Alma Mater Studiorum – Università di Bologna

DOTTORATO DI RICERCA IN

Scienze Zootecniche

Ciclo XXIV

Settore Concorsuale di afferenza: AGR/19

TITOLO TESI

USE OF BEHAVIOURAL PATTERNS TO ANALYZE TRANSPORT STRESS IN PIGLETS AND YOUNG BULLS. THE INFLUENCE OF INDIVIDUAL COPING CHARACTERISTICS IN STRESS RESPONSE.

Presentata da: Diego Magnani

Coordinatore Dottorato	Relatore
Chiar.mo Prof. Paolo Bosi	Chiar.mo Prof. Leonardo Nanni Costa

Esame finale anno 2012

ABSTRACT

Transport is a crucial practice in farm animal management. In modern farm systems the economic interests make reducing the risks related to transport practice an important goal. For these reasons an increasing attention is directed to the welfare of animals in transit, also considering the new existing facilities. In recent years the results coming from the study of animal farm behaviour were used as tool to assess the welfare, especially during handling and restrain situation.

In this thesis were analyzed behavioural patterns, jointly with blood variables, to evaluate the stress response of piglets and young bulls during transport. Since the animal behaviour could be very different between individuals and these differences can affect animal responses to aversive situations, the individual behavioural characteristics were taken in account.

Regarding young bulls, selected to genetic evaluation, the individual behaviour was investigated before, during and after transport, while for piglets was adopted a tested methodology classification and behavioural tests to observe their coping characteristics.

The aim of this thesis was to analyse the behavioural and physiological response of young bulls and piglets to transport practice and to investigate if coping characteristics may affect how piglets cope with aversive situations.

The thesis is composed by four experimental studies. The first one aims to identify the best existent methodology classification of piglets coping style between those that were credited in literature. The second one investigated the differences in response to novel situations of piglets with different coping styles.

The last two studies evaluated the stress response of piglets and young bulls to road transportation.

The results obtained show that transport did not affect strongly the behaviour and homeostasis of young animals which respond in a different way from adults.

However the understanding of individual behavioural characteristic and the use of behavioural patterns, in addition to blood analyses, need to be more investigated in order to be useful tools to assess the animal response in aversive situation, as transports.

Keywords: piglet, young bulls, transport, coping characteristics, stress response, animal welfare.

CONTENTS

BACKGROUND

Humans might induce stress in all farm animals, and in many different way, so we have to pay attention to their behaviour in order to develop new strategies to reduce their stress.

Evaluating the welfare of another species, often of our own, is not easy and the scientific assessment of welfare must be separated from any moral judgement. Welfare is a characteristic of an individual at the time of observation or measurement, so it can be assessed in an entirely objective way.

The farm animals are transported for century but only recently there has been questioned on the impact of a journey on their welfare.

The coping strategy adopted by the animal can have an effect on responses to the transport situation. In order to be able to improve the welfare of livestock which are being handled or transported, it is necessary to make measurements which allow the assessment of individual coping strategy and transport stress.

INTRODUCTION

1 INDIVIDUAL COPING CHARACTERISTICS

Psychosocial factors have long been recognized as important in health and disease both in man and in animals. It is not the physical characteristics of a certain aversive stimulus but rather the cognitive perception of that stimulus, which determines its aversive character and whether a state commonly described as stress is induced. The impact of aversive stimuli or stressors is determined by the ability of the organism to cope with the situation.

There are many definitions of "individual coping characteristics" in the literature, ranging from specific to general and more communally called "coping style".

In humans coping is "problem-solving efforts made by an individual when the demands he [or she] faces are highly relevant to his [or her] welfare (that is, a situation of considerable jeopardy or promise) and when these demands tax his [or her] adaptive resources." Lazarus et al. (1974).

For Wechsler (1995) "coping behaviour is a response to aversive situations.", Koolhaas et al. (1999) say that coping is "a coherent set of behavioral and physiological stress responses which is consistent over time and which is characteristic to a certain group of individuals".

Although worded differently, these definitions all view coping as a response by the individual to a disturbance. Since stress is defined as the disruption of homeostasis by an intrinsic or extrinsic, physical or psychological force (Avitsur et al., 2006), in this review coping refers to the response by the animal to stress.

Successful coping depends highly on the controllability and predictability of the stressor (Ursin et al., 1993; Weiss, 1968). A consistent finding across species is that whenever environmental stressors are too demanding and the individual cannot cope, its health is in danger. For this reason, it is important to understand the mechanisms and factors underlying the individual's capacity to cope with environmental challenges.

The possibility of distinct coping styles was investigated in domestic animal species, including pigs. The most successful results have been found in mice and rats, which show that individuals' responses fall into two distinct categories, referred to as coping styles (Koolhaas et al., 1999).

Henry and Stephens (1977) popularized the theory of the presence of two types of coping styles. Names of the two styles differ between articles, but characteristics of the styles are

consistent. Originally described as the "fight-flight response", one coping style is most commonly known as "active response" (Koolhaas et al., 1999). However, since some of the behavioural characteristics of "active response" style are not what some would consider active, this style is also referred to as "proactive" (Koolhaas et al., 1999). The second coping style is sometimes known as "conservation-withdrawal response" (Engel and Schmale, 1972). It is commonly referred to as "passive response" (Koolhaas et al., 1999). As with the first coping style, the notion of "passive" can be misleading, therefore, this coping style is also referred to as "reactive." For clarity, the two coping styles will be labelled proactive and reactive.

Proactive animals actively try to remove or escape stressors while reactive animals tend to freeze.

In accordance with their behaviours, proactive animals seem to activate more their sympathetic nervous systems in response to stress, preparing them to fight or flee the stress source.

The main behavioural characteristic that distinguishes proactive and reactive coping styles in mice and rats are show in Table 1.

Although studies are conflicting (Forkman et al., 1995; Erhard and Mendl, 1997; van Erp-van der Kooij et al., 2000), some scientists have found evidence that also in pigs, as in mice and rats, two different coping style are present (Hessing et al., 1993; Koolhaas, 2008; Koolhaas et al., 1999).

Table 1
Summary of the behavioural differences between proactive and reactive male rats and mice (Koolhaas et al., 1999).

Behavioural characteristics			
	Proactive	Reactive	References
Attack latency	Low	High	Oortmerssen et al., 1981
Active avoidance	High	Low	Driscoll et al., 1990; Benus et al., 1989
Defensive burying	High	Low	Sluyter et al., 1996
Nest-building	High	Low	Sluyter et al., 1995
Routine formation	High	Low	Benus et al., 1990
Cue dependency	Low	High	Benus et al., 1987; Sluyter et al., 1996
Conditioned immobility	Low	High	Benus et al., 1987
Flexibility	Low	High	Bohus et al., 1987

1.1 Behavioural test of Coping style

Several behaviour tests used in pigs measure characteristics similar to those that distinguish proactive and reactive coping styles in mice and rats.

One of the main characteristics distinguishing proactive and reactive coping styles in mice and rats is aggression (Benus et al., 1991). Procedures for testing pigs were adapted by Erhard and Mendl (1997) from aggression tests used for rodents.

A resident intruder test is commonly used to evaluate aggression in pigs. For the test, a portion of a litter's home pen is sectioned off via a solid divider to create a test area. Pigs from the home pen litter, "residents," are placed in the test area individually. An "intruder" pig from an unfamiliar litter is placed in the test area with the resident. Interaction of pigs is observed for up to 5 minutes, preferably no more than 3.5 minutes. As soon as an attack occurs, or when time expires, whichever occurs first, the pigs are separated and returned to their original pens. Attack occurrence and attack latency, time from first contact to attack, are used to classify pigs as aggressive or non-aggressive in many studies. Presence of attack and decreased latency were criteria to label pigs as aggressive (D'Eath and Pickup, 2002).

Response to novelty (which is one topic of Chapter2) is another characteristic that distinguishes rodents with different coping styles. Similar to mice and rats, an open field test can be used to measure this characteristic in pigs. For testing, pigs are removed from their home pens and individually placed in a novel environment. Behaviour is recorded for duration of the test, typically 5 to 10 minutes. Behaviours include locomotion, standing motionless, eating or drinking, urinating or defecating, and vocalizations (Giroux et al., 2000; Hessing et al., 1994; Jensen et al., 1995a).

A novel object test can also be used to measure response to novelty. This test can take place in a pig's home pen (van Erp-van der Kooij et al., 2002), a sectioned off portion of a home pen, similar to the area created for resident intruder testing (Forkman et al., 1995), or in a novel setting (Hessing et al., 1994; Jensen et al., 1995a; Spoolder et al., 1996).

Novel object tests are commonly performed in combination with open field and open door tests (Hessing et al., 1994; Jensen et al., 1995a; van Erp-van der Kooij et al., 2002). Tests can be performed with individual pigs (Hessing et al., 1994; Jensen et al., 1995a; Spoolder et al., 1996), or groups (van Erp-van der Kooij et al., 2002). For individual tests a pig is placed into the test area and allowed a short adjustment period, this time is typically the novel environment test. Behaviours of the pig(s) are recorded in response to novel object introduction for duration of testing, between 3 and 15 minutes (Forkman et al., 1995; Ruis et al., 2001). Behaviours recorded vary between

studies, but often include latency to contact object, duration of object exploration, and vocalization (Forkman et al., 1995; Hessing et al., 1994; Ruis et al., 2001; van Erp-van der Kooij et al., 2002).

These cross-species characteristics could be helpful to develop a reliable test of coping style in pigs and some researchers have already attempt to find a way.

It is important to emphasize that the differentiation in coping styles may not be expressed equally clearly in all challenging situations. In particular, tests that measure aspects of initiative or proactively seem to be most discriminative. This holds, for example, for latency measures such as the attack latency test in males or the defensive burying test, which allow the animal a choice between proactive and reactive coping.

If reliable tests of coping styles in pigs were found, perhaps lines with different coping styles could be created and producers would be able to select animals that would adapt well to their management conditions.

The most commonly used tests to identify the coping style in pigs are Backtest and Tonic Immobility Test, below a short review of both, while difference and strengths/weaknesses of both of them will be described in Chapter 1.

1.2 Backtest

The backtest is commonly used in studies to evaluate pig behaviour during a stressful situation, on an individual basis (Hessing et al., 1993; van Erp-van der Kooij et al., 2001). For this test a piglet is placed in the supine position for 60 seconds while gently restrained. For restraint, experimenter places one hand over upper body of the pig and the other gently on the hind legs. Each struggle of the piglet is counted as an escape attempt. The number of escape attempts is recorded to calculate backtest scores (Cassady, 2007; Hessing et al., 1993; van Erp-van der Kooij et al., 2000); duration of struggle attempts is also recorded in some studies (Cassady, 2007; Velie et al., 2009). In the first published study using backtests, piglets were backtested five times total over the first three weeks after farrowing (Hessing et al., 1993).

Classifications of piglets were: "high-resisting (HR)" if they made more than two escape attempts in a backtest, "intermediate" if exactly two attempts were made, and "low-resisting (LR)" if less than two attempts were made. Upon analysis of this study, it was found that only two backtests are needed to adequately categorize piglets as HR or LR. The two tests are generally

performed roughly one week apart (Cassady, 2007; van Erp-van der Kooij et al., 2000; van Erp-van der Kooij et al., 2001).

Even if the backtest is not comparable to any behaviour tests used to measure coping styles in mice and rats, past studies have attempted to investigate the backtest as an indicator of coping style.

This association between the backtest and coping styles originated from correlations between backtest scores and performance of piglets on behaviour tests, measuring characteristics thought to be similar to those distinguishing proactive and reactive coping styles in mice and rats (Hessing et al., 1993). Subsequent studies have produced both similar and opposing results, leading to the current debate.

1.3 Tonic immobility

Inhibition of motion in response to restraint is a phenomenon which is well-documented across the animal kingdom (Crawford, 1977; Maser and Gallup, 1977; Erhard and Mendl, 1999). Maser and Gallup (1977) found approximately 30 labels for this behaviour and expressed concern over the ongoing creation of new terms. 'Tonic immobility' (TI) is more descriptive and therefore a more neutral term for a very complex phenomenon (Gallup, 1974). What most of the behaviours described as TI have in common is some sort of physical restraint, and a reversible physical immobility, which is ended abruptly ''with the animal making an almost immediate transition from the immobile to a mobile state'' (Gallup, 1974).

The level or type of reaction is seen as a reflection of the level of the underlying emotion, fear.

In contrast to this, Klemm (1977) suggested that, at least in rabbits, fear was ''neither the sole nor necessary cause'' of the immobility. As an alternative interpretation of tonic immobility, a link between TI and 'emotionality' was proposed by McGraw and Klemm (1973) who reported an interrelationship between the ability of rats to learn a maze, exploration of new environments and TI and by Gallup et al. (1976) who suggested that differences in emotionality were the basis for the differences in immobility in chickens.

'Emotionality' in this context is used to describe a predisposition to react more or less strongly, quickly and lastingly to certain classes of stimuli (Savage and Eysenck, 1964). This definition of 'emotionality' is close to what Benus et al. (1991) called 'coping strategies'. One suggestion from research on coping strategies e.g., 'active/passive coping' strategies, (Benus et al., 1991; Hessing

et al., 1993) is that a given challenging situation will evoke specific responses, depending on the temperament or 'personality' of the individual involved.

Benus et al. (1991) identified 'active' and 'passive' types of mice. They found that individuals genetically selected over several generations for short attack latencies reacted in an active way e.g., fight/flight in response to an opponent, while those selected for long attack latencies reacted in a passive way e.g., immobility in response to an opponent. These strategies therefore had a genetic background and predicted the behaviour of individuals in response to various social and non-social challenges.

In the context of active/passive behavioural strategies sense (Benus et al.,1991), immobility can be said to represent a passive, and fight/flight an active response.

Erhard and Mendl (1997) reported the phenomenon of tonic immobility in pigs and suggested that the susceptibility to/duration of the immobility response in pigs may be seen as an indicator of the type of fear response (freezing vs. fight/flight) shown in a challenging situation rather than of fear itself (Boissy, 1995).

2 TRANSPORT STRESS

The monitoring of animal friendly production, including live animal transport, is becoming increasingly recognised as an important attribute of food quality and quality assurance schemes (Blokhuis et al., 2008, Veissier et al., 2008). Besides the animal welfare aspect, there is an increasing demand for traceability along the food chain that is related to food safety (Ruiz-Garcia et al., 2010).

Transport by its nature is an unfamiliar and threatening event in life of a domestic animal. It involves a series of handling and confinement situations which are unavoidable stressful and can lead to distress (Fraser, 1979), injury, and even death of the animal unless properly planned and carried out. Transport often coincides with a change of ownership whereby responsibility for the animal's welfare may be compromised.

Kenny and Tarrant (1987) divided the transport process into the component of repenning in a new environment (movement and restraint), loading/unloading (movement), confinement on a stationary vehicle (restraint) and confinement on a moving vehicle (restraint). More simply

transport involves two distinct type of actions: movement to new location and remaining stationary.

A sudden environmental change will usually elicit the movement of turning towards the source, a response called the orientation response, which may be followed by a startling response and defensive or flight reactions by the animal (Hemsworth and Barnett, 1987). As fear responses wane, the animal will also approach and examine the stimulus (Hinde, 1970), so the response to novel stimuli can be considered to contain elements of both fear and exploratory responses. The initial avoidance and subsequent exploration can be viewed as a consequence of the conflicting motivations of fear and exploration and the weaning of fear responses.

An animal can be stressed by internal and external environments; the loading procedures, facility design and the presence of unfamiliar animals can cause alarm.

An objective measurement of transport stress may be attempted, using behavioural, physiological and pathological indicators. Data on behaviour during transport are scarce, but are useful because they provide information about how the animals adapt and cope and show where modifications are necessary to improve transport equipment. More data are available on the physiological responses, at least for the more common transport situations. Dantzer and Mormède (1979) stated that the glucocorticoid content in blood is a good index for the reaction of animals to any environmental challenge and more in general changes in heart rate, blood composition (electrolytes, hormones, metabolites and enzymes) are used to assess the response of livestock to transport.

The European Union requires that journey times shall not exceed 8 h. However , this may be extended, if the transport vehicle meets additional requirements, to 24 hours or more, according to the species transported and making the required stops at control post (EFSA, 2011). Unfortunately not always resting facilities are adequate and animals are unloaded with care so rest stop may be counter-productive and serve only to prolong the overall transport time (Broom, 2007).

Observations suggest that it is not possible to recommend an "optimal" transport time. Moreover, recent findings confirm that the transport time "per se" is unlikely to be a risk factor but it becomes a risk when other aspects related to transport, such as animal fitness, fasting, vehicle design, driving style, stocking density, weather condition, ventilation, etc., are neglected (EFSA, 2011).

2.1 Pig Transport

The most common means of transport for pigs is by road vehicle.

During road transport, weather conditions (temperature, air velocity, humidity), loading density and duration of the journey are important factors influencing the condition of the animals (Broom, 2007).

Vehicle vibration and motion are known to have effects on humans. As well as generating motion sickness, health, comfort, postural stability, and ability to perform a task can be severely compromised. It is likely that similar responses occur in pigs; however the relevant ranges of frequencies could be different (Randall, 1992; 1996).

Pigs are usually transported in large trucks that may hold over 200 animals in 3 tiers with a compartment height of 90 cm.

In the EU, most of these trucks are equipped with a loading lift, in fact loading and unloading ramp is a cause off stress and injury for smaller animals, such pigs (Lambooij, 2007) and appears to be the most stressful component of the transport chain for pigs. For pigs climbing a loading ramp is difficult, since the situation is often psychologically disturbing. The animal may simply refuse to try and even turn their sides towards the ramps. The angle of the loading ramp should not be greater than 20° (EFSA, 2011)). Descending a loading ramp steeper than 20° is difficult for all animals and should be avoided (Grandin and Gallo, 2007).

In relation to the transport practices it is recommended that, wherever possible, animals should be kept in stable social groups. Pigs should be loaded onto vehicles in groups no greater than six. Sows and boars should be handled separately and transported in separate compartments. In the case of goats, groups should be kept stable, repeated regrouping should be avoided, and the introduction of new individuals should be monitored closely. The mixing of unfamiliar pigs at loading can increase both transport deaths and carcass damage (Gosalvez et al., 2006).

Averós et al. (2007) determined serum stress parameters in pigs transported to slaughter under Mediterranean conditions in different seasons. They found that stress reactions were largely determined by season (higher stress levels during winter) and genetics (depending on the halothane gene).

Assessment of mortality risk factors revealed that average temperature is more important than the duration of the journey. Mortality risk increased with average air temperature and was highest when pigs were not fasted (Averós et al., 2008).

Depending on the distance to the slaughterhouse or transport station, feeding should be stopped the night before transport, but water should be available.

In pigs, for journeys exceeding 24 hours, feed should be available every 24 hours at staging points followed by 6 hours rest. Weight loss attributable to withdrawal of food and water over long journeys represents an economic loss. The range of weight loss in pigs, even in short-term transport, is between 4-6% (Lambooij, 2007).

Increasing transport duration from 6 to 12 and 24 h increased fatigue in weaned piglets, but was also associated with some indicators of habituation, such as sitting and establishment of dominance hierarchy.

In a recent review, Lewis (2008) stated that the transport of early weaned piglets up to 24 h is not more detrimental than early weaning with respect to early feed consumption, as both transported and control piglets lose similar body reserves and recover at the same time. However, increasing transport duration from 6 to 12 and 24 h increased fatigue (Lewis and Berry, 2006). Increasing transport duration was also associated with increased drinking post-transport and higher haematocrits, indicative of rising levels of dehydration and thirst.

New research confirmed that pigs show maladaptation to stressful situations due to a relatively small heart size in relation to body mass. At low environmental temperatures pigs remain close together (huddle) during air transport (EFSA, 2011), even though they have plenty of space. Increased drinking post-transport and evidence of dehydratation indicate that water intake of pigs while vehicles are in motion is low, despite the fact that water is provided in the vehicle (Frotin et al., 2002).

Loading pigs onto a truck in groups of no more than 5 or 6 animals reduces the heart rates and takes the same amount of time as when larger group sizes are loaded. Both aggressive handling during loading and driving long distances, adversely affect rectal temperature and blood-acid balance (Knowles and Warriss, 2000). Transport of sows and/or entire boars together causes aggression and increases the risk of injury. 'Birth to slaughter' systems, where litters of pigs are kept together from birth to slaughter, including transport and pre-slaughter lairage, minimises skin damage (fighting).

2.2 Cattle Transport

There have been several recent studies on the effects of long journeys on physiological and behavioural indicators of cattle welfare. The behaviour of cattle on moving vehicles is of interest in view of the evidence that confinement on a moving vehicle is the most stressful step in the transportation process, at least for intensively reared cattle.

The response of cattle to transport varies, depending on the situation, and ranges from a moderate and readily identifiable stress response that may not cause concern about the animal's welfare to extreme responses that signify distress and cause major concern about welfare and economic losses.

According to Grandin (1997) both previous experience and genetic factors affecting temperament interact in complex ways to determine how fearful an animal may become when it is handled or transported.

Gupta et al. (2007) investigated the effect of 12 h transport by road of mature bulls that had been housed prior to transport at different space allowances (1.2, 2.7, 4.2 m^2/bull) on slatted floors for 97 days. Effects of loading bulls on a transporter, transporting for 12 h and subsequently unloading included body-weight loss, neutrophilia, eosinophilia, lymphopaenia and increased packed cell volume, red blood cell and haemoglobin levels. While transport increased cortisol and suppressed indicators of the immune response in the short-term, these changes were within normal physiological ranges, suggesting that 12 h road transport had no significant adverse effect on welfare over this period.

Adult cattle should not be transported on a journey of longer than 29 hours. After this time there should be a 24 hour recovery period with access to appropriate food and water.

Isolation of cattle induces struggling, vocalisation, increased heart rate and plasma cortisol levels (Færevik et al., 2006). The presence and sight of conspecifics are found to moderate the behavioural reaction of cattle to separation. Restlessness increased with social regrouping on the truck, but not with motion (Kenny and Tarrant, 1987). The calming effect of familiar animals should be taken into consideration during transport, handling and regrouping of cattle. Vocalisation can be a useful indicator of impaired welfare for both experimental and practical purposes (Watts and Stookey, 2000).

Partition of vehicles for cattle reduces the risk of injury, allows faster loading and unloading and allows animals to settle better during transport and thereafter at lairage.

Cattle should be provided with sufficient space to stand without contact with their neighbours and to lie down if the journey is more than 12 hours. Space allowances should be calculated according to an allometric equation relating size (Petherick and Phillips, 2009) to body weight. For cattle with horns, space allowance should be 7% higher (EFSA, 2011).

Cattle avoid contact with other individuals when they can and maintain their balance better when not touching other individuals (Cockram, 2007; Broom, 2008). Several authors have explored the possibility that giving cattle more space than required by the regulations may increase the risk of injury (Eldridge and Winfield, 1988; Tarrant and Grandin, 2000; Mounaix et al., 2011, in press). However, these results are equivocal and did not take account driving quality.

Water should be available during rest periods on journeys of 8 to 29 hours.

Repeated humane handling of cattle during rearing, and in particular immediately prior to transport, can minimise aversive reactions at the time of transport (Grandin, 2007).

Since there are many studies on the transport of cattle to fattening farms or to slaughterhouses, but few information were found on young bulls with different destination, major attention was devoted to the transport of young bulls delivered to the genetic test stations for selection. This will be the topic of the last chapter.

3 REFERENCES

- Augustini C.; 1976. ECG-und korper temperature messugen an schwenin wahrend der mast und auf dem transport. Fleischwirtschaft 56: 1133-1137.

- Averós X., Herranz A., Sanchez R., Comella J.X. and Gosalvez L.F.; 2007. Serum stress parameters in pigs transported to slaughter under commercial conditions in different seasons. Veterinari Medicina 528: 333-342.

- Averós X., Knowles T.G., Brown S.N., Warriss P.D. and Gosálvez L.F.; 2008. Factors affecting the mortality of pigs being transported to slaughter. Vet. Rec., 163, 386-390.

- Avitsur R., Padgett D.A., Sheridan J.F.; 2006. Social Interactions, Stress, and Immunity. Neurologic Clinics 24: 483-491.

- Benus R.F., Koolhaas J.M., van Oortmerssen G.A.; 1987. Individual differences in behavioural reaction to a changing environment in mice and rats. Behaviour 100:105–22.

- Benus R.F., Bohus B., Koolhaas J.M., Oortmerssen G.A.; 1989. Behavioral strategies of aggressive and non-aggressive male mice in active shock avoidance. Behav Processes 20: 1-12.

- Benus R.F., Den Daas S., Koolhaas J.M., Van Oortmerssen G.A.; 1990. Routine formation and flexibility in social and non-social behaviour of aggressive and non-aggressive male mice. Behaviour 112: 176-93.

- Benus R.F., Bohus B., Koolhaas J.M., van Oortmerssen G.A.; 1991. Behavioural differences between artificially selected aggressive and non-aggressive mice: response to apomorphine. Behavioural Brain Research 43: 203-208.

- Blokhuis H.J., Keeling L.J., Gavinelli A. and Serratosa J.; 2008. Animal welfares impact on the food chain. Trends in Food Science and Technology, 19, 79-87.

- Bohus B., Benus R.F., Fokkema D.S., Koolhaas J.M., Nyakas C., Van Oortmerssen G.A., Prins A.J.A., De Ruiter A.J.H., Scheurink A.J.W., Steffens A.B.; 1987. Neuroendocrine states and behavioral and physiological stress responses. In: De Kloet ER, Wiegant VM, De Wied D, editors. Progress in brain research, Amsterdam: Elsevier, p. 57–70.

- Boissy A.; 1995. Fear and fearfulness in animals. The Quarterly Review of Biology 70: 165-191.

- Broom D.M.;2007. Welfare assessment and welfare problem areas during handling and transport. In: T. Grandin (ed.) Livestock Handling and Transport. CABI Publishing, Wallingford, UK, 46-62.

- Broom D.M.; 2008. The welfare of livestock during road transport. In: Appleby, M.C., Garces, L. (eds.) *Long distance transport and welfare of farm animals*, 157-181. Wallingford, CABI.

- Cassady J. P.; 2007. Evidence of phenotypic relationships among behavioral characteristics of individual pigs and performance. J Anim Sci 85: 218-224.

- Cockram M.S.; 2007. Criteria and potential reasons for maximum journey times for farm animals destined for slaughter. Applied Animal Behaviour Science 106: 234-243.

- Crawford F.T.; 1977. Induction and duration of tonic immobility. The Psychological Record 27: 89-107.

- Dantzer R. and Mormède P.; 1979. Le Stress en Elevage Intensif. Masson, Paris.

- D'Eath R. B., and Pickup H. E.; 2002. Behaviour of young growing pigs in a resident-intruder test designed to measure aggressiveness. Aggress Behav 28: 401-415.

- Driscoll P., Demek J., D'Angio M., Claustre Y., Scatton B., 1990. A genetically-based model for divergent stress responses: behavioral, neurochemical and hormonal aspects. In: Pliska V, Stranzinger G, editors. Farm animals in biomedical research, Hamburg: Verlag Paul Parey, pp. 97–107.

- European Food Safety Authority (EFSA), 2011. Scientific Opinion Concerning the Welfare of Animals during Transport. EFSA Journal 2011; 9(1):1966

- Eldridge, G.A. and Winfield, C.G., 1988. The behaviour and bruising of cattle during transport at different space allowances. Australian Journal of Experimental Agriculture 28: 695-698.

- Engel G.L., Schmale A.H.; 1972. Conservation-withdrawal: A primary regulatory process for organismic homeostasis. Physiology, Emotion and Psycosomatic Illnes, Ciba Foundation Symposium, 1972, 8, 57-76.

- Erhard H. W., and Mendl M.; 1997. Measuring aggressiveness in growing pigs in a resident-intruder situation. Appl Anim Behav Sci 54: 123-136.

- Erhard H.W., and Mendl M.; 1999. Tonic immobility and emergence time in pigs—more evidence for behavioural strategies. Appl. Anim. Behav. Sci. 61: 227-237.

- Færevik G., Jensen M.B. and Bøe K.E., 2006. Dairy calves social preferences and the significance of a companion animal during separation from the group. Appl. Anim. Behav. Sci. 99: 205-221.

- Forkman B., Furuhaug I.L., Jensen P.; 1995. Personality, coping patterns, and aggression in piglets. Appl Anim Behav Sci 45: 31-42.

- Fraser A. F.; 1979. The nature of cruelty to animals. Appl. Anim. Ethol. 5: 1.

- Fraser A.F., Broom D.M.; 1990. Farm Animal Behaviour and Welfare. Baillière Tindall, London.

- Frontan P., Chevillon P., Rousseau P.; 2002. Systèmes d'abreuvement et comportement des porcs lors d'un transport de 24h. Techniporc 25: 15-24.

- Gallup G.G.; 1974. Animal hypnosis: factual status of a fictional concept. Psychological Bulletin 81: 836-853.

- Gallup G.G., Ledbetter D.H., Maser J.D.; 1976. Strain differences among chickens in tonic immobility: evidence for an emotionality component. Journal of Comparative and Physiological Psychology 90: 1075-1081.

- Gallup G.G.; 1977. Tonic immobility: the role of fear and predation. The Psychological Record 27:41-61.

- Giroux S., Martineau G.P., and Robert S.; 2000. Relationships between individual behavioural traits and post-weaning growth in segregated early-weaned piglets. Appl. Anim. Behav. Sci. 70: 41-48.

- Gosalvez L.F., Averós X., Valdelvira J.J. and Herranz, A.; 2006. Influence of season, distance and mixed loads on the physical and carcass integrity of pigs transported to slaughter. Meat Science 73: 553-558.

- Grandin T.; 1981. Livestock Trucking Guide. Livestock Conservation Istitute, Madison, Wisconsis, USA.

- Grandin T.; 1997. Assesment of stress during handling and transport. Journal of Animal Science 75: 249-257.

- Grandin, T. and Gallo, C.; 2007. Cattle transport in: T. Grandin (Editor) Livestock Handling and Transport, CABI International, Wallingford, Oxon, UK, pp 134-154.

- Gupta S., Earley B. and Crowe M.A.; 2007. Effect of 12-hour road transportation on physiological, immunological and haematological parameters in bulls housed at different space allowances. The Vet. J. 173: 605-616.

- Hails M.R.; 1978. Transport stress in animals: a review. Animal Regulation Studies 1: 289-343.

- Hemsworth P.J. and Barnett J.L.; 1987. Human-animal interactions. In: E.O. Price (Editor), Tile Veterinary Clinics of North America, Food Animal Practice. Farm Anita. Behav., 3: 339-356.

- Henry J.P., Stephens P.M.; 1977. The Social Environment and Essential Hypertension in Mice: Possible Role of the Innervation of the Adrenal Cortex. Progress in Brain Research 47: 263-276.

- Hessing, M. J. C., Hagelsø A.M., van Beek J. A. M., Wiepkema R.P., Schouten W. G. P., and Krukow R.; 1993. Individual behavioural characteristics in pigs. Appl Anim Behav Sci 37: 285-295.

- Hessing M. J. C., Hagelso A.M., Schouten W. G. P., Wiepkema P. R., and Vanbeek J. A. M.; 1994. Individual behavioral and physiological strategies in pigs. Physiol Behav 55: 39-46.

- Jensen P., Forkman B., Thodberg K., and Köster E.; 1995a. Individual variation and consistency in piglet behaviour. Appl Anim Behav Sci 45: 43-52.

- Kenny F.J. and Tarrant P.V.; 1987. The physilogical and behavioral responses of creossbred Friesian steers to short-haul transport by road. Livestock Production Science 17: 63-75.

- Klemm W.R.; 1977. Identity of sensory and motor systems that are critical to the immobility reflex ('animal hypnosis'). The Psychological Record 27: 145-159

- Knowles, T.G. and Warriss, P.D., 2000. Stress Physiology of Animals During Transport. In: Grandin, T. (ed.), Livestock Handling and Transport. 2nd edition, CAB International, Wallingford, UK, pp 230-242.

- Koolhaas J.M., Korte S.M., De Boer S.F., Van Der Vegt B.J., Van Reenen C.G., Hopster H., De Jong I.C., Ruis M.A.W, Blokhuis H.J.; 1999. Coping styles in animals: current status in behavior and stress-physiology. Neuroscience and Biobehavioral Reviews 23: 925-935.

- Koolhaas J.M; 2008. Coping style and immunity in animals: Making sense of individual variation. Brain, Behavior, and Immunity 22: 662-667.

- Lambooij E.; 2007. Transport of pig. In: T. Grandin (ed.) Livestock Handling and Transport. CABI Publishing, Wallingford, UK, pp 228-244.

- Lazarus R.S., Averill J. R., Opton E. M. Jr.; 1974. The psychology of coping: issues of research and assessment. In Coping and Adaptation, ed. G. V. Coehlo, D. A. Hamburg, J. E. Adams, pp 249-315. New York; Basic Books.

- Lewis N.J. and Berry R.J.; 2006. Effects of season on the behaviour of early-weaned piglets during and immediately following transport. Applied Animal Behaviour Science, 100, 182-192.

- Lewis N.J.; 2008. Transport of early weaned piglets. Applied Animal Behaviour Science, 110, 128-135.

- Maser J.D, Gallup G.G.; 1977. Tonic immobility and related phenomena: a partially annotated, tricentennial bibliography, 1636–1976. The Psychological Record. 1: 177-217.

- McGraw C.P., Klemm W.R.; 1973. Genetic differences in susceptibility of rats to the immobility reflex ('animal hypnosis'). Behavior Genetics 3: 155-161.

- Mounaix B., Dion J., Brun T., Dufour V., Perrin M., Rflegau T., Bruke A., Daivid V., Lucbert J. and Mirabito L.; 2011. Long distance cattle transport: impact of variations of space allowance on physiological and behavioural indicators of stress. (In press).

- Oortmerssen G.Av., Bakker T.C.M.; 1981. Artificial selection for short and long attack latencies in wild Mus musculus domesticus. Behav Genet 11:115–26.

- Petherick J.C. and Phillips C.J.C.; 2009. Space allowances for confined livestock and their determination from allometric principles. Applied Animal Behaviour Science 117, 1-12.

- Randall J.M.; 1992. Human subjective response to lorry vibration: implication for farm animal transport. Journal of Agricultural and Engineering Research 52: 295-307.

- Ruiz-Garcia L., Steinberger G. and Rothmund M.; 2010. A model and prototype implementation for tracking and tracing agricultural batch products along the food chain. Food Control, 21, 112-121.

- Ruis M. A. W, te Brake B., Engel J. H. A., Buist W. G., Blokhuis H. J., and Koolhaas J.M.; 2001. Adaptation to social isolation - Acute and long-term stress responses of growing gilts with different coping characteristics. Physiol Behav 73: 541-551.

- Savage R.D., Eysenck H.J.; 1964. The definition and measurement of emotionality. In: Eysenck, H.J. (Ed.), Experiments in Motivation, Oxford, Pergamon.

- Sluyter F., Bult A., Lynch C.B., Oortmerssen G.Av, Koolhaas J.M., 1995. A comparison between house mouse lines selected for attack latency or nest-building: evidence for a genetic basis for alternative behavioral strategies. Behavior Genetics 25:247-52.

- Sluyter F., Korte S.M., Bohus B., Van Oortmerssen G.A., 1996. Behavioral stress response of genetically selected aggressive and non aggressive wild house mice in the shock-probe/defensive burying test. Pharmacol Biochem Behav 54:113-6.

- Spoolder H. A. M., Burbidge J. A., Lawrence A. B., Simmins P. H., and Edwards S.A.; 1996. Individual behavioural differences in pigs: intra- and inter-test consistency. Appl Anim Behav Sci 49: 185-198.

- Tarrant V. and Grandin T.; 2000. Cattle transport, In: Grandin, T. (ed.), Livestock Handling and Transport, 2nd ed. CAB International, Wallingford, Oxon, UK, pp. 151–173.

- Ursin H., Olff M.; 1993. Psychobiology of coping and defence strategies. Neuropsychobiology 28: 66-71.

- van Erp-van der Kooij E., Kuijpers A.H., Schrama J.W., Ekkel E.D., and M. Tielen J.M.; 2000. Individual behavioural characteristics in pigs and their impact on production. Appl Anim Behav Sci 66: 171-185.

- van Erp-van der Kooij E., Kuijpers A.H., Van Eerdenburg F.J.C.M., and Tielen M.J.M. 2001. A note on the influence of starting position, time of testing and test order on the Backtest in pigs. Appl. Anim. Behav. Sci. 73: 263– 266.

- van Erp-van der Kooij E., Kuijpers A.H., Schrama J.W., van Eerdenburg F. J. C. M., Schouten W. G. P., and Tielen M. J. M.; 2002. Can we predict behaviour in pigs? Searching for consistency in behaviour over time and across situations. Appl. Anim. Behav. Sci. 75: 293-305.

- Veissier I., Butterworth A., Bock B. and Roe E.; 2008. European approaches to ensure good animal welfare, Applied Animal Behaviour Science 113: 279-297.

- Velie B. D., Maltecca C., and Cassady J.P.; 2009. Genetic relationships among pig behavior, growth, backfat, and loin muscle area. J Anim Sci 87: 2767-2773.

- Watts J.M. and Stookey J.M.; 2000. Vocal behaviour in cattle: the animal's commentary on its biological processes and welfare. Applied Animal Behaviour Science 67: 15-33.

- Wechsler B.; 1995. Coping and coping strategies: a behavioural view. Applied Animal Behaviour 43: 123-134.

- Weiss J.M.; 1968. Effects of coping responses on stress. J Comp Physiol Psychol 65: 251-260.

CHAPTER 1

BACK TEST vs TONIC IMMOBILITY TEST: BEHAVIOURAL RESPONSE IN TWODIFFERENT RESTRAIN SITUATIONS

Magnani, D., Cafazzo, S., Calà, P., Dall'Olio, S., Nanni Costa, L.

Dept. Protection and Valorisation of Agro-food, Unit of Animal Husbandry. Faculty of Agriculture, University of Bologna, Bologna, Italy.

Corresponding author: Tel.: +390245484143 E-mail address: diego.magnani@studio.unibo.it
(D. Magnani).

ABSTRACT

Backtest (BT) and Tonic Immobility Test (TIT) allow to classify piglets in different type of coping style (HR=High-Resisting; LR=Low-Resisting for the BT; non-TI= don't show tonic immobility and TI=showing tonic immobility for the TIT) on the basis of their reaction to restrain. There are no data concerning the relationship between TIT and BT responses in piglets. The aim of this study was to investigate this potential relationship. Sixty piglets of commercial crossbred (Duroc x (Landrace x Large White)) reared in the same farm were examined. The BT was performed on all piglets at 10 and 17 days of age; the TIT was carried out at 9 days (20 piglets), at 13 days (20 piglets) and at 19 days (20 piglets) of age. Results of the tests were not influenced by their reciprocal temporal sequence. There was not a correspondence between the BT and TIT categories.

This result suggests that the two tests measure reactions to different challenging stimuli and they may be used as indicators of different types of response to restrain. In the BT, the duration of vocalization, struggling, and relaxation were significantly different ($P<0.05$) between HR and LR piglets.

INTRODUCTION

Pigs vary largely in behavioural reactions when exposed to the same stressful situation. Individual differences in adaptive or coping reactions of pigs have received growing attention, as the identification of basic characteristics that predict (mal)adaptation to husbandry conditions could be relevant both for pig husbandry and pig welfare. In order to identify these characteristics, the Backtest (BT) (Figure 1a) and the Tonic Immobility test (TIT) (Figure 1b) have been used [4]. In both tests young piglets are restrained in supine position. The behavioural reaction of piglets in these tests is thought to reveal part of their "coping style" or 'personality'. Some piglets usually referred to as 'high-resisters' (HR), struggle a lot during the Backtest, whereas others respond with immobility, the so-called 'low-resisters' (LR) [6]. At a later age, HR and LR pigs have been shown to differ in their behavioural and neuroendocrine reactions to a variety of challenges [5, 6, 7, 9, 10, 11]. The response profiles of HR and LR largely resemble the diverging coping styles, often referred to as "(pro)active" versus "passive/reactive", respectively, that have been identified in pigs and in other species [1, 8]. Likewise, it has been proposed that the tonic immobility is one possible way of

assessing whether individual pigs are more likely to adopt a more active (low susceptibility/short duration of Tonic Immobility test, struggle, move fast) or a more passive behavioural strategy (high susceptibility to/long duration of Tonic Immobility test, tense, move more slowly) in a challenging situation [2, 3]. There are no data concerning the possible link between the Tonic Immobility and response to the Backtest in piglets. The aim of this study was to investigate this potential relationship. It can be hypothesized that f the tests measure the same behavioural characteristics, they should classify the same animal in the same category.

MATERIAL AND METHODS

Sixty piglets from 10 litters of commercial crossbred pigs (Duroc x (Landrace x Large White)) were tested. Male piglets were castrated at approximately 5 days of age. Sow and piglets were housed in conventional farrowing pens (1.5 m x 2.5 m) with the sows restricted in farrowing crates. The Backtest was performed at 10 and 17 days of age; the Tonic Immobility test was performed before (at 7 days of age - 20 piglets), between (at 13 days of age - 20 piglets) and after (at 19 days of age - 20 piglets) the Backtest. During the Backtest each piglet was restrained on its back by placing the right hand over the throat and the other loosely on the hind legs (Figure 1a). Classification of pigs was based on the number of escape attempts (i.e. bouts of struggling with at least the hind legs) they displayed during 60 s. A pig was classified as high-resisting (HR) if it performed more than four escape attempts in the two tests, with a minimum of two attempts in one test. If a pig struggled less than four times in two tests, with a maximum of two attempts in one test, it was labelled low resisting (LR) [1]. In the Tonic Immobility test the experimenter placed the piglet on its back onto a V-shaped wooden cradle (70 cm long, angle approximately 80°) (Figure 1b). Then the experimenter put a sand-filled cloth bag (15 x 20 cm2, ca. 500 g) on the piglet's chin, gently stretched its back legs and then let go of both the hind legs and the sand bag. If the pig became immobile, the duration of immobility was recorded from this point onwards (we call these pigs 'TI pigs'). As soon as the piglet struggled, the bag was removed and the duration of immobility recorded. If the piglet did not struggle within 5 min, the test was terminated, and duration of 300 s was allocated to this pig. Some piglets did not show the immobility response described above ('non-TI pigs') [2]. They struggle while they were being placed onto the cradle, or as soon as they touched the cradle. It was not possible to get them through the process described above. In this experiment, they were recorded as having duration of immobility of 0 s. Due to non-

normality of the data, non-parametric statistics were used for the analysis. Chi-square analysis was used to evaluate the effect of the reciprocal sequence of tests on the classification results for Backtest and Tonic Immobility test, and to analyse the relationship between Backtest scores and TIT. Mann-Whitney U Test was used to evaluate the differences in behaviour between High and Low resisters pigs and Low resisters pig.

RESULTS

The reciprocal sequence of the tests did not influence their results (Backtest: $\chi2=3.61$, d.f.= 2, n=60, P=0.16; Tonic Immobility test: $\chi2=1.08$, d.f.=2, n=60, P=0.58).

In the Backtest the duration of vocalization, struggling and relaxation were significantly different between HR and LR piglets, validating the methodology (Table 1).

Due to the Tonic Immobility test methodology, the differences between TI and non-TI piglets in the duration of struggling, relaxation and vocalizations were not analyzable. The 41.7% of tested subjects did not show tonic immobility response. Moreover, among "TI-pigs" we found a high variability in the time (mean ± SD in sec) of reaction (9.57 ± 9.21), relaxation (7.69 ± 13.72) and vocalizations (1.41 ± 3.79). There were no relationship between Backtest scores and susceptibility to immobility ($\chi2=1.15$, d.f.=1, n=60, P=0.29). Only 28.3% of "low resisters" pigs showed immobility response during the Tonic Immobility test and, on the other hand, only 20.0% of "high resisters" pigs struggled immediately afterwards placed onto a V-shaped wooden cradle.

DISCUSSION

Even if Backtest and Tonic Immobility test are present in literature for a long time [6, 7], there are no data concerning the relationship between their classifications. During Backtest and Tonic Immobility test piglets are subjected to a restrain situations and both tests adopt a bimodal classification creating two groups whose characteristics have been compare each other (HR as non-TI, LR as TI) [2, 3]. Nevertheless, our results indicated that behavioural response of an animal to one test was not predictive of the behavioural response of the same animal to the other test. It is important to underline that there are relevant differences between the two tests: the Backtest has a fixed duration while the duration of Tonic Immobility test is extremely variable. Moreover, in

the Backtest the animal is restrains by a human hand while in the Tonic Immobility test a piglet is restrain by means of a sand-filled cloth bag and V-shaped wooden cradle. These differences can affect the behavioural response of the animals which may perceive different emotions during the tests (e.g. fear of humans during BT, anxiety for the restrain situation during TIT) and therefore to show different reactions. The use of backtest as a valuable tool to be implemented by farmers for the formation of groups was suggested [9], nevertheless results remain inconclusive and their interpretation ambiguous depending in what the tests actually measure in the form they have applied.

CONCLUSIONS

There was not a correspondence between the BT and TIT categories. In particular, the "TI-pigs" class was heterogeneous and it included piglets with different coping styles. Based on our results it can be assumed that the two tests measure the reactions of pigs to different challenging stimuli and they may be seen as indicators of different types of response.

REFERENCES

1. BOLHUIS, J.E; SCHOUTEN, W.G.P.; SCHRAMA, J.W.; WIEGANT, V.M. (2005):
Individual coping characteristics, aggressiveness and fighting strategies in pigs. Anim. Behav. 69, 1085–1091.

2. ERHARD, H.W., MENDL, M. (1999): Tonic immobility and emergence time in pigs—more evidence for behavioural strategies. Appl. Anim. Behav. Sci. 61, 227-237.

3. ERHARD, H.W., MENDL, M., CHRISTIANSEN, S.B. (1999): Individual differences in tonic immobility may reflect behavioural strategies. Appl. Anim. Behav. Sci. 64, 31-46.

4. GEVERINK, N.A., SCHOUTEN, W.G.P.; GORT, G., WIEGANT, V.M. (2002): Individual differences in aggression and physiology in peripubertal breeding gilts. Appl. Anim. Behav. Sci. 77, 43–52.

5. GEVERINK, N. A., SCHOUTEN, W. G. P., GORT, G.; WIEGANT, V. M. (2003): Individual differences in behaviour, physiology and pathology in breeding gilts housed in groups or stalls. Appl. Anim. Behav. Sci. 81, 29–41.

6. HESSING, M.J.C., HAGELSØ, A.M., VAN BEEK, J.A.M., WIEPKEMA, P.R., SCHOUTEN, W.G.P., KRUKOW, R. (1993): Individual behavioural characteristics in pigs. Appl. Anim. Behav. Sci. 37, 285–295.

7. HESSING, M.J.C., HAGELSØ, A.M., VAN BEEK, J.A.M., WIEPKEMA, P.R., SCHOUTEN, W.G.P. (1994): Individual behavioural and physiological strategies in pigs. Physiol. Behav. 55, 39–46.

8. KOOLHAAS, J.M., DE BOER, S.F., BUWALDA, B, VAN RENEEN, K. (2007): Individual variation in coping with stress: A multidimensional approach of ultimate and proximate mechanisms. Brain Behav. Evol. 70, 218–226.

9. RUIS, M. A. W., TE BRAKE, J. H. A., VAN DE BURGWAL, J. A., DE JONG, I. C., BLOKHUIS, H. J., KOOLHAAS, J. M. (2000): Personalities in female domesticated pigs, behavioural and physiological indications. Appl. Anim. Behav. Sci. 66, 31–47.

10. RUIS, M.A.W., TE BRAKE, J.H.A., ENGEL, B., BUIST, W.G., BLOKHUIS, H.J., KOOLHAAS, J.M. (2002): Implications of coping characteristics and social status for welfare and production of paired growing gilts. Appl. Anim. Behav. Sci. 75 (3), 207– 31.

11. VAN ERP-VAN DER KOOIJ, E., KUIJPERS, A. H., SCHRAMA, J. W., EKKEL, E. D.; TIELEN, M. J. M. (2000): Individual behavioural characteristics in pigs and their impact on production. Appl. Anim. Behav. Sci. 66, 171–185.

Figure 1.

The Backtest **(1a)** and the Tonic Immobility Test **(1b)**.

1a

1b

Table 1.
Comparison between HR and LR reactions to the Backtest (Mean ± SD). Means are expressed in seconds.

Behaviour	HR	LR	U	P
Vocalization	49.76 ± 12.34	31.28 ± 9.98	150	0.02
Struggling	27.43 ± 11.56	13.57 ± 5.5	31	0.0001
Relaxation	8.13 ± 3.78	32.17 ± 13.78	35	0.005

CHAPTER 2

SEARCHING FOR DIFFERENCES IN THE BEHAVIOURAL RESPONSE OF PIGLET GROUPS SUBJECTED TO NOVEL SITUATIONS

Magnani, D., Cafazzo, S., Calà, P., Nanni Costa, L.

Dept. Protection and Valorisation of Agro-food, Unit of Animal Husbandry. Faculty of Agriculture, University of Bologna, Bologna, Italy.

Corresponding author: Tel.: +390245484143 E-mail address: diego.magnani@studio.unibo.it (D. Magnani).

ABSTRACT

The Backtest (BT), the Open Field (OF) and the Novel Object (NO) tests have been used to identify individual reaction patterns in piglets and to measure parameters that previous studies have shown to be correlated to the coping strategies of animals. The BT allows for the classification of piglets into two different "coping styles": high-resisting (HR) and low-resisting (LR), which respectively correspond to a (pro-) active and passive (or reactive) behavioural response.

During previous research, the subjects were tested singularly, so the aim of this study was to investigate if differences between HR and LR animals could be detected when piglets are tested in a group using the OP and NO tests.

A total of 132 piglets were subjected to the BT and then were housed in groups consisting of four individuals each so as to obtain HR pens, LR pens and mixed pens. We found differences in the pigs' behaviour during the OF and the NO tests and according to the type of group. Individual differences in the behavioural response of piglets to the Backtest were not predictive for the behavioural response of the animals subjected to the OF or to NO tests.

Our results show that there are no relevant differences between HR and LR piglets when they are subjected in a group to novel situations.

Keywords: coping style, group, novel situation, piglet.

1. INTRODUCTION

Over the past two decades, coping strategies have gained increasing attention in animal ethology. Livestock animals encounter many stressors during their life and coping with these stressors affects their health, welfare and production (Van Erp-Van der Kooij et al., 2002).

Previous research on coping characteristics, predominantly in rodents, have generally supported the existence of two distinct ''coping styles'' within populations, an active (or proactive) and a passive (or reactive) style (see Koolhaas et al., 2007 for a review). One of the most important difference between active and passive individuals lies in the way they use internal and external information to organize behaviour associated with a given stimulus (Koolhaas et al., 1997). Actively coping individuals create routines and seem to anticipate situations, whereas passive

individuals tend to react more to environmental changes (Benus et al., 1991; Koolhaas et al., 1997).

Aggressiveness in the resident-intruder test is central to much of the work on coping styles in mice and rats (Benus et al., 1991; Koolhaas et al., 2007), whereas in pigs, the Backtest is a more popular measure of coping and its outcomes have been compared with the responses of the same pigs in a variety of social tests (Hessing et al., 1993, 1994; Ruis et al., 2000; Van Erp-Van der Kooij et al., 2000, 2001; Geverink et al., 2003).

The behavioural reaction of piglets in this test is thought to reveal part of their "coping style", enabling two different behavioural strategies to be identified. During the test, a piglet is put on its back and restrained in this position for 1min, while the number of escape attempts is recorded. Some piglets, usually referred to as 'high resisters' (HR), struggle a great deal during the Backtest, whereas piglets at the other end of the distribution, the so-called 'low resisters' (LR), tend to respond with immobility. The response profiles of HR and LR piglets largely resemble the diverging coping styles, often referred to as (pro-) active versus passive/reactive.

Some attempts to categorize piglets into distinct personality types, as done for rodents, have not been successful and have shown divergent results (Lawrence et al., 1991; Jensen et al., 1995; Forkman et al., 1995; Spoolder et al., 1996). Moreover, the Backtest has also been criticised as being arbitrary in nature because it is not clear what motivational system it challenges (D'Eath, 2002). In fact, the piglet may perceive the handler as a predator, evoking escape, or behave as though in a fight with a conspecific, perhaps evoking an aggressive response (Jensen et al., 1995).

Moreover, Backtest responses do not fall into two distinct 'types' of responders as has been claimed for coping responses in other species (Koolhaas et al., 1997) because there is no clear evidence for a bimodal distribution characteristic of discrete styles (Forkman et al., 1995; Erhard and Mendl, 1999; van Erp-van der Kooij et al., 2000).

If differences in behaviour reflect coping characteristics, then behaviour in one situation should predict behavioural reactions in other situations and at other times. It is often assumed that individuals are relatively consistent in their response to an environmental challenge at different times (ages) and in different situations (Lawrence et al., 1991; Van Erp-Van der Kooij et al., 2000). These relatively stable individual characteristics that show some consistency over time and across situations are also referred to as temperament. Temperament refers to the particular configuration of behaviours that an individual expresses and is therefore a property of an individual (Bell, 2007).

In a study on individual behavioural characteristics in pigs (Hessing et al., 1994), it is stated that the complementary individual behavioural characteristics of active and reactive pigs under stress will result in a better socially integrated group. A socially well-integrated group will be more successful in solving their problems, which is beneficial for each individual and for the group as a whole. This is shown in two studies where active (HR) and reactive (LR) pigs were mixed. It was found that the most stable social relationship existed between HR/LR pairs where the LR animal was dominant (Ruis et al., 2002) and that HR/LR groups performed better in term of growth rate and weight (Hessing et al., 1994).

In previous research, pigs have often been tested at a later age (Lawrence et al., 1991; Brown et al., 2009) or singularly (Jensen et al., 1995; Spoolder et al., 1996; Wemelsfelder et al., 2000; Dalmau et al., 2009). With an eye to advancing knowledge about this subject, we studied piglets reared in small groups and tested them with their home pen mates to avoid behavioural responses due to isolation. Therefore, the aim of the present study was to investigate whether there is any consistency in individual variations in piglet behaviour in reaction to different challenges and whether group composition has any significant effect. For the purpose of identifying individual reaction patterns in pigs, the Backtest, the Open Field test and the Novel Object test were used. To evaluate the influence of group composition on the outcome of behavioural tests, different groups were created based on the Backtest scores and members of each group were tested all together. If group composition affects the ability of animals to solve their problems (e.g. cope with stressful situations), we would expect to find differences in behavioural response between groups consisting of both HR and LR individuals and groups with LR or HR pigs only.

2. MATERIALS AND METHODS

A total of 132 piglets from 37 litters of commercial crossbred pigs (Duroc x (Landrace x Large White)) were tested from April to September 2009 in four replicates of 33 subjects each.

Male piglets were castrated at approximately 5 days of age. All sows and their piglets were housed in conventional farrowing pens (1.5 m x 2.5 m), where each sow was restricted in a farrowing crate. At weaning (day 25±2) piglets were removed from the farrowing pens and mixed in two pens of about 3 m x 3 m. At 33 (±2) days of age all piglets were transported to the experimental farm of DIPROVAL, where they were housed in pens (1 m x 1 m with 4 animals) with slatted floors.

Each pig was fed two times a day from a five-space feeder containing a normal commercial feed and water was supplied ad libitum using a water-nipple.

2.1. Backtest

All piglets were tested twice during the suckling period, once at 10 days and again at 17 days. In this test each piglet was restrained on its back by placing the right hand over the throat and the other loosely on the hind legs (Hessing et al., 1993). Classification of pigs was based on the number of escape attempts (i.e. bouts of struggling with at least the hind legs) they displayed during 60 s. The classification of each individual was based on the outcome of these two Backtests. A pig was classified as high-resisting if it performed more than four escape attempts during the two tests, with a minimum of two attempts per test. If a pig struggled less than four times in two tests, with a maximum of two attempts per test, it was labelled low-resisting (Hessing et al., 1993). All remaining piglets were classified as "indefinite" and were excluded from the study, which included exclusively LR and HR subjects.

From the 196 piglets tested during the suckling period, we selected 66 HR and 66 LR individuals with similar body weights (6.36±2.05SD kg at 17 days of age), for a total of 132 (seventy-two male and sixty female) piglets. They were housed in groups of four, for a total of 12 HR pens, 12 LR pens and 9 mixed pens (2 HR and 2 LR piglets).

2.2 Open Field test and Novel Object test

The Open Field (OF) and Novel Object test (NO) were carried out when the piglets were between 42 and 46 days of age. Piglets housed in the same pen were tested together in a 3 m x 3 m isolated arena, so that they could not establish visual contact with other piglets in the same building. The arena had 2 circles painted on the centre of the floor, the first measuring 1 m in diameter (circle 1) and the second, which included the first one, measuring 1.5 m in diameter (circle 2). The four subjects were initially put into a waiting box (1 m x 1 m) close to the arena. Piglets stayed in the waiting box for 2 minutes. The OF test started when the box door was open and piglets could have access to the arena (Figure 1). After 10 minutes an object that was unfamiliar to the piglets (a 50 cm diameter blue ball) was dropped down into the arena and the NO test was started. The object fell exactly in the centre of the small circle painted on the floor, suspended 20 cm above the floor, without touching the floor or walls or making a noise; when the

ball fell, all piglets had a movement reaction. The NO test lasted 15 minutes so that the total duration of the two consecutive tests was 25 minutes.

All tests were video recorded with two Handy cams (DCR-HC42E, Sony). During video analysis, all behavioural patterns (for a description see Table 1) were recorded using the classical ethological methods (Altmann, 1974). In particular, during both tests the position of the animals in the arena, their activity (standing, lying, sitting and walking) and proximity within 1 meter to pen mates were recorded using the "instantaneous" sampling method (Altmann, 1974), with 60-second intervals, in order to obtain an estimation of the respective percentages of time that animals spent performing these behavioural patterns (Table 1). Explorative behaviours and affiliative and aggressive interactions among piglets were recorded using the "all occurrences" sampling method (Altmann, 1974), counting the total number of events. Moreover, during the NO test the latency and all occurrences of contact, exploration and attention to the object were recorded.

2.3 Statistical analysis

The normal distribution of residuals was checked for all variables by means of the Shapiro-Wilk test, using the PROC UNIVARIATE of SAS. The data of several variables were not normally distributed and were transformed in order to make them conform more closely to the assumption of analysis of variance (Fernandez, 1992). In determining whether a specific transformation value was appropriate for each data set variable, the Likelihood Ratio Test was used (Gurka et al., 2007). Measurements of time spent inside the circle, time spent walking and latency to leave the waiting box or to approach or contact the object were log10 transformed. Measurements of time spent outside the circle, lying and exploring mates were power transformed. The watching time variable was square root transformed. Aggressiveness, playing, return to the waiting box and exploring the object were not normalized using any of the transformations commonly used for this purpose and were analysed by means of a non-parametric test (NONPAR1WAY of SAS). The PROC MIXED procedure of SAS was used to analyse the normally distributed or normalized variables. The model included the random effect of subject within replicate, the fixed effects of the OF-NO test, Backtest, type of pen, sex, replicate and their respective interactions. Only the Backtest x Type of pen interaction was excluded because not estimable due to a confounding effect.

The same model was also used to compare the behaviour of LR piglets from homogeneous pens versus LR piglets from heterogeneous pens (the same for HR piglets).

All transformed variables were back-transformed for presentation in the tables to their original scale as arithmetic means (± standard error).

Correlations between behavioural variables in the OF or NO tests and Backtest were analysed using the Spearman rank correlation coefficient (r_s). The probability of rejecting the null hypothesis was set at 0.05.

3. RESULTS

Averaged over the two Backtests, HR pigs showed a mean 6.54±2.18 SE of escape attempts and LR pigs 1.64±0.97 SE. The correlation between Backtests conducted on different test days was 0.61 (p < 0.00001).

We did not find any significant correlations between the behavioural patterns displayed by piglets during the OF or NO tests and the Backtest scores (Table 2).

The effect of replicate was significant for almost of parameters, the only exceptions being events of watching, time spent inside circle 2, latency to leave the waiting box, and latency to contact the object. The interaction between replicate and OF-NO tests was significant (P<0.05) with respect to time spent outside or inside the circles, lying, sitting and exploring the area, while the other interactions were not significant (P>0.05).

HR and LR piglets did not differ in their individual behavioural response to either the OF or the NO test (Table 3). However, the piglets as a whole showed some differences in their behaviour during the OF and NO tests. Time spent outside the circles and lying and proximity were higher in the NO test, while time spent inside circle 1, time spent inside circle 2, sitting, walking, exploring the area and playing were higher in the OF test (Table 4). Moreover, we found that the type of pen influenced the time that piglets spent watching the object (piglets in LR pens watched the object more frequently than those in both the HR and mixed pens), as well as the time spent outside the circles (piglets in mixed pens spent more time outside the circles) (Table 5).

The sex of piglets did not affect their behavioural response during any of the tests, including the Backtest.

When comparing the behaviour displayed by piglets during the OF and NO tests we found very few significant correlations. The more time a piglet stayed in close proximity to its mates, the higher was the piglet's latency to contact the object (r_s=-0.52, p<0.001), to leave the waiting box and to contact the object (r_s=0.32, p<0.001). Moreover, the more often a piglet watched the

object, the higher was its latency to contact it (r_s=0.39, p<0.001), and, obviously, the slower its approach to the object was, the less frequent was its exploration (r_s=-0.66, p<0.001).

4. DISCUSSION

In accordance with the findings of other studies, the piglets' behaviour was consistent during the Backtests, since the correlation between days 10 and 17 was quite high (r_s= 0.61 versus r_s=0.47 and r_s=0.48 of Van Erp-Van der Kooij et al., 2000, 2002).

We did not find any differences between HR and LR piglets or among the different pens (HR, LR and mixed) in terms of behavioural reactions during the Open Field and Novel Object tests, except for time spent outside the circles and watching the object. These results do not support the view that HR and LR pigs differ in their ability to adjust their behaviour to a changing situation (Bolhuis et al., 2005).

Some correlations were found between the behavioural patterns observed during the OF and NO tests. The interpretation of "Latency to leave waiting box" depends to a large extent on the nature of the waiting box and of the arena. If the waiting box is the animal's home pen, the difference between this and the arena is the difference between familiarity (i.e. relative safety) and unfamiliarity (i.e. potential danger). If, however, the waiting box is novel to the animal, and if the animal belongs to a social species it may represent danger (unfamiliarity and social isolation) and is therefore an aversive stimulus (the animal is already in this situation). The arena, even though novel and thus potentially dangerous, represents the only way out of the waiting box, and therefore out of the already dangerous situation. The animal faces a choice not between a safe starting point (e.g. home pen), and a potentially threatening novel environment (e.g. arena), but between two fear-evoking situations, one already present and known (the waiting box), the other unknown (Misslin and Cigrang, 1986). In our OF test, the waiting box was of the same size and had the same type of floor as the home pen and all subjects were tested with their home pen mates, so unfamiliarity and social isolation were reduced, though probably not eliminated. For this reason, the "Latency to leave waiting box" could be considered a measure of fear or timidity in leaving the familiar environment (Erhard and Mendl, 1999), explaining why it correlated with the "Latency to contact object", which may be another indicator of fear. Moreover, piglets who took longer to approach and touch the object explored it few times, showing an inclination to avoid new stimuli.

Since in the Novel Object test "Latency to contact object" was also correlated with "Proximity", it is possible that subjects that were more affected by novelty of the object more frequently sought the proximity of their mates to feel safe. Moreover, they showed anxiety by spending a great deal of time watching the object, staying away from it and refraining from exploring it (Wemelsfelder et al., 2000).

Piglets probably displayed explorative behaviours and played more often during the Open Field than during the Novel Object test because the arena represented a novelty, larger than pens and therefore more suitable for playing. In the Open Field test, the piglets' tendency to investigate could naturally decrease over time; however, the habituation period in the arena test is generally neglected and arbitrarily fixed as a short period without any assessment of the habituation process and its potential effects on further responses (Forkman et al., 2007). The subsequent inactivity and tendency to seek the proximity of pen mates displayed by piglets during the Novel Object test might indicate a state of tension induced in the animals due to the sudden fall of the unknown object.

Our results suggest that individual differences between HR and LR piglets result in few behavioural occurrences when animals are subjected with their pen mates to novel situations.

If only two different behavioural "types" of pigs exist, as suggested by Hessing (1993, 1994), we would expect that when animals are tested in groups made up of subjects belonging to the same "coping style", more marked behavioural differences between these two categories would emerge because all group members should show the same behavioural patterns. Since we failed to find any relevant differences in behavioural response among the different groups of piglets, we might suppose that being in group could reduce individual behavioural differences. An alternative explanation is that the Backtest is a poor predictor of behaviour in other situations, regardless of whether pigs are tested in groups or singly, as other studies have shown (Forkman et al., 1995; Erhard et al., 1999; van Erp-van der Kooij et al., 2000). Moreover, as highlighted by several studies, it is possible that different tests measure different aspects of an animal's 'coping style' or different dimensions of the personality (Gosling and John, 1999; Van Erp-Van der Kooij et al., 2002), but in certain cases coping behaviour turns out to be situation specific, an observation which supports the opinion of those who challenge the existence of coping styles (Forkman et al., 1995; Spoolder et al., 1996).

The question of whether pigs can really be classified into distinct classes of individuals, differing consistently in their manner of reaction, is of central relevance to pig husbandry. Further research

will be useful to clarify if such individual differences can be identified, because such knowledge could potentially be used not only to increase the efficiency of pig production, but also as a useful tool for improving animal welfare.

Acknowledgments

REFERENCES

Altmann, J., 1974. Observational study of behavior: sampling methods. Behaviour 48, 227-265.

Bell, A.M. 2007. Future directions in behavioural syndromes research. Proceedings of the Royal Society B-Biological Sciences 274, 755-761.

Benus, R.F., Bohus, B., Koolhaas, J.M., van Oortmerssen, G.A., 1991. Heritable variation for aggression as a reflection of individual coping strategies. Experientia 47, 1008–1019.

Beattie, V.E., Walker, N., Sneddon, I.A., 1995. Effect of rearing environment and change of environment on the behaviour of gilts. Appl. Anim. Behav. Sci. 46, 57–65.

Bolhuis, J. E., Schouten, W. G. P., J. A., Schrama, J. W. & Wiegant, V. M. 2005. Behavioural development of pigs with different copyng characteristics in barren and substrate-enriched housing conditions. Appl. Anim. Behav. Sci. 93, 213-228.

Brown, J. A., Dewey, C., Delange, F.M., Mandell, I.B., Purslow, P.P., Robinson, J.A., Squires, E.J. Widowski, T., 2009. Reliability of temperament tests on finishing pigs in group-housing and comparison to social tests. Appl. Anim. Behav. Sci. 118, 28-35.

Dalmau, A., Fabrega, E., Velarde, A., 2009. Fear assessment in pigs exposed to a novel object test. Appl. Anim. Behav. Sci. 117, 173–180.

D'Eath, R.B., 2002. Individual aggressiveness measured in a resident-intruder test predicts the persistence of aggressive behaviour and weight gain of young pigs after mixing. Appl. Anim. Behav. Sci. 28, 264–283.

Erhard, H.W., Mendl, M, 1999. Tonic immobility and emergence time in pigs - more evidence for behavioural strategies. Appl. Anim. Behav. Sci. 61, 227–37.

Fernandez G.J.C. 1992. Residual analysis and data transformations: Important tools in statistical analysis. Hort. Science, 27, 297-300

Forkman, B., Furuhaug, I.L., Jensen, P., 1995. Personality, coping patterns, and aggression in piglets. Appl. Anim. Behav. Sci. 45, 31–42.

Forkman, B., Boissy, A., Meunier-Salaün, M.-C., Canali, E., Jones, R.B., 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. Physiology & Behav. 92, 340–374.

Geverink, N. A., Schouten, W. G. P., Gort, G. & Wiegant, V. M. 2003. Individual differences in behaviour, physiology and pathology in breeding gilts housed in groups or stalls. Appl. Anim. Behav. Sci. 81, 29–41.

Gosling, S.D., John, O.P. 1999. Personality dimensions in non-human animals: a cross-species review. Curr. Dir. Psychol. Sci. 8, 69-75.

Gurka, M.J., Edwards L.J.,, Nylander-French, L. 2007. Testing transformations for the linear mixed model. Comput. Stat. Data Anal, 51, 4297-4307.

Hessing, M.J.C., Hagelsø, A.M., van Beek, J.A.M., Wiepkema, P.R., Schouten, W.G.P., Krukow, R., 1993. Individual behavioural characteristics in pigs. Appl. Anim. Behav. Sci. 37, 285–295.

Hessing, M.J.C., Hagelsø, A.M., van Beek, J.A.M., Wiepkema, P.R., Schouten, W.G.P., 1994. Individual behavioural and physiological strategies in pigs. Physiol. Behav. 55, 39–46.

Jensen, P., Rushen, J., Forkman, B., 1995. Behavioural strategies or just individual variation in behaviour? A lack of evidence for active and passive piglets. Appl. Anim. Behav. Sci. 43, 135–139.

Koolhaas, J.M., De Boer, S.F., Bohus, B., 1997. Motivational systems or motivational states: behavioural and physiological evidence. Appl. Anim. Behav. Sci. 53, 131–143.

Koolhaas, J.M., de Boer, S.F., Buwalda, B, van Reneen, K., 2007. Individual variation in coping with stress: A multidimensional approach of ultimate and proximate mechanisms. Brain Behav. Evol. 70, 218–226.

Lawrence, A.B., Terlouw, E.M.C., Illius, A.W., 1991. Individual differences in behavioural responses of pigs exposed to non-social and social challenges. Appl. Anim. Behav. Sci. 30, 73–86.

Misslin, R., Cigrang, M., 1986. Does neophobia necessarily imply fear or anxiety? Behav. Processes 12, 45–50.

Ruis, M. A. W., Te Brake, J. H. A., Van de Burgwal, J. A., De Jong, I. C., Blokhuis, H. J., Koolhaas, J. M., 2000. Personalities in female domesticated pigs, behavioural and physiological indications. Appl. Anim. Behav. Sci. 66, 31–47.

Ruis, M.A.W., Te Brake, J.H.A., Engel, B., Buist, W.G., Blokhuis, H.J., Koolhaas, J.M., 2002. Implications of coping characteristics and social status for welfare and production of paired growing gilts. Appl. Anim. Behav. Sci. 75 (3), 207– 31.

Spoolder, H.A.M., Burbidge, J.A., Lawrence, A.B., Simmins, P.H., Edwards, S.A., 1996. Individual behavioural differences in pigs: intra- and inter-test consistency. Appl. Anim. Behav. Sci. 49, 185– 198.

Thodberg, K., Jensen, K.H., Herskin, M.S., 1998. A general reaction pattern across situations in prepubertal gilts. Appl. Anim. Behav. Sci. 63, 103–119.

Van Erp-Van der Kooij, E., Kuijpers, A. H., Schrama, J. W., Ekkel, E. D. & Tielen, M. J. M. 2000. Individual behavioural characteristics in pigs and their impact on production. Appl. Anim. Behav. Sci, 66, 171–185.

Van Erp-van der Kooij, E, Kuijpers, A.H., Van Eerdenburg, F.J.C.M. & Tielen, M.J.M. 2001. A note on the influence of starting position, time of testing and test order on the Backtest in pigs. Appl. Anim. Behav. Sci, 73 (4), 263– 266.

Van Erp-van der Kooij, E, Kuijpers, A.H., Schrama, J. W., Van Eerdenburg, F.J.C.M., Schouten W.G.P. & Tielen, M.J.M. 2002. Can we predict behaviour in pigs? Searching for consistency behaviour over time and across situations. Appl. Anim. Behav. Sci, 75, 293– 305.

Wemelsfelder, F., Haskell, M., Mendl, M.T., Calvert, S., Lawrence, A.B., 2000. Diversity of behaviour during novel object tests is reduced in pigs housed in substrate-impoverished conditions. Anim. Behav. 60, 385–394.

Figure 1.
The guillotine door on the Waiting box could be opened and closed through the use of an attached rope. Another attached rope was used to drop the ball, so the observers could not be seen by the animals
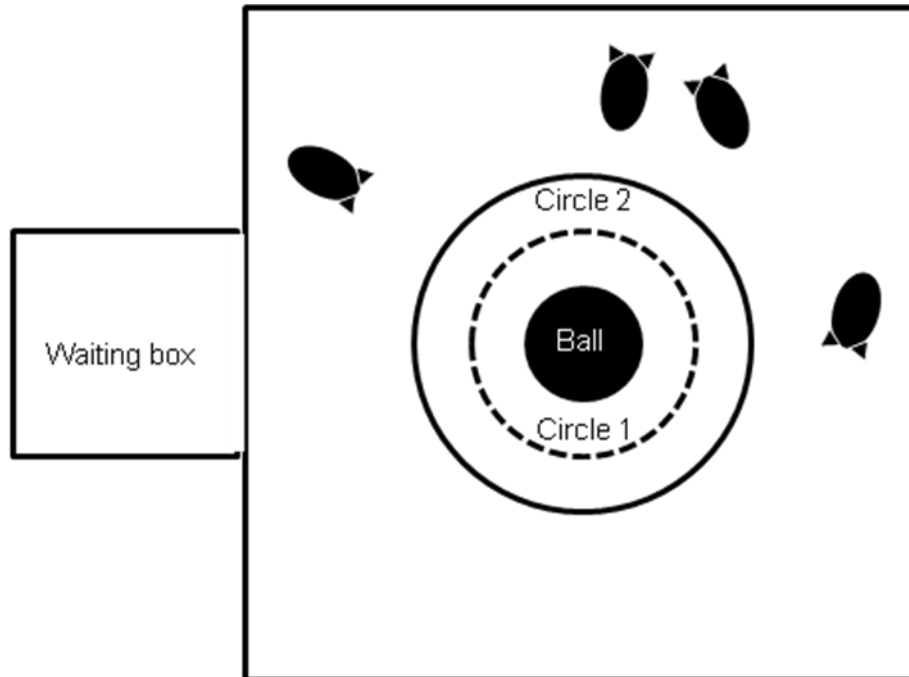
Table 1.
Behavioural patterns recorded during the Open field and Novel Object tests.
Variables marked by * were sampled by interval sampling in 60-s intervals. Variables marked by † were sampled only in NO. Latency to leave waiting box was sampled only in OP. All latencies were measured in seconds.

Behavioural patterns[a]	Description
Position*	% time spent out of circles, inside circle 1, inside circle 2.
Standing*	% time spent standing on its legs without exploring, with or without head movements.
Lying*	% time spent lying in a ventral or sternal position with or without exploring.
Walking*	% time spent walking with or without exploring.
Proximity*	% time spent within 1 m from one or more mates.
Exploring area	Smelling, biting, licking or touching the floor or the walls.
Exploring mate	Smelling, sucking, licking or touching one or more mates.
Aggressiveness	Biting, pushing, climbing one or more mates.
Playing	Running fast or slowly with vertical and horizontal bouncy movements, jumping around to face in a different direction.
Return in waiting box	Visiting the waiting box entering into and exiting from the box one or more times.
Latency to leave waiting box	Seconds until the body exited from the waiting box, the front legs cross the line of the door.
Watching[†]	Watching the ball without smelling or touching it.
Exploring object	Smelling, biting, licking or touching the ball.
Latency to approach 1[†]	Second until the front legs entered circle 1.
Latency to approach 2[†]	Second until the front legs entered circle 2.
Latency to contact object [†]	Seconds until the snout touched ball.

[a]Based on Thodberg et al. (1998), Spoolder et al. (1996), Beattie et al. (1995)

Table 2.
Correlation of behavioural patterns displayed during the Open Field (OF) test and the Novel Object (NO) test with the Backtest score.

Behavioural patterns	Backtest score	
	Open Field test	Novel object test
time spent out of circles	r_s= -0.008 p=0.92	r_s= -0.117 p=0.04
time spent inside circle 1	r_s= 0.051 p=0.55	r_s= 0.022 p=0.8
time spent inside circle 2	r_s= -0.032 p=0.71	r_s= 0.208 p=0.01
Lying	r_s= -0.063 p=0.47	r_s= 0.083 p=0.34
Sitting	r_s= -0.017 p=0.84	r_s= -0.127 p=0.14
Walking	r_s= 0.083 p=0.34	r_s= 0.060 p=0.49
Proximity	r_s= -0.113 p=0.19	r_s= -0.120 p=0.16
Exploring area	r_s= -0.066 p=0.45	r_s= 0.033 p=0.69
Exploring mate	r_s= -0.041 p=0.64	r_s= 0.002 p=0.97
Aggressiveness	r_s= 0.155 p=0.07	r_s= -0.157 p=0.07
Playing	r_s= 0.051 p=0.55	r_s= 0.051 p=0.55
Return in waiting box	r_s= 0.156 p=0.07	r_s= -0.042 p=0.63
Latency to leave waiting box	r_s= 0.099 p=0.25	-
Watching	-	r_s= -0.178 p=0.04
Exploring object	-	r_s= 0.057 p=0.51
Latency to approach 1	-	r_s= -0.095 p=0.27
Latency to approach 2	-	r_s= -0.153 p=0.07
Latency to contact object	-	r_s= -0.068 p=0.42

Table 3
Effect of Backtest on behavioural patterns.

Behavioural patterns	Backtest	
	HR (mean ± S.E.)	LR (mean ± S.E.)
Time spent out of both circles[†]	73.87 ± 20.00	76.37 ± 17.30
Time spent inside circle 1[†]	5.73 ± 11.37	4.98 ± 8.01
Time spent inside circle 2[†]	20.25 ± 15.25	18.49 ± 14.97
Standing[†]	48.74 ± 18.99	47.38±20.09
Lying[†]	20.02 ± 20.76	23.03 ± 22.33
Walking[†]	31.23 ± 18.68	29.59 ± 16.64
Proximity[†]	60.28 ± 19.99	65.25 ± 20.24
Exploring area[+]	1.25 ± 0.27	1.26 ± 0.60
Exploring mate[+]	0.12 ± 0.12	0.13 ± 0.12
Exploring object[+]	0.17 ± 0.27	0.16 ± 0.24
Latency to approach 1*	132.42 ± 279.98	128.89 ± 247.14
Latency to approach 2*	90.46 ± 234.29	96.64 ± 233.43
Latency to contact object*	158.17 ± 304.17	157.61 ± 304.37
Aggressiveness[+]	0.01 ± 0.04	0.01 ± 0.05
Playing[+]	0.59 ± 0.55	0.57 ± 0.55
Return in waiting box[+]	0.11 ± 0.12	0.10 ± 0.11
Latency to leave waiting box*	4.04 ± 6.13	3.60 ± 5.94
Watching[+]	0.16 ± 0.22	0.19 ± 0.24

* Seconds.
[†] % of time.
[+] Number of actions/minutes.
Means with different uppercase or lowercase superscript letters differ respectively for P<0.01 and P< 0.05.

Table 4.
Effect of Open Field - Novel Object on behavioural patterns.

Behavioural patterns	Open Field and Novel Object	
	Open Field (mean ± S.E.)	Novel Object (mean ± S.E.)
Time spent out of both circles[†]	68.78^A ± 17.77	81.71^B ± 17.07
Time spent inside circle 1[†]	7.04^A ± 11.44	3.58^B ± 7.05
Time spent inside circle 2[†]	24.01^A ± 15.07	14.54^B ± 13.60
Standing[†]	50.28^a ± 18.71	45.68^b ± 20.21
Lying[†]	10.97^A ± 14.92	32.39^B ± 22.09
Walking[†]	38.73^A ± 16.59	21.92^B ± 14.21
Proximity[†]	57.72^A ± 18.68	68.33^B ± 20.41
Exploring area[+]	1.67^A ± 0.45	0.84^B ± 0.37
Exploring mate[+]	0.13 ± 0.12	0.13 ± 0.11
Aggressiveness[+]	0.02 ± 0.05	0.01 ± 0.03
Playing[+]	0.98^A ± 0.05	0.18^B ± 0.18
Return in waiting box[+]	0.14 ± 0.14	0.7 ± 0.7

* Seconds.
[†] % of time.
[+] Number of actions/minutes.
Means with different uppercase or lowercase superscript letters differ respectively for P<0.01 and P< 0.05.

Table 5.
Effect of composition of pen on behavioural patterns.

Behavioural patterns	Composition of pen			Test
	LR (mean ± S.E.)	HR (mean ± S.E.)	Mixed (mean ± S.E.)	
Latency to leave waiting box*	3.56 ± 5.88	4.35 ± 6.99	3.27 ± 3.92	OF
Watching[+]	0.20^a ± 0.25	0.14^b ± 0.21	0.16^{ab} ± 0.21	NO
Exploring object[+]	0.16 ± 0.24	0.17±0.28	0.15 ± 0.21	NO
Latency to approach 1*	126.32 ± 273.30	132.82 ± 284.35	132.14 ± 272.73	NO
Latency to approach 2*	102.04^a ± 242.10	101.44^a ± 255.55	58.33^b ± 151.07	NO
Latency to contact object*	141.93 ± 293.54	155.40 ± 304.16	197.66 ± 330.36	NO
Time spent out of both circles[†]	76.86^{ab} ± 17.02	76.04^a ± 18.87	69.65^b ± 20.83	OF+NO
Time spent inside circle 1[†]	4.63 ± 7.43	5.52 ± 10.76	6.59 ± 11.95	OF+NO
Time spent inside circle 2[†]	18.30 ± 14.83	18.26 ± 14.10	23.75 ± 11.06	OF+NO
Standing[†]	48.11 ± 19.82	48.24 ± 18.97	47.15 ± 20.51	OF+NO
Lying[†]	23.48 ± 22.59	19.99 ± 21.62	20.55 ± 19.27	OF+NO
Walking[†]	29.39 ± 16.43	31.76 ± 19.29	32.29 ± 16.54	OF+NO
Proximity[†]	65.19 ± 19.89	60.10 ± 20.81	63.47 ± 19.64	OF+NO
Exploring area[+]	1.27 ± 0.61	1.22 ± 0.58	1.29 ± 0.53	OF+NO
Exploring mate[+]	0.13 ± 0.12	0.11 ± 0.12	0.15 ± 0.11	OF+NO
Aggressiveness[+]	0.01 ± 0.04	0.01 ± 0.04	0.02 ± 0.05	OF+NO
Playing[+]	0.56 ± 0.56	0.61 ± 0.58	0.57 ± 0.47	OF+NO
Return in waiting box[+]	0.10 ± 0.11	0.11 ± 0.11	0.12 ± 0.14	OF+NO

* Seconds.
[†] % of time.
[+] Number of actions/minutes.
Means with different uppercase or lowercase superscript letters differ respectively for P<0.01 and P< 0.05.

CHAPTER 3

EFFECT OF LONG TRANSPORT AND ENVIRONMENTAL CONDITIONS ON BEHAVIOUR AND BLOOD PARAMETERS OF PIGLETS WITH DIFFERENT GROUP COMPOSITION

Diego Magnani [a,], Simona Cafazzo [a], Pietro Calà [a], Elisabetta Razzuoli [b], Massimo Amadori [b] and Leonardo Nanni Costa [a*]

[a] *Dipartimento di Protezione e Valorizzazione Agroalimentare (DIPROVAL), Facoltà di Agraria, Università degli Studi di Bologna, via Fratelli Rosselli 107, 42100 Reggio Emilia, Italy*
[b] *Laboratorio immunologia cellulare, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "B. Ubertini", via Bianchi 7/9 - 25124 Brescia, Italy*

* Corresponding author: Tel.: +390522290515 E-mail address: leonardo.nannicosta@unibo.it (L. Nanni Costa).

Article *submitted*

.

ABSTRACT

In order to evaluate the effect of long transport on weaned piglets transported in warm weather condition with different group composition, one-hundred and forty-four piglets, previously submitted to back test, were monitored during four journeys of 14 hours carried out from May to September. During transport, truck air temperature, skin temperatures, postural and behavioural occurrences were recorded. Prior to and after transportation blood samples were taken for analysis of physiological measures and body weight was also recorded. Environmental conditions differed during transport and between journeys (THI values, $P<0.05$). In general, piglets showed more activities during the first 4 hour of transport (difference in standing position and exploring behaviours, $P<0.05$), especially when the temperature was around 20°C. Piglets behaviour was significantly influenced by the type of pen composition since some differences were found in mixed groups (differences in exploring behaviours and biting objects, $P<0.05$). Conversely no differences were found according the result of back test classification. Changes in the frequency of behaviours can be used as indicators of the immediate challenges faced by piglets during transport and as a measure of ability to cope with these stressors. Significant variation with respect the baseline were found only for glucose which decreased ($P<0.05$) and for urea which increased ($P<0.05$) as a result of the prolonged fasting. Haptoglobin showed differences(in what direction?) in pre- and in post-transport depending on the period of journey ($P<0.05$).

The results showed that journeys of 14 hours did not have consistent effects on physiological and behavioural parameters of weaned piglets.

1. INTRODUCTION

Transport represents a critical phase in animal production and is often considered as one of the main causes of stress (Mormede et al., 1982), with possible negative repercussions on health and welfare. Physical stress (fasting, fatigue, injury, and thermal extremes) and psychological stress (novelty, restraint and handling) could be experienced by the animals during transport inducing adaptive responses at the physiological and behavioural levels (Grandin, 1997).

As recently reviewed by Lambooij (2007), several and detailed researches were carried out to evaluate and indentify factors affecting the welfare of pigs during transport. A large part of these

studies are focused on commercial pigs destined to the slaughterhouse while there are not so many those concerning piglets. In general, the studies on piglets showed that long transportation can be a severe stressor causing weight loss due to dehydration and feed withdrawal (Berry and Lewis, 2001; Lewis et al., 2005). These negative effects can be exacerbated if temperature reaches 35° C (Lewis et al., 2003). Temperature is a crucial factor governing the amount of stress an animal experiences during transport. The application of acute cold and heat stress showed that piglets altered activity and resting times during attempts to cope with these stressors (Hicks et al., 1998). The European Food Safety Authority (EFSA) recommended a comfort zone between 20°C and 30 °C for piglets less of 10 kg and between 14°C to 32°C for pigs up to 30 kg live weight (EFSA, 2004).

Regarding the duration of transport, the Council Regulation (EC) No 1/2005 states that long journeys (> 8 h) are permitted if piglets are heavier than 10 kg. Where long transport of early weaned piglets (4-7 kg) is allowed, in journeys of 24 h the lying frequency increased and standing and sitting frequencies decreased after the first 12 h of transport (Lewis and Berry, 2006). Increasing transport duration was associated not only with some indicators of habituation but also with increasing drinking post transport and higher hematocrits, indicative of dehydration and thirst (Lewis and Berry, 2006).

Pigs vary largely in behavioural reactions when exposed to the same stressful situation. Individual differences in adaptive or coping reactions of pigs have received growing attention, as the identification of basic characteristics that predict (mal)adaptation to husbandry conditions could be relevant both for pig husbandry and pig welfare (Bolhuis, 2005) In order to identify these characteristics, the Backtest (BT) has been used. During the test a piglet is put on its back and restrained in this position for 1min, while the number of escape attempts is recorded. Some piglets usually referred to as 'high-resisters' (HR), struggle a lot during the backtest, whereas piglets at the other end of the distribution tend to respond with immobility, the so-called 'low-resisters' (LR). The response profiles of HR and LR piglets largely resemble the diverging coping styles, often referred to as (pro-) active versus passive/reactive. Though criticized for the problems with interpretation, and for the arbitrary cut-off points used to classify animals (Jensen et al., 1995), several studies have shown that these two extreme responders in the backtest differ in various behavioural and physiological variables (Bolhuis et al., 2000; Geverink et al., 2002; Hessing et al., 1995; Schrama et al., 1997). Despite the interest for the potential differences in adaptive or coping reactions between HR and LR subjects, there are not studies where these differences were tested during a stress situation such as long transport.

The aim of this study was to determine the effect of long transport in different warm weather conditions on the behavioural and physiological responses of weaned piglets with special emphasis on group composition based on the different reactivity to backtest. In particular, on the basis of previous research carried out in different stress situations, we would expect to find a higher level of stress in HR subject and in general in transports with aversive weather conditions.

2. MATERIALS AND METHODS

All animal procedures were approved by the University of Bologna Ethical Committee.

2.1 Piglet care and housing

From April to August 2009, four batches of 60 piglets from commercial crossbred pigs ((Landrace x Large White) x Duroc) were submitted to back test twice during the suckling period, once at 10 days and once at 17 days. Classification of pigs was based on the number of escape attempts (i.e. bouts of struggling with at least the hind legs) they displayed during 60 s. A pig was classified as high-resisting (HR) if it performed more than four escape attempts in the two tests, with a minimum of two attempts in one test. If a pig struggled less than four times in two tests, with a maximum of two attempts in one test, it was labelled low-resisting (LR) (Bolhuis et al., 2003). On the basis of the back test, four groups of 48 piglets (24 HR and 24 LR) were selected and identified by auricular marker (an ear tag). Groups were weaned at 25 ± 2 days of age. At 33 ± 2 days of age piglets were weighed (7.6 ± 1.2 kg) and transported for 1 hour to the experimental farm of DIPROVAL. Each group was housed in 12 pens (1 m x 1 m) with 4 piglets per pen. According to backtest result, 4 HR pens (H), 4 LR pens (L) and 4 mixed pens (M), the latter with 2 HR and 2 LR piglets, were arranged. The pens with rubber slatted floor were lift off the ground . The space allowance was 0.20 m^2 per head (Council Directive 2008/120/EC). Temperature was held at 27° C, each pen was fed two times a day with a post weaned commercial feed (CP 18.5%, Lysine 1.20%, 3500 Kcal ME/kg , G.I.Ma. S.p.a., Italy). Water was supplied at libitum using a water-nipple.

On the basis of their fitness for transport, 36 piglets (18 HR and 18 LR) for each group were placed on trial in one of the four journeys carried out in May, June, July and September 2009.The piglets were allowed to acclimate to their new environment and pen mates for one week before transportation began.

2.2 Trailer design and transport condition

A commercial multi-tier vehicle approved for long transportation (> 8 hours), equipped with mechanical ventilation and water supply systems was used. On the lower deck, eight pens whit wood shavings bedding were arranged. Space allowance was 0.15 m^2 per head as suggested by Lambooij (2007). Drinking system was changed after the first journey. Pipelines were used to move the nipples from the whole of the vehicle to inside the pens.

Piglets were loaded into pens of the lorry by a trolley keeping the same composition of pens as in the experimental farm except for front and rear pens where six piglets were mixed at loading (MAL) (fig. 1).

Once piglets were loaded onto the vehicle, the vehicle was driven for a period of 14 hours on the same highway route (830 Km) and by the same drivers before it returned to the experimental farm. Further details on journey characteristics are provided in Table 1. The piglets were loaded onto the lorry at 02.00 a.m. and the journey started one hour late. Unloaded was started around 05-00 p.m. In order to check the animals conditions, two stops of 30 minutes were carried out around 7.00 a.m. (Stop 1) and 12.00 p.m. (Stop 2).

Four cameras (CIR 14/68, Fracarro, Italy) were placed over the pens and connected to a DVR recording system (DVR 2036T/R, Fracarro, Italy). The positioning of the cameras required 1.80 m of distance between the floor and the ceiling. Thus, the available volume of the deck was very wide and, in order to reach the highest environmental temperature, both natural and mechanical ventilation were not used. Differences between experimental transport and commercial transport were accepted in order to have more control over the experimental parameters.

Air temperature and relative humidity were recorded by probes (Pro v2 logger, HOBO, USA) placed on the side of pens (approximately 40 cm above the floor). Thermal Humidity Index (THI) was calculated using the formula of Kelly and Bond (1971). Air quality was monitored using a probe for CO_2 and NH_3 (Drager x-am 5000, Germany) which was placed in the front part of the deck (fig. 1) where the ventilation is less efficient (Kettlewell et al., 2005).

Piglets were weighed around the 04.00 p.m. of the day before transport. The body weights (mean ± SD) of loaded piglets in May, June, July and September journeys were 9.8 ± 1.6 kg, 10.3 ± 2.0 kg, 10.7 ± 1.3 kg, and 8.5 ± 1.3 kg, respectively After weighing, the feed in the troughs was removed. Piglets were re-weighed within two hours from unloading. Piglets were not fed during transport while water was available continuously as requires by the EC Reg. No.1/2005.

2.3 Behavioural measures

Behaviour of transported subjects was recorded during transport. All cameras were connected to a DVR recording system and were fastened at the same angle and height, so that the recorded area was similar among pens. Twenty minutes was observed for each hour of transport. All behavioural patterns are described in Table 2. Behaviours were quantified using scan sampling (1 min intervals) for Exploring environment, Biting objects, Exploring mate and continuous sampling for Aggressiveness, Belly nosing and Drinking. The animal posture was identified by the following mutually exclusive behaviours: Standing, Resting and Sitting, all expressed in percentage. Only one observer previously trained to report consistent data from video tapes was used to evaluate behaviours.

2.4 Blood sampling

The day before transport piglets were blood sampled as baseline reference values. Blood sampling was repeated immediately after unloading. Blood samples were collected from the jugular vein in lithium heparin-coated tubes and in tubes without anticoagulant. One aliquot (1 ml) of whole blood was stored at 4°C. The remaining volume of heparinised blood was centrifuged at 2,000 rpm for 15 minutes and the resulting plasma was stored in aliquots at -20°C for subsequent analyses. Serum aliquots were stored at -80°C until analysis. Whole heparinised blood samples were used to determine the haematocrit by means of an impedance cell counter (Cell-Dyn 3500, ABBOTT, Chicago, Illinois, USA). Plasma samples were used to determine the concentrations of glucose (Kit Roche, code 11447513216), lactate (Kit Randox, code LC2389), creatine kinase (CK) (Kit Roche, code 12132524216), urea (Kit Roche, code 11489364216), using a multi-analyzer (Syncron CX5, Beckman, Fullerton, CA). Serum samples were used for assays of cortisol (Kit Cortisol Immulite, Medical System, code LKC01), haptoglobin (Kit Phase Haptoglobin, Celbio, code TP 801), albumin and total protein (Kit Roche code TP 1553836).

2.5 Skin temperatures

Maximum skin surface (a circular spot with 10 cm of diameter on the back of the animals) temperature was recorded by a thermo-camera Flir P640 (Flir System, Milan). Dorsal images of each piglet into the truck were recorded by the same operator before the start, during the two stops and at the end of journey from a distance of 0.5 m, settling the camera with an emissivity of 0.98. Camera was also set for temperature and relative humidity measured inside the vehicle by

portable Thermo-Hygrometer (HI9065, Hanna, USA). Images were processed using the software Therma CamPro2.9 Researcher (Flir System, Milan).

2.6 Statistical Analyses

For statistical analysis, the journey was divided in three periods, i.e. the first between departure and Stop 1 (Period 1), the second between the two stops (Period 2) and the third between Stop 2 to arrival (Period 3) which were expected to be characterized by different environmental condition. Position in the deck was considered classifying the pens as located in the front or in the rear. Concerning the behavioural data analysis, the percentages of time spent of resting, sitting and standing was normalized by log transformation and analysed using PROC MIXED of SAS (2006). The model included the fixed effect of journey (4 levels), Period (3 levels), backtest classification (2 levels), type of pen (4 levels), position in the deck (2 levels), sex (2 levels), the respective interactions, and the random effect of subject within journey. PROC GLIMMIX of SAS was used to generate least squares means of the other behavioural occurrences using the same model described above.

Data of blood parameters were analysed using PROC MIXED of SAS. The model included the fixed effect of journey (4 levels), sampling time (2 levels), backtest classification (2 levels), type of pen (4 levels) position in the deck (2 levels), sex (1-2), the respective interactions, and the random effect of subject within journey. The weight of piglets before the journey was included as covariate but it never reach the statistical significance (P>0.05); thus it was removed from the model. Data were transformed to meet assumptions of homogeneity of variance and normality of residuals. Cortisol, creatine kinase, lactate were subjected to log10 transformations while inverse, square and square root transformations normalized data of albumin, total protein and haptoglobin, respectively. All transformed estimates were back-transformed for presentation to their original scale as arithmetic means. Data of percent body weight loss and skin temperature recorded by thermo-camera were analysed using PROC MIXED of SAS using the same model used for the data of blood parameters but without the effect of sampling time. Data of THI recorded during the four journeys was analysed using PROC GLM. The model included the journey (1-4) and the period (1-3).

3. RESULTS

3.1. Environment conditions

Probe of CO_2 and NH_3 recorded a range from 0.04% to 0.10% and from 0 to 3.03 ppm respectively. The maximum levels recorded were below the thresholds of 0.15% CO2 and 10 ppm NH3 indicated (suggested) by Madec (2001). In general, the gaseous concentration slightly increased during the two stops, reaching the highest values during those occurred on July's journey.

THI and temperature values are shown in Table 3. THI variation during transport is shown in Figure 2, for all three periods, THI was significant higher in the journey of July than in other journeys. In all journeys, the lowest value of THI was recorded during Period 1 while the highest one during Period 3. During Period 3 of July journey the level of THI was over the limit of 75 indicated as Alert (NVSCR, 1976) and over the maximum limit of comfort zone indicated by EFSA (2004).

3.2. Body weight

Percent body weight loss was greater (P<0.05) after the journeys carried out in May and in September, compared with June and July journeys. In the former the weight loss reached 5.7 % and 5.8% respectively, while in the latter journeys it was 4.6% and 4.8% respectively.

3.3 Skin temperature

Mean of skin temperatures measured at the surface of the back in July's journey (37.8°C) were significantly higher than back temperature measured in May (34.8°C), June (36.7°C) and September (36.6°C).

3.4. Behaviour during transport

All behaviours included in Table 2 were displayed except for drinking that was never observed. The effect of journey was significant for all parameters with the only exception of Biting objects. Periods showed a significant effect on all Postures, on Exploration of environment and mates, and on Biting objects, while Pen compositions affected Standing, Exploring mate and environments, Biting objects and Aggressiveness. Back test classification, position in the deck and sex did not affect the behavioural response of the piglets during transport.

There was a significant interaction between journeys and periods for all postures, Exploration of environment and mates, and Belly nosing. In Table 4 are reported the last square means of interaction. Results show different activity rates between periods and journeys. With the only exception of September's journey, activities significantly decreased from the first 4 hours in transport (Period 1) to the last 10 h in transport (Periods 2 and 3) as indicated by a reduction in Standing and Exploring behaviours and by a concomitant increase of Resting. In the May's journey a reduction of activity involves also Sitting and Belly nosing which significantly decreased (P<0.05) from Period 1 to Period 2. In the September's journey the effect of period was not evident with the only exception of significant reduction of exploring mates between period 1 and period 2 (P<0.05).

A behavioural pattern related to different THI conditions between journeys was not apparent, even if several differences in behaviour occurrences were found within all three periods. Sitting and Belly nosing showed differences between journeys in the Period 1 only. In the July's journey, corresponding to the highest THI values, the highest value of Standing and Exploring environment and lowest value of Resting were observed during Period 1.

Piglets behaviour was significantly influenced by the type of pen (Table 5). The subjects mixed at loading (MAL) showed the highest value in Exploring mate (P<0.05) but the lowest one in Exploring environments and biting objects (P<0.05). Significant differences in behaviour between HR and LR were found only for the percentage of time spent standing which was lower for LR subjects. The piglets of M pens showed the highest level of aggressive behaviour.

3.5. Blood parameters

The effects of journey, sampling time and their interaction were significant for Hematocrit, Glucose, Lactate, Urea, Albumin, and total protein. The sampling time did not affect Cortisol, Creatin Kinase and Haptoblobin. The type of pen showed a significant effect only on Haptoglobin while the position of the truck (front/rear) affected the plasmatic level of Urea only.

Least squares means of blood parameters for each journey at different sampling time are reported in Table 6. Haematocrit showed significant but slight variations after the journeys, decreasing in June and September journeys and increasing in May's journey only. Glucose concentrations were lower (P<0.05) than baseline in all journeys except for the May's journey. Lactate was unchanged after July and September journeys while increased in May and decreased in June journeys. There was a difference (P>0.05) in baseline Cortisol concentration in May and

September journeys but not after transport. In May and July journeys Albumin concentration was higher and lower than baseline, respectively. Urea was higher ($P<0.05$) in post transport sampling on all journeys with the only exception of the journey carried out in June. Total protein concentrations were significant lower than baseline in June and July but not during the other journeys.

Piglets transported in May had the lowest values of glucose and lactate before the journey but the highest values of glucose, lactate, total protein and haematocrit at the arrival. In journeys carried out on July and on September, piglets showed lowest values of glucose and albumin and a higher value of urea. After the journey of September, the lowest values of albumin and haematocrit were found.

Haptoglobin showed differences in pre- and in post-transport depending on the period of journey. The highest and lowest values were found for both pre- and post-transport in the journeys carried out in May and July, respectively.

4. DISCUSSION

During all journeys and for almost the duration of the transports, truck air temperatures were within the range of the comfort zone for early-weaned piglets suggested by EFSA (2004). Only during the Period 3 in the July journey the maximum limit was passed. Even if natural and mechanical ventilation were not used, the concentration of CO_2 and NH_3 remaining always low. The numbers of piglets loaded into the vehicle did not lead to an increase of concentration of these gases. Piglets lost close to 5% of the body weight over the 14 hours of transport. This is the result not only of the prolonged fasting but also the failure of drinking water although available. The loss of weight recorded in the present study is less than showed by early weaned piglets (Lewis et al., 2006).

As expected, skin temperatures were found higher in the journey where the internal temperature of the truck was high such as July. These results agree with previous finding of Nanni Costa et al. (2012) showing that the pattern of skin temperature measured by infrared thermo camera strictly followed the change in ambient temperature. Similar hair temperature variations according to the internal temperature of truck were reported by Lewis et al. (2006).

Changes in the frequency of behaviours can be used as indicators of the immediate challenges faced by piglets during transport and as a measure of ability to cope with these stressors. It is

interesting to observe that the activity of piglets in standing and exploring was limited to the first hour of transport. Increased resting with duration of transport is a pattern similar to that observed either on early weaned piglets (Lewis et al., 2006) and in older pigs, and is consistent with fatigue as described by Lambooij (1998; 2000). Habituation to transport conditions may also have contributed to increased levels of resting in the last 5 hours of transport.

Different environmental condition showed to affect the behaviour of piglets mainly in the first hours of the journey. In the transport carried out in July, the higher temperature during the period 1 (more than 5°C respect to the other journeys (more than 20° in the first period) is probably the cause of the highest standing and exploring activity and lowest resting in comparison to the activities recorded during the corresponding period of the other journeys. The highest temperature and a THI over 75, recorded in the Period 3 during the July's journeys did not affect the activity of piglets which remain more elevated with respect the previous journey carried out with lower temperature. Pigs that were heat-stressed generally had a depressed activity level and pigs mainly engaged in lying behaviour, and thus had significantly fewer feeding and standing periods (Hicks et al., 1998), the high temperature recorded in the afternoon could having contributed to decrease in piglets' activity at the end of transport. The September's journey, even if carried out in environmental condition very close to those recorded in May and June, showed in Period 2 and 3a behaviour pattern very different with respect to the others journeys. In fact, the activity of piglets transported in September remained high as shown by the highest standing and the lowest resting and exploring activity. Other uncontrolled factors than the environmental condition could be responsible of this result.

Surprisingly, during the journey piglets did not show any attempt to drink, even when the drinking system was changed to be very similar to this available at the experimental farm. A strong reduction of drinking was recorded in slaughter pigs submitted to long journeys (Lambooij, 2007), while there is not available information on this behaviour on piglets. Lewis et al. (2006) showed in piglets early weaned an increase of drinking after a long transport with respect to un-transported piglets.

Exploring mates was higher in MAL pens than in others pens and there was less aggressive behaviour in the latter ones. This finding is in contrast with was observed in fattening pigs which, when mixing occurs, fight with unfamiliar individuals (Arey and Franklin, 1995; Fraser, 1974). The low level of aggressiveness observed in transport may therefore be indicative of a novelty situation enough to delay establishment of a dominance hierarchy, as observed by Lewis (2006). In

M pens aggressiveness was higher than in other pens, this result is in contrast with Ruis et al. (2002) results which show that the most stable social relationship existed between HR/LR pairs.

The results of blood analysis showed that journeys lasting 14 hours slightly affected the piglets' homeostasis. At the end of transport significant variation with respect the baseline was found only for glucose which decreased and for urea which increased as a result of the prolonged fasting. Transport of 24 h was found to reduce plasma glucose concentration in slaughter weight pigs (Bertol et al., 2005). Little variations in hematocrit percentage were found after transport. Similar slightly variations in hematocrit over journey time were observed on piglets by Averos et al. (2009) and on gilts by Brye et al. (2011). Despite the absence of water consumption during transit, the values of hematocrit indicate that piglets after the journeys were not dehydrated. This finding is supported by the absence of increase in total protein and albumin concentrations which were found to be associated to dehydration in gilts (Bryer et al., 2011).

Both initial and final Haptoglobin concentrations were lower than those found by Averos et al. (2009) on piglets subjected to short and long commercial transport. The absence of significant changes after transport could not permit to assess if an acute phase responses occurred.

5. CONCLUSION

Under the condition of the present study, the results obtained by means of different physiological and behavioural indicators show that journeys of 14 hours did not have consistent effects on weaned piglets. Even if any difference between HR and LR individuals were found, grouping and mixing the subjects may affect how piglets cope with transport. Further studies are essential to shed light on the effect of transport on the performance and welfare of early-weaned piglets and to bring up new questions about the stress response of this species under challenging situation. On the other hand, the present results suggest the importance to take in account the coping style classification and the effect of grouping and mixing procedure to evaluate the effect of long transport.

REFERENCES

Arey D.S., Franklin M.F., 1995. Effects of straw and unfamiliarity on fighting between newly mixed growing pigs. Applied Animal Behavioural Science 45, 23–30.

Averós, X, Herranz, A., Sánchez, R., Gosálvez, L.F., 2009. Effect of the duration of commercial journeys between rearing farms and growing–finishing farms on the physiological stress response of weaned piglets. Livestock Science 122, 339–344.

Bergeron, R. and Lewis, N. J., 1997. Farm animal transportation, health and welfare; Farm animal welfare in Canada: New technologies, research and world trade. The Lennoxville Symposium, Lennoxville, QC. 1997 Jun. 06.

Berry, R.J., Lewis, N.J., 2001. The effect of duration and temperature of simulated transport on the performance of early weaned piglets. Canadian Journal Animal Science 81, 199–204.

Berry, R. J. and Lewis, N. J. 2001b. Guidelines for the transport of early-weaned piglets based on field observations and experimental trials. Manitoba Pork Council Research News. Dec. 2001.

Bryer, P.J., Sutherland, M.A., Davis, B.L., Smith, J.F., McGlone, J.J., 2001. The effect transport and space allowance on the physiology of breeding age gilts. Science, Volume 137, Issues 1–3, 58-65.

Bolhuis, JE, Schouten, W.G.P., De Jong I.C., Schrama J.W., Cools A.R., Wiegant V.M., 2000. Responses to apomorphine of pigs with different coping characteristics. Psychopharmacology 152, 24– 30.

Bolhuis, J. E., Parmentier, H. K., Schouten, W. G. P., Schrama, J. W. Wiegant, V. M. 2003. Effects of housing and individual coping characteristics on immune responses of pigs. Physiology and Behavior 79, 289–296.

Bolhuis, J. E., Schouten, W. G. P., J. A., Schrama, J. W., Wiegant, V. M., 2005. Behavioural development of pigs with different copyng characteristics in barren and substrate-enriched housing conditions. Applied Animal Behavioural Science 93, 213-228.

Broom, D.M., 1986. Indicators of poor welfare. British Veterinary Journal 142, 524–526.

Broom, D.M., 2000. Welfare Assessment And Welfare Problem Areas During Handling And Transport. In: Grandin, T. (Ed.), Livestock Handling and Transport. 2nd ed. CABI Publ., Wallingford, pp. 43–61.

Canadian Agrifood Research Council. 1993. Recommended code of practice for the care and handling of farm animals: Pigs. Agriculture and Agri-Food Canada, Ottawa, ON. Publication 1898/E.

European Food Safety Authority (EFSA) (2004) Standards for the microclimate inside animal road transport vehicles. EFSA Journal, 122, 1-25.

Fraser D. 1974. The behaviour of growing pigs during experimental social encounters. Journal agricolar Science Cambridge, 82:147–163.

Geverink NA, Schouten WGP, Gort C, Wiegant VM., 2002. Individual differences in aggression and physiology in peri-pubertal breeding gilts. Applied Animal Behavioural Science 77, 43–52.

Grandin T., 1997. Assesment of stress during handling and transport. Journal of Animal Science 75, 249-257.

Hessing, M.J.C., Hagelsø, A.M., van Beek, J.A.M., Wiepkema, P.R., Schouten, W.G.P., 1994. Individual behavioural and physiological strategies in pigs. Physiology and Behaviour 55, 39–46.

Hessing, M.J.C., Coenen, GJ, Vaiman, C, Renard, C, 1995. Individual differences in cell-mediated and humoral immunity in pigs. Veterinary Immunology Immunopathology 45, 97– 113.

Hicks, T. A., McGlone, J. J., Wishant, C. S., Kattesh, H. G. And Norman, R. L., 1998. Behavioural, endocrine, immune and performance measures for pigs exposed to acute stress. Journal of Animal Science 76, 474–483.

Jensen, P, Rushen, J, Forkman, B., 1995. Behavioural strategies or just individual variation in behaviour? A lack of evidence for active and passive piglets. Applied Animal Behaviour Science 43, 135– 9.

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W., Blokhuis, H.J., 1999. Coping styles in animals: current status in behavior and stress-physiology. Neuroscience and Biobehavioral Reviews 23, 925–935.

Kettlewell, P., Mitchell, M., Harper, E, Villarroel, M., Levrino, G.M. 200.Thermal environments within transport vehicles. NJF seminar No. 361. Transport and handing of animals a Nordic Challenge. Alnarp, Sweden, 21-22 November 2005, pp. 24-33.

Lambooy, E., 1988. Road transport of pigs over a long distance: some aspects of behaviour, temperature and humidity during transport and some effects of the last two factors. Animal Production 46, 257–263.

Lambooij, B., 2000. Transport of pigs. In: Grandin, T. (Ed.), Livestock Handling and Transport. 2nd ed. CABI Publ., Wallingford.

Lambooij, B., 2007. Transport of Pigs. In: Livestock Handling and Transport. (3rd Edition). (T Grandin ed.), CABI International, Wallingford, UK, pp. 228-243.

Lewis, N.J., Berry, R.J., Wamnes, S., 2003. Transportation research: Transportation of early weaned piglets. Proc. Allen D. Leman Swine Conference 2003. p. 215-218.

Lewis, N. J., Berry, R. J., Crow, G. and Wamnes, S., 2005. Assessment of the effects of the season of transport on the performance of early-weaned piglets. Canadian Journal of Animal Science 85, 449–454.

Lewis, N.J., Berry, R.J., 2006. Effects of season on the behaviour of early-weaned piglets during and immediately following transport. Applied Animal Behaviour Science 100, 182–192.

Lewis, N.J., 2008. Transport of early weaned piglets. Applied Animal Behaviour Science 110, 128–135.

Madec, M., 2001. PMWS on-farm observations and preliminary analytic epidemiology. Proc. DNA viruses of plants, birds, pigs and primates. Saint-Malo France.

Mormede, P., Soissons, J., Bluthe, R.M., Raoult, J., Legarff, G., Levieux, D., Dantzer, R., 1982. Effect of transportation on blood serum composition, disease incidence, and production traits in young calves. Influence of the journey duration. Annales de Recherces Veterinaires 13, 369–384.

Nanni Costa, L., Magnani, D., Calà, P., Cafazzo, S., Dall'olio, S., Razzuoli, E., Amadori, M., 2012 Relationship between body temperature and coping style in post-weaned piglets, in: , Proceedings of the XVth International Congress of the International Society for Animal Hygiene, BRNO, Tribun EU s.r.o., 2011, pp. 1051–1054.

Schrama, J.W., Schouten, J.M., Swinkels, J.W.G.M, Gentry, J.L., De Vries Reilingh, G., Parmentier, H.K., 1997. Effect of hemoglobin status on humoral immune response of weanling pigs differing in coping styles. Journal of Animal Science 75(10), 2588– 2596.

Tarrant, V., Grandin, T., 2000. Cattle transport, In: Grandin, T. (Ed.), Livestock Handling and Transport, 2nd ed. CABI Publ., Wallingford, pp. 151–173.

Tarrant, V., Kenny, F.J., Harrington, D., Murphy, M., 1992. Long distance transportation of steers to slaughter: effect of stocking density on physiology, behaviour and carcass quality. Livestock Production Science 30, 223–238.

Robert, S., Weary, D. and Gonyou, H. W., 1997. Segregated early weaning and welfare of piglets. Pages 133–138 in S. Robert, ed. Lennoxville Symposium. Farm Animal Welfare in Canada: New Technologies, Research and World Trade. Lennoxville, QC. SAS Institute, Inc. 1999. SAS/STAT user's guide: Version 8.2. SAS Institute, Inc., Cary, NC.

Ruis, M.A.W., Te Brake, J.H.A., Engel, B., Buist, W.G., Blokhuis, H.J., Koolhaas, J.M., 2002. Implications of coping characteristics and social status for welfare and production of paired growing gilts. Applied Animal Behavioural Science 75 (3), 207– 31.

Warris, P. D. and Brown, S. N., 1994. A survey of mortality in slaughter pigs during transport and lairage. Veterinary Record 143, 513–515.

WSCR; 1976. Livestock hot weather stress. Regional operation manual letter C-31]76. National Weather Service Centra Region, USA.

Figure 1.
Trailer layout.
Piglets were transported in compartments with the same compositions of original pens or with a mixed composition.
Positions of video recording cameras are indicated with ▲
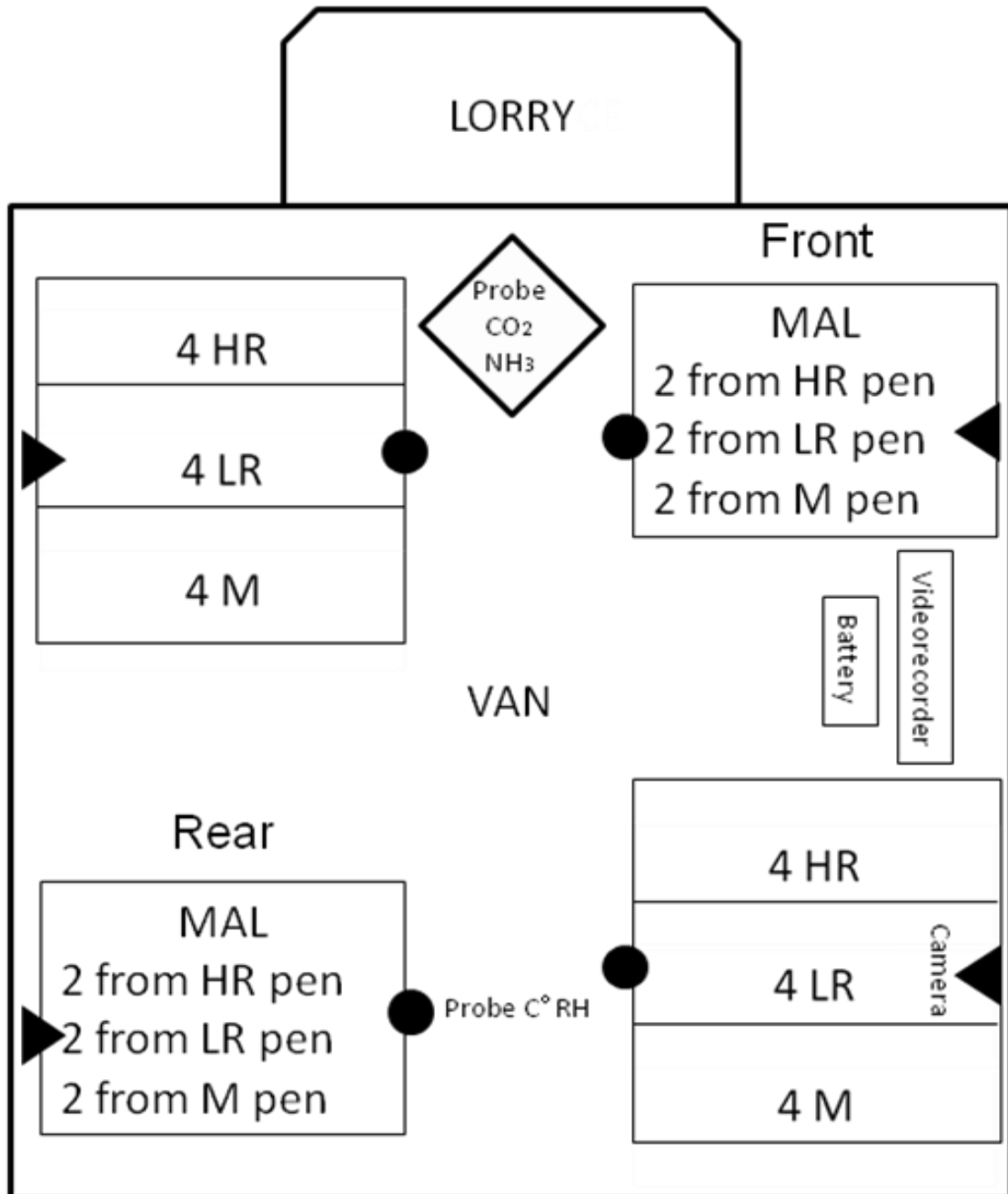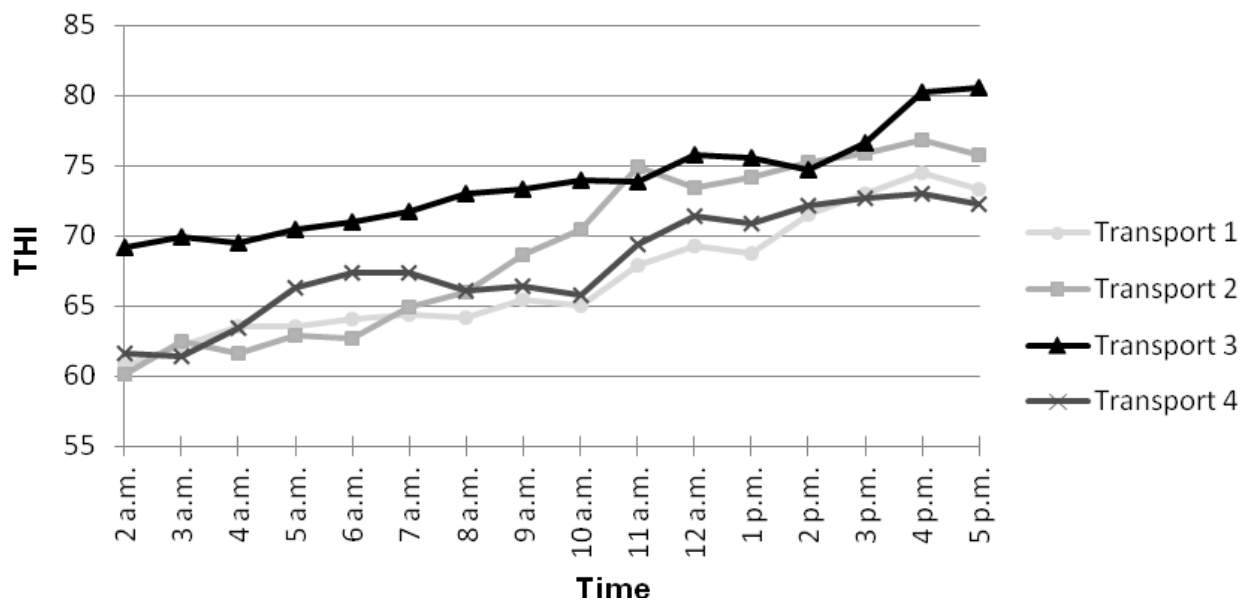Probes of temperature and humidity are indicated with ●

Fig. 2.
Fluctuation of temperature- humidity index inside the trailer during transport period for all journeys. Values between 75 and 80 belong to Attention Zone.

Table 1.
Journey characteristics and environmental condition inside the vehicle.

Journeys	Date	Duration (min)	Temperature (°C) (range)	Relative Humidity (%) (range)	THI[1] (range)
May	13/5/09	840	16.27-28.39	36.26-70.71	60.77-74.50
June	16/06/09	825	15.77-31.56	29.43-75.55	60.07-76.86
July	22/07/09	860	21.58-35.03	34.15-77.05	69.21-80.51
September	09/09/09	820	16.82-26.42	32.99-65.96	61.41-72.97

[1] THI: Thermal Humidity Index

Table 2.
Behavioural categories recorded.

Variable name	Registration
Standing	standing on its legs, with or without head movements.
Sitting	body supported on the front legs and hindquarters.
Resting	lying in a ventral or sternal position with or without head movements.
Exploring mate	Smelling, sucking, licking or touching one or more mates.
Exploring environment	Smelling, licking or touching floor or bars.
Biting objects	Biting floor or bars.
Aggressiveness	Biting, pushing, beating one or more mates.
Belly nosing	Strong and repeated movements of snout against to belly of mates.
Drinking	Piglets was in close proximity to the drinker and moving the mouth.

Table 3.
THI and Temperature values between periods and journeys.

	May		June		July		September		SE[1]	
	THI	T°	THI	T°	THI	T°	THI	T°	THI	T°
Period 1	62.7^{ax}	17.67	61.9^{ax}	17.04	70.0^{bx}	22.29	64.0^{cx}	18.90	0.19	0.51
Period 2	65.3^{ay}	19.65	68.9^{by}	22.78	73.2^{cy}	24.77	67.0^{dy}	21.03	0.19	2.66
Period 3	71.7^{az}	25.63	75.2^{bz}	29.57	77.2^{cz}	29.68	72.0^{dz}	26.38	0.17	3.09

[1] SE: Pooled standard error
Means within a row with different letters (a–c) differ ($P < 0.05$).
Means within a column with different letters (x–y) differ ($P < 0.05$).

Table 4.
Changes in behaviour of piglets observed during 14 hours of transport.

		May	June	July	September	SE[1]
Standing (%)	Period 1	$26.80^{a\ x}$	$29.51^{a\ x}$	$57.09^{b\ x}$	33.45^{a}	4.82
	Period 2	$8.82^{a\ y}$	$19.21^{ab\ y}$	$34.44^{b\ y}$	38.32^{b}	5.50
	Period 3	$11.26^{a\ y}$	$14.52^{a\ y}$	$23.75^{ab\ y}$	37.81^{b}	5.11
Sitting (%)	Period 1	$4.28^{a\ x}$	1.13^{b}	4.76^{a}	1.15^{b}	0.84
	Period 2	$1.46^{\ y}$	1.08	2.99	2.64	1.05
	Period 3	$0.74^{\ y}$	1.50	2.59	1.50	0.82
Resting (%)	Period 1	$68.92^{a\ x}$	$69.35^{a\ x}$	$38.15^{b\ x}$	65.40^{a}	4.92
	Period 2	$89.72^{a\ y}$	$79.71^{b\ xy}$	$62.57^{bc\ xy}$	59.05^{c}	5.07
	Period 3	$88.00^{a\ y}$	$83.98^{a\ y}$	$73.66^{b\ y}$	60.69^{b}	5.31
Exploring mate	Period 1	$2.25^{b\ x}$	0.67^{c}	$3.93^{a\ x}$	$4.79^{a\ x}$	3.03
	Period 2	$0.31^{c\ y}$	0.40^{b}	$0.88^{ab\ y}$	$1.62^{a\ y}$	1.11
	Period 3	$0.26^{b\ y}$	0.73^{a}	$0.97^{a\ y}$	$1.29^{a\ y}$	1.29
Exploring environment	Period 1	$2.70^{b\ x}$	$3.63^{ab\ x}$	$6.45^{a\ x}$	2.06^{b}	2.67
	Period 2	$0.73^{c\ y}$	$1.54^{b\ xy}$	$2.30^{ab\ y}$	3.67^{a}	3.09
	Period 3	$0.85^{b\ y}$	$1.50^{b\ y}$	$1.34^{b\ y}$	3.32^{a}	2.58
Belly nosing	Period 1	$1.86^{a\ x}$	0.00^{b}	2.67^{a}	0.52^{ab}	0.01
	Period 2	$0.47^{\ y}$	0.70	1.58	1.78	0.10
	Period 3	0.27^{y}	0.27	1.31	1.89	0.16

[1] SE: pooled standard error
Means within a row with different letters (a–c) differ ($P < 0.05$).
Means within a column with different letters (x–y) differ ($P < 0.05$).

Table 5.
Behavioural difference between composition of pens

	HR	LR	M	MAL	SE[1]
Standing (%)	30.18[a]	23.29[b]	29.98[a]	25.98[ab]	2.82
Exploring mate	1.08[b]	0.83[b]	1.00[b]	2.43[a]	0.92
Exploring environment	2.64[ab]	2.20[ab]	3.00[a]	2.02[b]	1.57
Biting objects	0.33[ab]	0.62[a]	0.39[ab]	0.25[b]	0.06
Aggressiveness	0.36[ab]	0.34[ab]	0.69[a]	0.30[b]	0.03

[1] SE: Pooled standard error
Means within a row with different letter (a-b) differ ($P < 0.05$).

Table 6.
Least Squares mean of blood parameter for each journey

		May	June	July	September	SE[1]
Haematocrit (%)	pre	32.54[ab x]	34.47[a x]	32.31[ab]	31.47[b x]	0.59
	post	35.10[a y]	31.13[bc y]	32.55[b]	29.24[c y]	0.49
Glucose (mmol/l)	pre	5.27[b]	6.13[a x]	6.17[a x]	6.76[a x]	0.17
	post	5.96[a]	4.86[b y]	3.20[c y]	3.34[c y]	0.17
Lactate (mmol/l)	pre	4.39[b x]	8.50[a x]	6.52[a]	7.83[a]	0.43
	post	10.22[a y]	5.60[c y]	7.88[ab]	6.54[bc]	0.49
Cortisol (nmol/ml)	pre	2.86[b]	3.41[ab]	3.64[ab]	4.52[a]	0.46
	post	4.13	3.40	4.26	3.15	0.45
Creatine Kinase (UI/l)	pre	1066.23	947.45	760.05	996.05	209.02
	post	1028.80	1031.03	855.75	808.53	191.33
Urea (mmol/l)	pre	1.54[ab x]	2.00[a]	1.89[a x]	1.25[b x]	0.38
	post	3.36[b y]	2.40[c]	4.62[a y]	4.44[a y]	0.76
Albumin (g/l)	pre	23.92[ab]	21.73[bc x]	26.25[a x]	18.17[c]	0.13
	post	25.56[a]	27.94[a y]	21.38[b y]	18.76[c]	0.14
Total Protein (g/l)	pre	51.00[b]	49.12[b x]	55.08[a x]	41.76[c x]	0.69
	post	53.11[a]	37.97[c y]	48.55[b y]	43.17[c y]	1.00
Haptoglobin (mg/ml)	pre	1.48[a]	0.84[b]	0.58[b]	0.78[b]	0.10
	post	1.00[a]	0.89[a]	0.70[b]	0.93[a]	0.11

[1] SE: Pooled standard error
Least square means within a row with different letters (a–c) differ ($P < 0.05$).
Least square means within a column with different letters (x–y) differ ($P < 0.05$).

CHAPTER 4

EFFECT OF SHORT ROAD JOURNEYS ON BEHAVIOUR AND SOME BLOOD VARIABLES RELATED TO WELFARE IN YOUNG BULLS

Simona Cafazzo [a, †,*], Diego Magnani [a,†], Pietro Calà [a], Elisabetta Razzuoli [b], Gabriele Gerardi[c], Daniele Bernardini[c], Massimo Amadori [b] and Leonardo Nanni Costa [a]

[a] *Dipartimento di Protezione e Valorizzazione Agroalimentare (DIPROVAL), Facoltà di Agraria, Università degli Studi di Bologna, via Fratelli Rosselli 107, 42100 Reggio Emilia, Italy*
[b] *Laboratorio immunologia cellulare, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "B. Ubertini", via Bianchi 7/9 - 25124 Brescia, Italy*
[c] *Dipartimento di Scienze Cliniche Veterinarie, Università degli Studi di Padova, Viale dell'Università, 16 Agripolis 35020 – Legnaro, Padova, Italy*

[†]These authors contributed equally.

* Corresponding author: Tel.: +390672670591 E-mail address: simona.cafazzo@inwind.it (S. Cafazzo).

ABSTRACT

Many studies have focused on the transport of cattle to fattening farms or to slaughterhouses but there is little information concerning the transport of young bulls delivered to the genetic test stations for selection. Due to the high expected value of young AI candidate bulls, their transport conditions could be very different from those intended for other cattle categories. The objective of the present study was to examine the effect of transport on behavioural response and on some blood variables of 26 young Holstein bulls (mean weight kg 278 ± 64) before, during and after short journeys (duration < 8 hours) to a genetic test centre. Behavioural patterns such as agonistic and affiliative interactions, environmental and conspecific explorative behaviour were recorded by means of an instantaneous sampling method at the farms of origin, into the truck and at the genetic test station. Moreover, standing orientation and postures during transport were recorded. Blood samples were collected by jugular venipuncture before transport (- 4 days, T -4), immediately after transport (day 0, T 0) and at 4 days (T +4) relative to time day 0. The space allowance during transport, ranging from 1.50 m^2 to 3.75 m^2 per head, enabled the animals to move quite freely and adopt comfortable positions. Young bulls did not show any preferences in their standing orientation during journeys and they were observed in a lying position for short time periods; they were also observed to ruminate, especially during the stationary periods of the journey. Mixing of animals during journeys did not affect their behaviour. The agonistic behaviour rate was higher during transport ($P= 0.049$) and in the first two hours after unloading ($P= 0.003$) than on the rearing farms. Four days after transport, agonistic behaviour decreased compared to the level observed during the first two hours after unloading ($P = 0.003$), whereas affiliative behaviours increased ($P= 0.023$); however, since the hourly rate of agonistic interactions remained higher than the rate observed on the farms of origin ($P = 0.001$), we postulate that hierarchical relationships were not well established yet. After unloading, all animals spent more time interacting with others than exploring their new pen. Plasma glucose and non–esterified fatty acid (NEFA) concentrations both increased significantly after transport. There were no changes in the activity of plasma creatine kinase (CK) after transport, suggesting that the journeys did not cause physical exertion. The results indicate that the transport conditions adopted for the AI candidate bulls only slightly affected the behavioural response and blood variables examined here and could be considered satisfactory for their welfare.

1. INTRODUCTION

Transport represents a critical phase in animal production and is often considered as one of the main causes of stress (Mormede et al., 1982), with possible negative repercussions on health and welfare. Novelty, fatigue, handling and mixing of animals during transport induce stress, which is expressed by specific physiological and behavioural changes. Other factors such as the duration of transport, stocking density and ventilation may also contribute to the impact of transport (Tarrant et al., 1992; Tarrant and Grandin, 2000; Buckham Sporer et al., 2007; Gupta et al., 2007).

Changes in behaviour can occur when an animal is coping with handling or transport (Broom 2003). Some of these help to show which aspects of the situation are aversive. When male adult cattle are mixed during transport or in lairage, they may fight, and this behaviour can be recorded directly (Kenny and Tarrant, 1987). Calves of 6 months of age may also fight (Trunkfield and Broom, 1991). Swanson and Morrow-Tesch (2001) reviewed cattle transport literature and concluded that: (a) factors involved with "transport stress" include pre-transport management, noise, vibration, novelty, social regrouping, crowding, climatic factors (temperature, humidity and air composition), restraint, loading and unloading, time of transit, and feed and water deprivation; (b) young calves are especially vulnerable to "transport stress" with problems of morbidity (e.g. diarrhoea, pneumonia and "shipping fever") and mortality; (c) agonistic behaviour seems to be decreased by crowding and the motion of the truck. Tarrant and Grandin (2000) found that restlessness can result in changes in posture triggered by social interactions, such as chin resting and mounting, as well as by driving events like cornering while the truck is travelling. On long journeys, the standing orientation is most commonly perpendicular to the direction of travel and cattle tend not to lie in trucks when they are travelling; obviously, the maintenance of balance heavily depends on driving events (Tarrant and Grandin, 2000).

There are several blood variables influenced by transport (Crookshank et al., 1979) that are used as stress indicators. Stressors are conducive to both physical and neuro-endocrine changes, like the activation of the sympathetic-adrenal (Saunders and Straub, 2002) and hypothalamic pituitary-adrenal (HPA) axis (Haddad et al., 2002); as a result, the plasma cortisol concentration is widely used as stress indicator. Additionally, changes in blood glucose and muscle enzyme activities such as creatine kinase (CK) may be of some importance, the latter being associated with muscle tissue injury (Stephens, 1980; Tripp and Schmitz, 1982). Other blood parameters such as total protein and albumin have been proposed as physiological indicators of dehydration during transport (Knowles and Warriss, 2007). Acute phase proteins (APP) are a group of molecules

whose concentrations vary in animals subjected to internal or external challenges such as infection, inflammation, surgical trauma or stress (Eckersall, 2000; Murata et al., 2004). Haptoglobin (Hp), and the positive acute phase proteins in cattle may be useful to evaluate the influence of transport on cattle welfare (Murata and Miyamoto, 1993; Arthington et al., 2003).

Although numerous studies have investigated the effects of transport on cattle destined to finishing farms or to slaughterhouses, to our knowledge there are no reports concerning the transport of young dairy bulls to genetic test stations. As a result, basic information on young breeding bulls during road transport could contribute to improving the economic and welfare aspects of transportation. (what aspects of economy and welfare?) Therefore, the aim of this study was to investigate some specific behavioural and blood variables of young AI candidate bulls during short journeys to a genetic test centre.

2. MATERIALS AND METHODS

2.1 Animals and transportation procedures

A total of twenty-six Holstein bulls from seven different dairy farms were monitored during journeys from farms to the genetic test station of the Italian Holstein Breeders Association (ANAFI). The animals were 242 ± 42 days old and their weight was 278 ± 64 kg. At the farms of origin, the bulls were fed with grass hay and concentrate. They were mostly housed two per pen on straw-bedded floors with water always available at a space allowance ranging from 1.5 to 3.75 m^2/head. Only three subjects were found to be housed alone in their pens. We monitored five journeys at loading, during transport and at unloading between December 2008 and July 2009. The journeys examined were planned by the genetic station in order to collect candidate bulls from farms located in an area of the Piedmont region located about 300 km from the genetic test station. A commercial truck with a standard loading deck (length 6.60 m, width 2.50 m and height 2.50 m) was used. In this type of vehicle, natural ventilation is provided by 9 windows (length 0.75 m, height 0.35 m) per side. Mechanical ventilation during stops is provided by 9 fans placed close to the windows on the right side. Video recording equipment and batteries were placed close to the front wall in a space (0.6 m x 2.50 m) separated by a partition. Thus, the total space allowance for the animals was 15.0 m^2. During all journeys another partition was placed in the middle of the deck leading to a front and a rear compartment with the same space allowance. The floor of both compartments was deep bedded with straw for each journey. The space allowance ranged from

1.50 m^2 to 3.75 m^2 per head. Information on the space allowance per compartment/journey and on transport conditions is shown in Tables 1 and 2. The truck and driver were the same for all journeys. Eleven bulls coming from different farms were mixed in the same truck compartment (see Table 2), as the established protocol of the genetic station does not exclude this practice. Air temperature and relative humidity (Table 2) were recorded every 30 sec throughout the journey by two sensors (Pro v2 logger, HOBO, USA) placed in each compartment and positioned on the side of the partition at a height of 1.5 m from the floor.

Transportation started immediately after loading of bulls. Due to a vehicle stop occurring during the third journey, data of three subjects were not considered because the transport time exceeded the 8-hour limit for short-distance journeys established by EU regulation 1/2005. Each journey was tracked by means of a GPS System (Truckstick II, Telespial Sys., USA) so as to monitor the route, stops and travelling speed. The truck travelled mainly on national highways at an average speed of 70 km/h. After arriving at the genetic station, all bulls were immediately unloaded and placed in a straw-bedded quarantine pen (8.0 m x 4.5 m), where hay (DM= 90.0%, Net Energy/kg DM=3.56MJoule, crude protein % DM= 13.0) and water were available *ad libitum*. The groups of bulls located in the quarantine pen also included subjects arriving the same day from other farms, so that the total number ranged from 12 to 14 subjects. Two days after the arrival, the hay was supplemented with 2.0 kg of a commercial concentrate (Net Energy/kg DM =7.82MJoule, crude protein % DM = 22.3) per animal per day.

2.2 Behavioural measures

Behavioural data were collected by video recording before, during and after the journey. In each cattle farm, four days before the journey (T-4), the bulls' behaviour was recorded by manual video recording (DCR-HC42E Sony Handy Cam) for two hours. Digital cameras (CIR 14/68, Fracarro, Italy) were placed in each compartment of the truck to record the behaviour and postures of bulls throughout the journey. All cameras were connected to a DVR recording system (DVR 2036T/R, Fracarro, Italy) and were fastened at the same angle and height, so that the recorded area was similar among compartments. Recording of behaviour began as soon as the bulls were loaded and the ramp was raised and continued until the truck arrived at the genetic centre. Finally, at the genetic centre the behaviour of bulls was recorded for two hours after the journey (T0) and 4 days later (T+4).

During video analysis, all behavioural patterns (for a description see Table 3) were recorded using the "all occurrences" or "instantaneous" sampling method. In particular, agonistic and affiliative behaviours, environment and conspecific explorative behaviours and restlessness were recorded by the "all occurrences" sampling method (Altmann, 1974). The standing orientation of animals (diagonal, perpendicular and parallel orientations) and their postures during the journey (lying down and standing) were recorded using the "instantaneous" sampling method (Altmann, 1974). The bulls were identified on the basis of differences in coat spots.

The observation time varied among animals because the journeys had different durations. Therefore, when comparing the behaviour of all individuals under different conditions (before, during and after the journey) we corrected the individual measures of behavioural patterns based on animal observation time, so as to obtain the hourly rate of each behavioural pattern considered. All behaviours were compared distinguishing between stationary and travelling vehicle situations.

Only one observer was used to evaluate behaviours: she had been previously trained to report consistent data from video tapes.

2.3 Collection and processing of blood samples

Ten ml blood samples were collected from the jugular vein in lithium heparin-coated tubes and in tubes without anticoagulant. The first blood sample (T-4) was collected four days before the journey to provide baseline values. Two further samples were collected immediately after the bulls were unloaded from the truck (T0) and four days (T+4) after the arrival at the Genetic Centre. T-4 and T+4 samples were taken at the same hour. T0 sample depended to arrival time. One aliquot (1 ml) of whole blood was stored at 4°C. The remaining volume of heparinised blood was centrifuged at 2,500 rpm for 15 min and the resulting plasma was stored in aliquots at -20°C for subsequent analyses. Non–heparinised blood samples were stored at room temperature for 2 hours and centrifuged at 2,500 rpm for 15 min; serum aliquots were stored at -80°C until analysis.

2.4 Blood analyses

Whole heparinised blood samples were used to determine haematocrit by means of an impedance cell counter (Cell-Dyn 3500, ABBOTT, Chicago, Illinois, USA). Plasma samples were used to determine the concentrations of glucose (Kit Roche, code 11447513216), lactate (Kit Randox, code LC2389), creatine kinase activity (CK) (Kit Roche, code 12132524216), urea (Kit Roche, code

11489364216), non–esterified fatty acids (NEFA) (Kit Randox, code FA115) and β-hydroxybutyrate (BHB) (Kit Rambut, Randox, code RB1007) using a multi-analyzer (Syncron CX5, Beckman, Fullerton, CA). Serum samples were used for assays of cortisol (Kit Cortisol Immulite, Medical System, code LKC01), haptoglobin (Kit Phase Haptoglobin, Celbio, code TP 801) and total protein (Kit Roche code TP 1553836).

2.5 Statistical Analyses

Chi-squared test was used to look for differences among standing orientations (perpendicular, parallel, and diagonal) of the animals during journeys. The Wilcoxon signed rank test was used to compare the hourly rates of behavioural categories observed at each sampling time and the behaviour of animals under the different conditions (before, during and after the journey); in the second analysis to adjust for multiple comparisons (3 comparisons: T-4 vs T0, T-4 vs T+4, T0 vs T+4) the significance level α was adjusted using the Bonferroni method (dividing a by the number of tests: 0.05/3 = 0.016; Sokal and Rohlf 1995). Mann-Whitney U test was used to detect the effect of mixing on behavioural patterns. Due to some problems during video recording, behavioural data concerning some animals under different conditions were missing; therefore, the number of animals in each comparison is not the same (see Table 1 for details). All non-parametric tests (two-tailed) were performed by using the STATISTICA package, 7.1 edition (StatSoft Italy s.r.l. 2005).

Blood parameters data were analysed using PROC mixed for repeated measures of SAS (2006). The model included the fixed effect of sampling time, journey, farm of origin, sampling time × journey and sampling time × farm of origin interactions and the random effect of subject within journey. In addition, the weight and the age of calves, the space allowance in compartments and journey duration were included as covariate terms in the model. None of these covariates were statistically significant ($P>0.05$); thus they were removed from the model. Data were transformed to meet assumption of homogeneity of variance and normality of residuals. Cortisol, NEFA, creatine kinase and haptoglobin data were subjected to log10 transformations. All transformed estimates were back-transformed for presentation to their original scale as arithmetic means.

3. RESULTS

3.1. Behaviour during the journey

There was no preference in standing orientation with respect to the long axis of the truck either in stationary (χ^2 = 2.67, *n* = 19, d.f. = 2, P = 0.26) or travelling periods (χ^2 = 0.06, *n* = 21, d.f. = 2, P = 0.97) of journeys. The bulls spent about the same amount of time in perpendicular (travelling period: 33.77 ± 19.07%; stationary period: 37.74 ± 21.80%), diagonal (travelling period: 32.53 ± 12.95%; stationary period: 30.86 ± 12.53%), and parallel standing orientations (travelling period: 33.70 ± 15.57%; stationary period: 31.31 ± 16.79%). Bulls spent 7.20% of the journey lying down and there was no difference in time spent standing while the vehicle was stationary or travelling (*t* = 9, *z* = 0.85, *n* = 19, P = 0.40). Some bulls (N=12) were observed to ruminate for a longer time while the vehicle was stationary (16.70%) as compared to during travel (6.88%), although the difference was not significant (*t* = 13, *z* = 1.78, *n* = 12, P = 0.08).

The hourly rate of restlessness (*t* = 67, *z* = 1.27, *n* = 19, P = 0.26), social interactions (agonistic behaviour: *t* = 65, *z* = 0.89, *n* = 19, P = 0.37; affiliative behaviour: *t* = 20, *z* = 0.76, *n* = 19, P = 0.44) and explorative behaviours (environment: *t* = 55, *z* = 1.61, *n* = 19, P = 0.11; conspecific: *t* = 71, *z* = 0.26, *n* = 19, P = 0.79) did not differ between stationary and travelling periods. Therefore, data concerning the two periods were processed together for the subsequent analyses.

Mixing of animals in the truck compartments did not affect the hourly rate of social interactions (Mann-Whitney U Test; agonistic behaviour: U = 45, *n1* = 10, *n2* = 11, P = 0.48; affiliative behaviour: U = 39, *n1* = 10, *n2* = 11, P = 0.22), and explorative behaviours (environment: U = 50, *n1* = 10, *n2* = 11, P = 0.73; conspecific: U = 32.5, *n1* = 10, *n2* = 11, P = 0.11).

3.2. Behaviour in rearing farms and during the journey

To compare the bulls' behaviour during the journey with that observed on farm, subjects previously housed alone in their pens (n = 3) were excluded.

Bulls interacted agonistically and explored the environment more frequently during the journey than on farm (*t* = 30, *z* = 1.96, *n* = 16, P = 0.049; *t* = 8, *z* = 3.10, *n* = 16, P = 0.002 respectively). There was no significant difference regarding the affiliative behaviour (*t* = 31, *z* = 1.65, *n* = 16, P = 0.099), even if it was more frequent on farms. Moreover, there were no differences in conspecific exploration between the two sampling times (*t* = 48, *z* = 1.034, *n* = 16, P= 0.30).

3.3. Behaviour before and after the journey

Bulls showed the highest hourly rate of agonistic behaviour during the two hours after unloading (T0vsT+4: t = 11.5, z = 2.92, n = 17, P = 0.003; T0vsT-4: t = 6, z = 3.58, n = 19, P = 0.003; Fig. 1). Four days after the journey, the rate of agonistic behaviours was lower than during the first two hours after unloading, although it remained significantly higher than that observed in the farms of origin (T+4vsT-4: t = 0, z = 3.41, n = 16, P = 0.0006; Fig. 1). In contrast, affiliative behaviours were more frequently observed in farms compared with the genetic centre during the first two hours after unloading (T-4vsT0: t = 22.5, z = 2.35, n = 19, P = 0.019; Fig. 1), although the difference was not significant. After four days we observed a slight increase in affiliative behaviours among bulls (T0vsT+4: t = 20, z = 2.27, n = 17, P = 0.023; Fig. 1) but they remained lower than those observed on the farms of origin, although the differences were not significant (T-4vsT+4: t = 29.5, z = 1.44, n = 16, P = 0.15; Fig. 1).

Conspecific explorative behaviours recorded among bulls at the genetic centre during the first two hours after unloading were higher than those recorded after four days, although the difference failed to reach a statistically significant level (T0vsT+4: t = 18.5, z = 1.89, n = 17, P = 0.059; Fig. 1); moreover, the hourly rate of these behaviours did not differ between farms and the genetic centre (T0vsT-4: t = 75.5, z = 0.44, n = 19, P = 0.66; T+4vsT-4: t = 24.5, z = 1.47, n = 16, P = 0.14; Fig. 1).

At the genetic centre bulls showed a lower frequency of environment exploration than at farms (T0vsT-4: t = 26, z = 2.78, n = 19, P = 0.006; T+4vsT-4: t = 3.5, z = 3.34, n = 16, P = 0.0009; Fig. 1). Moreover, at the genetic centre the exploration of the environment was more frequently displayed during the first two hours after unloading than after four days, although the difference failed to reach a statistically significant level (T0vsT+4: t = 22.5, z = 1.88, n = 17, P = 0.06; Fig. 1).

We analysed each sampling time independently in order to compare the hourly rates of all behaviours observed. The statistical significance of the comparisons within each sampling time is reported in Table 4, while the distribution of acts per hour for each type of behaviour is shown in Figure 2. On the farms (T-4), the most frequent behaviour was exploration of the environment, while affiliative behaviour was more frequently displayed than agonistic behaviour, which was observed at the lowest frequency (Fig. 2).

During the first two hours after unloading (T0), agonistic and affiliative behaviours were the most and the least frequent behaviours respectively (Fig. 2). Agonistic behaviours remained the most frequent also four days after the arrival at the genetic centre; nevertheless, hourly rates of all behaviours considerably decreased (Fig. 2).

We considered all agonistic behaviours occurring at the genetic centre (T0 and T+4) to evaluate whether each individual was more aggressive towards unfamiliar subjects. As expected, we found a higher level of agonistic behaviours among unfamiliar bulls than among familiar ones (t = 11, z = 2.41, n = 13, P = 0.016).

3.4. Blood parameters

In the current study, sampling time had a significant effect (P<0.05) on haematocrit, urea, glucose, lactate, BHB and NEFA, with tendencies toward significance for CK (P=0.068), haptoglobin (P=0.053) and cortisol (P=0.061). The journey had a significant effect (P<0.05) on haematocrit, albumin, total protein, creatine kinase and glucose, while the farm of origin of the transported bulls did not show any significant effects (P>0.05) on the plasma and serum parameters under study. There was a significant interaction between sampling time and journey for plasma concentrations of albumin, total protein, urea, glucose, lactate and BHB. Significant interaction between sampling time and farm of origin was found for glucose, lactate and BHB.

The least square means of blood parameters at different sampling times are shown in Table 5. Haematocrit was not increased by transport and was lower than T0 four days later at the genetic centre. Transport showed significant effects on some metabolic parameters such as glucose and NEFA which were higher (P < 0.05) at unloading as compared with the levels observed in the pre-transport period and at 4 days after transport. There were no significant differences in Urea and BHB concentrations between samples collected pre-transport and immediately after unloading. Pre-transport concentration of plasma lactate were higher (P < 0.05) compared with the levels at arrival and 4 days later. Although not significant, a similar trend toward an increase immediately after unloading and a decrease four days after transport was observed for cortisol and haptoglobin concentrations. No significant changes in albumin or total protein were observed between the different sampling times.

As regards the effect of journey (data not shown), which was related to the variation of environmental conditions between journeys, haematocrit significantly decreased (P<0.05) from the second (35.90 ± 0.64) to the fifth (30.03 ± 0.43) journey. Glucose and NEFA showed a similar trend, decreasing from 5.50 ± 0.14 mmol/l to 3.73 ± 0.12 mmol/l and from 0.39 ± 0.04 milleq/l to 0.22 ± 0.048 milleq/l, respectively, from the first to the fifth journey. The journey affected the concentrations of total protein and haptoglobin which, without any apparent cause, were significantly lower (P<0.05) in the second and third journeys, respectively. Regarding the effect of interactions between sampling time and journey on plasma concentrations of albumin, urea,

glucose, lactate and BHB (data not shown), there was no apparent meaningful association, even though the response of some groups of loaded subjects was slightly different from that of other groups. Similar results were found by analyzing the interaction between sampling time and farm of origin on glucose, lactate and BHB. Individual variations in response to the sampling procedures during shipment were probably the causes of such interactions.

4. DISCUSSION

The behaviour of bulls in transit was not affected by the movements (roll on and vibration) of vehicle during transport, as suggested by the absence of any significant differences in the hourly rates of all analysed behavioural categories during stationary versus travelling periods. Moreover, they did not show any preferences in standing orientation, irrespective of whether the vehicle was stationary or moving. This finding is in contrast with previous evidences showing that the preferred orientations during commercial cattle transport are parallel to or perpendicular to the direction of movement (Eldridge et al., 1986; Kenny and Tarrant, 1987; Tarrant et al., 1992; Nanni Costa et al., 2003; Tarrant and Grandin 2000). It is possible that the large space allowance during transport let the animals move easily in order to balance and maintain stability in a travelling vehicle, thereby making a particular standing orientation unnecessary.

In the present study, bulls were observed in lying position during both stationary and travelling periods of the journey, although only for a short time. Kent and Ewbank (1983) observed six-month old bulls lying down during a journey of about six hours while the vehicle was stationary or travelling along a motorway. We also observed bulls ruminating during journeys, especially during the stationary period. Usually, the ruminating reflex is inhibited during journey (Kent and Ewbank, 1983; Grigor et al., 2004) and is thought to occur only when the animal is in a relaxed state (Trunkfield and Broom, 1990). In our study, bulls coming from different farms were loaded in the same compartment of the truck. Grouping of unfamiliar animals is found to increase aggressions and social stress (Tennessen et al., 1985; Bøe and Færevik, 2003; Færevik et al., 2007). In transit, bulls interacting agonistically and exploring the environment more frequently than in their farms. However, the mixed bulls did not show higher hourly rate of agonistic behaviour than no-mixed ones. Moreover, we did not detect any influence of mixing on all other of the behavioural categories considered. As reviewed by Swanson and Morrow-Tesch. (2001), a reduction of the

level of social and agonistic behaviours on a moving vehicle was often observed during cattle transportation.

In order to assess cattle welfare during transport, the behaviour of cattle after transport should be analysed. In the present study particular attention was devoted to the changes concerning social behaviours of animals.. During the first two hours after unloading, bulls spent more time interacting with others than exploring the new pen. Moreover, a considerable increase of agonistic behaviour was observed; bulls displayed mounting and fighting especially towards unfamiliar individuals, probably in order to establish a dominance hierarchy. Four days after transport we observed a decrease of agonistic behaviour and a slight increase of affiliative behaviours. However, since the hourly rate of agonistic behaviour remained higher compared with what was observed on the farms of origin, probably the hierarchical relationships were not well established yet.

Bulls undergoing transportation to genetic centre showed changes in blood clinical chemistry values, within normal pre-transport physiological ranges (Jain, 1986; Kaneko, 1989). In the current study, there was no significant change in haematocrit levels at off-loading compared with baseline values before transport. *Ad libitum* access to water in the farm's pen prevented the animals from showing signs of dehydration such as an elevated haematocrit. In this study, journeys not longer than 6 hours increased the concentration of glucose and NEFA and decreased lactate concentration. Plasma glucose concentration increased after the journey in response to the increase in cortisol released during the stress of transport (Kent and Ewbank, 1983; Shaw and Tume, 1992). Activation of the hypothalamic-pituitary-adrenal axis (HPA) due to transport stress also may result in an increased plasma concentration of NEFAs (Swanson and Morrow-Tesch., 2001). NEFA was found to increase in calves after a simulated short journey (Sartorelli et al., 1990) while no significant difference of NEFA levels before and after transport was detected in Holstein calves delivered to the slaughterhouse (Van de Water et al., 2003). After journey, the lactate concentration was lower than the pre-transport baseline level. Probably, some physical activity related to the blood sampling at the farms of origin is probably the cause of the difference detected.

It is of interest that bulls had significantly lower concentrations of glucose and higher concentrations of NEFA and BHB four days after the journey compared with the pre-transport baseline values. A rise in circulating fatty acid and ketone bodies is associated with a mobilization of fat reserves in response to an energy deficiency (Penicaud et al., 2000). Probably, due to the

change of diet at the genetic station a transient deficit of energy occurred.. On the other hand, the significant decrease of urea concentration from T0 to T+4 suggests that the new diet was adequate for protein needs .

Although there was no difference in CK plasma activity before and after the journey, it is not possible to exclude that animals did not experience any physical stress. Variations in haptoglobin concentration during and after transport have been detected in previous investigations (Early et al. 2010; Sporer at al., 2008), but the findings are inconsistent. In the present study, the change in haptoglobin between sampling times was very close to the statistic significance . Its concentration increased immediately after unloading with respect to pre-transport baseline and slightly decrease four 4 days later.

Increase of environmental temperature inside transport road vehicles is considered a risk factor for animal welfare (AHAW, 2002). In the present study, the change of environmental temperature during the series of journeys was not negligible (from + 5.6 °C to + 28.4°C. Despite the wide range of temperatures experienced by the bulls, the extremes values were far from lower (LCT) and upper critical temperature (UCT) values for this category of cattle (AHAW, 2002).

5. CONCLUSION

Under the conditions of the present study, the stress imposed on AI candidate bulls by the journey to the genetic station caused slight changes in behavioural and blood parameters. Agonistic behaviours observed under the new housing conditions at the genetic centre were probably related to the natural tendency of bulls to establish dominance relationships and their decrease a few days after the formation of new groups may indicate that the animals reached in a short time a certain degree of social stability . Changes in the concentration of several blood variables immediately after the journey were transitory and related to the stress of transport, while those observed after 4 days were associated to the adaptation of the new social and rearing conditions.

In conclusion, our results indicate that the conditions of pre-transport, transport and post transport of AI candidate bulls investigated in the present study may be considered satisfactory for their welfare.

Acknowledgments

REFERENCES

Altmann, J., 1974. Observational study of behavior: sampling methods. Behaviour 48, 227-265.

Arthington, J.D., Eicher, S.D., Kunkle, W.E., Martin, F.G., 2003. Effect of transportation and commingling on the acute-phase protein response, growth, and feed intake of newly weaned beef calves. J. Anim. Sci. 81, 1120–1125.

Buckham Sporer, K.R., Burton, J.L., Earley, B., Crowe, M.A., 2007. Transport stress in young bulls alters expression of neutrophil genes important for the regulation of apoptosis, tissue remodeling, margination, and anti-bacterial function. Vet. Immunol. Immunop. 118, 19–29.

Bøe, K.E., Færevik, G., 2003. Grouping and social preferences in calves, heifers and cows. Appl. Anim. Behav. Sci. 80, 175–190.

Broom, D.M., 1986. Indicators of poor welfare. Br.Vet. J. 142, 524–526.

Broom, D.M., 2003. Causes of Poor Welfare in Large Animals During Transport. Vet. Res. Comm. 27 (1), 515–518.

Crookshank, H.R., Elissalde, M.H., White, R.G., Clanton, D.C., Smalley, H.E., 1979. Effect of transportation and handling of calves upon blood serum composition. J. Anim. Sci. 48, 430–435.

Earley, B., Murray, M., Prendiville, D. J., 2010. Effect of road transport for up to 24 hours followed by twenty- our hour recovery on live weight and physiological responses of bulls. BMC Vet. Res. 6, 38.

Eckersall, P.D., 2000. Recent advances and future prospects for the use of acute phase proteins as markers of disease in animals. Rev. Med. Vet. 151, 577–584.

Eldridge, G.A., Bamett, J.L., Warner, R.D., Vowles, W.J., Winfield, C.G., 1986. The handling and transport of slaughter cattle in relation to improving efficiency, safety, meat quality and

animal welfare. Research Report Series No. 19, Victoria Department of Agricultural and Rural Affairs, Australia.

Færevik, G., Andersen, I.L., Jensen, M.B., Bøe, K.E., 2007. Increased group size reduces conflicts and strengthens the preference for familiar group mates after regrouping of weaned dairy calves (*Bos taurus*). Appl. Anim. Behav. Sci. 108, 215–228.

Grigor, P.N., Cockram, M.S., Steele, W.B., Le Sueur, C.J., Forsyth, R.E., Guthrie, J.A., Johnson, A.K., Sandilands, V., Reid, H.W., Sinclair, C., Brown, H.K., 2001. Effects of space allowance during transport and duration of mid-journey lairage period on the physiological, behavioural and immunological responses of young calves during and after transport. Anim. Sci. 73, 341–360.

Grigor, P. N., Cockram, M. S., Steel, W. B., McIntyre, J., Williams, C. L., Leushuis, I. E., 2004. A comparison of the welfare and meat quality of veal calves slaughtered on the farm with those subjected to transportation and lairage. Livest. Prod. Sci. 91(3), 219-228.

Gupta, S., Earley, B., Crowe, M.A., 2007. Effect of 12-hour road transportation on physiological, immunological and haematological parameters in bulls housed at different space allowances. Vet. J. 173, 605–616.

Haddad, J.J., Saadé, N.E., Safieh-Garabedian, B., 2002. Cytokines and neuro-immune-endocrine interactions: A role for the hypothalamuspituitary-adrenal revolving axis. J. Neuroimmunol. 133, 1–19.

Jain, N.C., 1986. Schalm's Veterinary Hematology. 4th ed. Lea & Febiger, Philadelphia, USA.

Jarvis A.M, Harrington D.W.J., Cockram M.S., 1996. Effect of source and lairage on some behavioural and biochemical measurements of feed restriction and dehydration in cattle at a slaughterhouse. App. Anim. Behav. Sci. 50, 83–94.

Kaneko J.J.,1989. Clinical Biochemistry of Domestic Animals. 4th ed. Academic Press Inc. San Diego,

Kenny, F.J., Tarrant, P.V., 1987. The reaction of young bulls to short-haul road transport. Appl. Anim. Behav. Sci. 17, 209-227.

Kent, J.E., Ewbank, R., 1983. The effect of road transportation on the blood constituents and behaviour of calves. I. Six months old. Br. Vet. J. 139, 228.

Knowles, T.G., Warriss, P.D., Brown, S.N., Edwards, J.E., 1999. Effects on cattle of transportation by road for up to 31 hours. Vet. Rec. 145, 575–582.

Knowles, T.G., Warriss, P.D., 2000. Stress physiology of animals during transport, In: Grandin, T. (Ed.), Livestock Handling and Transport, 2nd ed. CABI Publ., Wallingford, pp. 385–407.

Lensink, B.J., Fernandez, X., Cozzi, G., Florand, L., Veissier, I., 2001. The influence of farmer's behavior on calves' reactions to transport and quality of veal meat. J. Anim. Sci. 79, 642–652.

Loerch, S.C., Fluharty, F.L., 1999. Physiological changes and digestive capabilities of newly received feedlot cattle. J. Anim. Sci. 77, 1113–1119.

Marahrens, M., Von Richthofen, I., Schmeiduch, S., Hartung J., 2003. Special problems of long-distance road transports of cattle. Dtsch. Tierärztl. Wschr. 110, 120–125.

Moberg G.P., 1985. Biological response to stress: key to assessment of animal well-being? In: Moberg GP (Ed.). Animal Stress. Bethesda, Maryland: American Physiological Soc., pp. 27-49.

Mormede, P., Soissons, J., Bluthe, R.M., Raoult, J., Legarff, G., Levieux, D., Dantzer, R., 1982. Effect of transportation on blood serum composition, disease incidence, and production traits in young calves. Influence of the journey duration. Ann. Rech. Vet. 13, 369–384.

Murata, H., Miyamoto, T., 1993. Bovine haptoglobin as a possible immunomodulator in the sera of transported calves. Br. Vet. J. 149, 277–283.

Murata, H., Shimada, N., Yoshioka, M., 2004. Current research on acute phase proteins in veterinary diagnosis: An overview. Vet. J. 168, 28–40.

Nanni Costa, L., Lo Fiego, D.P., Cassanelli, M.G., Tassone, F., Russo, V., 2003. Effect of journey time and environmental condition on bull behaviour and beef quality during road transport in Northern Italy. Dtsch. Tierärztl. Wschr. 110, 107-110.

Penicaud, L., Cousin, B., LeLoup, C., Lorsignol, A., Casteilla, L., 2000. The autonomic nervous system, adipose tissue plasticity, and energy balance. Nutrition 16, 903-908.

Sartorelli P.,. Dominoni, S. Agnes F., 1992 Influence of Duration of Simulated Transport on Plasma Stress Markers in the CalfJournal of Veterinary Medicine Series A, 3*9, 401-403*.

SAS, 2006. User's Guide. Cary, NC: SAS Institute Inc.

Saunders, V.M., Straub, R.H., 2002. Norepinephrine, the β-adrenergic receptor and immunity. Brain Behav. Immun. 16, 290–332.

Shaw, F.D., Tume, R.K., 1992. The assessment of pre-slaughter and slaughter treatments of livestock by measurement of plasma constituents – a review of recent work. Meat Sci, 32, 311-329.

Siegel, S., Castellan, N.J., Jr., 1988. Non-parametric Statistics for the Behavioural Sciences, 2ndedn. McGraw-Hill, Singapore.

Sokal, R.R., Rohlf, F.J., 1995. Biometry. W.H. Freeman, New York.

Stephens, D.B., 1980. Stress and its measurement in domestic animals. A review of behavioural and physiological studies under field and laboratory situations. Adv. Vet. Sci. Comp. Med. 24, 179–210.

Swanson, J.C., Morrow-Tesch., J., 2001. Cattle transport: Historical, research and future perspectives. J. Anim. Sci. 79 (E. Suppl.):E102-E109.

Tarrant, P.V., 1990. Transportation of cattle by road. Appl. Anim. Behav. Sci. 28, 153-170.

Tarrant, V., Grandin, T., 2000. Cattle transport, In: Grandin, T. (Ed.), Livestock Handling and Transport, 2nd ed. CABI Publ., Wallingford, pp. 151–173.

Tarrant, V., Kenny, F.J., Harrington, D., Murphy, M., 1992. Long distance transportation of steers to slaughter: effect of stocking density on physiology, behaviour and carcass quality. Livest. Prod. Sci. 30, 223–238.

Tennessen, T., Pricea, M.A., Berga, R.T., 1985. The social interactions of young bulls and steers after re-grouping. Appl. Anim. Behav. Sci. 14, 37-47.

Tripp, M.J., Schmitz, J.A., 1982. Influence of physical exercise on plasma creatine kinase activity in healthy and dystrophic turkeys and sheep. Am. J. Vet. Res. 43, 2220–2223.

Trunkfield, H.R., Broom, D.M., 1990. The welfare of calves during handling and transport. Appl. Anim. Behav. Sci. 28, 135-152.

Trunkfield, H.R., Broom, D.M., 1991. The effects of the social environment on calf responses to handling and transport. Appl. Anim. Behav. Sci. 30, 177.

Van de Water, G., Verjans, F., Geers, R., 2003. The effect of short distance transport under commercial conditions on the physiology of slaughter calves; pH and colour profiles of veal. Livest. Prod. Sci. 8, 171–179.

Warriss, P.D., 1990. The handling of cattle pre-slaughter and its effects on carcass and meat quality. Appl. Anim. Behav. Sci. 28, 171.

Warriss, P.D., Brown, S.N., Knowles, T.G., Kestin, S.C., Edwards, J.E., Dolan, S.K., Phillips, A.J., 1995. Effects on cattle of transport by road for up to 15 hours. Vet. Rec. 136, 319–323.
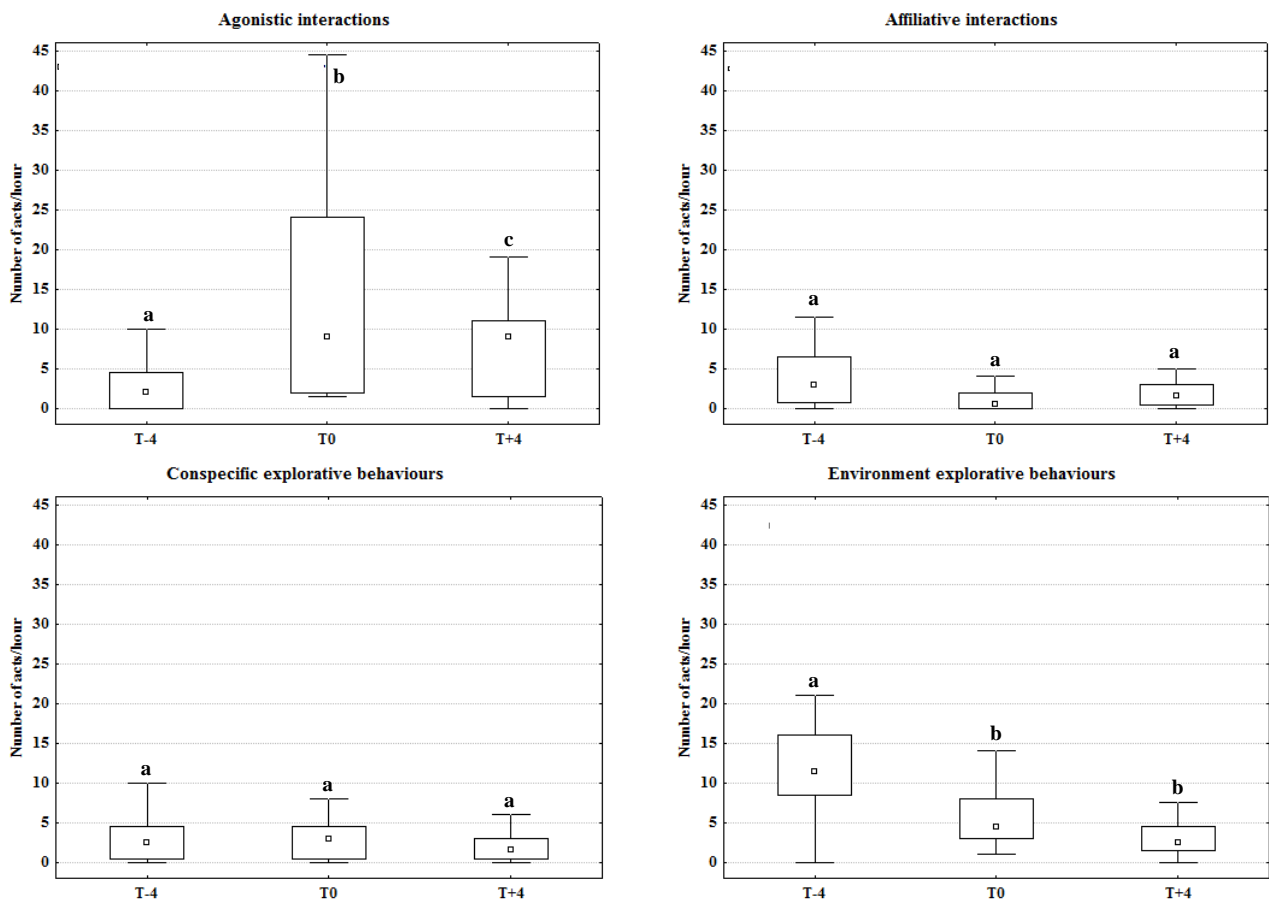
Fig. 1.
Comparison among the three contexts analysed (T-4, T0, T+4) for each behavioural category (different superscript letters indicate significant differences at P < 0.016 for the corresponding time point).
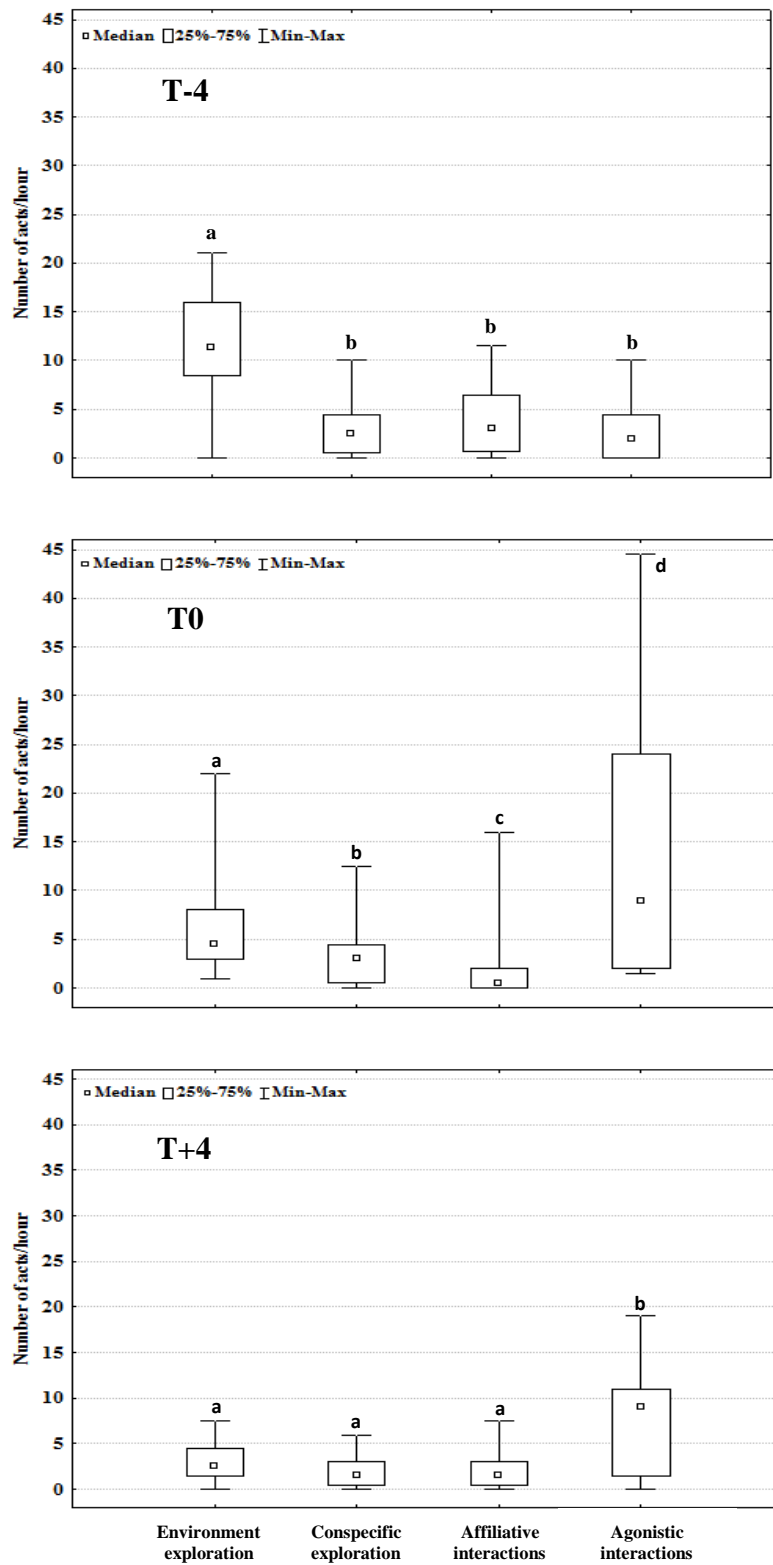Median (and 1st/3rd quartile) are given; whiskers show the minimum and maximum values.

Fig. 2.
Comparison among behavioural categories analysed for each context (different letters are different at P < 0.05). Median (and 1st/3rd quartile) are given; whiskers show the minimum and maximum values.

Table 1
Information about animals transported for each journey

Journey	Date	Farm of origin	N. of animals in the front compartment	Space allowance (m²/head)	Farm of origin	N. of animals in the rear compartment	Space allowance (m²/head)	Total animals transported
1	01/12/08	A	2#	3.75	F	2°	3.75	4
2	26/01/09	B C	**3** **2**	1.50	F	2	3.75	7
3	20/04/09	D E	**1*** **2***	2.50	G A	**2** **1**	2.50	6
4	03/06/09	F	2	3.75	F	2	3.75	4
5	13/07/09	F	2•	3.75	F	3	2.37	5

Bold numbers indicate the animals coming from different rearing farms and mixed into the same truck compartment
*Data of these subjects were not considered because transportation exceeded the 8-hour limit of short-distance journeys of EU regulation 1/2005.
°Data during travelling and stationary phases and at T+4 are missing for these animals.
Data during stationary phase are missing for these animals.
• Data at T+4 are missing for these animals.

Table 2
Transport condition for each journey

Journey	Date	Journey duration (means±SD)	Stationary phase duration (means±SD)	T (means±SD)	RH (means±SD)
1	01/12/08	321±56	56±9.13	7.04±1.44	77.21±6.38
2	26/01/09	275±1	48.25±14.70	5.89±2.07	78.32±7.83
3	20/04/09	510±266	173.8±19,73	14.05±1.14	81.84±5.61
4	03/06/09	230	40	27.96±2.03	34.27±6.99
5	13/07/09	245	96	28.41±2.60	51.68±7.96

Duration, Temperature (T) and Humidity (RH) are respectively expressed in minutes, centigrade and percentage.

Table 3
Behavioural categories recorded

Behavioural patterns	Description
Agonistic behaviour	Head to head pushing, sometimes followed by head to neck pushing, attempt to mount, mounting, pressing the muzzle on the another individual's back.
Affiliative behaviour	Sniffing, touching and rubbing against the muzzle of another individual; licking another individual's head, neck and/or shoulder areas.
Environment explorative behaviours	Sniffing substrate and air, sniffing, liking and biting an object in the environment.
Conspecific explorative behaviours	Sniffing the genitals of another individual sometimes followed by flehmen; sniffing body.
Restlessness	Changing from an orientation to another one (perpendicular, parallel and diagonal) during transport.
Body posture	Lying down or standing.
Standing orientation	Perpendicular, diagonal or parallel orientation to the long axis of the lorry.

Table 4
Comparison of the hourly rates of behavioural categories observed at each sampling time.

	T-4 (n = 19)	T0 (n = 21)	T+4 (n = 17)
Environment exploration vs Conspecific exploration	$t = 0$, $z = 3.62$***	$t = 60$, $z = 1.93$	$t = 39$, $z = 1.50$
Environment exploration vs Affiliative behaviour	$t = 4$, $z = 3.43$***	$t = 23$, $z = 3.22$***	$t = 46.5$, $z = 1.11$
Environment exploration vs Agonistic behaviour	$t = 1$, $z = 3.57$***	$t = 52$, $z = 2.21$*	$t = 18$, $z = 2.59$**
Conspecific exploration vs Affiliative behaviour	$t = 45$, $z = 1.19$	$t = 36.5$, $z = 2.35$*	$t = 47.5$, $z = 0.71$
Conspecific exploration vs Agonistic behaviour	$t = 36$, $z = 1.36$	$t = 12.5$, $z = 3.58$***	$t = 1$, $z = 3.35$***
Affiliative behaviour vs Agonistic behaviour	$t = 29$, $z = 2.02$*	$t = 0$, $z = 3.82$***	$t = 11$, $z = 3.10$**

We used the Wilcoxon Signed Rank test to compare the hourly rates of all behavioural categories observed in each context. *P≤0.05; **P≤0.01; ***P≤0.001.

Table 5.
Least square means of blood parameters at different sampling time.

	T-4	T0	T+4	SEM(*)
Hematocrit (%)	35.66^A	34.03^{AB}	31.71^B	0.9639
Albumin† (g/l)	32.36	31.44	31.40	1.1343
UREA† (mmol/l)	2.83^a	2.91^A	2.04^{bB}	0.2392
Total Protein (g/l)	62.18	61.78	62.54	2.0870
CK† (UI/l)	145.83	132.43	228.01	39.6758
Glucose† (mmol/l)	4.83^A	5.52^B	4.35^C	0.1447
Lactate† (mmol/l)	2.14^a	1.36^b	1.41^b	0.2603
BHB† (mmol/l)	0.32^A	0.34^A	0.47^B	0.0350
NEFA† (milleq/l)	0.18^a	0.47^b	0.32^c	0.0869
Haptoglobin (mg/ml)	4.07	5.96	5.48	0.1108
Cortisol (nmol/l)	20.41	41.18	12.34	0.2132

(*) Standard Error of Means of transformed data. Means with different lowercase and uppercase superscripts are different at $P<0.05$ and $P<0.01$, respectively.
Parameters marked with (†) were measured in plasma, others parameters were measured in serum.

CONCLUSION

The aim of this thesis was to analyse the behavioural and physiological responses of young bulls and piglets to transport practice and to investigate if coping characteristics may affect how pigs cope with aversive situations.

In the last years several evidences emerged on the importance of behavioural patterns in detecting animal stress response. For that reason, tools allowing to identify changes in behaviour and in individual behavioural characteristic are of major interest and cover a pivotal role in this thesis.

Regarding the research on pigs, Chapter 1 could be considered a preliminary study. Two different immobilization tests already used to identify different coping styles such as Backtest and Tonic Immobility Test were examined. Experimenters have been trained to execute these tests before to select the subjects candidate to be used in further experiments.

Although in literature these two tests are described as to be predictive for coping style, from the study reported in Chapter 1 emerged that the two tests classified in different way the tested subjects independently to the order of the tests.

Because the large use and the more relevant evidences in literature for Backtest than Tonic Immobility Test, and considering the excessive heterogeneity between different Tonic Immobility-class, the Backtest was chosen to classify piglets involved in the further experiments.

The study reported in Chapter 2 shows how piglets were tested to search the behavioural differences between HR and LR subjects as classified by Back test during open field and novel object tests . Even if this study is presented before that carried out on piglet's transportation, the subjects were part of the same experimental group. Since the two studies were carried out several days apart, the transport did not influence the results of behavioural tests.

Open field test and Novel object test are two of most used tests in behavioural investigation, unfortunately they were unsuccessful to detect difference between HR and LR, and on the other hand they were useful to observe correlations between some behavioural patterns.

In chapter 3 and 4 transport stress is evaluated.

Chapter 3 shows the effect of transport on piglets with a live weight around 10 kg and close to the threshold indicated by the regulation to be considered fitted for long transport (> 8 h). Even if

several researches were carried out on fattening pigs, data on piglets transported with different group composition based on Backtest classification are missing.

The environment conditions during transport were also considered. Collected data show a correlation between temperature and animal activity, even if the transport duration played an important role in animals' fatigue. However, the results obtained on blood analyses show that transport did not affect piglets in a significant way, diverging from many results obtained in fattening pigs. It is possible to suppose that young individuals are more adaptable and their physical resistance is still understated. These doubts should encourage further research to better understand the reason of this difference.

Another approach was used for cattle. Unfortunately there is not a test similar to Backtest or Tonic Immobility test for ruminants. Moreover, we had not the same control over the animal collection and transport condition as happened for the piglets. For these reasons animal behavioural and blood parameters were taken into account in order to evaluate the effect of the transport on a special category of young cattle. The changes observed in all parameters measured indicate that the condition of transport may be considered satisfactory for the welfare of young bull selected for genetic evaluation in a test station.

It was interesting to observe that agonistic behaviour increased when the subjects changed their environment and/or their mates but it decreased after few days. This change is part of the adaptation to the new social and rearing condition which also involved some change in blood parameters. To avoid misunderstand in cause/effect relationship a multifactor approach considering both behavioural and blood analyses is necessary and is also useful to evaluate the stress response of animals.

The topics presented in this thesis focused on the stress response of young individuals , a farm animal category not often object of investigations. Moreover, the results presented contribute to the knowledge on behavioural analysis as approach for a better investigation on animal welfare during transport.

ACKNOWLEDGMENTS