EFFECTS OF PLAY EXPERIENCE ON FEAR-RELATED BEHAVIOR OF CHICKENS

By

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EFFECTS OF PLAY EXPERIENCE ON FEAR-RELATED BEHAVIOR OF CHICKENS

Abstract

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The welfare of laying hens is a topic of concern to the public and egg producers, since poor welfare may reduce consumer confidence and decrease production. Play behavior is a rewarding activity thought to have benefits including increased coping ability during novel, unanticipated events. Play, mainly studied in mammals, has received less attention in birds, especially poultry. In this thesis, literature on play, chickens, and welfare assessment is reviewed to explore the possibility of stimulating play in chickens, to enhance well-being, productivity, and public perception.

Running with inedible worm-like objects and repeatedly jumping on swinging perches in safe environments are possible forms of play in chickens. I hypothesized that stimulating chicken play by providing worm-like objects and swinging perches in home-cages would reduce fear in future unanticipated situations and enhance body condition. To test this hypothesis, 8 cages each containing 9 female White Leghorn chicks were assigned to one of two ‘worm’ treatments (‘worms’ for 30 min/day or no ‘worms’ provided) and one of two perch treatments (permanent swinging perch in cage or no perch provided) in a randomized block design. At 5 and 10 weeks of age, effects of ‘worm’ and perch provision on fear-related responses of 4 chickens/cage were investigated using standardized fear tests: open field, novel object, handling, and tonic immobility tests. At 10 weeks, the same birds were subjected to previous tests while 4 other chickens/cage were subjected to variations of these tests. All chickens were weighed and examined for skin lesions weekly. Chickens provided with ‘worms’ weighed significantly more at 10 and 11 weeks, and exhibited longer durations in the center of the open field and higher rates of wing flapping during handling. Chickens provided with a swinging perch weighed significantly more at 7 weeks of age. Differences in behavior at 5 versus 10 weeks were consistent with an interpretation of reduced fear with age whereas effects of the ‘worm’ and perch treatments on fear-related behavior were less robust. In conclusion, the results suggest that there could be benefits to providing ‘worms’ to improve productivity and well-being of chickens.

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Dedication

To my family, for always maintaining a playful mood.

INTRODUCTION

Recently, bans on use of conventional cages for the housing of laying hens in the European Union and in California show that chicken welfare is important to the public. Cage bans are worrisome for some producers because, although conventional cages are thought to reduce welfare in some ways, they have prevented injurious pecking from conspecifics. A mild form of this is feather pecking, which damages the plumage of birds leading to skin being uncovered. A more severe form of injurious pecking is cannibalism, where birds cause wounds in a target bird. Besides the worry of injurious pecking in a more spacious housing system allowing increased mobility, is a more general problem at the root of many welfare issues—fear. Fear may present an increased problem in a system allowing more movement because chickens are able to exhibit stronger behavioral responses to fear-inducing stimuli, which can result in them piling up in a corner such that birds at the bottom of the pile become smothered. Therefore, methods to reduce cannibalism and fearful behavior in enriched cages and non-cage systems are needed.

Although certain forms of foraging enrichment have been shown to reduce ‘redirected’ pecking, they alone may be insufficient to prevent problems. Inducing additional engrossing and biologically relevant, positive behaviors may help to prevent the development of cannibalism, reduce fear, and increase welfare in laying hens, which would be beneficial not only for the birds but also, by improving public perception, for sustainability of the poultry industry. Such behavior would have to be rewarding and highly motivated. Behaviors commonly associated with these conditions are feeding and mating. However, providing feed ad libitum (as is typical in poultry production) is not sufficient to prevent welfare problems. Furthermore, although a few males can be housed with hens in egg laying flocks in non-cage housing, this approach is less feasible in cages due to space limitations. A rewarding behavior less commonly considered in animals, especially in chickens, is play behavior. By inducing play behavior, which may not only be highly biologically motivated but may have developmental benefits, chickens may exhibit reduced fearful behavior during times of stress as well as superior body condition.

In this thesis, a literature review covering the above themes is followed by a description of an experiment involving two behaviors interpreted as play in chickens—worm running and balancing on swinging perches, respectively induced by the provision of 'worm objects' and swinging perches. Standardized fear tests were used to examine the immediate and eventual effects of play on fearful behavior and body condition of White Leghorn pullets, thus assessing their value in enriching the cage environment during the rearing period.

LITERATURE REVIEW - PLAY BEHAVIOR, CHICKENS, AND WELFARE

I. Play behavior - evidence and hypotheses thus far

What is play?

Play behavior has been studied scientifically for over 100 years but, due to its lack of obvious biological function and its subjective association with pleasure and leisure, play has posed the difficulty of not possessing a clear, objective definition (Bekoff, 1972; Ficken, 1977; Burghardt, 2005). The most common definition of play, from an external view, is that it is a 'purposeless' behavior lacking immediate benefits, appearing to lack a serious intention or specific goal, and often resembling modified adult behavior (Fagen, 1977; Bekoff, 1984; Lewis, 2005).

Forms of play behavior

Play may be characterized in three main categories based on form and number of participants: locomotor play, object play, and social play (Fagen, 1981; Smith, 1985).

The most widespread, and speculated original, form of play (Byers, 1985) is locomotor play, which appears as rapid and spontaneous movement. It may take the form of running, jumping, bucking, and sliding, and often incorporates rotational components such as twisting and rolling (Brownlee, 1954; Wilson and Kleiman, 1974). It is often considered a rudimentary form of play as it may be the first and, for some species, the only form of play exhibited (Byers, 1985). Locomotor play tends to comprise repeated elements and may resemble reordered prey capture or escape from predators (Lancy, 1980; Byers and Walker, 1995). Locomotor play may be combined with other forms of play (Bekoff and Allen, 1998) or may be performed by itself. Sometimes it occurs in synchrony with other animals (Watson, 1998).

Often linked with exploration (Hall, 1998), object play occurs when an animal directs behaviors towards an object, such as pawing, nosing, chewing, and leaping on the object, in an exaggerated and repetitive manner (Fagen, 1981). Most often the object is inanimate, but it may also be animate, for example, when chimpanzees manipulate the bodies of hyraxes (Hirata et al., 2001). This form of play resembles exploration but is distinguished by its energetic and cyclic interactions (Burghardt, 1985). Object play may be readily induced by objects with tactile similarities to a food or prey item (Negro et al., 1996; Hall and Bradshaw, 1998; Hall et al., 2002) and typically occurs in a single animal. However, the interaction may draw the attention of conspecifics to the object, resulting in defense or play between the animals and the object (Biben, 1982).

Finally, there is social play, the variety most commonly reported (Pellis and Pellis, 1998b) possibly due to its more obvious form and ease of induction in rats, the favored laboratory species for the study of play. In this form of play, behavior is directed towards one or more individuals actively engaged in a play bout (Fagen, 1981). This category may include play fighting, resembling adult fighting, rough and tumble, commonly known as wrestling, and other forms of physical contact involving one or more actively participating partners. Social play may resemble agonistic behavior but harmful actions of an agonistic pattern are modified so no damage is inflicted (Fagen, 1981) and, in some cases, the body targets are completely different (Pellis and Pellis, 1998b; Diamond and Bond, 2004; Kamitakahara et al., 2007). It has been hypothesized that this form of play entails more advanced cognitive abilities and evolved later than other forms of play (Bekoff and Allen, 1998). This is due to the fact that social play involves soliciting a partner and engaging in what might appear to be fighting. Yet, for the play bout to continue, the participants must self-handicap to avoid harming one another (Bekoff, 1995). The possibility of play being mistaken as aggression may make it necessary to communicate good-natured intent to facilitate cooperation, possibly involving theory of mind in some species (Bekoff and Allan, 1998). When a partner refuses social play, self directed play may occur (Bekoff, 1972) such as tail chasing (Biben, 1998).

Although play is often categorized into locomotor, object and social play, play may incorporate features from two or all three categories (Fagen, 1981; Diamond and Bond, 2003).

Conditions and characteristics of play

Play appears to be ubiquitous in mammals, with numerous species in every order of mammalia having been reported to exhibit some form of play behavior (Burghardt, 1985, 2005). Play has been reported to a lesser extent in birds, but has been described in twelve orders in over one-hundred species (Ortega and Bekoff, 1987; Diamond and Bond, 2004; Burghardt, 2005). It has also been proposed that rudimentary play exists in ectotherms and invertebrates such as reptiles and octopuses (Burghardt et al., 1996; Burghardt, 2005; Kuba et al., 2006) although it is less clear if the alleged play activities actually constitute play.

Play is typically exhibited by juveniles (Fagen, 1981; Bekoff, 1984), and occurs at similar rates in both males and females during infancy. Later in development, males, especially those of polygynous species, will play more often (Špinka et al., 2001), more roughly, (Pellis and Pellis, 1998a; Foroud and Pellis, 2003) and longer into their life than females (Lancy, 1980). Although infants may initially engage in social play with their mothers (Bekoff, 1972), social play thereafter is most common between animals of the same age (Mendoza-Granados and Sommer, 1995; Thompson, 1996; Biben, 1998). Partner preference may be based on sex, age, social rank, and reciprocity shown during play (Govindarajulu et al., 1993; Thompson, 1996; Biben, 1998). Adult play exists in some species, but may have a more aggressive appearance (Foroud and Pellis, 2003), making it more difficult to distinguish from serious behavior (Hall et al., 1998), especially when the play bout is terminated by a harmful event.

Play typically occurs in a familiar or safe environment, often in the presence of the mother or adult group members (Lancy, 1980; Vanderschuren et al., 1997). Optimal climatic conditions and levels of resources such as food and water also support the exhibition of play (Bekoff, 1972). When essential resources are in short supply, play is typically depressed or vanishes completely (Müller-Schwarze et al., 1982; Lancy, 1980; Burghardt, 2005; Shimozuru et al., 2007). On the other hand, among predatory species an individual briefly deprived of food may exhibit higher levels of object play in comparison to an individual that has just eaten (Negro et al., 1996; Hall and Bradshaw, 1998).

Another aspect of the environment that influences play is social setting. When the group dynamics of a young animal are disrupted, for example during weaning or isolation, play may decrease or vanish (Fagen, 1974; Donaldson et al., 2002; Shimozuru et al., 2007). Levels of play may return to baseline or rebound at higher levels after animals habituate to their new social setting (Donaldson et al., 2002). Member composition in group-dwelling animals also has an effect on playfulness of the young—when groups have a broader range of differently aged members, juveniles may have richer play repertoires (Thompson, 1996). Furthermore, timing of birth in a social group can influence play levels, for example, due to presence or absence of older juveniles and seasonal differences in availability of resources (Bekoff, 1972; Thompson, 1996).

A novel component in the surroundings of the animal, such as a new object or environmental feature may induce play, usually after exploration (Wood-Gush and Vestergaard, 1991; Špinka et al., 2001; Hall et al., 2002). There may be a continuum between exploration and play, making it difficult to differentiate where exploration ends and play begins. In these cases play is differentiated from exploration due to its manipulative and exaggerated quality (Fagen, 1977), and the appearance of a more relaxed, confident as opposed to fearful mood (Panksepp, 1998). Using neuroscientific techniques, play can be distinguished from exploration based on arousal of different motivational systems with activation of different neural circuitry (Panksepp, 1998).

Play may be further recognized behaviorally by the performance of specific play signals before and during play (Bekoff, 1972; Bekoff and Allan, 1998). Preceding a play bout, species-specific gestures may be used by one animal to solicit play from a potential playmate. These play signals may take the form of body postures, facial expressions, and vocalizations (Bekoff, 1972, 1984). Examples include the characteristic play face in chimps, body lowering in lions, the play bow in dogs, and laughter in chimpanzees and rats (Bekoff, 1995; Panksepp, 1998; Burghardt, 2005). These signals may be repeated throughout the duration of the play bout (Bekoff, 1972). As the organism gets older, these signals as well as other aspects of play may become more species-typical and ritualized (Bekoff, 1972). Learned, idiosyncratic play signals have also been described, for example, in dogs playing with humans (Rooney et al., 2001). It is hypothesized that play signals communicate playful rather than serious intent, reducing the risk of aggression and helping to maintain play (Bekoff, 1972; Bekoff and Allan, 1998).

Another distinguishing property of play is role reversal, in which no single animal is continually in the ‘winning’ position (Bekoff, 1984). Animals that are dominant and the strongest outside of play will not actively pursue this position during the play bout. Rather, a dominant animal will take on the submissive role and stronger animals will self-handicap themselves for a length of time, allowing an animal that is weaker and submissive outside of play to be temporarily in the ‘winning’ position (Špinka et al., 2001). This characteristic of play has been called the 50/50 rule—whereby each player is in a dominant position 50% of the time, and a submissive position

50% of the time (Aldis, 1975). The 50-50% rule probably does not apply to most cases of play since playing individuals are rarely identically matched and the stronger individual may only self-handicap to the degree necessary to encourage continuation of play by the weaker individual. Thus, some degree of inequality during play is probably the norm (Biben, 1998). An example of this is seen in rat rough and tumble play—it is typical for one partner to be on top (in the pinning position) 70% of the time and underneath (in the pinned position) 30% of the time while the reverse is true for the other partner; often the partner that ends up on top will be slightly larger (Pellis and Pellis, 1991; Panksepp, 1998). Self-handicapping by the stronger individual, and some degree of role reversal, appear to be necessary for play to continue (Lewis, 2005).

During play, motor patterns that occur in ‘serious’ situations such as mating, fighting, and stalking prey are incorporated in a modified manner (Bekoff, 1984). These ‘borrowed’ motor patterns may appear in an animal’s play repertoire prior to the development of the ‘real’ behaviors that clearly have functional biological consequences (Bateson, 2005). The altered motor patterns occurring in play have detectable differences, tending to be repetitive, exaggerated, incomplete, and ordered in alternate sequences in comparison to the non-play behaviors (Lancy, 1980; Burghardt, 1985). Although social play may resemble ‘serious’ behavior, movements may be slower and are often performed in a less energetically efficient manner (Panksepp, 1998; Pellis and Pellis, 1998b). Additionally, animals may orient to different body targets during play than those seen in serious fighting. For example, during play both rats and golden hamsters exhibit an increased proportion of contacts oriented towards the rump of the partner whereas in fighting the target is the more vulnerable neck region (Fagen, 1981; Pellis and Pellis, 1998b). Furthermore, damaging behaviors, such as biting and scratching, are modified or absent (Aldis, 1975). Play bites usually do not lead to injury—if a player does seriously injure the partner, the play bout will end.

Occasionally, real aggression ensues, in which case, the prospect of future play between these individuals may be unlikely (Fagen, 1981).

As previously mentioned, play is socially facilitated—one or two playing animals can initiate play in many animals or will at least attract their attention. Therefore, one or two playing animals may act as the stimulus to initiate play in many other animals on an immediate, apparently contagious, basis. Not surprisingly, this is common in group-living animals (Ficken, 1977; Špinka et al., 2001; Diamond and Bond, 2004). Depending on the form of play, animals may play socially (i.e., with physical contact) or separately in parallel (Ficken, 1977). Additionally, when conspecifics that play at different levels are housed together, they may influence each other's levels of play. In rats, for example, an individual that plays at high levels may induce higher levels of play in a partner whereas a rat that plays at low levels may reduce levels of play in a partner (Pellis and McKenna, 1995).

Many researchers suggest that play in animals is a voluntary and positive experience (Ficken, 1977; Lancy, 1980; Burghardt, 2005). After undergoing an aversive event such as brief isolation, animals may show increased levels of play following reunion, and, during isolation, animals will often be willing to work for an opportunity to gain access to a playmate (Humphreys and Einon, 1981; Niesink and van Ree, 1982, 1989; Normansell and Panksepp, 1989). Furthermore, it has been found that social play increases opioid receptor binding in reward areas of the brain (Vanderschuren et al., 1995; Vanderschuren et al., 1997). Although general social activity is also known to increase opioid peptide release (van den Berg et al., 1999), it appears that social contact may not be the primary rewarding experience of play. Social play in rats seems to be rewarding if actions are reciprocated; a non-responsive playmate may be aversive (Pellis and McKenna, 1995). As mentioned above, when rats perform rough and tumble play, one partner consistently ends up on top 70% of the time and on the bottom 30% of the time (Panksepp, 1998). However, proportions of time spent either on the bottom or top during a play bout can be manipulated chemically by addition of small doses of opiate agonists and antagonists. The rat receiving the opiate agonist will occupy the top position for most of the play bout while the rat receiving the opiate antagonist will be pinned more often (Panksepp, 1998). It has also been found that, in rats, brain cholecystokinin, previously associated with social defeat, is correlated with the amount of time the animal is in the pinned position during play (Burgdorf et al., 2006), suggesting that play would not be a positive experience unless the top position is reciprocated.

There is no criterion establishing that a specific number of the above characteristics must be present for a behavior to be considered play (Bekoff, 1984). Play varies between and among species; usually playful-seeming behaviors are readily accepted as play in mammals while closer scrutiny is given to non-mammalian species (Burghardt, 2005). Nevertheless, subjectively, playful mood in mammals and birds is recognizable by most observers (Heinrich and Smolker, 1998; Špinka et al., 2001), especially younger individuals (Panksepp, 1998). Evidence comes through the phenomenon of interspecies play (Lancy, 1980). Panksepp (1998) hypothesizes that interspecies recognition of play in mammals is facilitated by homologous subcortical brain structures found in all mammals (Panksepp, 1998). Homology between avian and mammalian brain structures has now been recognized (The Avian Brain Nomenclature Consortium, 2005), allowing this hypothesis to be extended to interspecific play between birds and mammals.

The costs and benefits of play

It is estimated that, on average, juveniles may only spend 1-10% of their daily time budget (Fagen, 1981), and 6-15% of their daily energy (Martin, 1982), on play. Furthermore, the period during ontogeny in which animals exhibit play may be very short in comparison to their total lifespan (Lancy, 1980) and, when the previously discussed conditions for play are violated, play may completely disappear from the animals' repertoire (Fagen, 1981; Burghardt, 2005). This leads some researchers to conclude that play is not costly and not vital for fitness (Martin and Caro, 1985; Siviy and Atrens, 1992).

Other researchers argue that this amount of time and energy spent on an activity without obvious immediate biological returns is moderate for a young animal who instead could dedicate the time and energy to growth (Fagen, 1977; Martin and Caro, 1985). Furthermore, there is evidence that play can be a risky activity given that, during play, animals have an increased risk of predation, acquiring injuries, and becoming separated from their mother and natal group (Fagen, 1977; Byers, 1985; Biben, 1998; de Oliveira et al., 2003). Because of these potential immediate negative consequences of play on fitness, coupled with the animals' high motivation to play under optimal conditions and following recovery from a stressful event, many researchers agree that play must have functional benefits from an ultimate perspective even if play gives the impression of being purposeless from a proximate perspective (Burghardt, 2005; Lewis, 2005).

Why do animals play? Hypotheses on the functions of play

Although play traits may be similar across species, the precise forms of play and the rates at which it is performed differ (Fagen, 1981). Nevertheless, the existence of play throughout mammalian lineages allows the inference that play has had a long evolutionary history. These points lead to the hypothesis that play had a single function in a common mammalian ancestor but has been modified by different selective pressures on separate species resulting in the variety of play we see at present (Bekoff, 1984; Špinka et al., 2001; Burghardt, 2005). Therefore, play may no longer serve a single purpose (Byers and Walker, 1995; Thompson, 1996), with locomotor play, object play, and social play possibly serving different functions.

The most obvious method to investigate the functionality of play is to prevent it—or to compare the fitness of play-deprived animals with that of play-experienced animals. As a result, a plethora of studies has been conducted on the effects of play deprivation with a focus on the effects of social play deprivation. Results show that animals prevented from engaging in social play exhibit higher levels of aggression and fear (Suomi and Harlow, 1972; Byrd and Briner, 1999), severe deficits in social and sexual function (Einon and Potegal, 1991; Hol et al., 1999; Pellis et al., 1999; Arakawa, 2003), exaggerated stress responses (da Silva et al., 1996;.van den Berg et al., 1999), inability to cope with new environments (Suomi and Harlow, 1972), and reduced cognitive performance (Harlow et al., 1965; Einon et al., 1981; von Frijtag et al., 2002). Although not extended to assessment of fitness, it is plausible that such deficits would adversely affect survival and reproductive success under natural conditions as opposed to the protected, short-term conditions of an experimental study.

These studies are often criticized because they disrupt the social environment of the juveniles, confounding the effects of play deprivation with social deprivation in a more general sense (Bekoff, 1976; Špinka et al., 2001). Nevertheless, these detrimental effects are generally most severe in species with extensive social play (Einon et al., 1981) and can usually be avoided with brief play episodes throughout the isolation period (Einon et al., 1978). Additionally, it has been found that re-socialization following isolation may reverse detrimental effects of isolation, with more play interactions indicating greater social recovery (Harlow et al., 1965). Nevertheless, in rats and rhesus monkeys, it has been found that play deprivation directly after weaning can permanently alter the behavior of an animal, resulting in a more aggressive and socially incompetent animal (Harlow and Suomi, 1971; Einon and Morgan, 1977). After weaning, levels of social interactions among peers are high, suggesting that this period of time may represent a sensitive period for the effects of social play (Hol et al., 1999).

In contrast to play deprivation, playing at high versus low rates appears to have few net benefits. Some examples of evidence for benefits of higher versus lower rates of play are reduced social tension before resource sharing in chimpanzees (Palagi et al., 2004) and improved coping after weaning as measured by levels of play in piglets (Donaldson et al., 2002). Higher rates of play may also override positively-selected aggressive traits in monkeys (Suomi, 1991). However, these findings are sparse and species specific. Other researchers testing hypotheses for the function of play have found no evidence of benefits with increased rates of play. For example, it has been reported that meerkats that played at higher levels did not obtain an increased rank later in life (Sharpe, 2005a) or exhibit enhanced social cohesion to their peers (Sharpe, 2005b), and that cats playing at relatively high levels did not have increased hunting success (Martin and Caro, 1985). Although play deprivation has shown costs, additional play often shows no increased benefits although such findings may result from low statistical power when comparing relatively rare events. This has led to dispute on why play exists and how it helps animals.

Some of the many hypotheses for the function of play are as follow: to burn surplus energy (Burghardt, 1985), to practice adult behaviors (Burghardt, 1985), to exercise muscles (Brownlee, 1954), to create social hierarchies (Bekoff, 1972), to enhance social bonding (Sharpe, 2005a), for motor training (Byers, 1985), for self assessment (Thompson, 1996), a side effect of surplus resources (Burghardt, 2005), and to generate novel behavior patterns (Fagen, 1974; Pellegrini et al., 2006). Although many of these hypotheses are rational, they lack evidence and bring up the question: why would an animal play to achieve these functions if similar results were available using less costly behavior (Byers and Walker, 1995; Byers, 1998)?

A few other hypotheses provide explanations that avoid this problem. One is a modified version of the motor training hypothesis - that play modifies cerebellar synaptogenesis and muscle fiber type differentiation (Byers and Walker, 1995). This hypothesis proposes that play influences neural growth in the cerebellum and allows adaptive modification of muscle and bone used during play, resulting in increased bodily and behavioral plasticity over time. Accordingly, the brain and body of the animal are shaped by play, eventually enhancing motor performance (Byers and Walker, 1995). Evidence for this hypothesis comes from the fact that the terminal phase for synapse formation corresponds to the peak of play seen in cats (Byers and Walker, 1995). Furthermore, during the ages at which murids and rats play, synaptophysin, a protein associated with synapse formation, and c-fos, a marker for gene activity, are found at elevated levels in cells of the cerebellum (Byers and Walker, 1995).

There is evidence for other modifications in brain activity during play. Rats allowed to play immediately prior to sacrifice for brain analysis exhibited significantly higher activation in many brain regions in comparison to animals not allowed to play (Gordon et al., 2002). Using c-fos as a gene expression marker, it was found that the deep and dorsolateral tectum, inferior colliculus, dorsal periaqueductal gray, ventromedial hypothalamus, dorsal and ventral striatum, and somatosensory cortex were significantly more activated in animals that had played versus those that had not (Gordon et al., 2002). In another rat study, recent play experience was characterized by a unique pattern of heightened c-fos expression in the medial thalamus (parafascicular area), hippocampus and somatosensory cortex (Siviy, 1998). These findings indicate that play has differential effects on specific brain regions, which may enhance adaptive behavior. Moreover, Gordon et al. (2003) found that rats allowed to play prior to sacrifice exhibited significantly increased levels of mRNA transcription of brain derived neurotrophic factor in the amygdala and dorsolateral frontal cortex. Brain derived neurotrophic factor is a key modulator of neuronal development, and its presence provides evidence for a role of play in modulating brain shaping processes (Gordon et al., 2003).

Another hypothesis that addresses this question is based on behavioral plasticity (Fagen, 1981). This hypothesis predicts that play allows animals to exhibit more flexible behavior in general, preventing them from setting on a substandard method for completing a task (Bateson, 2005). Since this hypothesis is so general, a good deal of evidence falls under it. For instance, animals induced to exhibit behavior associated with play, such as running, exhibit enhanced learning and acquire heavier brains due to neuronal growth (van Praag et al., 1999; van Praag et al., 2005). It has also been found that play allows animals that have been bred for aggressive traits to become more socially inclined (Suomi, 1991).

A more refined hypothesis with specific predictions is the ‘training for the unexpected’ hypothesis (Špinka et al., 2001). It postulates that the disorienting and clumsy behavior an animal voluntarily performs during play allows it to experience unpredictable conditions similar to those encountered in future life and death situations. During play, animals can practice recovering from unusual conditions in a safe environment with a relatively low risk of harmful consequences. In the future during unanticipated events with potentially severe consequences, animals that have experienced play may exhibit improved coping, both physically and emotionally, due to their play experience (Špinka et al., 2001). Although most predictions arising from this hypothesis have not been directly tested, some evidence from existing literature seems to fit. For example, piglets raised in play-stimulating environments exhibited reduced levels of aggression during subsequent food competition (Chaloupkova et al., 2007) and preschool boys who were more adept at switching behavior during rough and tumble play scored higher when later tested on social problem solving ability (Pellegrini, 1995).

Why do some animals play and others do not?

As indicated above, there is evidence for various functions of play but, if it is an important behavior with viable function, why do some species exhibit it at varying levels while other species may not exhibit it at all (Baldwin and Baldwin, 1974; Bekoff, 1984)?

Species predicted to exhibit higher rates of play are k-selected, have a high encephalization quotient, live in unpredictable environments, experience a long period of immaturity, and receive extended parental care (Fagen, 1977; Bekoff, 1984; Špinka et al., 2001). This description fits most animals considered to play vigorously, but some species, such as rats, may not meet all the expectations but still play vigorously (Panksepp, 1998; Pellis, 2002). Although these conditions predict the presence of play, evidenced by the ubiquitous presence of play in large-brained mammalian orders, they cannot predict the complexity of play exhibited, since intraorder comparisons do not yield stable correlations between brain size and the expression of play (Iwaniuk et al., 2001).

Some of the most common examples of play come from domesticated animals or wild animals in captivity that may perform higher rates of play behavior longer into their life than wild animals (Ficken, 1977; Fagen, 1981; Burghardt, 2005). Common examples of such domesticated animals are horses and dogs while examples of captive wild animals would be chimpanzees and orangutans. Their high rates of play are hypothesized to occur due to a lack of constraints found in the wild, such as procuring food and avoiding predators. Therefore, these animals take up their ‘free time’ with play (Burghardt, 2005). Further evidence is found in domestic mammals with relatively large brains such as dogs, which exhibit high levels of play compared to their non-domesticated ancestors (Burghardt, 1985). It is hypothesized that these elevated rates of play are due not only to the absence of predators and ready availability of food, modeling parental care, but also because these animals, such as dogs, have been inadvertently selected for juvenile traits that allow for easier management in captivity (Price, 1999).

Despite the conditions in which play arises, it is hypothesized that play behavior is more widespread in the animal kingdom than previously thought (Diamond and Bond, 2003; Burghardt, 2005). Identification of play in non-mammalian taxa may be restricted as their play may be externally dissimilar from what we recognize as play (Fagen, 1981; Burghardt, 2005). Some researchers suspect that play exists in ectotherms and invertebrate species such as sturgeons, the soft-shelled Nile turtle, and the common octopus (Burghardt et al., 1996; Burghardt, 2005; Kuba et al., 2006). The presence of play in complex invertebrates would suggest that play behavior has arisen multiple times in evolution, as the common ancestors of invertebrates and vertebrates were too simple to exhibit play (Kuba et al., 2006). Birds and mammals are the only groups that display obvious play, although avian play is not considered to be as widespread as mammalian play (Ficken, 1977; Fagen, 1981). Because their evolutionary histories are distant, it is hypothesized that play behavior did not arise from a common reptilian ancestor, but has arisen convergently (Diamond and Bond, 2003).

Play in birds - the current situation

Avian play has been a challenging field of study due to the difficulty of recognizing playful behavior in birds. In the wild, playful looking avian behavior may later be discovered to have an immediate biological function, such as a mating ritual or territorial display (Ficken, 1977). In captivity, playful looking behavior may comprise forms of redirected behavior occurring in the absence of appropriate stimuli (Ficken, 1977). This ambiguity has resulted in a more critical approach to the subject, resulting in fewer reports of avian play that are more critically examined. This difficulty has led to relative neglect of the subject, except in species in which play is obvious. To date, play in birds has been reported in twelve orders: Anseriformes, Psittaciformes, Cuculiformes, Galliformes, Strigiformes, Apodiformes, Piciformes, Passeriformes, Musophagiformes, Columbiformes, Bucerotiformes, and Ciconiiformes (Ortega and Bekoff, 1987; Negro et al., 1996; Diamond and Bond, 2003; Burghardt, 2005). Within these orders, reports of play may be scarce and exist for only a few species. Undisputed evidence of play comes only from a few orders (Ortega and Bekoff, 1987; Diamond and Bond, 2003). Two of these are Psittaciformes and Passeriformes—within which members of the parrot and corvid families, respectively, exhibit the most obvious play behavior (Heinrich and Smolker, 1998; Brazil, 2002; Diamond and Bond, 2004). Reports of play exist in other families but descriptions are mostly anecdotal, ambiguous, and may be describing behaviors other than play (Ficken, 1977; Diamond and Bond, 2003, 2004).

Avian play occurs under similar conditions as mammalian play and manifests in the same forms (locomotor, object, and social play); object and locomotor play are most commonly reported (Ortega and Bekoff, 1987; Diamond and Bond, 2003). Like mammals, all three forms of play may occur separately or may be combined (Diamond and Bond, 2003, 2004) and share previously mentioned characteristics of mammalian play. For example, locomotor play may take the form of ravens repeatedly sliding down inclines and performing aerial acrobatics (Heinrich and Smolker, 1998; Brazil, 2002), and vultures exhibiting play chasing (Blumstein, 1990). For object play, predatory birds, such as kestrels, toy with their incapacitated victim before eating (Negro et al., 1996) while corvids and gulls may repeatedly drop a non-food object from a high place, each time watching it fall (Heinrich and Smolker, 1998). Avian social play is hypothesized to occur in species with relatively high levels of sociality, extensive exploratory behavior, high intelligence, and flexible foraging behavior (Ortega and Bekoff, 1987; Diamond and Bond, 2003, 2004). Social play may take the form of parrots play-chasing (Diamond and Bond, 2003) while corvids have been observed attempting to gain and keep possession of a non-food object from conspecifics while standing on top of a mound of dirt (Heinrich and Smolker, 1998). Play signals are also recognized despite the fact that birds cannot exhibit commonly recognized facial and tail postures associated with mammalian play. For example, keas in the wild perform head cocking, a hopping approach, and rolling onto their back in the presence of a play partner, behaviors which may also be used to induce play from a partner. Additional play behaviors observed in captive keas include stiff legged walking, non-directional object throwing, lying on their back with head between legs, and lifting a foot while ducking and touching (Diamond and Bond, 2004).

The functions, as well as the conditions predisposing animals to exhibit play, are thought to be similar between birds and mammals (Diamond and Bond, 2003). Juveniles of avian species predicted to exhibit play, but not necessarily complex play, interact with adults' longer, have altricial development, experience longer periods of sexual immaturity, are highly social, and have large brain to body ratios (Fagen, 1981; Ortega and Bekoff, 1987; Diamond and Bond, 2003). Also, Diamond and Bond (2003) report a general trend for larger birds that develop more slowly such as keas and ravens to show what they consider the most complex social play (e.g., combined social and object play; play invitations) whereas smaller-bodied, more rapidly growing birds such as spectacled parrotlets and dusky lories exhibit what they consider simpler social play (e.g., play chasing and play fighting). The above conditions exist in many avian species that have not been reported to play, suggesting that avian play is more widespread but has yet to be characterized in these species (Diamond and Bond, 2003).

Lack of evidence for avian play may stem from a few factors: as mentioned previously, it may be difficult to discern ritualistic behavior from playful behavior (Ficken, 1977) or avian play may differ externally from observer expectations (Fagen, 1981). It may also be difficult to observe juveniles in play-conducive environments—in captivity, birds may be stressed and, in the field, it is often easier to observe adults than juveniles (Ficken, 1977; Diamond and Bond, 2003). Additionally, there is dispute as to what constitutes play in birds. Some researchers attribute any non-biological interaction with objects or conspecifics as play while others have more rigid standards (Negro et al., 1996; Diamond and Bond, 2003). Due to lack of interest, resources, or ability to recognize or induce play, the study of avian play has been neglected. A probable solution to this dilemma is to observe domestic birds that are common, inexpensive, and accustomed to captive environments and human presence. A method to reliably induce play behavior would be an additional advantage. Most of the above conditions are seemingly present in *Gallus gallus domesticus*—or the humble domestic chicken.

II. The chicken

Life history, past and present

The wild ancestor of the domestic chicken is hypothesized to be the Red Jungle Fowl (*Gallus gallus*) with some degree of hybridization with the Grey Jungle Fowl (*Gallus sonneratii*) (Eriksson et al., 2008). The Red Jungle Fowl is native to the jungles of South Asia, inhabiting forested areas, thick vegetation, and abandoned burned clearings (Collias and Collias, 1967;

Collias and Saichuae, 1967). Red Jungle Fowl are medium-sized ground-dwelling birds weighing between 800-1000 grams, and are omnivorous foragers (Appleby et al., 1992) feeding on a variety of vegetation, insects, carrion, and small animals (Collias and Saichuae, 1967). These birds spend a majority of time in areas providing vegetative cover, coming out into more open areas only to forage or drink (Collias and Saichuae, 1967; Appleby et al., 1992).

Jungle fowl are territorial and travel in small flocks typically composed of 5-6 adult hens with male and female offspring, and a dominant male (Collias and Saichuae, 1967; Collias and Collias, 1995). Jungle fowl have a dominance hierarchy, with adult males dominant over adult females, and, within the sexes, one individual dominant over all other members of their respective sex (Kim and Zuk, 2000; Johnsen et al., 2001). Dominance confers increased mating success and access to resources (Kim and Zuk, 2000; Pizzari and Snook, 2003). Individuals within a group recognize flock members and will attack a stranger that wanders into their group (Collias and Collias, 1995). Despite this, membership is not permanently fixed and females and young males may move between flocks, but are initially pecked and displaced from resources (Collias and Collias, 1995). Although shy and intolerant of humans, jungle fowl often live in the wake of human activity and exhibit a good deal of physiological and behavioral plasticity; these factors have predisposed them to domestication and have permitted their global spread (Collias and Saichuae, 1967; Appleby et al., 1992).

Chickens are thought to have been domesticated multiple times in various regions of south and southeast Asia, starting at least 8000 years ago (Kerje et al., 2003; Eriksson et al., 2008). In the Roman Empire, they were initially favored for their loud crowing and fighting abilities and were later prized primarily as food animals. Large-scale chicken breeding and production did not emerge until its adoption in America during the 19th century (Appleby et al., 1992).

Most chickens used for food production are now bred and maintained in an intensive environment (Muir and Craig, 1998). Breeders aim to produce birds that generate a maximum quality and quantity of eggs and meat. This has resulted in two main varieties of chickens; broilers, selected for high meat production, and layers selected for high egg production (Appleby et al., 1992). Although much research has been done on both varieties, references to domestic chickens in the following review are primarily based on research on chickens of layer strains.

Differences in behavior: chickens vs. jungle fowl

Physically speaking, domestic chickens are considerably different from jungle fowl. They are heavier, lay more eggs, and are unable to fly far, traits that have been selected for by humans (Collias and Saichuae, 1967; Kerje et al., 2003). Chickens still share behavioral parallels with jungle fowl ancestors (Appleby et al., 1992)—that is, both jungle fowl and chickens forage on the ground and exhibit anti-predator behavior (Collias and Saichuae, 1967). Despite these similarities, domestication has altered the behavior of chickens resulting in the dampening of wild traits (Andersson et al., 2001; Lindqvist, 2008). Although behavior has not been a primary selection factor in more recent artificial selection, there has been indirect selection for or against behaviors that are directly related or genetically linked to production traits (Schütz et al., 2001, 2002; Lindqvist, 2008). Thus, increased growth and egg production of domestic chickens under commercial housing conditions has been correlated with reduced fear and anti-predatory behavior (Andersson et al., 2001; Schütz et al., 2001; Håkansson et al., 2007). Related to reduced fear, there is some evidence that domestic chickens adopt less costly foraging strategies and are more willing to eat novel food (Andersson et al., 2001; Schütz et al., 2002). Additionally, they exhibit reduced foraging, exploration, and social interactions compared with jungle fowl (Jensen and Andersson, 2005). Even jungle fowl bred in captivity over several generations exhibit reduced fear responses compared with wild-raised jungle fowl (Håkansson and Jensen, 2005; Håkansson et al., 2007).

Overall, domestic fowl exhibit reduced fear-related behavior in comparison to jungle fowl —however, fearful behavior is not completely absent in domestic chickens. Fear responses are evoked in production environments even in protected indoor environments free from (non-human) predators. Under these conditions, chickens also exhibit social traits, perching, foraging, and other behaviors genetically conserved from their evolutionary history when these behaviors were vital for survival (Appleby et al., 1992).

In an outdoor environment where domestic chickens and jungle fowl are under the threat of predation, group living is their primary form of defense, (Appleby et al., 1992), presumably due to safety in numbers and many eyes leading to faster detection of predators. Related to this instinctual social tendency, modern chickens housed in small flocks exhibit recognition of flock mates, form a dominance hierarchy, and may attack unfamiliar birds (Appleby et al., 1992). When isolated, chickens are highly motivated to reestablish contact with their flock and may remain in a state of panic until contact is made; if contact is not made over an extended period, chicks may exhibit a failure to thrive and die (Panksepp, 1998).

Another remnant of anti-predatory behavior still seen in domestic chickens is perching. At dusk, chickens of lightweight strains, once old enough to fly, will roost in trees outdoors, or on perches indoors, and not return to the ground until morning (Newberry et al., 2001). Perches are also used for resting and preening during the day (Newberry et al., 2001). Before night, the birds will tussle and displace each other, vying for a central position in the roosting group, not spacing themselves evenly but squeezing together and preferring upper rather than lower perches (Newberry et al., 2001). If denied access to perches, chickens that habitually use perches exhibit frustrated behavior (Olsson and Keeling, 2000) even though perching is not necessary for safety from predators in an indoor production environment.

Chickens are also highly motivated to forage. Jungle fowl and domestic fowl will root through vegetation and soil with their beak and claws to find food (Collias and Collias, 1967). Although chickens in a production environment have their food presented in feeders and do not need to forage to find food, they still go through the motions of scratching for food, sometimes making scratching movements with their claws while feeding from a trough, and they also spend time searching in the litter (Appleby et al., 1992). If the ability to forage is denied, it is thought chickens of some strains may instead redirect their foraging to various cage structures or, in some cases, the feathers of other birds (Jones and Carmichael, 1998; Jensen et al., 2005; Newberry et al., 2007).

Laying hens in the production industry

Eggs are a leading animal product in the American food industry, with over 349 million layers producing over 1,823 million dozen eggs in 2007 (USDA, 2009). The layer industry is, for the most part, owned by large-scale producers in countries such as the United States, China, Brazil, and India, and about 98% of hens are kept in cage housing environments at high density due to economic pressures for production efficiency (Appleby et al., 1992). Buildings are ventilated to control ammonia from feces. Depending on the photoperiod, laying hens reach maturity and start laying eggs at around 18 weeks of age. In the US, they are typically kept in production for about a year, put through a molt by feeding a low nutrient diet and shortening the photoperiod, and then brought back into lay for a second production cycle, after which they are considered spent.

Although cage housing is most common, various alternative systems are increasing in popularity due to consumer interest in cage-free eggs. In a free range system, hens have access to the outdoors but are usually kept indoors at night for safety from predators and during the morning when the majority of eggs are laid. This type of system may have higher mortality of birds, but is often perceived as a more humane way to raise chickens (Appleby et al., 1992). Percheries or aviaries contain birds indoors and have foraging substrates along with more vertical room and perches (Appleby et al., 1992). A favorable aspect of these systems is that they allow more natural behavior than in a cage, but deleterious behavior such as cannibalism and feather pecking may occur (Colson et al., 2008).

Conventional cages contain birds within a limited space and low bird number per cage in a relatively barren environment, with an overall high stocking density (Appleby, 1991). This system has been the most commonly used in production settings as it maximizes production while minimizing disease and behavioral problems. Birds are separated from their droppings and, due to space restriction and low group size, cannibalism is reduced (Appleby, 1991; Appleby et al., 1992). However, public perception of this type of system is poor and natural behavior is restricted leading to concerns that hens in these conditions may suffer reduced well-being (Appleby, 1991; Appleby and Hughes, 1991; Appleby et al., 1992). Directive 1999/74/EC banning conventional cages in the European Union has resulted from these concerns (van Horne and Bondt, 2003). This directive requires that hens be provided with material for foraging and dustbathing, a nest area, and a perch. These resources can be supplied in furnished cages as well as cage-free systems.

Problems with laying hen well-being and behavior in intensive production

Welfare is a measure of the physical and emotional well-being of the animal—a healthy animal experiencing positive emotions may be defined as an animal experiencing good welfare (Dawkins, 2006). Specific physical conditions are indicative of poor welfare, such as low body weight, disease, i1njury, deformity, poor rates of reproduction, and high morbidity (Wechsler, 1995; Dawkins, 2006). Behavioral parameters may provide additional indicators allowing researchers to judge an animal's internal state of well-being prior to the development of physical maladies (Dawkins, 2006).

Concern for the welfare of laying hens in cages comes from the fact that hens are prevented from performing behaviors such as laying their eggs in an enclosed location, perching off the ground to avoid danger, foraging and dustbathing, leading to frustration in those individuals that are highly motivated to perform these behaviors. Stress associated with fear may also lead to poor body condition, abnormal behavior, lower production levels, and public discontent (Jones, 1996; Blokhuis et al., 2005). The root of this problem may stem from the fact that chickens have been primarily selected for production traits and not behavior.

Additionally, individual selection for high productivity in grandparent stock has allowed undesirable behavioral traits such as cannibalism and feather pecking to persist in the population due to absence of expression when breeding hens are housed individually (Schütz et al., 2001; Blokhuis et al., 2005). Unless hens are beak trimmed to control feather pecking and cannibalism, these behaviors manifest in reduced well-being of the commercial layers in group housing, leading to reduced production (Wechsler, 1995). This problem is being rectified as breeding companies now practice group selection against mortality, which has resulted in strains that are less cannibalistic when housed in groups (Muir and Craig, 1998).

When a hen is exposed to an alarming stimulus, the sympathetic nervous system is activated, resulting in the release of stress hormones such as glucocorticoids and catecholamines and activating fight or flight behavior (Appleby et al., 1992). In the wild, this behavior has been selected for as it allows birds to evade predators (Wechsler, 1995). In captivity, hens housed in cages cannot escape perceived negative stimuli that are encountered as part of the management routine (Dawkins, 2006). When hens are unable to escape stressors or engage in other biologically motivated behavior, they may become increasingly stressed or frustrated. This may culminate in the birds experiencing a continuous state of anxiety, eventually resulting in detrimental effects (Appleby et al., 1992) that may even affect progeny (Janczak et al., 2007). When birds are continuously expending metabolic resources on fear responses or attempting to achieve a biologically motivated behavior, resources are drained from secondary bodily functions. This may not show any dramatic consequences initially but may later result in morbidity such as a weakened immune response and susceptibility to illness due to prolonged stress and possible mortality (Appleby et al., 1992).

Some common examples of behavioral abnormalities that indicate poor welfare in laying hens are feather pecking, cannibalism, stereotypies, and vacuum behavior, which may be indicative of increased fear or suboptimal use of brain capacity in an attempt to cope with a suboptimal environment (El-Lethey et al., 2000). Researchers hypothesize that many of these abnormal behaviors develop over time in conditions where animals cannot perform their full range of natural behavior (Broom, 1991; Dawkins, 2006; Krause et al., 2006). Therefore, abnormal behaviors may result from attempts to cope with stress induced by a suboptimal environment (Broom, 1991; Wechsler, 1995; Dawkins, 2006). Coping behavior is classified in two main categories: active coping in which animals continue to attempt to avoid negative stimuli, associated with high activity levels, and passive coping (sometimes equated to learned helplessness), in which animals stop reacting behaviorally to stimuli even though the stimuli continue to be perceived as negative, associated with low activity levels (Wechsler, 1995; Jensen et al., 2005). Animals may adopt a combination of active or passive coping strategies to deal with stressors in their environment (Wechsler, 1995).

When animals exhibit coping behavior, this does not mean their condition will improve (Wechsler, 1995; Dawkins, 2006) even if they show physiological benefits such as reduced cortisol (Mittleman et al., 1991). Over time, active coping behavior can become more prevalent and stereotyped in form (Scheepens et al., 1991), and, depending upon its form, can result in a variety of injuries (e.g., skin lesions, injuries resulting from ingestion of wood splinters, etc.). When directed repetitively towards the body of other animals, the behavior may injure the other animals as well (Wechsler, 1995; Jensen et al., 2005).

Genetic selection against undesired behaviors of laying hens has begun (Blokhuis et al., 2005), but only so much change can occur in a given timeframe. Behavioral research may be able to provide more immediate solutions to problems resulting from inadequate environments by identifying methods for improving the environment through environmental enrichment.

III. Assessing levels of welfare

In welfare testing, there are three main categories by which to measure welfare: physical, physiological, and behavioral measures. As previously mentioned, physical conditions that are indicative of poor welfare may include, low body weight, disease, injury, deformity, poor rates of reproduction, and high morbidity (Wechsler, 1995; Dawkins, 2006). These conditions are obvious indicators of current poor welfare—but animals may not always show physical symptoms of poor welfare. Physiological measures can detect ‘hidden’ symptoms by measuring immune parameters, levels of stress hormones, and autonomic responses such as heart rate (Wechsler, 1995; Dawkins, 2006). Although these are objective methods, their true meaning may be uncertain (Appleby et al., 1992). For instance, elevated heart rate and levels of stress hormones may be present under both seemingly negative and positive situations involving high arousal (Dawkins, 2006). Furthermore, obtaining these measures may be invasive and involve handling, and may ultimately show effects of collecting the sample rather than the underlying baseline of welfare (Appleby et al., 1992). Expense may also be an additional limiting factor in analyzing these variables.

Behavior, on the other hand, when carefully tested in an objective manner, may provide an accurate and immediate measure of welfare (Dawkins, 2006). Although we can never know exactly what another organism is capable of feeling, we can infer underlying emotional states via behavior. Behavior may be an indicator of emotional states because it is assumed that behavior humans perform during certain emotional states will be similar in other animals sharing homologous brain features (Panksepp, 1998; Dawkins, 2006). In addition to homologous brain regions, humans and animals, especially mammals, also share genes that code for the same hormones, neurotramsmitters, and receptors in similar regions of the brain. When non-human species and humans share homologous brain regions, share similar hormones, have similar genes coding for similar neurotransmitters and receptors in similar brain regions, and exhibit similar behavior when exposed to specific stimuli, it can be inferred that subjective emotional experiences in human and animals may be similar (Panksepp, 1998).

Common tests to measure fear in poultry

Because fear may be a welfare problem at the root of many behavioral abnormalities, several methods to detect fear-related behavior in poultry are frequently used and partially validated (Jones, 1996; Forkman et al., 2007). One of the most common is the open field test, originally used for rats (Suarez and Gallup, 1984), for which it is considered a measure of anxiety (Takahashi et al., 2006). In this test, an animal is taken from its home cage by a handler and placed into a novel, testing arena that is larger than the home cage, after which the human handler moves out of view of the bird until the end of the test (Suarez and Gallup, 1985). The arena may be circular or rectangular, and may or may not have lines drawn on the floor, used to delineate the field into central and peripheral areas (Jones and Carmichael, 1997).

The variables most commonly recorded in the open field test are latency to vocalize, latency to ambulate, number of lines crossed, and time spent in the center of the open field (Gallup and Suarez, 1980). It is assumed that reduced underlying fear is indicated by more time spent in the center of the open field (Lowndes and Davies, 1996), shorter latency to vocalize and ambulate (Suarez and Gallup, 1983), and higher levels of line crossing (Suarez and Gallup, 1985). Increased fear, on the other hand, is assumed to be indicated by increased freezing behavior (Vallortigara and Zanforlin, 1988), high latencies to ambulate and vocalize (Suarez and Gallup, 1985), low rates of ambulation, measured by line crossing (Suarez and Gallup, 1983), and increased time near the wall of the arena (Lowndes and Davies, 1996). It is hypothesized that the process of a human taking the bird from its home cage and placing it in an unfamiliar environment away from its flock and without anywhere to hide simulates a predatory encounter (Suarez and Gallup, 1982); the resulting behavior of the bird is assumed to reveal levels of fear under a perceived, lurking predatory threat (Suarez and Gallup, 1981, 1983). It is additionally assumed that the animal is experiencing two conflicting motivations: either to evade further predatory contact or reinstate social contact with its group members (Suarez and Gallup, 1981).

Because handling by a human is necessary for the open field test, it is assumed that the open field test will measure fear due to predatory simulation via human handling prior to testing (Forkman et al., 2007). Evidence that isolated animals are seeking social reunion comes from the fact that chicks tested in pairs take longer to vocalize than a single bird (Suarez and Gallup, 1983). Therefore, measures such as increased defecation, latency to move, and freezing behavior are commonly considered indicators of higher fear while increased distress calling and attempts to escape are thought to measure social reinstatement motivation (Forkman et al., 2007).

There is no single, standardized open field test procedure used for poultry, and various factors may lead to variable results in open field tests (Jones, 1987; Vallortigara and Zanforlin, 1988; Jones and Carmichael, 1997). The size of the arena is one such factor, with a larger arena considered to be more fear inducing (Suarez and Gallup, 1985). Treatment of birds with aversive stimuli such as electric shock before the test induces higher levels of fear-related behavior (Suarez and Gallup, 1983; Vallortigara and Zanforlin, 1988; Forkman et al., 2007). Novelty of the arena is also a factor, with fear responses being greater on the first than on subsequent exposures to the arena (Jones, 1987). Sex of the tested birds also has an effect. Males tend to exhibit a longer latency to vocalize and ambulate, often interpreted as being more cautious, while females often begin vocalizing sooner and may walk sooner (Jones and Faure, 1981). These findings may be interpreted as increased fear in the males but this explanation seems unlikely given that males tend to exhibit less fearfulness than females in other contexts (Jones 1987; Vallortigara and Zanforlin, 1988). They are more easily explained by females exhibiting higher social motivation and, thus, beginning to 'search' for flock mates sooner (Gallup and Suarez, 1980; Schütz et al., 2004).

There are reports of heritable components mediating behavior in the open field (Buitenhuis et al., 2003; Rodenburg et al., 2003). For example, the heritability of the frequency of flying is around 0.2 while the heritability of the number of steps is about 0.5 (Rodenburg et al., 2003). Furthermore, quantitative trait loci (QTL) have been identified that are correlated with fear-related behavior in the open field (Buitenhaus et al., 2003). For example, line crossing at 5 weeks was significantly correlated with a QTL on chromosome GGA4, and a suggestive QTL on chromosome GGA2. However, for line crossing at 29 weeks of age, a different QTL on chromosome GGA4, and two suggestive QTLs on chromosome GGA1 and GGA10, were detected. These findings suggest that fear behavior in chicks and adults has different genetic modulation (Buitenhaus et al., 2003).

A novel object test is performed by introducing an unfamiliar object to a flock or a single bird located in a novel arena or in the home cage. A greater latency to approach the novel object is generally considered indicative of greater underlying fear (Hegelund and Sorenson, 2007). There is no standard procedure for this test and a variety of objects have been used as the novel object, placed either in the center or periphery of the living quarters or a novel test arena (Miller et al., 2006; Forkman et al., 2007). For instance, novel objects in previous experiments are usually relatively inedible objects such as zucchini, pinecones, pencils, and plastic cups (Schütz et al., 2004; Miller et al., 2006). Results from the test may be affected by size and other characteristics of the novel object. The test may be repeatable across days or weeks but, due to the lack of standardization, interpretations across studies may be ambiguous (Forkman et al., 2007). For example, the human observer may hide behind a screen, leave the area, or watch the response in sight of the birds (Forman et al., 2007). Housing factors such as cage tier level may influence latency to approach the novel object as well as increasing other fear responses although these effects may be negated if birds are equally exposed to humans (Scott et al., 1997). However, responses to a novel object appear not to be influenced by the handling of the birds suggesting that the test may be a useful measure for fear levels in the birds' home environment (Forkman et al., 2007). The novel object test has also been conducted on birds in a novel environment, such as after an open field test (Lowndes and Davies, 1996). This kind of test can have an initial startle component, reflecting the bird's immediate reaction to the novel object, followed by assessment of danger and emergence of fear-provoked immobility, flight or attack, or it may elicit exploratory investigation of the object.

Startle tests are also conducted which test a chicken's startle response to a sudden, unexpected stimulus. There are many variations for this test using a variety of methods to produce the startle effect (e.g., puff of air in eyes, sudden opening of an umbrella, air horn), but a commonly used one in birds is the predator surprise test. In this test, a bird is isolated in a novel arena. A simulated 'predator,' which is typically a flat silhouette of a hawk or predatory bird with a bell attached to it, is suddenly pulled across the top of the arena, which typically instigates a fear response in the bird. Commonly measured variables are duration of freezing, latency to resume ambulation, and flight distance (Miller et al., 2006). This test has been found to have good test-retest validity with a partial correlation coefficient of 0.63 (Miller et al., 2006).

In human handling or interaction tests, a bird is physically handled by a human and behavioral responses are measured. The method of handling varies between experiments as do the measures (Forkman et al., 2007). In general, increased movement or escape attempts are interpreted as increased levels of fear of human presence (Jones et al., 1994). However, this measure may not be completely valid as increased struggle may be dependent on a bird's coping style (active or passive), and may also depend on type of handling, with more struggle occurring in situations where escape is possible while freezing or immobility may occur under more immediate threat. Additionally, there is the possibility of habitation--birds may habituate to a handler, but a change of clothing may induce a renewed fear response (Forkman et al., 2007). Investigations of the validity and repeatability of handling tests for poultry are lacking from the literature.

Finally, the tonic immobility testing is a fear test that simulates a predatory encounter. Tonic immobility refers to the unlearned state of motor inhibition elicited by temporary restraint by a human handler (Suarez and Gallup, 1981). To perform this test, a human handler typically turns the bird on its back in a u-shaped cradle with its head hanging down over the end of the cradle, presses gently on its sternum to restrain the bird and prevent it from standing up, waits fifteen seconds (Jones and Faure, 1981; Jones, 1987), and then moves away behind a screen. If the bird comes out of tonic immobility by righting itself before ten seconds have elapsed when the handler moves away, it is not considered a successful induction and the process of inducing tonic immobility is repeated (Jones and Faure, 1981). Variables measured are number of inductions and righting time after release from restraint (Jones and Faure, 1981; Janczak et al., 2007). Fewer inductions and a longer righting time are interpreted as increased fear (Forkman et al., 2007). This behavior is hypothesized to be an anti-predatory mechanism of ‘death feigning’ that deactivates the predator’s hunting response, allowing the bird to escape (Forkman et al., 2007). Birds that undergo tonic immobility attempt escape when they have a reduced chance of experiencing further predatory attack (Sargeant and Eberhardt, 1975; Thompson et al., 1981). Evidence that this test measures underlying fear comes from the fact that longer tonic immobility may be induced when preceded by aversive stimuli hypothesized to simulate a predatory attack (Suarez and Gallup, 1981).

The tonic immobility test may be performed in a variety of ways. For example, researchers may put the bird on a flat surface rather than on a cradle. They may wait varying lengths of time before releasing the bird, before deciding that tonic immobility has been induced, and before ending the test (Hocking et al., 2001; Albentosa et al., 2003; Hocking et al., 2005). They may either stand beside the bird in visual contact (Janczak et al., 2007) or hide behind a screen, which is relevant because the presence of an observer, or even false eyes ‘staring’ at the bird, lengthens the duration of tonic immobility (Suarez and Gallup, 1981). Testing birds in their home pen results in shorter durations of tonic immobility than testing birds while isolated (Bilcik et al., 1998). When methods are standardized within a study, the tonic immobility test is repeatable (Forkman et al., 2007), but birds exhibit reduced tonic immobility as they age (Hocking et al., 2001) and length of immobility may be influenced by strain and genetic variation within strain. All QTL that have been correlated with the tonic immobility response are on chromosome one. For duration in tonic immobility, QTL have been found at 67 and 237 cM, and for number of inductions, at 236 cM (Albentosa et al., 2007; Schütz et al., 2004).

Although the above tests may measure underlying fear, they may not all test the same internal mechanism (Suarez and Gallup, 1981) as levels of behavioral responsiveness measured in different tests often do not correlate and may be stimulus specific (Hegelund and Sorenson, 2007). The tests presumably vary in the extent to which they evoke sub-cortical startle, fear, panic, and attack responses, and in the degree to which higher order (cognitive) assessment and decision-making responses are involved, suggesting that varying neural circuitries will be involved. For example, tonic immobility is an anti-predatory adaptation that may be relatively instinctual or ‘automatic’ but the outcome is influenced by assessment of the possibility of escape. Similarly, behavior in the open field, although anti-predatory, appears to involve learned and decision making components (Forkman et al., 2007).

Methods to improve welfare—measures already taken for laying hens

The above methods are focused on assessment of a negative emotion - fear. Duncan (1998) suggests that, to understand the complexity of animal well-being, positive emotions should also be evaluated. As previously discussed, the level of play exhibited may provide a useful indicator of positive well-being (Duncan, 1998; Donaldson et al., 2002) given that play behavior occurs in healthy animals in an optimal environment, suggesting that animals experiencing higher states of welfare are more likely to exhibit play.

Housing parameters and conditions affect the behavior and welfare of chickens (Dawkins et al., 2004; Albentosa et al., 2007). For example, when housed in non-cage environments with more room to move and with substrate on the floor, hens can acquire more feather damage from pecking by other birds as well as foot infections (Greene et al., 1985). Therefore, welfare may be a compromise based on housing style: giving birds more room to move as well as substrate to forage in is thought to improve some aspects of welfare, but may reduce others. Problems with non-cage housing can be reduced by finding methods to control feather pecking, such as genetic selection against this behavior (Muir and Craig, 1998), as well as careful management of litter to prevent accumulation of moisture (Berg, 2001). Thus, animal welfare is simultaneously dependent on the behavior of the birds and consumer willingness to pay more for eggs, offsetting costs of more expensive systems that, to the public, appear to house birds under more humane conditions.

Methods to improve welfare by manipulating various housing factors are often referred to as environmental enrichment. There is a good deal of evidence for the positive effects of environmental enrichment for laying hens as well as other types of animals in laboratories, zoos, and production environments. Animals in enriched environments tend to have increased hippocampal neurogenesis and enhanced survival of newborn neurons in the dentate gyrus (van Praag et al., 2000; van Praag et al., 2005), better body condition (Appleby et al., 2002), more varied behavior (Kempermann et al., 1997; Bizeray et al., 2002), and reduced fear responses (Friske and Gammie, 2005; Forkman et al., 2007). Environmental enrichment should, in some way, be biologically relevant to the animal to have benefits (Newberry, 1995). Something that may look interesting and enriching to us may be meaningless to members of other species (Newberry, 1995; Kells et al., 2001). Examples of environmental enrichment that have shown positive effects include foraging substrates (Martrenchar et al., 2001), food variety (Visalberghi et al., 2002), and environmental complexity (Wolfer et al., 2004).

In poultry, environmental enrichment is achieved by manipulating various aspects of the environment such as cage complexity, space available per bird, availability of nest boxes and perches, and foraging and dust bathing substrates (Kells et al., 2001; Arnould et al., 2004). These environmental features are biologically relevant and stimulate more varied and natural behavior (Newberry, 1995). For example, it has been found that provision of vertical cover can provide a safe refuge and stimulate more even distribution of birds throughout the available pen space, that provision of litter attracts pecking behavior thus reducing feather pecking and cannibalism, and that novel foraging substrates can increase locomotion and reduce leg deformities (Newberry and Shackleton, 1997; Cornetto and Estevez, 2001; Kells et al., 2001). Additionally, these complex environments may allow chickens to better adapt to new housing systems into which they are moved when they become older (Colson et al., 2008). Birds from enriched environments exhibit reduced latency to explore a novel environment, interpreted as reduced fear, as well as improved short term learning (Krause et al., 2006).

The presence of perches is a major form of environmental enrichment. Perching is a basic anti-predatory behavior that enables birds to avoid ground predators (Newberry et al., 2001). Perches increase leg bone strength of caged hens as they encourage jumping and locomotion (Hughes and Appleby, 1989) and increase the effective space available to the birds (Heikkilä et al., 2006). They can reduce rates of feather pecking and cannibalism and provide a place for submissive birds to escape aggressive conspecifics (Cordiner and Savory, 2001). Rearing chicks with access to perches stimulates locomotion, jumping, and balancing, allowing birds to develop their spatial skills (Gunnarsson et al., 2000).

Novel objects and experiences also provide sources of enrichment. Chickens that are handled and exposed to novel stimuli exhibit reduced fear responses (Forkman et al., 2007). Furthermore, novel objects and foraging substrates are attractive to chickens and stimulate locomotion and exploration (Newberry, 1999; Kells et al., 2001). Novel objects may additionally prevent redirection of foraging to conspecifics, potentially preventing feather pecking and cannibalism (Jones et al., 2000). Birds may have color preferences for novel objects dependent on learning and context (Jones and Carmichael, 1998).

Although these forms of enrichment have benefits, positive effects on welfare may be outweighed by the cost and inconvenience of having them brought in. Simple, inexpensive ways of enriching the environment for chickens in cages would be of value. If chickens play, enrichments that enhance play behavior may not only improve the welfare of chickens, but increased play would also provide a measure of improved welfare.

IV. Play behavior in chickens?

Do chickens play?

Although chicken behavior has been studied for over a century, few researchers have suggested that chickens play. This may be due to the concerns of misclassifying avian ritualistic behavior as play along with a general conception of chickens being humble, simple creatures. Chickens fit some avian predictors of play, but not others. For instance, chickens are precocial whereas play is predicted to be most prevalent in altricial birds (Diamond and Bond, 2003). However, chicks learn about different sources of food from the hen and they fit the prediction for play in species in which the young stay with the mother and their social group for an extended period (Diamond and Bond, 2003). Chickens are not renowned for having large brains, an attribute predicted in avian species that play (Ortega and Bekoff, 1987). However, they are highly social, group living birds that exhibit complex social behavior such as recognition of flock mates and pecking order relationships. Diamond and Bond (2003) have observed that social play is exhibited in species with flexible foraging behavior and high sociality, and, additionally, conclude that the best evidence of object play is when the object cannot be used for food or to meet other immediate needs. All these conditions are met in chickens: not only are they social birds that forage on a wide variety of food, but a playful-appearing behavior which they exhibit, worm running, is often directed towards non-food items (Collias and Saichuae, 1967; Cloutier et al., 2004).

Although some may be skeptical of play in a species commonly perceived to be simple and stupid, there are mammalian species that also defy some predictions for play. The best example is the laboratory rat. Rats are fast developing, short lived animals, not famous for having high intelligence—yet they have been one of the main species used to study play (Panksepp, 1998;

Pellis and Pellis, 1998b). Therefore, just because an animal species is common and does not meet all predictions of play does not mean it is incapable of exhibiting play behavior. As such, chickens are likely candidates for the exhibition of avian play.

A few researchers have mentioned seemingly playful behavior in young domestic fowl. Kruijt (1964) examined sparring behavior in young roosters as well as worm running. Dawson and Siegel (1967) reported on several behaviors in chicks that could possibly be characterized as play such as frolicking and streaming. Furthermore, chickens have been observed to repeatedly attempt perching on unstable swinging structures despite having access to stable perches (Newberry et al., 2001). These behaviors are rapid, disorienting, and do not appear to serve an immediate purpose, making them candidates for play behavior (Bekoff, 1972; Špinka et al., 2001).

Worm running as play

Worm running (sometimes also referred to as food running) is a behavior stimulated by the presence of a worm-like object, which may or may not be a food item (Cloutier et al., 2002, 2004). These worm-like objects protrude from either side of a chick's beak. A chick will pick up the 'worm object' and begin peeping and running. The behavior is socially facilitated and the entire flock may join. All chicks chase the bird with the 'worm object,' peeping rapidly and engaging in 'tugs-of-wars' with the worm-object. Possession of the worm changes throughout the duration of the worm running bout and different members of the flock may temporarily gain possession of the 'worm.' This may carry on for some time until the worm-object is eaten, lost, or other stimuli occupy the chicks (Cloutier et al., 2002). Worm running occurs in chicks as early as 2 days of age and persists to adulthood.

There are a few hypotheses for the function of worm running. Kruijt originally speculated that worm running is the reaction chicks have when encountering a large food particle they cannot break up and is caused by the chicks' conflicting motivation to eat and flee. This behavior manifests as worm running and eventually attracts the attention of the hen, who breaks it into smaller food particles, making it available to eat (Kruijt, 1964). In adulthood, Kruijt speculated that worm running is a way to keep other chickens from stealing foraged prey. Later, Hogan (1965, 1966) used isolated chicks under various conditions, to assess the conflict between feeding and fear during worm running. He hypothesized that chicks would first peck at the worm based on spontaneous arousal but then flee due to the onset of fear, which manifested in worm running behavior (Hogan, 1965). He observed that, instead of being a fearful stimulus, the worm was a positive stimulus for chicks that interacted with it the most, and that conditions of increased fear reduced the frequency of worm running (Hogan, 1966), concluding that worm running is not representative of an approach-withdrawal conflict, but caused by a different drive (Hogan, 1965, 1966).

Another hypothesis is that worm running is linked to aggression and social rank (Rogers and Astiningsih, 1991). Cloutier et al. (2004) hypothesized that success in worm running rank would be positively correlated with social rank due to aggression later in life but when the data were analyzed, no correlation between success in worm running and social rank based on aggression was found. They concluded that worm running fits some of the criteria for play behavior (Cloutier et al., 2004).

Worm running exhibits various features and conditions that correspond with obvious cases of play behavior. First of all, it is a behavior that does not necessarily have any immediate biological effect, as it can occur in the absence of a hen and in isolation. However, in groups, it is contagious—when one bird seizes the worm object and begins running, other flock members join in. Furthermore, social conditions are optimal, as Hogan (1966) found that many birds did not run with the worm in isolation. Worm running is repetitive and reciprocal; birds will sequentially seize the worm object from another bird and run, with many chicks getting the chance to temporarily possess the worm. The worm object may transfer between birds multiple times and birds may continue running repeatedly until other stimuli attract their attention. Furthermore, the item used in the worm running bout is often a non-food item with a shape and size resembling a food item. These criteria are also associated with increased object play in other avian and non-avian species (Negro et al., 1996; Hall and Bradshaw, 1998; Hall et al., 2002). Worm running also resembles a modified form of feeding behavior. Although birds peck and carry the worm, they manipulate and pass it repeatedly, often engaging in 'tug of wars' with the item, often not eating the worm object, but leaving it and attending to other stimuli after the worm running bout. This feature resembles play behavior in parrots and corvids, who may also engage in 'tug of war' interactions with non-food items (Heinrich and Smolker, 1998; Diamond and Bond, 2003).

Worm running may be easily stimulated in a group of chicks by providing a rod-like object (Cloutier et al., 2004). If worm running is play, then this is a reliable way to induce play, which has been difficult to accomplish in other laboratory species aside from rats (Panksepp, 1998). Because worm running is reliably induced, it may provide a simple and inexpensive method to provide environmental enrichment. String is one safe, low cost candidate as an enrichment material to stimulate worm running because it has been shown to elicit frequent pecking by chicks and hens and does not appear to lose its novelty (Jones and Carmichael, 1998). Providing worm running objects may be a way to stimulate exercise to help build bone strength in chicks destined for table egg production and could also be beneficial for preventing leg problems in broiler chickens.

Additionally, if play enhances flexible behavior and improves ability to cope with future unexpected events (Špinka et al., 2001), inducing worm running in chickens may help them adjust to future stressful situations. If the types of worm objects provided are varied, they would provide novelty which may help to reduce fearful behavior. Providing worm objects may also promote a positive human-chicken interface, inducing positive feelings in both chickens and caretakers. Finally, worm running is a fascinating behavior that interests general audiences; the idea of inducing play in chickens using worm running objects is a novel idea that may intrigue the general public and induce a better view of production settings.

V. Conclusions

Because play is not only an indicator of welfare but also may have developmental benefits, a method by which to induce it using a simple stimulus would be advantageous in a production system lacking natural substrates to enrich the environment, such as cage housing used for rearing layer chicks. Although some researchers may casually acknowledge play in chickens (Duncan, 1998), it has never previously been investigated for immediate or delayed benefits to chickens. Based on the evidence reviewed above, I propose that ‘worm running’ is a form of play in chickens when directed towards worm-like non-food objects. I also propose that locomotory behavior directed towards unstable surfaces such as swinging perches is a form of play in chickens. Given the multiple potential benefits of play outlined above, an experimental study investigating the value of play experience in reducing fear and improving body condition is warranted.

EFFECTS OF PLAY EXPERIENCE WITH WORM RUNNING OBJECTS AND SWINGING PERCHES ON FEAR RESPONSES OF CHICKENS IN NOVEL SITUATIONS

(Manuscript in preparation)

1. Introduction

The welfare of animals used in agriculture has been a topic of increasing concern, not only to the public but also to animal producers, since poor welfare may decrease production and reduce consumer confidence (Martrenchar, 1999; Pryce et al., 2001; Bowell et al., 2003). Body condition and levels of play behaviour are useful indicators of animal well-being (Donaldson et al., 2002). It has been observed that levels of play exhibited are influenced by the environment; poor conditions reduce or abolish play while optimal conditions stimulate it (Fagen, 1981; Siviy and Panksepp, 1985; Chaloupkova et al., 2007). Animals experiencing stressful situations such as weaning, feed restriction, social isolation, exposure to novelty, and mixing with strangers show reduced levels of play behaviour, along with other unfavourable traits such as weight loss, poor body condition, morbidity, and mortality (Müller-Schwarze et al., 1982; Weary et al., 1999a, b; Donaldson et al., 2002). Because of these provisional conditions under which play occurs, it is reasonable that levels of play could be used as a welfare measure, with higher levels of play indicating higher welfare (Spruijt et al., 2001).

In addition to being an indicator of well-being, it has been hypothesized that play behaviour itself has beneficial effects. First of all, neurobiological studies suggest that play behaviour is rewarding, given that play increases opioid binding in reward centres of the brain (Calcagnetti and Schechter, 1992; Vanderschuren et al., 1995; Knutson et al., 1998). Also, due to the disorienting and exaggerated movements of play, it has been hypothesized that play provides training for the unexpected, allowing young animals to develop their ability to recover both physically and emotionally from novel and unexpected situations (Špinka et al., 2001). Although some studies have not found effects of play treatments later in life (Caro, 1980; Martin and Caro, 1985; Potegal and Einon, 1989; Sharpe, 2005a, b), others have reported increased boldness, social skills, coping, and an overall enhancement of behavioural plasticity in animals allowed to engage in play (Baldwin and Baldwin, 1974; Suomi, 1991; Plagi et al., 2004; Dudink et al., 2006). Furthermore, repeated exposure to novel objects has been reported to reduce fear-related behaviour in many species including fowl (Jones, 1982; Roy et al., 2001).

Although play behaviour has been identified in several avian species (Brazil, 2002; Diamond and Bond, 2003; Ricklefs, 2003), it has not been typified in chickens. However, frolicking, play fighting, and worm running are playful-appearing behaviours described in fowl (Kruijt, 1964; Dawson and Siegel, 1967; Cloutier et al., 2004). In particular, worm running is characterized by a chicken running and peeping while holding a long thin worm-like object protruding from each side of the beak. Worm running is seen in chicks as early as 2 days old and may persist into adulthood, although at reduced levels, and occurs with both edible and inedible objects (Hogan, 1966; Rogers and Astiningsih, 1991; Cloutier et al., 2002). It is easily stimulated in groups of chickens simply by providing an inedible worm-like object (Cloutier et al., 2004). Participating members of the flock chase after the chicken currently carrying the ‘worm’ and engage in ‘tugs-of-war’ in their attempts to gain control of it, resulting in a rapid succession of different ‘worm’ holders. Chickens advertise their possession of a worm with loud, rapidly repeated peeping, leading Kruijt (1964) to suggest that worm running behaviour functions to attract other chickens and the mother hen to pull at a large piece of food, resulting in it being torn into pieces small enough for chickens to consume. However, this explanation seems unlikely given that it is elicited by a variety of non-food items and, when others approach, the ‘worm’ holder runs away (Rogers and Astiningsih, 1991; Cloutier et al., 2004).

Worm running meets criteria for play behaviour (Bekoff, 1972; Fagen, 1981; Byers and Walker, 1995). It consumes more energy than apparently necessary, and, given that it is easily induced by inedible objects throughout the juvenile period, appears to serve no clear purpose. It also lacks seriousness, considering that control of the worm object reciprocates among different flock members and is not correlated with social dominance based on aggression (Cloutier et al., 2004). As such, we propose this behaviour to be play when induced by provision of inedible worm-like objects.

Another form of behaviour observed in young domestic fowl that meets criteria for play is repeatedly jumping onto, and balancing unsteadily on, wires, ropes, and other unstable structures despite having access to secure perches and in the apparent absence of any threat necessitating escape off the floor (Newberry et al., 2001; personal observations). Precariously swinging and balancing on an unsteady surface such as a swinging perch provides conditions that are disorienting and unpredictable, similar to those occurring in other forms of play (Špinka et al., 2001). Therefore, we propose provision of low, swinging perches as a method for inducing play in chickens.

If play provides chickens with training for the unexpected (Špinka et al., 2001), it is possible that play experience gained from interacting with worm-like objects and swinging perches would reduce fearfulness and enable more adaptive responses when exposed to fear-inducing situations. This hypothesis could be investigated by exposing chickens to standardized “fear tests” hypothesized to measure underlying fear in a variety of contexts (Forkman et al., 2007). Tests to measure fear include the open field test (Suarez and Gallup, 1981, 1985; Vallortigara and

Zanforlin, 1988; Buitenhuis et al., 2004), novel object test (Lowndes and Davies, 1996; Uitdehaag et al., 2008), handling test (Graml et al., 2008), and tonic immobility test (El-Lethey et al., 2000; Schütz et al., 2004). All of these tests are used to measure fear in chickens, although they may evoke different components (e.g., startle reactions, anxiety, panic) to varying extents. For example, in rodents, behaviour in the open field is considered a measure of anxiety (Takahashi et al., 2006). In chickens, the open field test is hypothesized to measure fear given that human handling before the test simulates a predatory encounter while isolation afterwards is hypothesized to leave the chicken in a state of susceptibility to attack, although the resulting behaviour may be more complex and involve learned components (Forkman et al., 2007). The novel object test and modified novel object test (a version of novel object test involving the sudden appearance of a large or looming object) incorporate a startle component and test for fearful behaviour resulting from addition of a novel object (Forkman et al., 2007). Tonic immobility and handling tests are also considered to test for fear resulting from an immediate threat and include responses towards a stimulus that is direct and cannot be escaped (Forkman et al., 2007). The tonic immobility test additionally allows assessment of the rate of recovery from the fear generated by capture, following release in a situation where danger may still be present (Forkman et al., 2007). Given that domestic fowl evolved from group-dwelling jungle fowl subject to predation, it is assumed that isolation, sudden novelty, capture and restraint are reliable inducers of fear (Jones, 1987; Forkman et al., 2007). Therefore, these tests can be expected to reveal differences in fear resulting from differences in play experience.

We hypothesized that play experience reduces fear and increases adaptability in domestic chickens when exposed to novel and unanticipated situations. Specifically, we predicted that chickens with enhanced play experience as a result of being reared in an environment enriched with both worm-like objects and swinging perches would exhibit reduced behavioural signs of fear in standardized tests than chickens reared without these forms of enrichment, and that chickens reared with only one of these two forms of enrichment would show intermediate responses. Also, because play is hypothesized to be a measure of well-being, we predicted that increased play activity would be associated with physical benefits. Specifically, we predicted that chickens receiving "play enrichment" would exhibit superior body condition and increased body weight in comparison to control groups.

2. Materials and Methods

2.1 Subjects and housing

We obtained 72 female White Leghorn chicks, unvaccinated with untrimmed beaks, from a commercial hatchery, and randomly divided them into 8 flocks of 9 individuals. From 0-6 weeks of age, chickens were housed in brooder cages (100 cm long x 35 cm wide x 23.5 cm high), with four groups randomly assigned to cages in each of two vertically tiered brooder units. Each brooder cage had a main compartment (68 cm long) and a heated compartment (32 cm long), separated by a plastic curtain. The wire floor was covered with brown paper for the first week only, to prevent the legs of chicks from becoming caught in the wire floor. Groups were visually isolated by spacing them out between two levels and two sides of each brooder unit. From 6-12 weeks of age, groups were housed in visually isolated grower cages (91.5 cm long x 91.5 cm wide x 37 cm high). Groups were distributed across two vertically tiered grower cage units in the same configuration as when in the brooder cages.

Brooders were set at 30° C in the first week, with the heat being lowered by 2° C weekly to 6 weeks of age when chickens were moved to the grower cages. Room temperature was maintained at an average of 23.2 ± 0.1° C and room humidity was maintained at an average of 40 ± 0.8 %. During the first week, the photoperiod was 22 hours (lights on at 0800 h). Subsequently, light was reduced by 1 hour at the end of each day until there was a photoperiod of 12 hours light. Light intensity was maintained at the same level throughout the experiment. Chickens were provided food *ad libitum* (Purina Start and Gro crumbles, Purina Mills, LLC, St. Louis, MO) and water was continuously available from automatic push cups.

For individual recognition in video recordings, all chickens within each group were marked with a unique colour combination on their wings using non-toxic coloured markers. Members of a group were given a leg band of the same colour on the same leg, but with different numbers. Every individual in the study had a unique leg band/wing mark combination.

Chickens were visually inspected daily for health problems. Any chicken with minor injuries was isolated in a hospital cage until fully recovered, and then returned to the flock. One chick died early in the study.

2.2 Treatments

There were two ‘worm’ treatments (none or provided daily) and two swinging perch treatments (absent or present continuously) arranged in a 2 x 2 factorial design to produce four treatment combinations (control, worm, perch, and both). Groups were assigned to treatments in a randomized complete block design with two blocks.

For groups on the ‘worm’ treatment, inedible worm-like objects held the basic description of a long thin object that would protrude from either side of a chicken’s beak when carried. Groups on the ‘worm’ and ‘both’ treatments were presented with a ‘worm’ in their feed trough for a maximum of 30 min daily during the period between 0900 and 1000 h, which could be picked up and used in worm running. Older chickens grabbed the worm directly from the experimenter's fingers. If the 'worm' was lost from the cage (e.g., by falling through the wire floor), a new one was supplied. The worm was removed after 30 min. Worm object type was changed weekly to retain a degree of novelty, under the assumption that this would maintain a high level of worm running activity. To retain a similar worm object weight: body weight ratio, the size and weight of the worm objects was increased as the chickens grew (Table 1).

Groups on the 'perch' and 'both' treatments had a swinging perch permanently present in the centre of the cage, parallel to the cage front. The perch was a metal rod 20.5 cm long and 1.5 cm in diameter. In the brooders (0-6 weeks), the perch was suspended by chains at a height of 4 cm above the wire floor and, in the grower cages, it was suspended 6 cm above the floor from 6-8 weeks, and 12 cm from 8-11 weeks. The height was adjusted as the chickens grew to keep the perch level at approximately the same height relative to the height of the chickens.

2.3 Data collection

2.3.1 *Play behaviour*

Between 0900 and 1130 h on the second and seventh day of each week, a 15-min video recording was made of each cage using two Sony DCR-HC21 cameras (Sony Corporation, Tokyo, Japan) with Mini DV tapes. For Weeks 1-6, cameras were placed 81 cm away from the centre of the cage front. For Weeks 7-11, the camera was set 136 cm from the front of the cage. Extra light was directed into the cage during recordings to enhance picture quality, using a 13-Watt florescent light placed about 90 cm from the cage. In Weeks 1-6, a 35 x 27 cm piece of cardboard, secured with clips, was placed across the plastic flap to block entrance to the heated region of the brooder cage and prevent chickens from going out of sight during recording. Chickens were given about 5 minutes to acclimate to the light and cardboard barrier prior to recording. The order of recording of each cage was pre-determined via Latin square. On recording days, groups receiving worm objects were given their daily ‘worm’ at the start of their 15-min recording. An observer stood quietly out of sight to one side of the cage, watching the birds via the camera monitor and, if the ‘worm’ was lost, threw a new one into the feed trough without disturbing the birds or blocking the camera. Behavioural data extracted from the recordings indicated elevated levels of locomotory and object play in the cages provided with worm objects and perches (Massey, 2009).

2.3.2 *Fear tests*

In Weeks 5 and 10, standardized fear tests were performed on individual chickens from each group in a balanced order across the four treatments and two blocks using a Latin square design. All tests were conducted between 1300 and 1700 h in the room in which the birds were housed. In Week 5, four randomly-selected chickens in each group were subjected to a 10-min tonic immobility test. Four days later, the same four chickens per group were placed individually into an open field arena for a 5-min open field test. At 5 minutes, a novel object was introduced into the centre of the open field and the chicken was observed for an additional 2 min (novel object test).The chicken was then removed from the arena and held inverted by both legs for a 30-s handling test, and then returned to its home cage. The same chickens (Set 1) were re-tested in these same four tests in Week 10. In addition, four previously untested chickens from each group (Set 2) were tested in Week 10 in a 10-min tonic immobility test and then, four days later, in a modified open field test for 5 min, followed by a 2-min modified novel object test in the same arena, and then a 30-s handling test

Behaviour during the tonic immobility tests was recorded by direct observation from behind a screen whereas all other tests were recorded on Mini DV tapes using a Sony DCR- HC21 camera and subsequently analyzed using The Observer version 5.50 (Noldus, Wageningen, The Netherlands).

2.3.2.1 *Tonic immobility test*

Following a modified method of Jones and Faure (1981), each chicken was removed from her cage and placed on her back in a U-shaped wooden cradle (46 cm long x 25 cm wide), located 20 cm above the floor in a corner of the room behind a cardboard screen (230 cm long x 115 cm high) with a viewing slit. The screen was folded in the centre and positioned so as to ‘box’ the cradle. To induce tonic immobility, the handler restrained the chicken for 15 s with one hand over the chicken’s eyes and the other hand over the sternum and then moved out of sight behind the screen. If the bird righted herself within 10 s, induction was repeated until a state of tonic immobility lasting more than 10 s was reached. The test ended when the subject righted herself or when the maximum time (10 min) elapsed. The number of inductions was recorded and the duration of tonic immobility was measured to the nearest 1 s using a stopwatch. The numbers of vocalizations emitted during tonic immobility were also recorded (see Table 2 for ethogram).

2.3.2.2 *Open field test followed by novel object test*

The open field was located in the same room in which the birds were housed and consisted of a 91.5 x 91.5 cm arena with 115-cm-high cardboard walls and brown paper on the floor divided into nine equal squares using a black marker. To increase the quality of video recordings, a 13-Watt florescent light was placed 102 cm above the test arena. The video camera was placed above the arena so that the full area was visible. Following the method of Gallup and Suarez (1980), each chicken was carried from the home cage to the arena in an upright position with hands around her body. The 5-min test started when the bird was placed in the central square of the arena. The handler remained quiet and out of sight during the test. Behaviour variables measured from video recordings were observed in three categories: 1) position variables including latency to cross the first line, number of lines crossed, number of times the central square was entered, and time spent in the central square (Table 3), 2) behavioural states, including latencies and durations of immobile behaviour (freezing), escape behaviour (fleeing), ambulation (pacing), immobile but moving head (vigilant), and exploratory behaviour (Table 4), and 3) behavioural events, including latencies and frequencies of pecks at the floor, scratches at the floor, distress vocalizations, jumps, and defecations (Table 5).

Using methods modified from Ennaceur and Delacour (1988), the 2-min novel object test started immediately following the open field test when the handler opened the door, dropped a small novel object in the central square of the arena, then closed the door and remained quiet and out of sight. If the bird was in the central square at the time, the worm was dropped next to the bird, within or close, to the central square. The novel object was a king mealworm (*Tenebrio molitor*) for tests in Week 5, and a larger species of mealworm, called a super worm (*Zophobas morio*) for tests in Week 10. If the bird ate the mealworm another was thrown in—this was repeated until the end of the test. Behaviour variables measured were the same as for the open field test and, additionally, latency to peck the mealworm, number of pecks at the mealworm, latency to eat the first mealworm, and the number of mealworms eaten (Table 6).

2.3.2.3 *Modified open field test followed by modified novel object test*

The modified open field test was conducted in the same arena and with the same procedure as described above but with the addition of a window screen (15 cm long x 61 cm high) hung in the middle of the back left corner square of the arena, and a swinging perch (a 20.5 cm long x 1.5 cm diameter metal rod), hung 25 cm above the middle of the back right corner square of the arena (Fig. 1). The perch was placed at a greater height than that in the home cage of groups with a perch, to incorporate an element of novelty for all tested chickens. Behaviour variables measured from the video tapes were as described above for the open field test.

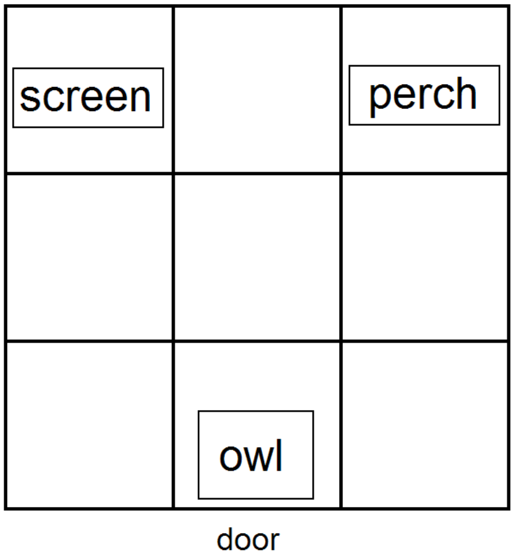

Fig. 1. Diagram depicting the elements in the modified open field and novel object tests. The window screen and swinging perch were present throughout both tests. After the 5-min modified open field test, a plastic great horned owl model was introduced, initiating the 2-min modified novel object test.

The 5-min modified open field test was followed immediately by the 2-min modified novel object test, a modified version of the novel object test in which, instead of a mealworm, the chicken was presented with a simulated predator (a plastic Great Horned owl model about 30 cm in diameter and 45 cm high) placed in the middle square by the wall closest to the door (Fig 1). Behaviour variables measured from the video tapes included those for the open field test and, additionally, latency to perch, and latency to move behind the screen (Table 7).

2.3.2.4 *Handling test*

The chicken was taken out of the open field arena, held by both legs in one hand, turned upside down, and held out of contact with the handler's body with her head about 102 cm from the ground and 30.5 cm away from the camera. Behaviour was videotaped for 30 seconds. Afterwards, the chicken was righted and returned to her home cage. Behaviour variables measured from the video tapes were number of wing flaps and number of distress calls (Table 8).

2.3.3 *Body weight and skin lesions*

All chickens were weighed weekly to the nearest gram. During weighing, chickens were physically examined and a score from 1 to 3 was assigned for skin lesions (1=skin intact, 2=minor scabs and scratches, 3=moderate to extensive skin damage).

2.4 Statistical analysis

The behavioural, and skin lesion, data were not normally distributed and thus both untransformed and ranked data were analysed. Similar results for the two analyses indicated reliability of the analysis of ranked data (Zar, 1999). Only results from the analyses of the ranked data are presented for these variables. Behavioural, skin lesion, and body weight data were analyzed using the Mixed procedure of SAS 9.2 (SAS, 2008). The model for behavioural variables from tests conducted on chickens in both Weeks 5 and 10 (Set 1) included worm, perch and their interaction, block, age, and interactions with age. Age was a repeated measure, group was a random effect, and individual chickens were the subjects. A similar model without age effects was used for behavioural data from chickens tested only in Week 10 (Set 2), and for body weights and skin lesion data, where each week was analysed separately. Due to low variation, the analyses on skin lesions did not meet convergence criteria. Means comparisons were based on least-squares means using the Tukey option in Proc Mixed to adjust for multiple comparisons. For birds subjected to tests at 5 and 10 weeks of age, test-retest reliability was assessed using the Wilcoxon matched pairs test (data not shown). The same method was used to assess inter-observer concordance (data not shown). For all statistical tests, significance was set at $p<0.05$.

3. Results

3.1 Fear tests on four birds/group in Weeks 5 and 10 (Set 1 birds)

3.1.1 *Tonic immobility test in Weeks 5 and 10*

Chickens with ‘worm’ experience exhibited longer durations of tonic immobility than birds that did not receive ‘worms’ ($F=4.66$, df=1, 52, $p=0.036$) whereas provision of perches did not have a significant effect (Fig. 2). Fewer vocalization were given ($F=41.54$, df=1, 52, $p<0.001$), while more inductions were needed to induce tonic immobility ($F=17.23$, df=1, 52, $p< 0.001$), at 10 than 5 weeks (Fig. 2).

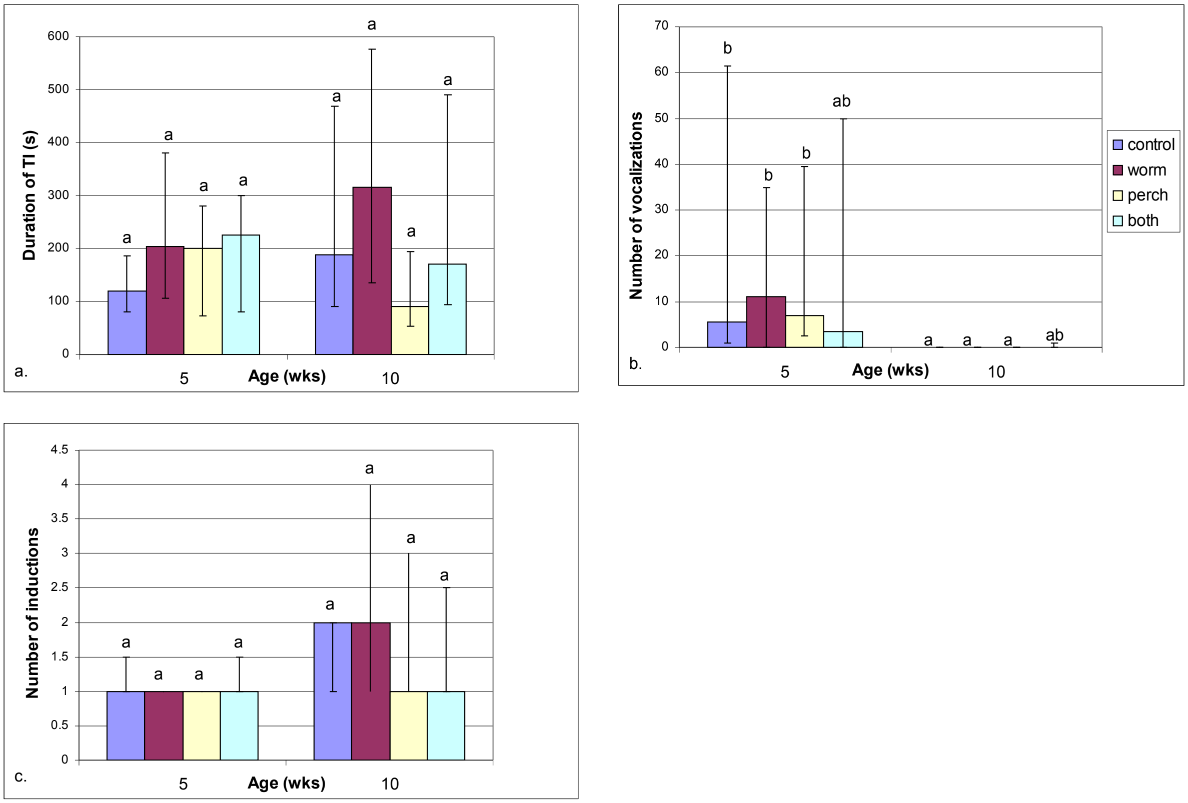

Fig. 2. Median (± interquartile range) (a) duration of tonic immobility ('worm' effect), (b) number of vocalizations (age effect) and (c) number of inductions (age effect) during tonic immobility tests at 5 and 10 weeks of age. The maximum duration permitted was 10 min. Letters denote significant differences in pairwise means comparisons on ranked data ($p<0.05$).

3.1.2 *Open field test in Weeks 5 and 10*

Chickens with 'worm' experience spent a significantly longer duration in the central square of the open field than birds lacking 'worm' experience (F=6.95, df=1, 52, p=0.011; Fig. 3).

Chickens with perch experience had a lower latency to freeze (F=6.25, df=1, 52, p=0.016; Fig. 3), in the open field than those lacking perch experience. They also showed higher rates of floor pecking (F=4.17, df=1, 52, p=0.046), duration of exploration (F=4.03, df=1, 52, p=0.049), and a longer latency to cross the first line ($F=4.85$, $df=1, 52$, $p=0.032$), in the open field at 10 than 5 weeks of age when compared with the chickens lacking perch experience (Fig. 3).

There were also numerous main effects of age on behaviour in the open field. In general, the chickens spent more time in the central square ($F=41.48$, $df=1, 52$, $p<0.001$), and exhibited a higher rate of floor pecking ($F=5.69$, $df=1, 52$, $p=0.021$), a higher latency to cross the first line ($F=28.89$, $df=1, 52$, $p<0.001$) and lower rate of line crossing ($F=15.32$, $df=1, 52$, $p<0.001$), at 10 than 5 weeks of age (Fig. 3). Additionally, they exhibited a higher latency to pace ($F=16.15$, $df=1, 52$, $p<0.001$), a lower duration of pacing ($F=11.13$, $df=1, 52$, $p=0.002$), a lower latency to give a first distress call ($F= 8.32$, $df=1, 52$, $p=0.006$), and reduced rates of distress calling ($F=21.17$, $df=1, 52$, $p<0.001$), defecating ($F=9.40$, $df=1, 52$, $p=0.003$), and jumping ($F=11.01$, $df=1, 52$, $p=0.002$), at 10 versus 5 weeks of age (Fig. 4).

Medians for variables unaffected by ‘worm,’ perch or age effects during the open field test are presented in Table 9.

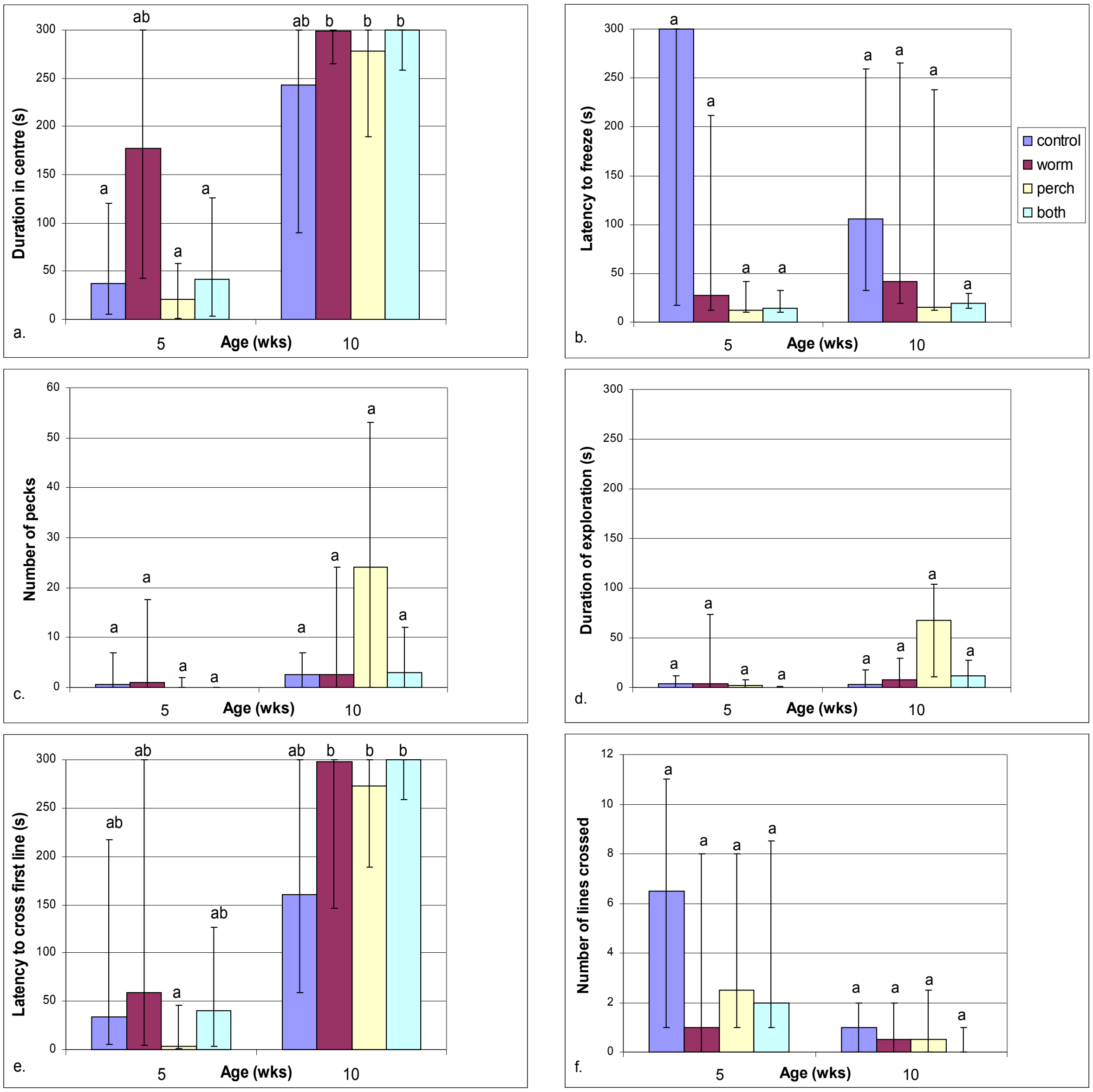

Fig. 3. Median (± interquartile range) (a) duration in the centre ('worm' and age effects), (b) latency to freeze (perch effect), (c) number of floor pecks (perch x age, and age, effects), (d) duration of exploration (perch x age effect), (e) latency to cross the first line (perch x age, and age, effects), and (f) number of lines crossed (age effect) during a 5-min open field test. Letters denote significant differences in pairwise means comparisons on ranked data ($p<0.05$).

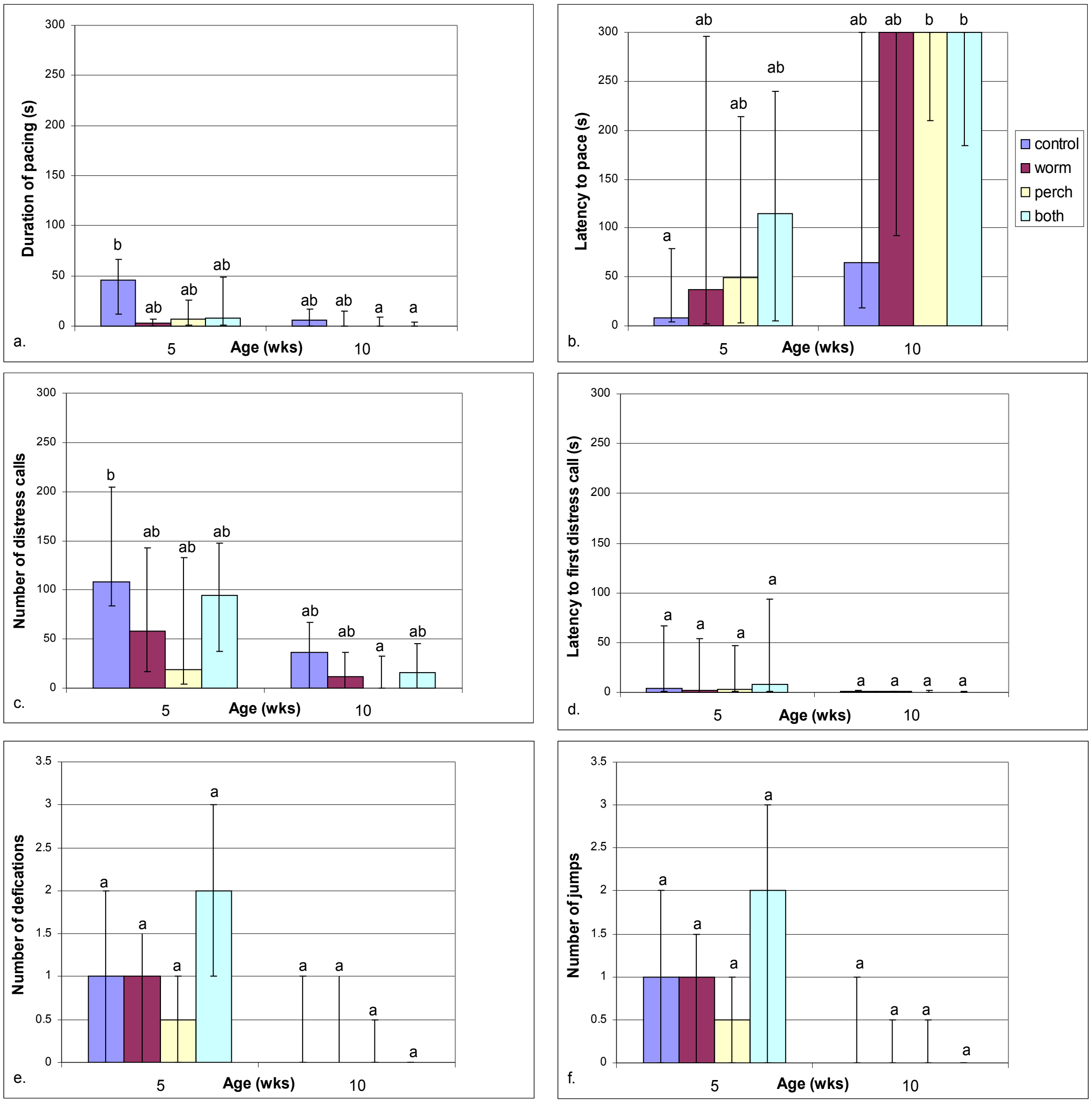

Fig. 4. Median (± interquartile range) (a) duration of pacing, (b) latency to pace, (c) number of distress calls, (d) latency to first distress call, (e) number of defecations, and (f) number of jumps during a 5-min open field test (a-f all affected by age). Letters denote significant differences in pairwise means comparisons on ranked data (p<0.05).

3.1.3 *Novel object test in Weeks 5 and 10*

After a mealworm was thrown into the centre of the open field, chickens with perch experience were counted more times in the centre (F=7.26, df=1, 52, p=0.01), and spent a longer duration in the centre and closer to the mealworm (F=5.89, df=1, 52, p=0.019; Fig. 5), at 10 than 5 weeks compared with chickens without perch experience.

In general, there were age changes in responses in the novel object test. Chickens spent more time in the central square (F=5.38, df=1, 52, p=0.024), and exhibited an increased latency to cross the first line (F=7.44, df=1, 52, p=0.009), and reduced rates of line crossing (F=7.17, df=1, 52, p=0.01), jumping (F=5.21, df=1, 52, p=0.027), and distress calling (F=7.47, df=1, 52, p=0.009) at 10 than 5 weeks of age (Fig. 5).

Medians for variables unaffected by ‘worm,’ perch or age effects during the novel object test are presented in Table 10.

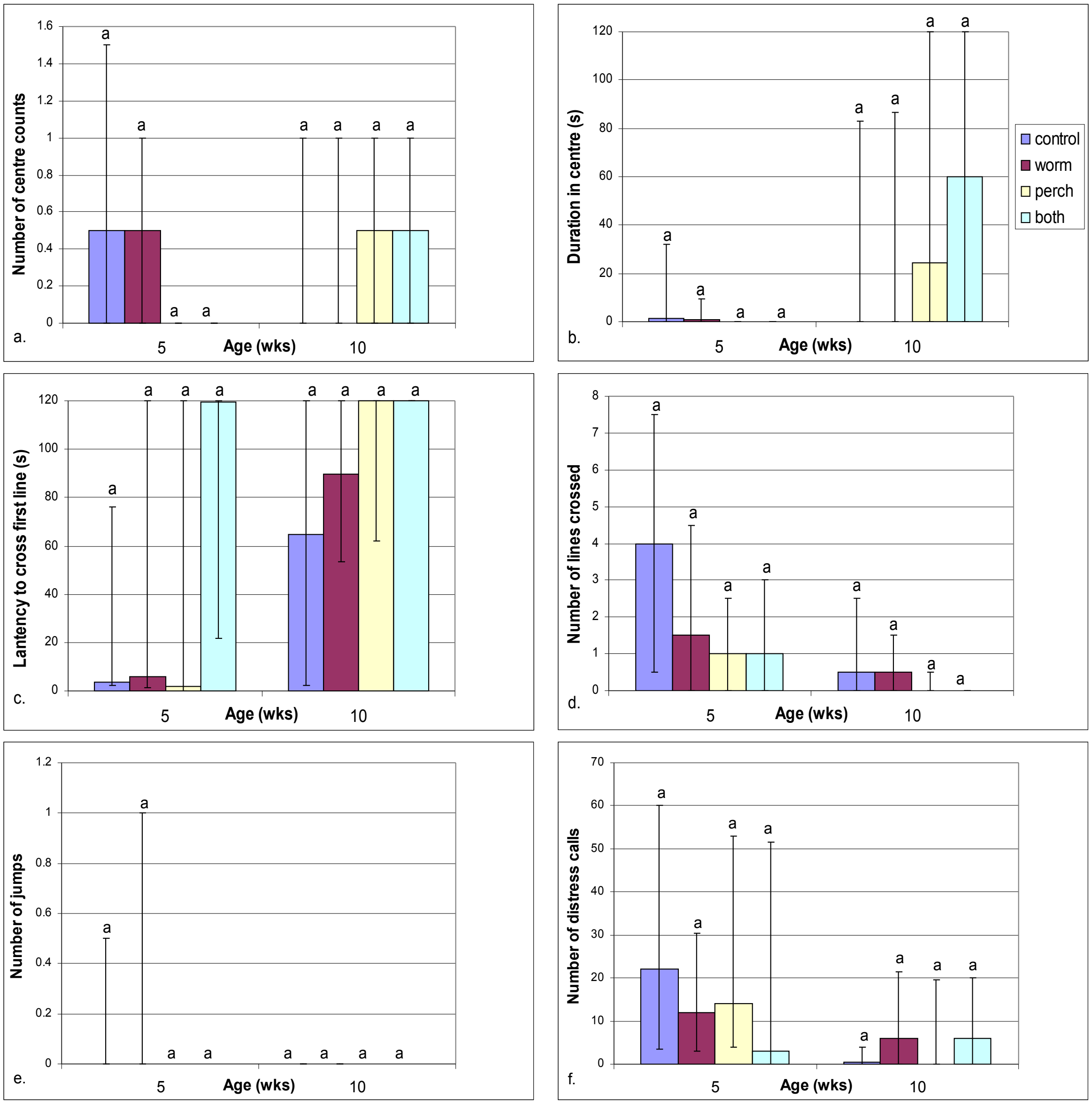

Fig. 5. Median (± interquartile range) (a) number of times that the central square was entered (perch x age effect), (b) duration in the central square (perch x age effect), (c) latency to cross the first line, (d) number of lines crossed, (e) number of jumps, and (f) number of distress calls during a 2-min novel object test (b-f affected by age). The novel object was a mealworm. Letters denote differences in pairwise means comparisons on ranked data ($p<0.05$).

3.1.4 *Handling test in Weeks 5 and 10*

Chickens with ‘worm’ experience exhibited a higher rate of wing flapping than birds in groups that were not exposed to ‘worms’ (F=4.04, df=1, 52, p=0.0498; Fig. 6). When handled at 10 weeks of age, chickens from all groups exhibited a higher rate of wing flapping (F=8.85, df=1, 52, p=0.004), and more distress calling (F=6.85, df=1, 52, p=0.012), compared with 5 weeks (Fig. 6).

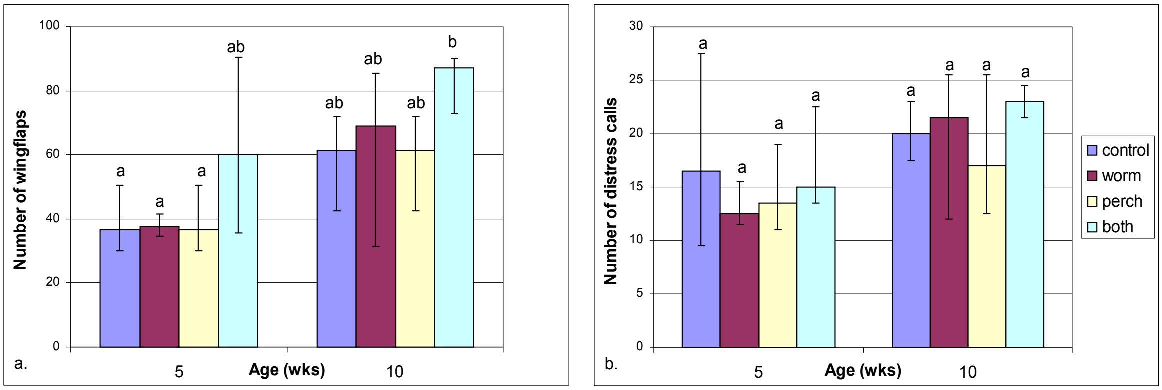

Fig. 6. Median (± interquartile range) (a) number of wing flaps (‘worm’ and age effects), and (b) number of distress calls (age effect), during a 30-s handling test. Letters denote differences in pairwise means comparisons on ranked data ($p<0.05$).

3.2 Fear tests on four previously untested birds/group in Week 10 (Set 2 birds)

3.2.1 *Tonic immobility test in Week 10*

There were no significant ‘worm’ or perch effects on duration of tonic immobility, number of inductions or number of vocalizations given during tonic immobility in the Set 2 birds tested in Week 10 (see Table 11 for overall medians).

3.2.2 *Modified open field test in Week 10*

When compared with chickens that were not given ‘worm’ objects, chickens with ‘worm’ experience exhibited a lower latency to freeze (F=5.31, df=1, 24, p=0.030) when tested for the first time at 10 weeks (Fig. 7). Chickens with perch experience exhibited fewer entries into the centre (F=4.27, df=1, 24, p=0.0497), and a reduced latency to peck the floor (F=5.16, df=1, 24, p=0.032), than chickens reared without a perch (Fig. 7). No birds used the perch or hid behind the screen. See Table 12 for medians of variables unaffected by 'worm' or perch.

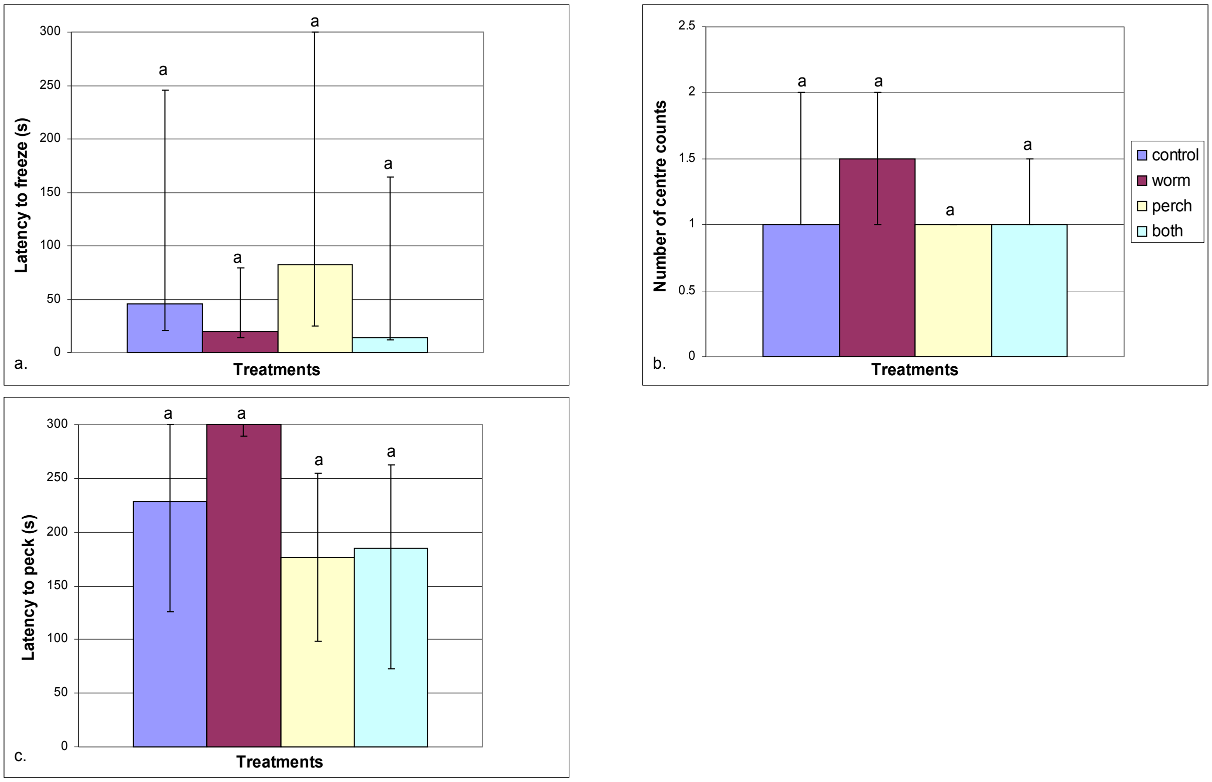

Fig. 7. Median (± interquartile range) (a) latency to freeze ('worm' effect), (b) number of times counted in centre (perch effect), and (c) latency to peck the floor (perch effect) during the 5-min modified open field test. Letters denote significant differences in pairwise means comparisons on ranked data (p<0.05).

3.2.3 *Modified novel object test in Week 10*

At 10 weeks of age, there was a significant worm x perch interaction for duration of explore, with control birds exhibiting a higher duration of exploration than birds with 'worm' or perch experience when exposed to the plastic owl (F=5.79, df=1, 24, p=0.024; Fig. 8). No birds perched or hid behind the screen. Also, birds with worm experience exhibited increased latencies to pace (F=4.79, df=1, 24, p=0.039; Fig. 8). Medians of variables that were not affected by ‘worm’ or perch are shown in Table 13.

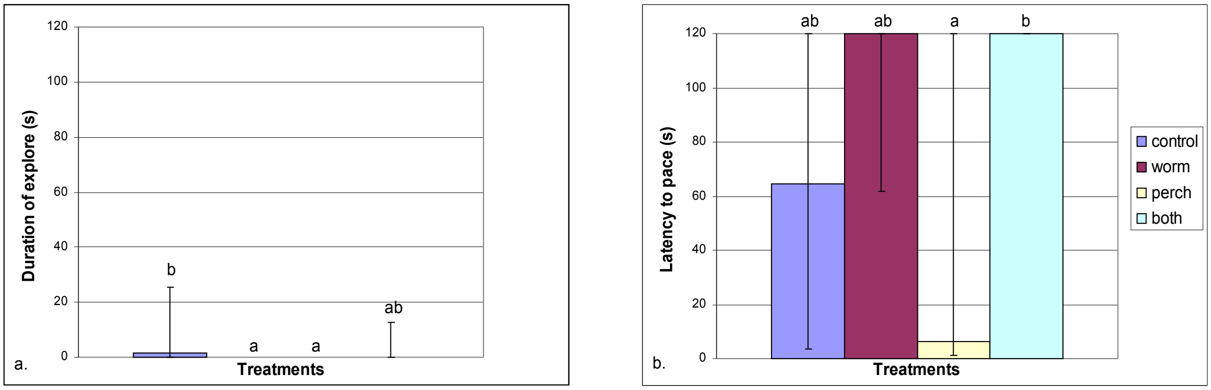

Fig. 8. Median (± interquartile range) (a) time spent exploring (‘worm’ x perch effect), and (b) latency to pace (‘worm’ effect), during 2-min modified novel object test. The novel object was a plastic owl model. Letters denote significant differences in pairwise means comparisons on ranked data ($p<0.05$).

3.2.4 *Handling test in Week 10*

There were no significant ‘worm’ or perch effects on number of wing flaps number, or vocalizations, given by the previously untested Set 2 birds when handled at 10 weeks (see Table 11 for overall medians).

3.3 Weekly body weights and skin lesions

Chickens given ‘worms’ weighed more than chickens without ‘worm’ experience at 10 (F=5.37, df=1, 64, p=0.024) and 11 (F=5.38, df=1, 64, p=0.024) weeks of age (Fig. 9). In addition, chickens reared in cages with a perch weighed more than those in cages without a perch at 7 weeks of age (F=4.05, df=1, 64, p=0.048; Fig. 9).

There were so few cases of birds exhibiting skin lesions (scores 2 and 3) that it was not possible to analyze the data statistically. From examination of the numbers, there was no evidence of any 'worm' or 'perch' effects on skin lesions but there was a tendency for more birds to have skin lesions with increasing age.

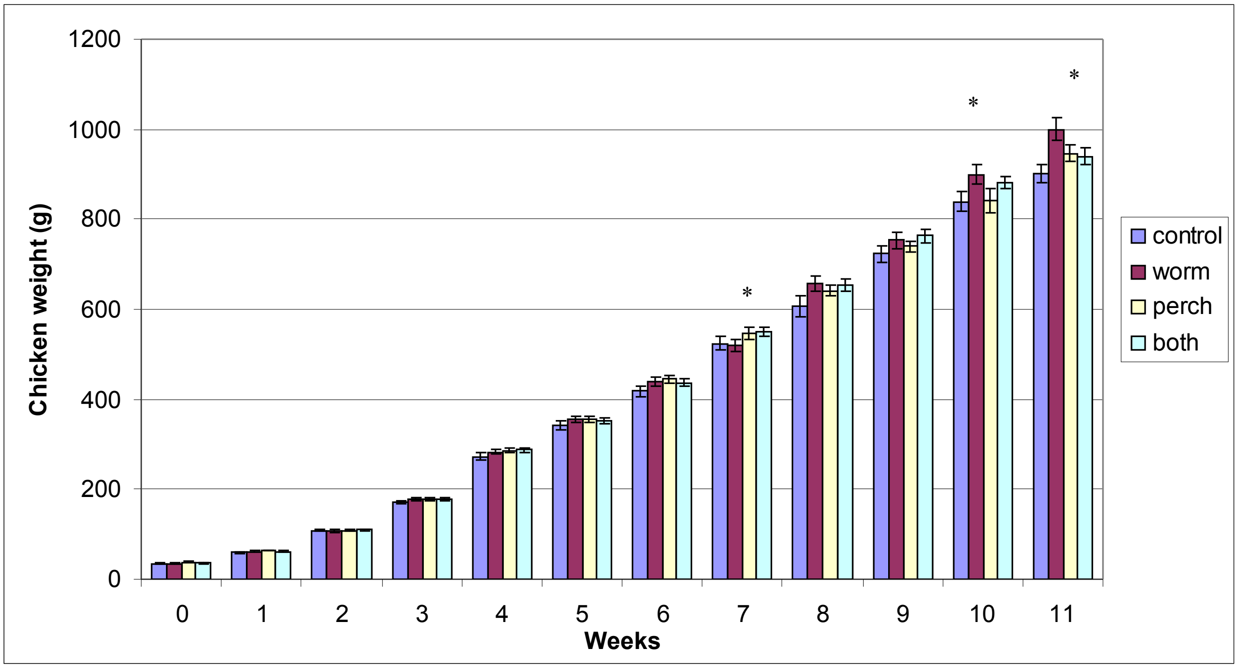

Fig. 9. Mean (± SE) body weight. A star denotes a significant treatment effect at that age ($p<0.05$). At 7 weeks, birds with perches were heavier than birds without perches. At 10 and 11 weeks, birds given 'worms' were heavier than birds without 'worms.'

4. **Discussion**

We had predicted that birds induced to play would exhibit superior body condition measured by increased body weight and a reduction in skin legions. Groups receiving 'worms' weighed significantly more at 10 and 11 weeks and exhibited a general trend of being heavier from 8 weeks on, supporting the hypothesis that playing animals experience higher states of well-being in comparison to those that do not play. Because body condition of birds, measured by skin lesions, was not significantly different, differences in weight were likely due to benefits of inducing play rather than redirecting pecking or reducing aggression. It was found that birds with worms participated in increased activity (Massey, 2009) suggesting that the increase in weight was due to increased feed intake stimulated by running during play activity. It is known that feed deprivation reduces play (e.g. Müller-Schwarze et al., 1982). However, we provided feed ad libitum so lower growth rates in chickens in the control groups cannot be explained by feed deprivation but are more likely due to reduced interest in food (a sign of depression in humans) or reduced need of additional food due to lack of exercise. This result is dissimilar to findings by other authors who report no benefits for increased levels of play. This may be due to the fact that other studies have either not examined effects on body weight, or possibly because a cage environment such as the one used in our study may diminish natural play, allowing for clearer effects when play was induced with worm objects. Additionally, groups with perches exhibited significantly higher body weights at 7 weeks, and exhibited a general trend of being heavier from 7 weeks on. This suggests that the presence of a perch somehow influenced the chickens. However, it was found that chickens did not jump on or over the perch frequently (Massey, 2009). Therefore, the perch may have been beneficial by some other mechanism than increased play directed specifically towards the perch. Because the perch was a large swinging object that moved unpredictably when displaced by one bird or by other flock mates, it is possible the presence of a perch made it necessary for birds to move more and manoeuvre more to reach various parts of the cage, also increasing activity and therefore stimulating more feeding.

We had also hypothesized that play experience would reduce fear and increase adaptability in chickens exposed to novel and unpredictable situations. Specifically, we predicted that birds provided with both worm objects and swinging perches would exhibit the most drastic reduction in fearful behaviour during standardized fear tests while groups with only worm objects or perches would exhibit milder reductions of fearful behaviour. However, we found few robust effects and the results often conflicted.

Contrary, to our predictions, worm groups exhibited some behaviour indicative of increased fear while other variables pointed to reduced fear. For birds tested at 5 and 10 weeks, birds in the worm treatments exhibited higher durations of tonic immobility and more wing flaps, interpreted as being more fearful. There was no ‘worm’ by age effect, indicating that these effects were present at both ages. These results may indicate greater fear associated with ‘worm’ experience, given that fear is thought to be the primary motivator underlying an adaptive anti-predator response under immediate threat of predation. However, why the ‘worm’ treatment would cause birds to be more fearful in the tonic immobility and handling tests is difficult to explain. Possibly birds were more disturbed by the unexpectedly ‘rough’ handling than the other birds because of a greater violation of expectations, given that they may have previously learned to associate humans only with positive events such as provision of ‘worms’.

On the other hand, there may be alternative explanations for the effects of ‘worm’ experience on behaviour in the tonic immobility and handling tests besides increased fear. For example, it is possible that the increased durations of tonic immobility were due not to greater fear but to a more ‘intelligent’ response in evaluating danger. For example, there is evidence from wild animals that ‘death feigning’ increases the chance of evading predation when captured and released from restraint while the predator remains nearby (Sargeant and Eberhardt, 1975). Additionally, an increased struggle when only restrained by the legs, as simulated by the handling test, may be an adaptive mechanism to thwart a predator, as the animal may have an increased chance of escape. It is possible that the 'worm' birds were more aware of their surroundings and better able to inhibit fleeing behaviour when actively immobilized by a predator and better able to recognize when to escape. They may also have been able to better recognize a greater chance of escape by struggling when dangling from the grip of the (human) predator in the handling test. It is also likely that birds on the 'worm' treatment were more physically fit and, thus, able to struggle for longer than those on the control treatment, given that these birds were observed to perform more wing flapping and running in their home cage (Massey, 2009). The struggle shown by the wing flapping of 'worm-experienced' birds may also reflect a more active coping response by these birds, which could indicate lower fear than in more passive birds if the latter went more quickly into an immobile 'frozen' state. After an initial bout of wing flapping, all birds stopped flapping before the end of the 30-s test. We did not record physiological data which could help to determine whether birds were relaxed, exhausted, or frozen in a tense state at this time.

Data for birds tested at 5 and 10 weeks are not consistent with results for previously untested birds tested only at 10 weeks, which showed no significant results for wing flaps or duration of tonic immobility. Additionally, there were no significant treatment effects on number of tonic immobility inductions, which would be expected to be lower in the 'worm' treatment birds if they were more fearful, or on number of calls emitted during the test, which could be higher if birds were more fearful, although this measure is not generally reported. The lack of consistent results across subsets of birds and across different variables, as well as the lack of significantly higher tonic immobility durations in the groups with 'worm' experience in the pairwise means comparisons, suggests that the effects of ‘worm’ experience on behaviour in these tests was not very robust.

On the other hand, birds tested at 5 and 10 weeks in the worm treatment exhibited significantly longer durations of time in the central square of the open field, interpreted as a sign of reduced fearfulness, given that animals of prey species such as chickens are at greater risk of predation if they venture into open areas and away from the safety of cover. When animals have freely available food (as was the case in our experiment) and are not driven to emerge into the open by hunger, it is assumed that individuals spending more time in the centre are bolder whereas those that are more fearful prefer to stay close to the walls. In the open field, prior handling is considered to simulate a predatory attack. After this, when the bird is placed into the novel arena alone, it is assumed that the larger and more unlike the home cage the arena, the more fearful chickens will be. Overall, despite the simulated predatory threat posed by handling, it is the novelty of the open field that is hypothesized to be the most fear inducing component of this test (Jones, 1987).

Increased time spent in the central square by birds with ‘worm’ experience when tested at 5 and 10 weeks may have resulted from an increased behavioural plasticity to unexpected situations as a result of experience in responding rapidly to the relatively unpredictable events transpiring in worm running. However, none of the other variables measured indicated significant ‘worm’ effects in either the 5-min open field test or in the subsequent novel object test. The latter finding is particularly surprising since we expected that ‘worm’ experience would reduce fear of a live mealworm relative to birds without this experience. This result shows that the responses to inedible novel objects in the home cage were not generalized to novel objects in a different location. Furthermore, results from the open field and novel object tests on one subset of birds were not consistent with results from the other subset of birds tested in the modified open field (with screen and perch) and novel object (owl) tests at 10 weeks of age. In these birds, ‘worm’ experience resulted in a shorter latency to freeze in the modified open field test and longer latency to pace after introduction of the owl, which could be indicative of increased fear if the ‘freeze’ response (differentiated from vigilance, which is assumed to be a less fearful state) was associated with fear and not simply lack of movement due to calmness. These inconsistencies between subsets of chickens, between variables, and in responses across similar tests, along with a lack of consistent ‘worm’ effects in pairwise means comparisons once again suggest that the effects of ‘worm’ experience on fear-related behaviour were not robust.

We do not know of any previous studies on the effects of swinging perches in poultry. However, there has been some research examining effects of standard perches on fear. Campo et al. (2005) and Tauson et al. (1999) found no effect of the presence of perches on duration of tonic immobility, consistent with our findings. Other experiments have demonstrated improved spatial skills with increased use of perches (Gunnarsson et al., 2000; Heikkilä et al., 2006), which could be predicted to alter responses when suspended upside down but we found no significant effects of perch experience in the handling test.

Although we had expected birds with swinging perch experience to exhibit reduced symptoms of fear, we found that birds with access to a perch exhibited lower latencies to freeze in the open field test when tested at both 5 and 10 weeks of age, suggestive of greater fear. However, they performed more pecking at the floor, spent more time exploring, and took longer to move out of the central square, when tested at 10 weeks, suggestive of reduced fear. In the novel object test, they moved into the centre fewer times, and spent less time in the centre, when tested at 5 weeks, suggestive of more fear, whereas the opposite occurred at 10 weeks. For birds tested only at 10 weeks, those with perch experience entered the centre square fewer times, suggestive of greater fear, but had shorter latencies to peck the floor, suggestive of less fear, and in the presence of the large novel (owl) object, they spent more time exploring if they also had 'worm' experience but less time exploring if they had perch experience alone. The birds did not make much use of the swinging perches and only interacted briefly with them (Massey, 2009). Therefore, these perches did not appear to stimulate play to the extent intended. The perch was present continuously in the cage so lacked novelty. However, it did move in a somewhat unpredictable manner depending on how it was contact by different birds. Considering that birds with perches weighed more at 7 weeks of age, this may show that even though the swinging perch did not induce the predicted amount of playful behaviour, the birds gained benefits from manoeuvring around it and becoming accustomed to its random movements when displaced by themselves or flock mates. Nevertheless, it appears that, in general, swinging perch experience did not have a strong effect on fear when birds were tested outside the familiar home cage.

Our results on effects of enrichment with worm objects and swinging perches are in contrast to other studies showing that early environmental enrichment can increase walking, jumping and vocalization, reduce inhibition in the open field and reduce the duration of tonic immobility (Jones, 1982; Jones et al., 1991; Jones, 2004). However, it is difficult to make comparisons across studies due to differences in methodological details and strains of birds used. It should also be noted that play may not be stimulated by all types of environmental modification labelled as enrichment. In the current study, the specific goal of the enrichments selected was to stimulate play, whereas previous studies on enrichment for poultry have not identified play as the goal.

Some responses could be interpreted as showing that the birds were more fearful when tested at 10 than 5 weeks of age, including crossing fewer lines, taking longer to start pacing, and spending less time pacing in the open field. They also had a higher rate of wing flapping and calling in the handling test but these results may, alternatively, be interpreted as reduced fear, if the birds continued struggling rather than falling into an immobile state similar to tonic immobility, or as greater physical fitness, if they were stronger and had the physical strength to struggle longer at the older age. However, the majority of findings suggest that the birds were less fearful at 10 than 5 weeks of age, consistent with previous studies, although for different variables (Hocking et al., 2001; Albentosa et al., 2003). In our study, the birds spent a greater duration in the centre of the open field, had a higher rate of floor pecking, took longer to move from the centre, exhibited less defecation, began vocalizing sooner, and vocalized less frequently in the open field test at 10 than 5 weeks of age, all interpreted as indicators of reduced fear. Similarly, they spent more time in the centre, and performed fewer jumps and calls, in the novel object test, and required more tonic immobility inductions and called less in the tonic immobility test, at 10 than 5 weeks, consistent with reduced fear and social reinstatement motivation at the older age. Hocking et al. (2001) reported reduced durations of tonic immobility in older than younger chickens, consistent with our results, but no less fear shown during open field tests. This may be due to various factors. First, our birds were of a layer strain whereas they were observing broilers. Also, our birds were tested in the same room in which they were housed, in auditory and olfactory contact with conspecifics, which may have reduced their fearfulness relative to birds tested in complete social isolation (Bilcik et al., 1998). Additionally, at 10 weeks, our birds were tested in an arena the same size as their home cage and half of them had prior experience of the open field from testing at 5 weeks. These factors could be expected to have fear-reducing effects.

Although the open field is favoured because it is an easy test to perform, the results from this test are controversial because it is difficult to know exactly what is being measured—fear or social motivation. Another difficulty in interpreting results of tests of fear-related behaviour is that, rather than responses being linear, birds may show a more active response at intermediate than at low or high levels of fear. In this experiment, we suggest that the open field test was not a very decisive measure of fear and may not have been performed under strong enough fear inducing conditions. Use of a novel room and a larger arena with a higher ceiling might have allowed more clear interpretations. Because our other tests, such as the handling, modified open field, and modified novel object test, were somewhat different from those used in previous studies, the exact procedures that we used have not been validated by other researchers. The modified open field, in particular, did not produce the expected hiding behind the screen or use of the perch. It might have been a better test if the perch had been higher and sturdier, as birds that jumped during this test appeared to try and perch on top of the screen. Additionally, changing the screen to a more familiar feature previously associated with safety may increase the chance that the birds would react to novel stimuli by hiding behind it.

Looking at the significance of these results, the only relatively robust effects appear to be for the age and body weight effects. Because we had to rank the data due to lack of normal distributions, it is possible that some more subtle effects were not detected, as some variables were only marginally significant while, for other variables, no significant results were found. For relatively rare behaviours, a lack of significance cannot be interpreted as a definitive result as we lacked the statistical power to detect significant differences. This concern applies especially to the following behaviours: pecking the worm, eating the worm, and scratching the floor. These results were surprisingly low—perhaps due to the fact that these behaviours are associated with feeding whereas birds in the ‘worm’ treatment, which we predicted would exhibit more interaction with a real, moving worm, did not associate an inanimate worm-like object with food but with play.

5. Conclusions

There were inconsistent effects of the ‘worm’ and perch treatments on behavior in the different tests, some suggestive of increased fear and others suggestive of reduced fear. Most of these effects were not very robust. In contrast, a majority of the differences in behavior in the tests at 5 versus 10 weeks of age were consistent with an interpretation of reduced fear and social motivation at the older age. Based on increased body weights at some ages, and an effect of ‘worm’ experience in increasing time spent in the center of the open field, it is concluded that there could be some benefits to providing ‘worms’ and swinging perches to improve productivity and well-being of caged chickens being reared for egg production.

6. Tables

Table 1. Worm-like objects provided daily in each week to stimulate worm-running behaviour in treatment groups receiving ‘worms.’

Age (Weeks)	‘Worm’ type	Weight (g)	Length (cm)	Description
1	Pipe cleaner	0.10	5	Pink, fluffy threads; wire through middle
2	Foam strip	0.10	6	Orange fine pore foam
3	Embroidery floss	0.20	7	Orange/pink acrylic threads; braided
4	Yarn	0.25	8	Red/yellow acrylic yarn; braided
5	Fluffy yarn	0.25	9	Multicolour acrylic yarn; braided
6	Hemp	0.25	10	Brown hemp threads; braided
7	Craft feather	0.25	10	Yellow dyed craft feather
8	Paper	0.30	10	Sea green paper; twisted
9	Rope	0.45	10	Red plastic rope
10	Crepe paper	0.40	10	Purple textured paper; twisted
11	Cloth worm	0.50	10	White knit cloth cut into strips; knotted

Table 2. Ethogram for tonic immobility test. Maximum test duration is 10 minutes.

Variable	Description	Assumed fear level if value higher
Number of induction attempts	Number of times that bird is placed on her back until she remains on her back for > 10 s	Less fear
Duration of tonic immobility (s)	Total time taken from induction of tonic immobility until the bird rights herself (i.e., stood up)	More fear
Frequency of distress calling	Number of distinct high pitched vocalizations	More fear and/or more social motivation

Table 3. Ethogram for 5-min open field test - position variables. Test starts when bird is placed in central square.

Variable	Description	Assumed fear level if value higher
Latency to cross first line (s)	Time before moving into another square with both feet (main part of foot, not including toes)	Less fear, unless frozen
Number of lines crossed	Number of times that lines on arena floor are crossed with both feet	Less fear
Number of times central square entered	Number of times central square was entered after walking out of it the first time	Less fear
Duration in central square (s)	Total time spent with both feet in central square	Less fear, unless frozen

Table 4. Ethogram for 5-min open field test - behavioural state variables. Test starts when bird is placed in central square.

Variable	Description	Assumed fear level if value higher
Latency to freeze (s)	Time to first bout of freezing (remaining completely motionless except for breathing for > 5 s)	Less fear
Duration of freezing (s)	Total duration of all bouts of freezing	More fear
Latency to flee (s)	Time to first running bout (running rapidly for > 0.5 s)	Less fear, unless frozen
Duration fleeing (s)	Total time spent in running bouts	More fear, unless frozen
Latency to pace (s)	Time elapsing from being placed in central square until first pacing bout (walking rapidly with head up for > 0.5 s)	Less fear
Duration of pacing (s)	Total time spent in pacing bouts	More fear, unless frozen
Latency to vigilance (s)	Time to first bout of vigilance (body still but head moving for > 0.5 s)	Less fear, unless frozen
Duration of vigilance (s)	Total time spent in vigilance bouts	Less fear than freezing
Latency to explore (s)	Time to first bout of exploration (walking calmly examining floor and walls of arena for > 0.5 s)	More fear
Duration of exploring (s)	Total time spent in exploration bouts	Less fear

Table 5. Ethogram for 5-min open field test - behavioural event variables. Test starts when bird is placed in central square.

Variable	Description	Assumed fear level if value higher
Latency to ground peck (s)	Time to first peck at floor with beak	More fear
Frequency of ground pecking	Total number of ground pecks	Less fear
Latency to ground scratch (s)	Time to first scratch at floor with foot	More fear
Frequency of ground scratching	Total number of scratching movements with each foot against floor	Less fear
Latency to give distress calls (s)	Time to first distress vocalization (high pitch call, may be emitted occasionally or in rapid sequences)	Less fear and/or less social motivation
Frequency of distress calling	Total number of distress calls given	More fear and/or social motivation
Frequency of jumping	Total number of jumps	More fear, unless frozen
Frequency of defecation	Number of faecal boli produced	More fear

Table 6. Ethogram for 2-min novel object test. Additional measures (all other behaviours same as for open field test). Test starts when mealworm lands in central square.

Variable	Description	Assumed fear level if value higher
Latency to peck worm (s)	Time elapsing from arrival of worm to first peck at worm, making beak contact	More fear
Frequency of worm pecking	Total number of pecks at worm	Less fear
Latency to eat worm (s)	Time elapsing from arrival of worm to devouring worm	More fear
Frequency of worm eating	Total number of worms eaten	Less fear

Table 7. Ethogram for 5-min modified open field test and 2-min modified novel object test. Additional measures (all other behaviours same as for open field test). Modified novel object starts when plastic owl model placed in arena.

Variable	Description	Assumed fear level if value higher
Frequency of jumping on perch	Number of attempts to jump onto the swinging perch (jump with both feet off ground in direction of perch)	More fear unless frozen
Duration of hiding (s)	Total time spent behind hanging screen with more than half of the body obscured	More fear unless frozen

Table 8. Ethogram for 30-s handling test. Test starts when bird is suspended by legs in inverted position.

Variable	Description	Assumed fear level if value higher
Frequency of wing flapping	Total number of synchronized downward movements of both wings, where each downward movement is preceded by raising both wings	More fear, unless exhausted
Frequency of distress calling	Total number of distress calls given	More fear and/or social motivation

Table 9. Median, upper and lower quartile values for open field test variables unaffected by ‘worm,’ perch or age effects (p>0.05) - birds tested in both Weeks 5 and 10.

Variable	Median	75% quartile	25% quartile
Number of times central square entered	1	1	1
Duration of freezing (s)	34	159	3
Latency to flee (s)	0.2	300	0
Duration fleeing (s)	1.3	2.5	0
Latency to vigilance (s)	0.5	2.2	0
Duration of vigilance (s)	195	231	109
Latency to explore (s)	271	300	130
Latency to ground peck (s)	300	300	150
Latency to ground scratch (s)	300	300	300
Frequency of ground scratching	0	0	0

Table 10. Median, upper and lower quartile values for novel object test variables unaffected by 'worm,' perch or age effects (p>0.05) - birds tested in both Weeks 5 and 10.

Variable	Median	75% quartile	25% quartile
Latency to freeze (s)	34	120	12
Duration of freezing (s)	17	75	0
Latency to flee (s)	61	120	0
Duration fleeing (s)	0.2	1.9	0
Latency to pace (s)	120	120	6.6
Duration of pacing (s)	0	3.7	0
Latency to vigilance (s)	0.3	2.5	0
Duration of vigilance (s)	73	99	36
Latency to explore (s)	120	120	63
Duration of exploring (s)	0	11	0
Latency to ground peck (s)	120	120	111
Frequency of ground pecking	0	1	0
Latency to ground scratch (s)	120	120	120
Frequency of ground scratching	0	0	0
Latency to give distress calls (s)	120	120	120
Frequency of defecation	0	0	0
Latency to meal worm peck (s)	120	120	118
Frequency of meal worm pecks	0	0.5	0
Latency to eat mealworm (s)	120	120	120
Number of meal worms eaten	0	0	0

Table 11. Median, upper and lower quartile values for tonic immobility test and handling test variables unaffected by 'worm' or perch effects (p>0.05) - birds tested in Week 10 only.

Variable	Median	75% quartile	25% quartile
Duration of tonic immobility (s)	170	323	65
Number of vocalizations	0	0	0
Number of inductions	1	2	1
Number of wing flaps	57	76	41
Number of vocalizations	19	24	15

Table 12. Median, upper and lower quartile values for modified open field test variables unaffected by ‘worm’ or perch effects (p>0.05) - birds tested in Week 10 only.

Variable	Median	75% quartile	25% quartile
Latency to cross first line (s)	155	281	29
Number of lines crossed	4	2	0.5
Duration in central square (s)	173	299	68
Duration of freezing (s)	20	119	1.2
Latency to flee (s)	0	300	0
Duration fleeing (s)	0.9	1.7	0
Latency to pace (s)	115	279	3.4
Duration of pacing (s)	2.8	9.1	0.5
Latency to vigilance (s)	0.9	1.7	0
Duration of vigilance (s)	186	242	127
Latency to explore (s)	151	300	71
Duration of exploring (s)	26	60	0
Frequency of ground pecking	4.5	19	0
Latency to ground scratch (s)	300	300	300
Frequency of ground scratching	0	0	0
Latency to give distress calls (s)	1.5	63	0
Frequency of distress calling	17	50	0
Frequency of jumping	0	0	0
Frequency of defecation	0	1	0

Table 13. Median, upper and lower quartile values for modified novel object test variables unaffected by ‘worm,’ perch or age effects (p>0.05) - birds tested in Week 10 only.

Variable	Median	75% quartile	25% quartile
Latency to cross first line (s)	111	120	1.1
Number of lines crossed	0.5	3	0
Number of times central square entered	0	1	0
Duration in central square (s)	0	1.8	0
Latency to freeze (s)	18.1	59.4	10.1
Duration of freezing (s)	92.2	110.9	8.7
Latency to flee (s)	0.6	120	0
Duration fleeing (s)	1.4	1.7	0
Duration of pacing (s)	0	1.7	0
Latency to vigilance (s)	1.1	3.4	0
Duration of vigilance (s)	38.0	110.7	9.5
Latency to explore (s)	120	120	120
Latency to ground peck (s)	120	120	120
Frequency of ground pecking	0	0	0
Latency to ground scratch (s)	120	120	120
Frequency of ground scratching	0	0	0
Latency to give distress calls (s)	8.5	120	0.5
Frequency of distress calling	4.5	24.5	0
Frequency of jumping	0	1	0
Frequency of defecation	0	1	0

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