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Effects of analgesic intervention on behavioural responses to Low Atmospheric Pressure Stunning

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ABSTRACT

Worldwide, more than 50 billion chickens are killed annually for food production so their welfare at slaughter is an important concern. Low Atmospheric Pressure Stunning (LAPS) is a novel approach to pre-slaughter stunning of poultry in which birds are rendered unconscious by gradually reducing oxygen tension in the atmosphere to achieve a progressive anoxia (hypobaric hypoxia). Advantages of this approach over electrical stunning are that birds are not shackled while conscious and all birds are reliably and irreversibly stunned. However, concerns remain that birds undergoing LAPS could experience discomfort or pain. Here we investigated whether subjecting birds to LAPS with and without administration of an opioid analgesic (butorphanol) affected behavioural responses. A blocking design was used in which pairs of birds receiving either analgesic or sham treatment were allocated to three types (analgesic/analgesic, analgesic/sham, or sham/sham). In line with previous studies, birds showed a consistent sequence of behaviours during LAPS: ataxia, loss of posture, clonic/tonic convulsions, leg paddling and motionless. Overall, administration of butorphanol had no effect on the range and patterning of behavioural responses during LAPS, but there were some differences in behaviour latencies, counts and durations. For example, latencies to ataxia, mandibulation and deep inhalation were delayed by analgesic treatment, however the duration of ataxia and other behaviours related to loss of consciousness were unaffected. Fewer birds receiving analgesia showed jumping and slow wing flapping behaviour compared to controls, which suggests these may be pain related. These behaviours after the onset of ataxia and the results may reflect a smoother induction to unconsciousness in analgised birds. Collectively, the results do not provide convincing evidence that birds undergoing LAPS are experiencing pain. While there were effects of analgesia on some aspects of behaviour, these could be explained by potential sedative, dysphoric and physiological side effects of butorphanol. The behavioural responses to LAPS appear to be primarily related to exposure to anoxia rather than hypobaric conditions, and thus in terms of welfare, this stunning method may be equivalent to controlled atmosphere stunning with inert gases.

1. Introduction

Low Atmospheric Pressure Stunning (LAPS) is a novel approach to pre-slaughter stunning of poultry in which birds are rendered unconscious by gradually reducing air pressure and thus oxygen tension to achieve a progressive hypobaric hypoxia. LAPS shares many of the welfare advantages of controlled atmosphere stunning (CAS) systems, which use exposure to hypoxic and/or hypercapnic gas mixtures, reliably and irreversibly stunning birds in their transport crates (Vizzier-Thaxton et al., 2010; Johnson, 2013). A major benefit of CAS systems and the LAPS system is that they avoid the considerable stress and pain of shackling of conscious birds (Sparrey and Kettlewell, 1994; Gentle and Tilston, 2000) and 100% of the chickens are rendered insensible before shackling and bleeding. By contrast, electrical stunning is associated with various welfare issues such as shackling of conscious birds, pre-stun

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shocks and the risk of inadequate stunning (Raj, 2006). LAPS is in routine commercial use at a poultry processing plant in Arkansas, having been given 'no objection' status by both the United States Department for Agriculture (USDA) in 2010 and the Canadian Food Inspection Agency in 2013. While there has been much research to determine humane gas mixtures for CAS (e.g. McKeegan et al., 2007; Johnson, 2013; Joseph et al., 2013), less is known about the welfare impact of LAPS.

Previous work investigating the induction of unconsciousness in hypoxic gas environments (Woolley and Gentle 1988; Raj et al., 1991) suggests that the approach has promise, and the gradual nature of LAPS avoids obvious concerns related to the welfare consequences of rapid decompression (Close et al., 1996; AVMA 2013). Previously, Purswell et al. (2007) identified process variables for a suitable decompression and some aspects of behaviour, corticosterone responses, meat quality and pathology have been investigated (Battula et al., 2008; Vizzier-Thaxton et al., 2010). Electroencephalogram (EEG) and electrocardiogram (ECG) responses of broilers undergoing LAPS were reported by McKeegan et al. (2013), where the process was associated with changes in the EEG pattern (highly significant increases in total power, decreases in median frequency and progressive increases in slow wave activity), indicating a gradual loss of consciousness. Recently, a detailed behavioural study described the responses of broilers undergoing LAPS and reported a consistent sequence of behaviours: ataxia, loss of posture, clonic and tonic convulsions and leg paddling (Mackie and McKeegan, 2016). Additional responses were observed in a proportion of birds such as mandibulation (repetitive and rapid opening and closing of the bill, 32% of birds), headshaking (76% of birds) and open bill breathing (74% of birds). Based on loss of posture (on average at 84 s), the data suggest that birds are in a conscious state for longer during LAPS than in controlled atmosphere stunning with inert gases (McKeegan et al., 2007a; Abeyesinghe et al., 2007),other behavioural responses are equivalent. Given that headshaking, mandibulation and open bill breathing are all seen during exposure to anoxic gases (normobaric hypoxia) and LAPS (hypobaric hypoxia), it is difficult to conclude whether they are a response to hypoxia or decompression, or both. Concerns remain that some of the behavioural responses observed could be pain related, possibly resulting from painful expansion of trapped air in body cavities. Vizzier-Thaxton et al. (2010) noted that the anatomy and function of the avian respiratory tract with interconnecting airsacs and lungs makes it unlikely that significant amounts of gas would be trapped in the abdomen, while hemorrhagic lesions were found in the lungs, brain, and heart of animals undergoing rapid decompression (Van Liere, 1942).

Pain is difficult to assess as it cannot be measured directly, but behaviour is the parameter most often used to assess animal pain (Rutherford, 2002) and signs of stress during stunning in poultry include head shaking (Erhardt et al., 1996), gasping (Raj and Gregory, 1990), yawning (Erhardt et al., 1996), vocalisation (Zeller et al., 1988), sneezing (Hoenderken et al., 1994) and defecating (Morton et al., 1998). Some of these signs may also indicate pain or varying degrees of discomfort, or may reflect physiological responses. Quantitative differences may be significant from a welfare point of view, as well as the time at which they occur during the stunning process.

Analgesic intervention has been widely used in a range of contexts in animal welfare research, for example to examine pain associated with lameness (e.g. Hocking et al., 1997). It is widely recognised that the abolition of suspected pain related behaviour with analgesic is circumstantial evidence of pain (Rutherford, 2002; Walker et al., 2013). However, analgesic drugs may have behavioural effects unrelated to pain and nociception, and some also have general sedative or side effects. Thus, care must be taken with the choice of agent and the dose applied. The primary objective of this study was to investigate whether subjecting birds to LAPS with and without administration of an opioid analgesic would affect their behavioural responses, especially those suspected to relate to pain and discomfort. Butorphanol was chosen for this trial, as it is a Kappa opioid receptor agonist and a mu opioid receptor antagonist with characterised pharmacokinetics (Guzman et al., 2014) and is the currently recommended opioid for use in birds (Paul-Murphy and Fialkowski, 2001; Hawkins and Paul-Murphy, 2011; Paul-Murphy, 2013). We used a low-moderate dose (Paul-Murphy, 2013) to minimise sedation and side effects. Broilers were exposed to LAPS in pairs to maximise visibility of their reactions to the process while eliminating isolation stress. A blocking design was used in which birds receiving analgesic or sham treatments were randomly allocated to three types of pairs (analgesic/analgesic, analgesic/sham, or sham/sham)). This robust design, random allocation and blinding of behavioural observers to pair type allowed us to reliably determine the effects of analgesic intervention on behaviour during LAPS, and thus contribute to a thorough welfare assessment of the process.

2. Material and methods

2.1. Animals and housing

Ninety Cobb 500 male broiler chickens (*Gallus gallus domesticus*) from the female breeder line were used in this study. They were sourced from a commercial hatchery and were wing tagged at 4 weeks of age. The birds were housed at the University of Arkansas poultry facilities within a larger single flock split into three groups, reared in three identical environmental chambers (measuring 3.05 × 3.05 m, approximately 100 birds per pen resulted in a stocking density of ∼30 kg/m2). Clean pine shavings were used for litter. Single-pass ventilation was maintained at a constant rate of 6 m3/min in all chambers. The photoperiod was 23L:1D for d 1–4, and 16L:8D thereafter. Chambers were equipped with 2 rows of nipple waterers, and 2 hanging feeders and birds had ad libitum access to feed (standard commercial starter and grower diet) and water. Environmental controls for climate were maintained to follow recommended management practices (Cobb-Vantress, 2012). Birds and environmental controls were monitored twice daily by trained staff. The trials were undertaken in Arkansas, USA, and therefore were not subject to UK legal requirements through DEFRA or Home Office regulations. The experimental design and animal husbandry was performed following the EU Directive on the Protection of Animals used for Scientific Purposes (EU 2010/63) for guidance. The experiments were specifically authorized by the University of Arkansas Institutional Animal Care and Use Committee (Protocol 15031).

2.2. LAPS process

The LAPS chamber was developed by Technocatch LLC in Mississippi, USA the system and the pressure curves applied by the process are patented (Cheek and Cattarazzi, 2010). The chamber, it's monitoring and control systems used in the current study is a scaled down research unit, but is otherwise identical to those used commercially except for manual door operation. The chamber is cylindrical (2.2 m in length and 1.8 m in diameter) and is designed to accommodate a reduced scale transport module (153 cm × 121 cm × 102 cm, three tiers each 23 cm height). The required decompression curve is automatically applied and controlled by a computer and once started, can only be stopped in the case of an emergency. An infra-red camera (130° camera with (2.1 mm lens) 18 infra-red illuminators, Model #RVS-507, RVS Systems) was fitted into the chamber to observe the birds (fixed