Veterinary endocrinology and reproduction. 1st Ed., Lea and Febiger, Philadelphia, USA.

Napolitano, F.; Marino, V.; De Rosa, G. and Bordi, A. (1995): Influence of artificial rearing on behavioural and immune response of lambs. Appl. Anim. Beav. Sci., 45: 245-253.

Radostits, O.M.; Leslie, K.E. and Fetrow, J. (1994): Herd health. 2dn Ed., UK

Rhind, S.M.; Reid, H.W.; Mc Millen, S.R. and Palmarini, G. (1998): The role of cortisol and B-endorphin in the response of the immune system to weaning in lambs. Anim. Sci., 66: 397-402.

Rushen, J. (1987): A difference in weight reduces fighting when unacquainted newly weaned pigs first meet. Can. J. Anim. Sci., 67: 951-960.

SAS (1995): Statistical analysis system. User's Guide : Statistics. Version 6, 2nd Ed., SAS Inst. Inc. , Cary, NC.

Scott, G.H. (1981): What is animal stress and how is measured ?, J. Anim. Sci., 52: 150-153.

Sevi, A.; Taibi, L.; Albenzio M.; Muscio A.; Dell S and Napolitano F. (2001): Behavioral, adrenal, immune and productive responses of lactating ewes to regrouping and relocation. J. Anim. Sci., 79: 1457-1465.

Tennessen, T.; Price, M.A. and Berg, R.T. (1985): The social interaction of young bulls and stress after re-grouping. Appl. Anim. Behav. Sci., 14: 37-47.

Varner, M.A. and Johnson, B.H. (1983): Influence of adrenocotricotropin upon milk production, milk constituents and endocrine measures of dairy cows. J. Dairy Sci., 66: 458-465.

Varner, M.A.; Johnson, B.H. and Mochrie, R.D. (1983): Influence of herd relocation upon production and endocrine traits of dairy cows. J. Dairy Sci., 66: 466-474.