Reducing visual stimulations in European hares (Lepus europaeus Pallas) captured for translocation

Gisella Paci,1 Marco Ferretti,2 Marco Bagliacca2
1Dipartimento di Patologia Animale, Profilassi e Igiene degli Alimenti, Università di Pisa, Italy
2Servizio Agricoltura, Patrimonio Naturale ed Ittio-faunistico Gestione Aree Protette, Ufficio della Provincia di Pistoia, Italy
3Dipartimento di Scienze Fisiologiche, Università di Pisa, Italy

Abstract

Introduction

The brown hare (Lepus europaeus) is common throughout Europe except in the northern most part where the mountain hare (Lepus timidus) occurs. The brown hare is an important game animal. To restore the right population density in low densities free hunting territories it is often necessary to introduce wild or reared foreign subjects.

A valuable technique for restocking, inside low densities free hunting territories, is the release of brown hares captured in high-density protected areas (Ferretti et al., 2010) and/or in large enclosures (Santilli et al., 2004). Capture for translocation (transport and release in different areas, presenting more or less similar habitats) may cause direct death or stress that can play a role in the development of future complications in the released hares. The stress may be light, with no physiological consequences; it may be heavy and cause physiological alterations, or it may be extremely heavy and cause also cell deaths (Paci et al., 2006). In any case stress which alters the normal physiology of the animals can reduce the immune response (Spraker, 1993; Ranucci et al., 1996; Williams and Thorne, 1996; Diverio et al., 1998; Montané et al., 2003; Paci et al., 2006; Paci et al., 2011). Stress can be suppressive (if prolonged) or stimulatory (if transient acute) on the immune response (Amadori et al., 2009). The capture could be considered as a transient acute stress which might be associated with a better immune response and, consequently, as nature’s adjuvant in natural condition. Symptoms are generally not obvious at the time of capture but myopathy should be of concern. Death may occur almost immediately or up to 1 month after handling (Bartsch et al. 1977; Chalmers and Barrett, 1982). For this reason, we performed a study to evaluate the effect of the use of a blindfold to reduce stress during the capture operations of the hares.

Materials and methods

The samples were collected from brown hares that were caught in Tuscany (central Italy) during 2007-2009. A total of 119 hares were sampled in 14 different non-hunting high-density areas where hares are usually captured for subsequent release in free hunting low-density areas (Paci and Bagliacca, 2003). The hares were captured by coursing with 3-4 dogs (greyhounds or lurchers). The samples were collected from brown hares that were caught in Tuscany (central Italy) during 2007-2009. A total of 119 hares were sampled in 14 different non-hunting high-density areas where hares are usually captured for subsequent release in free hunting low-density areas (Paci and Bagliacca, 2003). The hares were captured by coursing with 3-4 dogs (greyhounds or lurchers).
and 0.5×16 mm needles). Body temperature (by digital thermometer introduced in the rectum), as well as heart (mediated auscultation by phonendoscope) and respiratory rate (by visual observation), were measured in each hare.

Blood samples were divided into two sub-samples:
- The first sub-samples (0.5-1 ml EDTA-plasma) were analyzed within 24 h by automatic complete blood counter (Seac Heoco Vet C, Calenzano, FI, Italy). The following analyses were performed: white blood cell (WBC); red blood cell (RBC); haemoglobin (Hgb); haematocrit (Hct); mean blood cell volume (MCV), mean corpuscular haemoglobin concentration (MCHC); red blood cell distribution width (RDW); platelets (Pit); mean platelet volume (MPV); platelet haematocrit (Pct); platelet distribution width (PDW).
- The second sub-samples (0.5-1 mL of Li-heparin plasma,) were frozen at -20°C and the following analyses were performed on plasma with AU400 Olympus using Olympus Diagnostics Kits: Glucose (GLU): colorimetric determination with oxidase-peroxidase (Sclavo, Siena, Italy); Cholesterol: enzymatic colorimetric determination (Sclavo); Triglicerides: enzymatic colorimetric determination (Poli Diagnostici, Italy); Creatine kinase or creatine phosphokinase (CK): kinetic colorimetric method (Sclavo); Alanine amino Transferase (ALT): kinetic UV test IFCC (Sclavo); AST: aspartate amino Transferase (AST): kinetic UV test IFCC (Sclavo); Non-Esterifies Fatty Acids (NEFA): enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany); Blood Urea Nitrogen (BUN): enzymatic colorimetric method (Intermedical, Grassobbio, BG, Italy); Total protein: colorimetric, Endpoint determination (Sclavo); Albumins: colorimetric method (SEAC, Radim Group, Calenzano, FI, Italy); Globulins: electrophoresis of serum protein carried out by Microtech 648R (Interlab, Roma, Italy); Cortisol by radioimmunoassay (SEAC). The relationship between the blood biochemistry of the hares equipped with the blindfold vs the hares of the control group was analyzed by one-way ANOVA and confirmed by non-parametric test (Wilcoxon rank sums test) in the bordering values. Sub-clinically stressed hares were discriminated from non-stressed hares by the use of the discriminant function based on CK, AST and Glucose determined by Paci et al. (2006). The different incidence of sub clinical stress within the hares equipped with the blindfold vs the hares of the control group was tested by χ² (SAS, 2009).

Results and discussion

Results showed that temperature, respiratory rate and heart rate were significantly increased in the hares without blindfold compared to the hares equipped with blindfold (Table 1). Both hearth rates however were within the limit observed for healthy hares (Nicro et al., 2007; Paci et al., 2007; Noszczyk-Nowak et al., 2009). In particular, the highest level of temperature, respiratory and heart rate in hares without blindfold can be considered as the response to difficult situations perceived by individuals (Broom and Johnson, 1993; Diverio et al., 1996): the captured animals without blindfold perceive the constriction to be a dangerous situation more than the hares with blindfold.

Also the haematic parameters determined on Li-plasma were comparable to those observed in previous observations (Paci et al., 2007; Massányi et al., 2009). However, even if within physiological values, significant differ-

![Figure 1. The hood used to blindfold the hares.](image-url)

Table 1. Physiological and haematic parameters of the hares after capture.

| Parameter                  | Hares with blindfold | Hares without blindfold | Prob.>F |
|----------------------------|----------------------|-------------------------|---------|
| Temperature, °C            | 23 36.98 0.0864      | 83 37.93 0.164          | <0.0001** |
| Respiratory rate, n/min    | 23 72 2.19           | 83 68 4.17              | 0.0032**  |
| Hearth rate, n/min         | 23 109 4.39          | 83 133 8.35             | 0.0147*   |
| Glucose, mg/dL             | 38 161 7.1           | 64 198 9.2              | 0.0019**  |
| Cholesterol, mg/dL         | 38 137.0 18.9        | 62 172 14.8             | <0.01**    |
| Triglycerides, mg/dL       | 38 67.1 8.84         | 64 83.0 6.66            | 0.1501     |
| CK, U/L                    | 38 2220 669          | 81 4476 458             | 0.0063**  |
| ALT (or GPT), U/L          | 38 49.6 4.16         | 81 63.9 2.85            | 0.0052**   |
| AST (or GOT), U/L          | 36 123 18.3          | 81 171 12.2             | 0.0303*    |
| NEFA, mg/L                 | 38 43.2 1.00         | 65 38.2 1.30            | 0.0031**   |
| BUN, mg/dL                 | 38 44.5 2.31         | 81 53.9 1.58            | 0.001**    |
| Total protein, g/dL        | 38 5.70 0.171        | 64 6.30 0.132           | 0.0063**   |
| Albumins, g/dL             | 38 4.26 0.128        | 64 3.74 0.098           | 0.0016**   |
| Globulins, g/dL            | 36 1.29 0.157        | 81 2.01 0.091           | <0.0001**  |
| A/G ratio                  | 22 4.45 0.759        | 15 3.88 0.919           | 0.6342     |
| Cortisol, μg/dL            | 38 12.7 1.35         | 67 12.4 1.01            | 0.8749     |

SE, standard error; CK, creatine kinase; ALT, alanine amino transferase; AST, aspartate amino transferase; NEFA, non-esterifies fatty acids; BUN, blood urea nitrogen. *P<0.05; **P<0.01.
ences were observed between the hares equipped with the blindfold and the hares captured and kept with the naked eyes for glucose, cholesterol, CK, ALT (or GPT), AST (or GOT), NEFA, BUN, total protein, albumins and globulins. The captured hares without blindfold presented higher glucose value and lower level of NEFA than hares with blindfold (198 mg/dL vs 161 mg/dL, P<0.01; 38.2 mg/dL vs 43.2 mg/dL, respectively), this can be explained by the hyperglycaemic effect of catecholamines and glucocorticoids released during stress involved in the hares captured without blindfold (Spraker, 1993).

Cholesterol concentration resulted higher in the hares without blindfold (172 mg/dL vs 137 mg/dL, P<0.01); even if the level of cholesterol can vary with the diet and the time of year also other Authors have found higher concentrations of cholesterol in captured animals and attributed higher cholesterol levels to the effects of catecholamines and corticosteroids (Marco et al., 1997; Marco and Lavin, 1999). ALT, AST and CK showed significantly higher values in hares without blindfold, as reported by other Authors these enzymes result high in many stressed wild animals that have been running and tends to continue rising even after the end of the action of the stressing factor (Bateson and Bradshaw, 1997; Montané et al., 2003). The high significant differences between the two groups of hares could be explained by the over-exposition to the stress. In our case the physical restraint associated with the handling operations to remove the hares from the net and the restriction to take the blood are very stressful experiences for hares, particularly for the animals that are also subjected to the visual presence of the human predator. In the real practice only a sample of hares is subjected to blood drawing for sanitary monitoring, so that the effect of the stress may be less strong in most of hares, which suffer the removing operation from the net. The increase level of urea and total protein in hares without blindfold could be attributed to the effect of glucocorticoids that induce changes in the protein metabolism.

The haemocromo-cytometry of the hare showed greater concentrations in the captured hares without the blindfold than the captured hares with blindfold (Table 2). The RBC count was comparable to those relieved in Poland by Nicpoń et al. (2007) in hares caught alive in their natural environment. The effect of the chase can explain the differences between Authors. For this reason the data of Marco et al. can be used as reference values only for captive hares, while our data with those of Nicpoń et al. (2007) better express reference values for the wild European brown hares.

Table 2. Haemocromo-cytometric parameters of the hares after capture.

| Parameter | Hares with blindfold N=38 | Hares without blindfold N=81 | Prob. > F |
|-----------|---------------------------|-----------------------------|----------|
| WBC, K/μL | 7.14 ± 0.271              | 8.26 ± 0.188                | 0.0008** |
| RBC, M/μL | 8.53 ± 0.115              | 9.03 ± 0.101                | 0.0655** |
| Hgb, g/dL | 18.24 ± 0.537             | 20.17 ± 0.372               | 0.0120*  |
| Hct, %    | 46.91 ± 0.984             | 49.69 ± 0.683               | 0.0222*  |
| MCV, FL   | 54.99 ± 0.314             | 55.03 ± 0.217               | 0.9166   |
| MCH, Pg    | 21.38 ± 0.559             | 22.34 ± 0.388               | 0.1610   |
| MCHC, g/dL | 38.88 ± 1.092             | 40.59 ± 0.757               | 0.2005   |
| RDW, %    | 12.850 ± 0.0476           | 12.912 ± 0.0330             | 0.2931   |
| Plt, K/μL | 457.3 ± 9.398             | 466.7 ± 6.21                | 0.4160   |
| MPV, FL   | 8.953 ± 0.0197            | 8.933 ± 0.0137              | 0.3955   |
| Pct.      | 0.040610 ± 0.000525       | 0.04153 ± 0.000572          | 0.4028   |
| PDW, %    | 9.853 ± 0.0215            | 9.907 ± 0.0357              | 0.3947   |

Table 2. Haemocromo-cytometric parameters of the hares after capture.

Table 2. Haemocromo-cytometric parameters of the hares after capture.

With blindfold

| Parameter | Hares with blindfold | Hares without blindfold |
|-----------|----------------------|-------------------------|
| WBC, K/μL | 7.14                 | 8.26                    |
| RBC, M/μL | 8.53                 | 9.03                    |
| Hgb, g/dL | 18.24                | 20.17                   |
| Hct, %    | 46.91                | 49.69                   |
| MCV, FL   | 54.99                | 55.03                   |
| MCH, Pg    | 21.38                | 22.34                   |
| MCHC, g/dL | 38.88                | 40.59                   |
| RDW, %    | 12.850               | 12.912                  |
| Plt, K/μL | 457.3                | 466.7                   |
| MPV, FL   | 8.953                | 8.933                   |
| Pct.      | 0.040610             | 0.04153                 |
| PDW, %    | 9.853                | 9.907                   |

Figure 2. Different incidence of non-clinical stress in hares with or without blindfold.
The stress due to the run of the hares chased by 3-4 (greyhounds or lurchers) coursing dogs for different times and tracks, their consequent trapping and handling before being blindfolded or not cannot be affected by the blindfolding. On the other hand, a short track, combined with quick and good handling of an experienced wildlife-officer, game wardens or hunter, can allow a significant reduction of the subclinical stress sustained by the hares captured and handled with naked eyes (Paci et al., 2006).

Conclusions

Capture and handling procedures for translocation of hares should be based on protocols designed to minimize risk of myopathy to animals and risk of injury to humans. We demonstrated that the use of a blindfold applied to the hares immediately after their trapping in the net determine a significant reduction of the stress which may be heavy and directly cause the deaths of the animals and alters the normal physiology of the animals. For this reason the procedures designed for the hares translocation must always include the use of blindfolding, which should be applied as soon as possible after the trapping of the hares.

References

Amadori, M., Stefanoff, B., Sgorlon, S., Farinacci, M., 2009. Immune system response to stress factors. Ital. J. Anim. Sci. 8(Suppl.1):287-299.
Bartsch, R.C., McConnell, E.E., Imes, G.D., Schmidt, J.M., 1977. A review of exertional rhabdomyolysis in wild and domestic animals and man. Vet. Pathol. 14:314-324.
Bateson, P., Bradshaw, E.L., 1997. Physiological effects of hunting red deer (Cervus elaphus). P. Roy. Soc. B-Biol. Sci. 264:1707-1714.
Broom, D.M., Johnson, K.G., 1993. Assessing welfare: Short-term responses. In: D.M. Broom and K.G. Johnson (eds.) Stress and animal welfare. Chapman and Hall, London, UK, pp 87-114.
Chalmers, G.A., Barrett, M.W., 1982. Capture myopathy. In: G.L. Hoff and J.W. Davis (eds.) Noninfectious diseases of wildlife. Iowa State University Publ., Ames, IO, USA, pp 84-94.
Diverio, S., Goddard, P.J., Gordon, J.L., 1996. Use of long acting neuroleptics to reduce the stress response to management practices in red deer. Appl. Anim. Behav. Sci. 49:83-88.
Diverio, S., Morgante, M., Pelliccia, C., Lucaroni, A., 1998. Stress responses to different restraining procedures in farmed fallow deer (Dama dama). pp 322-324 in Proc. 4th Int. Deer Biology Congr., Kaposvar, Hungary.
Ferretti, M., Paci, G., Porrini, S., Galardi, L., Bagliacca, M., 2010. Habitat use and home range traits of resident and relocated hares (Lepus europaeus, Pallas). Ital. J. Anim. Sci. 9:e56.
Marco, I., Cuenca, R., Pastor, J., Velarde, R., Lavin, S., 2003. Hematology and serum chemistry values of the European brown hare. Vet. Clin. Pathol. 32:195-198.
Marco, I., Lavin, S., 1999. Effect of the method of capture on the haematology and blood chemistry of red deer. Res. Vet. Sci. 66:81-84.
Marco, I., Vinas, L., Velarde, R., Pastor, J., Lavin, S., 1997. Effects of capture and transport on blood parameters in free-ranging mouflon (Ovis Ammon). J. Zoo. Wildlife Med. 28:428-433.
Massainyi, P., Slameèka, J., Lukáè, N., Capcarová, M., Mertin, D.A., Jurèìk, R., 2009. Seasonal variations in the blood biochemistry of brown hare. Medycyna Wet. 65:390-393.
Montané, J., Marco, I., Lopez-Olvera, J., Perpinan, D., Manteca, X., Lavin, S., 2003. Effects of acepromazine on capture stress in Roe deer (Capreolus capreolus). J. Wildlife Dis. 39:375-386.
Nicpoñ, J., Sawuta, P., Nicpoñ, J., Noszczyk-Nowak, A., 2007. Hematological, biochemical and acid-base equilibrium parameters of the European brown hare. Medycyna Wet. 63:1239-1241.
Noszczyk-Nowak, A., Nicpoñ, J., Nowak, M., Sawuta, P., 2009. Preliminary reference values for electrocardiography, echocardiography and myocardial morphometry in the European brown hare (Lepus europaeus) Acta Veterinaria Scandinavica 51:1-6 (doi:10.1186/1751-0147-51-6).
Paci, G., Bagliacca, M., 2003. La lepre e l’ambiente agricolo. Large Anim. Rev. 9:47-55.
Paci, G., Bagliacca, M., Lavazza, A., 2006. Stress evaluation in hares (Lepus europaeus Pallas) captured for translocation. Ital. J. Anim. Sci. 5:175-181.
Paci, G., Lavazza, A., Ferretti, M., Bagliacca M., 2007. Relationship between habitat, densities and metabolic profile in brown hares (Lepus europaeus Pallas). Ital. J. Anim. Sci. 6:241-255.
Paci, G., Lavazza, A., Ferretti, M., Santilli, F., Bagliacca, M., 2011. Relationship between anti-European Brown Hare syndrome serological titers and Brown Hare (Lepus europaeus Pallas) densities. Int. J. Zool. Article ID 436193.
Ranucci, S., Morgante, M., Diverio, S., Costarelli, S., Beghelli, D., Durante, E., 1996. Blood constituents and clinical findings in captured fallow deer (Dama dama L.). Suppl. Ric. Biol. Selvaggina 25:209-217.
Santilli, F., Mazzoni, R., Guerrini, A., Mori, L., Bisogno, G., Bagliacca, M., 2004. Factors affecting brown hare (Lepus europaeus Pallas) production in fenced areas in Italy. pp 103-104 in Proc. 6th Int. Symp. sur l’Utilisation Durable de la Faune Sauvage, Paris, France.
SAS, 2009. Jmp 8 User’s Guide, 2nd ed. SAS Inst. Inc., Cary, NC, USA.
Spraker, T.R., 1993. Stress and capture myopathy in artiodactyls. In: M.E. Fowler (ed.) Zoo and wild animal medicine. Current therapy 3. Saunders Publ., Philadelphia, PA, USA, pp 481-488.
Williams, E.S., Thorne, E.T., 1996. Exertional myopathy. In: A. Fairbrother, L.L. Locke and G.L. Hoff (eds.) Noninfectious diseases of wildlife. Manson Publ., London, UK, pp 181-193.
