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## Does stocking density modify affective state in pigs as assessed by cognitive bias, behavioural and physiological parameters?



<sup>a</sup> School of Agriculture, Food and Rural Development, Newcastle University, Newcastle upon Tyne NE1 7RU, UK
<sup>b</sup> Department of Animal Medicine, Production and Health, Padova University, Legnaro, 35020 Padova, Italy

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### ABSTRACT

Recent studies suggest that emotional state can affect cognitive abilities of humans and non-human animals, determining biases in information processing. Negative mental states, such as anxiety or depression, induce pessimistic judgments of ambiguous stimuli. These assumptions may be used to derive indicators of emotional state in captive animals, providing a novel approach to the assessment of animal welfare. This study used a spatial judgement task, in which farmed pigs were trained to expect food inside a bowl in one location and not in another, to determine whether pigs housed in ways that might be expected to result in relatively positive or negative emotional states respond differently to ambiguous stimuli of intermediate spatial locations. Forty growing pigs were housed in groups of 10 at different density for 8 weeks prior to the start of the test. After training, the pigs successfully discriminated between the rewarded and the unrewarded locations as assessed by increased latency to arrive at the unrewarded location, with no rearing treatment difference. Then, pigs were tested on 3 days in which three ambiguous locations, intermediate between the known rewarded and the unrewarded sites, were introduced and latency recorded. In order to compare the novel cognitive bias task with other welfare indicators, during the 8 weeks of the study four behavioural observations, two measurements of skin lesions, two salivary samples for cortisol and  $\alpha$ -amylase, and six individual weights to assess growth were collected.

Considering the mean of the three test days, there was no difference between the treatments in the pigs' judgement of the three ambiguous locations. However, the latency trend during the testing days led to difference between treatments on the third day (P=0.026). Pigs housed a higher density seemed to learn faster that the ambiguous stimulus near the unrewarded location was also not reinforced by a reward and showed a higher latency to approach on day 3 (44 vs 15.6 s). These animals were also observed to have a higher frequency of sitting posture (P=0.01), and more total skin lesions (P=0.035) due to aggressiveness, in particular at the ear location (P=0.009), but did not differ in other physiological parameters.

Although the results showed no immediate effect of stocking density on cognitive bias, differences in latencies to reach the bowl over the three testing days suggest a different learning process between treatments.

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\* Corresponding author at: University of Padova, Animal Medicine, Production and Health, viale dell'Università 16, 35020 Legnaro, Italy. Tel.: +39 49 827 2620/+39 349 242 1613; fax: +39 49 827 2669.

E-mail addresses: annalisa.scollo@studenti.unipd.it, anna.polly@libero.it (A. Scollo).

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### 1. Introduction

The study of the emotional state of animals is increasingly attracting the attention of researchers and in recent years has also been reflected in public concern about animal welfare (Dawkins, 2006; Mendl and Paul, 2004). Furthermore, the recently adopted EU Strategy for the Protection and Welfare of Animals 2012–2015 (European Commission, 2012) highlights the desirability of using scientifically validated animal-based indicators to complement the prescriptive requirements in EU legislation (EFSA AHAW panel, 2012).

Currently it is not possible to obtain direct and objectively measurable observations of the emotional experience of animals, and the investigation of emotions focuses mainly on the assessment of physiological and behavioural indicators of stress and welfare (Abou-Ismail et al., 2007; Burman et al., 2007). However, interpretation of these indicators is complicated by the fact that a particular measure could reflect both a positive or negative emotional state. For example, an increase in locomotory activity or in heart rate can be associated both with escape from predation (aversive activity, negative valence) and with sexual behaviour (pleasurable activity, positive valence). Furthermore, changes in physiological indicators of stress such as cortisol, could indicate an alteration of the psychological state but also pathological states or inflammation (Dawkins, 2001). Often the method of collection for biological samples (e.g. blood) or the testing of spontaneous behaviour in an open field can cause emotional reactions in animals that may confuse the results (Broom and Johnson, 1993).

For these reasons, the development of alternative methods for the assessment of emotional states for which behavioural and physiological measures may be inconclusive or imprecise has been considered, not only by animal welfare researchers but also in neuroscience and psychopharmacology (Lawrence, 2008; Rolls, 2005). One of these alternatives is the study of cognitive bias, a term used for the first time by Shettleworth (1998) to indicate 'the mechanisms by which animals acquire, process, store and act on information from the environment'. The literature on human psychology provides the main source of information about the effects of background emotional states on cognitive processes, influencing attention, memory and judgement (Paul et al., 2005). However, the 'gold standard' indicator of the subjective emotional state in humans is verbal report, which animals are unable to provide, thus making evaluation more uncertain. Furthermore, interpretation of tests, and translating them from one specie to another, could be difficult and provide some uncertainties in research findings (Cryan and Mombereau, 2004; Janczak et al., 2002).

The first attempt to explore cognitive biases in animals was conducted by Harding et al. (2004) in rats. Animals experiencing an unpredictable housing treatment showed a reduced anticipation of a positive event, similar to pessimistic cognitive bias seen in depressed humans. These initial results were followed by a number of studies carried out in different species, confirming the assumptions also in starlings (Bateson and Matheson, 2007) and dogs (Mendl et al., 2010). In 1999, Laughlin et al. stated that cognitive processes could be involved in welfare problems also in farmed animals, where housing and husbandry procedures may result in stress or increased arousal of the animals. An initial experiment demonstrated that the degree of environmental enrichment in the housing of pigs could influence cognitive bias in a go/no-go discrimination task (Douglas et al., 2012). However, this task involved lengthy training and staff effort. In the present study we therefore decided to investigate the promising approach of cognitive bias in pigs using a location judgement bias task based on the absence/presence of a food reward.

A spatial location task was used because of its relevance to foraging behaviour, a common cognitive task for animals (Thorpe et al., 2002; Wood et al., 1999). Furthermore, Held et al. (2002) showed that pigs have well-developed spatial memory abilities. To induce a mild stress, we housed pigs at two different stocking densities. Reduced space allowance is reported to have negative effects in pigs and is detectable in both behavioural and physiological indicators of stress (Averós et al., 2010) and space is one of the most compromised needs in commercial housing systems in the interests of efficiency of building utilization (Turner et al., 2000).

In order to compare results of the cognitive bias task with other commonly used indicators of affective state, concurrent behavioural and physiological measures were taken. Among the behavioural parameters, we decided to record vocalizations as a spontaneous behaviour indicating the expectation of the food in the rewarded location, as reported by Waitt and Buchanan-Smith (2001) in pigs before the meal delivery. Vocal expression of apparently valenced affective states has been identified in several species and it is reported by Manteuffel et al. (2011) as a potential indicator of reduced welfare in pigs. Moreover, we measured salivary cortisol and  $\alpha$ -amylase. The latter has become an emerging biomarker for sympathetic nervous system activity related to depression in humans. Ishitobi et al. (2010) suggested that  $\alpha$ -amylase may be a state-dependent marker of major depressive disorder in addition to salivary cortisol, but its role in non-human animals has not yet been investigated.

It was hypothesized that pigs with a lower space allowance would show a statistically significant difference in latency to approach an ambiguous stimulus as compared to pigs with a larger space allowance. Such difference would be indicative of a negative or pessimistic emotional state. Moreover, it was also predicted that this difference in assumed emotional state would be associated with significantly different behavioural and physiological parameters measured during housing in the home pen.

#### 2. Materials and methods

#### 2.1. Animals, facilities and management

All experimental procedures and animal care were carried out in accordance with the minimum standards for the protection of pigs laid down by law (Council of The European Union, 2008), and were approved by the Ethical Review Committee of Newcastle University. Download English Version:

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