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Enriched housing conditions in rearing male chickens differing in growth performance:

Effects on animal behaviour and animal welfare

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ANIMALS LIKE NOVELTY IF THEY CAN CHOOSE TO INVESTIGATE: THEY FEAR NOVELTY IF YOU SHOVE IT IN THEIR FACE.

(Temple Grandin)

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List of abbreviations

D	dark period
d	days
diva	digital value
DTB	Deutscher Tierschutzbund
Dual	Lohmann Dual
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization of the United Nations
GS	gait score
h	hour
L	light period
LB	Lohmann Brown
LME	linear mixed model
LW	week of life
n	number
n/a	not available
OECD	Organisation for Economic Co-operation and Development
Ross	Ross 308
rps	rotation per second
SEM	standard error of the mean
TierSchNutztV	Tierschutz-Nutztierhaltungsverordn

CHAPTER I

General introduction

Current situation in poultry production

The history of the domesticated chicken goes back more than 8000 years (West and Zhou, 1989). The ancestor of the domestic fowl (*Gallus gallus* f. dom.), the Red Junglefowl (*Gallus gallus*), lives in the Southeast Asian jungle and was widely spread by traders and seafarers (West and Zhou, 1989). In the early centuries, chickens were kept in small backyards for self-sufficiency. Due to the steady increase of the world population and in per capita consumption, the number of animals is still rising and will continue to do so in the future (OECD, 2018). To date, the global chicken population, with over 22 billion birds (FAO, 2018), is the leading species of poultry worldwide and important supplier of meat and eggs and byproducts like feathers and dung (McGovern, 2000).

The increasing demand for chicken products led to a separation of egg and meat production, because high egg and meat performance could not be unified as one breeding target due to a highly negative correlation between fattening and laying performance. Hence, the optimization and increasing performance led to highly specialized chicken strains since the 1950s. The layer strains show high quality and large numbers of eggs. In contrast, the meat chicken strains show an efficient muscle growth performance. While the female chickens of meat chicken strains can also be used for meat production, the male layers can only be reared and marketed economically to a very limited extent. The main part of the male layer strains is already sorted out as day-old chicken and killed in accordance with animal welfare regulations. This problem does not only occur in conventional egg production but also in organic livestock farming. However, the killing of male day-old layer chickens increasingly came into the public focus and is no longer accepted socially and politically due to ethical issue (Buhl, 2013).

In order to avoid the killing process, a practicable and economically efficient alternative is sought. There are already alternatives such as the rearing of "coquelets" (Hörning et al., 2010; Koenig et al., 2012), the male layer chicken for meat production ("laying hen brother") or the sex determination *in ovo* (Krautwald-Junghanns et al., 2017). The rearing of "laying hen brothers" is very expensive compared to conventional broilers, as the reduced fattening performance and the resulting high feeding costs (Koenig et al., 2010) do not produce a satisfactory yield. Moreover, there is still no sufficient infrastructure for these chickens on the market in Germany, which means that there are no suitable slaughterhouses or sales markets. The "laying hen brothers" can hardly be sold as whole body products but only as finished products (Koenig et al., 2012).

There are various methods for sex determination *in ovo*, of which none is yet practically applicable in conventional hatcheries (Krautwald-Junghanns et al., 2017). For the sex determination *in ovo*, there are two different approaches: the non-optical (e.g. endocrinological (Clinton et al., 2016)) and the optical method (e.g. spectroscopic (Galli et al., 2016)). The advantage of both methods is that the egg of the male chick has no longer to be bred until hatch but can be sorted out beforehand. However, a very important difference is the application of the incubation day: The spectroscopic approach can already determine the sex on the 4^{th} day of incubation (Galli et al., 2017); in contrast, this is possible only after the 8^{th} day of incubation using the endocrinological method (Weissmann et al., 2014). From the 7^{th} day onwards, sensitivity and pain may already have developed in the chicken embryo (Eide and Glover, 1997). However, both types of hatching eggs count as industrial eggs (Commission Regulation (EC) No 589/2008) and belong to animal by-products. No distinction between incubation days and their following use are made (Regulation (EC) No 1069/2009).

Another approach to avoid the killing of male day-old chicks is the keeping of dual-purpose chicken strain (Krautwald-Junghanns et al., 2017). Dual purpose chickens are commercial cross-breedings between layer and meat strain, which have a moderate performance in both egg and meat production (Hörning et al., 2010). Although the egg performance of the dual-purpose hen (e.g. "Lohmann Dual" approx. 250 eggs/ year) cannot keep up with a layer hybrid hen (approx. 320 eggs/ year), the dual-purpose rooster proves to be much more effective in feed conversion compared to the "laying hen brother" (Icken and Schmutz, 2013). In comparison to the dual-purpose strain that were used until the 1950s, the "new" strain has almost the same egg performance, but the meat performance has been doubled (Nagel, 1962). Furthermore, the dual-purpose rooster reaches a live weight of 2 kg in 8 weeks, much faster than the "laying hen brother" (Mueller et al., 2018). However, this is almost twice the time a fast-growing broiler needs to gain the same weight (Mueller et al., 2018). Therefore, from an economic point of view, dual-purpose chickens are gainless in both sexes in comparison to conventional specialized breeds (Damme et al., 2015). However, special niches and sales markets can be found in the organic sector (Lambertz et al., 2018), according to its philosophy of animal husbandry and profitability is different.

In contrast to "laying hen brothers" or dual-purpose cockerels, in commercial meat production both sexes were used and classified in slow- and fast-growing chickens. The difference between the growth rates is the number of fattening days to reach a living weight of 1.5 – 2.5 kg. To the slow-growing strains, chickens were allocated with a fattening period of not less than 56 days (Commission Regulation (EC) No 543/2008). Fast-growing chickens are kept from an age of 28 till 42 days (Berk, 2014) and these birds are the commonly used meat chickens worldwide. Animal welfare issues based on intensive genetic selection such as leg problems, contact dermatitis, ascites, and sudden death syndrome are linked by fast growth rates of chickens (Bessei, 2006). Lighting regime, litter management, dietary deficiencies, air quality and temperature as environmental risk factors are also affecting the welfare of chickens. It has to be taken into account that interactions between genetic predisposition, environmental factors and intensive selection of fast growth rate due to poor animal welfare. In matters of skeletal disorder, high body mass, muscle disorder, and unbalanced body center fast growing chickens are more susceptible to above-mentioned issues (EFSA, 2010).

In this regard the dual-purpose strain has a great advantage with respect to animal welfare: the dual-purpose rooster is a moderate-growing chicken because of its lower growth rate. Thus, it has less problems with lameness, skeletal disorder or contact dermatitis (Bessei, 2006) compared to fast-growing broilers. The dual-purpose hens also show advantages in animal welfare aspects as well as a reduced risk of feather pecking and cannibalism compared to conventional laying hybrids (Giersberg et al., 2017). By reducing animal welfare problems of both sexes mortality and injuries can be reduced and thus costs can be saved. Dual-purpose strains are commercial cross-breeding, which can be seen as a compromise between inadequate and high-performance strains with relevant issues for animal welfare.

In summary, in chicken meat production strains can be kept with different growth rates concerning such as efficiency, ethical problems or animal welfare issues. In the present thesis, these aspects were considered by using male layer chickens (slow-growing, Lohmann Brown Plus/ Classic), dual-purpose strain (medium-growing, Lohmann Dual) and commercial meat strain (fast-growing, Ross 308, see Fig. 1). To investigate and evaluate the differences in animal-related indicators between the three strains the baseline situations (housing condition, assessment tools of animal welfare etc.) and possible improvements of the situation has to be clarified. The following paragraphs provide an overview about the mentioned points.

Figure 1 Image of the three different strains of this thesis at the 5th week of age: male layer chicken (Slow-growing, Lohmann Brown Plus), dual-purpose strain (Medium-growing, Lohmann Dual), and commercial meat strain (fast-growing, Ross 308) (from left to right).

Housing conditions in meat production

After hatching, the chickens are housed in different systems depending on the type of production. The types of housing systems can be differentiated into cages, barn, free range, and organic production (Commission Directive 2002/4/EC; Commission Regulation (EC) No 543/2008). Free-range systems allow the chickens to freely use the stable and an outside pasture. The size of the outside area has to be at least 1 to 2 m^2 per chicken (Commission Regulation (EC) No 543/2008). In addition, a shelter must be present to protect chickens against raptors and weather conditions. This type of husbandry is mandatory for organically managed farms (Commission Regulation (EC) No 889/2008), but is also used in conventional sectors. In contrast to free-range systems, semi-intensive systems provide only access to an outdoor climate area, which must offer for example at least 20 % of stable floor area (DTB, 2017). Both systems are often combined and have the advantage that chickens are given the opportunity to act out their natural behaviour such as comfort behaviour or foraging (described in the next subchapter) and to have access to fresh air. Contrary to the described systems, intensive system stables are fully automatically ventilated and the chickens have no access to an outside area. The stable equipment can vary depending on the type of production, the age of birds and the regulations of the respective country.

In Germany, meat chickens and pullets (growing female laying chicken), as well as broiler breeders are kept in floor systems. However, pullets are also kept in aviary system with several tiers or broiler breeders are offered deep pit and perch racks to the litter area additionally. For pullets and meat chickens environmental structuring is not subjected to any regulations. However, the essential aspects such as appropriate drinking and feeding facilities must be offered (Council Directive 2007/43/EC). Furthermore, the bedding should be loose, dry and consist of natural material (Council Directive 2007/43/EC). There is a lack of legal regulations regarding the stocking density in the stable of broiler breeders and pullets, but there are recommendations for pullets by the Federal Ministry of Food and Agriculture (BMEL, 2015). In meat chickens, there are clear regulations for the stocking density in the European Union, which differ depending on the type of farm: organic farm – 21 kg living weight/ m^2 (Commission Regulation (EC) No 889/2008); commercial farms – general in the EU: 33 up to 42 kg living weight/ m^2 (Council Directive 2007/43/EC), and in Germany: 35 kg living weight/ m^2 for chicken less than 1600 g (TierSchNutztV, 2006). However, a maximum stocking density of 39 kg living weight/ m^2 can be achieved in agreement with the competent authority but is less applicable (TierSchNutztV, 2016). Structural elements such as perches, prescribed for laying hens (Council Directive 1999/74/EC), are not mandatory according to the legal requirements for meat chicken. However, there are certain programmes and labels, which require elevated structures for meat chickens, but it must be taken into account that strains differing in growth performance are used (see Tab. 1).

In a governmental program in Switzerland ("Besonders tierfreundliche Stallhaltungssystem (BTS)"), meat chickens must be offered elevated structures, mostly plastics grids. This area is counted by the BLV (Bundesamt für Lebensmittelsicherheit und Veterinärwesen) as a walk-area and thus as additional allocable floor area. Hence, 10 % more chickens can be kept if the requirements of 30 cm width, 50 cm free space above and an angle of inclination of less than 12 % are met (BLV, 2018).

Table 1 shows requirements by different labels in Germany. Most of them require the use of meat chickens of a moderate or slow growth rate. Fast growing chickens are slaughtered after 35 days at an approximate weight of 2 kg. The feed conversion of the fast-growing chickens is approximately 1.6 kg/kg (Aviagen, 2014). In contrast, slow-growing chickens reach the weight of 2 kg after a fattening period of 56 days. Also their feed conversion is much lower with about

2.5 – 4.5 kg/ kg (Keppler et al., 2009). Slow-growing chickens thus have clear deficits in performance, which leads to a strong economic and ecological disadvantage (Koenig et al., 2012).

There is a rising tendency to improve the housing conditions in meat chickens focussed on structuring the environment, using strains with slower growth or reducing the stocking density. To assess the effect of these aspects regarding to animal welfare, animal behaviour and animal health has to be quantified. Suitable methods and procedures are described in the following section.

Table 1 Overview of various meat chicken husbandry and their requirements regarding rearing facilities in Germany

Farming system	commercial		
	Tierschutznutztier-haltungsverordnung (TierSchNutztV, 2006)	Tierschutzlabel First Level (DTB, 2017)	Initiative Tierwohl (ITW) – Prerequisite QS programm (ITW, 2019)
Strain	All meat chicken strains	Moderate and slow growing meat chicken strains	All meat chicken strains
Total number of chickens in one stable	n/a	max. 30,000	n/a
Fattening period	Up to an age of 28 days of life	At least 56 days	Up to an age of 28 days of life
Stocking density [kg LW/ m^2]	39 or 35 for living body weight less than 1,600 g	25	39 or 35 for living body weight less than 1,600 g
Environmental enrichment	n/a	3 straw bales/ 2,000 chickens; 1 Pecking object/ 1,000 chickens; 15 m perches/ 1,000 chickens – 10 or 30 cm above the ground	Supplementary material for forage or peck at least 10 % more house floor – additional level possible
Farming system	**organic**		
	Commission Regulation (EC) No 889/2008	Bioland e.V. (Bioland, 2018)	Demeter (Demeter, 2015)
Strain	Moderate and slow growing meat chicken strains	Moderate and slow growing meat chicken strains	Slow growing meat chicken strains
Total number of chickens in one stable	max. 4,800	max. 4,800	max. 2,500
Fattening period	At least 81 days	Between 70 – 90 days	At least 81 days
Stocking density [kg LW/ m^2]	21	21	21
Environmental enrichment	n/a	Perches, according to body size and age	4 cm/ kg LW perches

Welfare assessment of the housing environment

In order to evaluate the husbandry environment and the suitability of the different strains concerning the animal welfare status, scientific measurement methods can be used. Different protocols can be found that are used for such evaluations. However, a balance must be found for the application in experimental and field studies. First of all, animal welfare has to be clarified and, which aspects are influencing animal welfare. There are different concepts to define animal welfare such as reductionist, animals' adaptability and animals' feelings, and consequently different definitions and measurements (Weber and Valle Zárate, 2005). This thesis focussed on animals' adaptability. According to Broom (1991) welfare is mirrored by the state of an individual to cope with its environment. Environmental challenges demand coping strategies from an animal to control its physical and mental stability (Broom, 1986). Welfare can divided in poor up to good welfare and included failure or difficulty to cope which may lead to stress, injuries or diseases, changes in emotional states such as pleasure, fear or frustration (Broom, 2001b). To assess these multidimensional aspects, influences of, for example, the husbandry environment, animal health parameters, interactions of animals with the environment and their conspecifics can be investigated. On this basis, the "Welfare Quality® Assessment protocol for poultry" has been developed. It includes many different aspects evaluating the welfare of poultry and other species like cattle or pigs. The application of this protocol is specially designed for on-farm assessments. The 12 different welfare criteria are arranged in four principles to get a thematic overview (Tab. 2). Some of the indicators listed in Table 2 can be objectively assessed and subsequently evaluated such as number 1, 2, 5 or 7. For other parameters (e.g., (3) plumage cleanliness or (6) lameness), scoring systems must be used that are scientifically validated and easy to apply in practice. However, these assessment systems require training of the respective observer, as the results and the assessment must be consistent in order to allow comparability between different animal flocks and their husbandry systems. The assessment is based on a subjective estimation of behaviour and physical condition and their subsequent classification in a scoring system. Particularly worth mentioning is the assessment of the walking ability. It is based on a 6-scale-scoring systems according to Kestin et al. (1992). For the assessment of walking ability, animals in a small group are circled in a spiral-shaped area and individually returned to the flock by a small passage. The walking movement is observed. In the best case, the chicken should show its individual movement possibilities, but factors such as new situation, fear, and separation from the group play an important role (Forkman et al., 2007). The influencing factors can lead to misjudge of the walking ability or can affect the walking ability, which in turn can lead to a poor or good assessment of animal welfare, for example the scoring system from Kestin et al. (1992), which is not only recommended in Welfare Quality® but also in other existing protocols (Knierim et al., 2016). Specially in fast-growing meat chickens, the gait is hardly to assess based on the unbalanced body center (EFSA, 2010), which can lead to a waddling gait and short stride frequency (Paxton et al. 2013) and consequently a wrong assessment of the walking ability. The reliability of the assessment of walking ability could profit from a more objective method.

Table 2 Overview of the classification of criteria and their implementation after Welfare Quality ® Assessment protocol for poultry (Welfare Quality®, 2009)

	Welfare Criteria		Measures
Good feeding	1	Absence of prolonged hunger	This criterion is measured at the slaughterhouse
	2	Absence of prolonged thirst	Drinker space
Good housing	3	Comfort around resting	Plumage cleanliness, litter quality, dustbathing, sheet test
	4	Thermal comfort	Panting, huddling
	5	Ease of movement	Stocking density
Good health	6	Absence of injuries	Lameness, hock burn, foot pad dermatitis
	7	Absence of disease	On farm mortality, culls on farm
	8	Absence of pain induced by management procedures	This criterion is not applied in this situation
Appropriate behaviour	9	Expression of social behaviours	As yet, no measures are developed for this criterion
	10	Expression of other behaviours	Cover on the range, free range
	11	Good human-animal relationship	Avoidance distance test (ADT)
	12	Positive emotional state	Qualitative behavioural assessment (QBA)

In poultry farms, methods have to be found with few effort, economical time management and an objective method. The already described method of assessing the walking ability can be used to validate objective methods (Sandilands et al., 2011). Motor coordination and resulting balance can be affected by physical problems (LeBlanc et al., 2016), which can lead to changes in the walking ability specially in meat chickens (Bessei, 2006). In order to test motor coordination and its change, a rotarod test is used in rodents in the pharmaceutical sector (Hamm et al., 1994). The animals are placed on a rotating bar and the time until they leave is measured (Lynch and Mittelstadt, 2017). The longer the animals perform this test, the better their motor coordination and therefore their balance (Hamm et al., 1994). This approach was applied in the presented study and is described in more detail in Chapter II.

Effects of environmental enrichment

General points to environmental enrichment

Since the book "Animal Machines" by Ruth Harrison was published in 1964, the society was more and more interested in the intensity of animal husbandry. People focussed on the housing conditions and their requirements. Of course the farmers could not keep the animals in an almost natural environment because of the intensification of livestock farming after the Second World War. To improve the environment for housed animals it must be known which biological functions are important for animal health and animal welfare. Newberry (1995) defined "environmental enrichment as an improvement in the biological functioning of captive animals resulting from modifications to their environment". There are different types of stimuli to enrich the environment of animals for example socially (Rault, 2012), physically (Bailie et al., 2013), sensory (Wells, 2009), and nutritionally (Bizeray et al., 2002c). All approaches share the same aim: animals should be able to benefit for their species-specific behaviour while minimizing the cost of housing them. Animal needs and benefits have to be balanced with the technical and financial implementations. In this thesis, the economic aspect of the various enrichments will not be discussed as it focuses on the positive and negative effects of environmental enrichment on the welfare of domesticated farm animals.

For farm animals, types of environmental enrichment can be divided into two categories (Riber et al., 2018). The first category includes "point-source-objects", which are limited in size and not usable by all animals in the same area at the same time (e.g. bite or pecking objects, elevated structures, foraging materials). The second category is called "more complex enriched environments" (Riber et al., 2018). This category includes objects to trigger more specific natural behaviour like access to an outside area or combinations of different objects and structural function areas.

Investigations and publications on the topic of enriched housing increased over the last 30 years (de Azevedo et al., 2007). To offer a suitable environment for domesticated animals, knowledge about their natural behaviour patterns, diurnal rhythm, physical conditions at different age stages and natural habitat is needed. The enrichment can be beneficial as well as detrimental (Newberry, 1995). Wrong or barren environment can cause abnormal behaviour (feather pecking, tail biting etc. Brunberg et al. (2016)) or even severe diseases (Koolhaas et al., 1999). The following objectives need to be focused: "(1) increase behavioural diversity, (2) reduce the frequencies of abnormal behaviour, (3) increase the range or number of normal behaviour patterns, (4) increase positive utilization of the environment, (5) increase the ability to cope with challenges in a more normal way" (Young (2013), modified according to Shepherdson (1989); Chamove & Moodie (1990)).

Existing studies, especially on domesticated farm animals, deal with the structural enrichment to divide the artificial environment in the functional areas of comfort, activity, feeding behaviour and resting behaviour (Kells et al., 2001; Ventura et al., 2012; Baxter et al., 2017; Campbell et al., 2018). Comfort behaviour can be supported and improved by straw as additional bedding material in pigs or cattle (Tuyttens, 2005). In addition, straw can increase other behaviour patterns like exploration and foraging (Baxter et al., 2017) and improves animal welfare overall. Skeletal condition or muscle growth (Bessei, 2006) can be improved by increasing the activity of meat chickens (Bizeray et al., 2002a), which stimulates the locomotor activity for exploration or foraging behaviour (Bailie et al., 2013). These two behavioural patterns can also be promoted by enriching the environment with feed (e.g. corn or wheat) added to the litter (Jordan et al., 2011). These results show that different types of enrichment can improve the same behaviour patterns. Exploration and foraging behaviour are very important for rearing animals to learn to cope with new environmental elements (Brunberg et al., 2016). Especially in laying hens, coping is important, since they are kept in different housing systems for the rearing and laying period, which differ considerably in complexity (Rodenburg et al., 2013). At an early age, adaption to new objects or functional areas can help reducing stress in later living stages (de Jong et al., 2013).

Structuring the environment can be implemented in different farm animals by elevated platforms. Elevated structures can contribute to reduce stocking density, disturbance of resting animals (Martrenchar et al., 1999), and agonistic situations (Cordiner and Savory, 2001) and improve species-specific behaviour. Fraser and Phillips (1989) investigated the acceptance of "two-level pens" in pigs that have shown a willingness to use the elevated platforms, while no negative effect of weight gain or feed intake was found. Even though wild boars do not usually have the opportunity to go up, elevated levels can lead to more physical exercise and can mainly reduce the stocking density in the captive environment. Vermeij et al. (2003) have shown that fattening pigs used more frequently the elevated platforms with increasing age, which is in line with the results of Fraser and Phillips (1989). The use of elevated structures in pigs is unusual, unlike in chickens. However, compared to chickens similar advantages can be achieved.

As already described in the previous paragraph, only offering elevated perches for laying hens are mandatory (Council Directive 1999/74/EC). But it has been shown that elevated structures are not only used by laying hens; also pullets (Newberry et al., 2001), meat chickens (Kaukonen et al., 2016), heavy non-laying hens like broiler breeders (Gebhardt-Henrich et al., 2017) or turkeys show a motivation to "perch on tree" (Martrenchar et al., 2001), especially for night roosting (Marks, 2017). Also young and heavy chickens use appropriate elevated structures (McBride et al., 1969; Estevez et al., 2002).

Previous studies on environmental enrichment in rearing and meat chickens

Knowledge about behavioural needs of chickens and how they can best be acted out, is important for an optimized environment. This paragraph focusses on functionality of enrichment as well as an optimized environment and its improvement for animals. The environment should offer options to cover individually a wide range of biological functions like social interactions, thermoregulatory processes, comfort behaviour etc. (Broom, 2001a). In general, chickens have a wide repertoire of behavioural patterns with which they interact with their environment. For meat and rearing chickens, behavioural repertoire can be described in Table 3.

The intensity and frequency of performing the listed behaviour repertoire are particularly dependent on the age, the strain and the housing environment. On wired floor systems without littered areas, it is not possible for chickens to forage for food (Lay et al., 2011). Nevertheless, they may try to scratch or forage. It has been observed that layer chickens spend 40 % (Klein et al.,

2000) up to 60 % (Dawkins, 1989) of their day activity time budget searching for food on the ground.

Table 3 Overview about natural behaviour of chickens (modified after Nicol (2015) and Bokkers and Koene (2003)

Natural behaviour	Performing time	Description
Feeding and foraging	Day	Searching for food on the ground, pick movements and scratching with the feet and subsequent feed intake; foraging does not lead directly to ingestion of food
Preening	Day	Grooming of own feathers with the beak (Bokkers and Koene, 2003)
Dustbathing	Day	Performed with fluffed feathers while lying, head rubbed on floor, wings opened, scratching at ground (Bokkers and Koene, 2003)
Comfort behaviour	Day	Summarized as stretching, preening, dustbathing (Shimmura et al., 2007)
Locomotion	Day	Movement in the form of walking or running (Nicol, 2015)
Perching and roosting	Day and night	Grasping with toes of elevated structures for resting or sleeping (Nicol, 2015)
Rest and sleep	Day and night	Sitting or lying with hocks resting on ground without any other activity either with eyes open or closed (Bokkers and Koene, 2003)
Play	Day	Combination of spontaneous running, jumping with raising wings in early life (Guhl (1968)cited by Nicol (2015))
Exploration	Day	Inspection of novel areas or objects (Nicol, 2015)

However, this does not apply to all chicken strains. In meat chickens, depending on the growth rate and the performance, a rapid inactivity or reduction in locomotor activity can occur (de Jong et al, 2012). This aspect appears in all fast-growing meat chicken strains, which show a lower activity and higher lying frequency by increasing age and weight in comparison to the layer type or slow-growing strains (Bessei, 2006). Not only foraging behaviour is affected by the reduced activity but also dustbathing, locomotion, comfort behaviour as well as playing or exploration (de Jong et al., 2012). With regard to meat chicken type, previous study have shown that the activity of chickens could be increased by enriching the environment (Ventura et al., 2012; Bailie et al., 2013). For example, barriers between food sources like a feed trough could increase activity, as the chickens were either forced to climb over or go around them (Ventura et al., 2012). The low activity of fast growing chickens, especially at the end of the fattening period, may be caused by poor leg health or footpad health (Bessei, 2006). Not only the long lying frequency but also the combination of the high stocking density with the high feed conversion and the resulting excretion rate leads to moist bedding (Bessei, 2006). High litter moisture (>30 %, (Wu and Hocking, 2011)) may cause inflammation and lesions of the footpads and hocks, creating access ports for pathogens (Ekstrand et al., 1997). It may also inhibits behaviour such as dustbathing, which is only possible with a loose dry litter (Baxter et al., 2017). Furthermore, a high stocking density makes it almost impossible for broilers to rest or sleep, as disturbances occur repeatedly due to conspecifics and the small space between food and drinking lines (Dawkins et al., 2004). As far as possible, broiler like to lie near walls or on the edge of the housing environment in order to avoid these disturbances (Buijs et al., 2010).

Other behavioural patterns like perching or roosting are less considered, especially for rearing chickens, than for laying hens where elevated structures like perches are required (Council Directive 1999/74/EC). However, already from the first week of life, chickens show a high motivation to use elevated structures for exploring, resting or sleeping (McBride et al., 1969; Roden and Wechsler, 1998; Riber et al., 2007). Elevated structures do not only support species-specific behaviour, but also the stocking density can be reduced if these elements are installed in the housing environment. This could result in better drying of the litter material, which improves the health of foot pads and hocks (Hongchao et al., 2014). Furthermore, elevated elements offer a structure of the housing environment in activity and resting areas for chickens (Riber et al., 2018). Hence, disturbances during sleep are reduced (Ventura et al., 2012). Another advantage is the avoidance of dominant conspecifics and agonistic interactions (Cordiner and Savory, 2001). By using the elevated structures and the motivation to move, it can also trigger a training of the musculoskeletal system resulting in better leg health (Reiter and Bessei, 2009). The task is to develop a suitable optimal accessible elevated structure, which meets the requirements of rearing chickens, and especially those of meat chickens. A couple of studies have already investigated the effect of elevated structures in chickens (Su et al., 2000; Ventura et al., 2010; Zhao et al., 2012; Bailie and O'Connell, 2015; Norring et al., 2016). Nevertheless, only few studies dealt with an optimal, animal-friendly design of the elevated structure (Oester et al., 2005; Kaukonen et al., 2016). However, perches and grids are commercially offered in different shapes, heights and angles by manufacturers. In previous studies, fast-growing chickens have shown a very low usage of elevated structures compared to other strains with lower growth rate, especially if perches were offered (Bokkers and Koene, 2003; Groves and Muir, 2013). Broilers showed an increase in the use of elevated structures until the third or fourth week of life, and then the usage decreased (Zhao et al., 2012; Hongchao et al., 2014). For choosing a suitable elevated structure, it must be taken into account that in fast-growing chickens, the body center shifts and the balance is cranially moved (Duggan et al., 2017). This makes it more difficult for meat chickens of fast-growing strains to keep a balance on a perch, as those offered to laying hens (e.g. metal perches). In studies with elevated platforms, a better acceptance as well as use could be observed (Oester et al., 2005; Kaukonen et al., 2016).

In summary, environmental enrichment like elevated structures have proven to support different species-specific behavioural patterns of chickens and give them the opportunity to act them out. Elevated structures can contribute to the improvement of animal welfare in the conventional and also in the organic husbandry system of chickens. However, age, genetics, and the husbandry system must be taken into account.

Aim of the thesis and objectives

Environmental enrichment has positive effects on animal behavior and welfare of chickens, i.e. physical activity and leg and footpad health. However, suitable elements must be provided to enable the respective chicken strain to perform species-specific behaviour. In particular for meat chickens, elevated structures can support the chicken to perch during the resting phases and to explore. Furthermore, the environment can be structured into activity and resting areas, stocking density on the floor can be reduced and litter quality improved. Although chickens of different strains share their behavioural repertoire, it must be taken into account that different breeding goals, especially strains for meat, differ in growth performance and resulting in different phenotypical characteristics and, thus, their ability to use environmental enrichment.

In order to assess the effects of enrichment elements on animal behaviour and animal health, animal-related indicators such as walking ability and locomotor activity, also leg and footpad health and plumage cleanliness can be used. Assessment methods for the walking ability like the common gait score system indicated some weakness in objectivity as this system strongly depends on the observers' assessment and can lead to wrong conclusions. The walking ability is closely linked with the motor coordination of the body system. The rotarod test, which is a common test for motor coordination in rodents, probably can be used to assess walking ability in meat chicken.

The first aim of this thesis was to develop and validate an appropriate objective method for assessing the walking ability, the rotarod test. The second aim of the present thesis was to further design and validate elevated structures as an enrichment for housing of chickens differing in growth rates. In respect of all aims, three different strains were used for the following studies (slow-growing: Lohmann Brown Plus/Classic; medium-growing: Lohmann Dual; fast-growing: Ross 308). The objectives for this thesis were:

(1) To modify the rotarod test for chickens in order to assess objectively their walking ability, to test its applicability and validate it with the gait score system (Chapter II).

(2) To evaluate suitable elevated structures that differ in shape and height, and examine chicken use and space requirements (Chapter III).

(3) To evaluate suitable elevated structures differing in heights with animal-related indicators in comparison to conventional housing system (Chapter IV).

CHAPTER II

Based on:

Is the rotarod test an objective alternative to the gait score for evaluating walking ability in chickens?

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Abstract

Walking ability is related to motor coordination, which in rodents can be assessed by an established test in pharmacological studies - the rotarod test. The purpose of this study was to evaluate a modified rotarod test for chickens and its relation to the often-used gait score system. At the end of their rearing period, we tested 138 male chickens (Gallus gallus domesticus) from three differing growth performance strains: Ross 308 (fast-growing, n = 46), Lohmann Dual (medium-growing, n = 46) and Lohmann Brown Plus (slow-growing, n = 46). First, the chickens' gait scores were assessed and, immediately following this, they were place gently onto a steady rod. The velocity of the rotating rod gradually increased, and the latency to leave the rod recorded. By using a linear mixed model, we were able to show that the latency to leave the rotating rod was significantly affected by the gait score. Fast-growing chickens had shorter durations on the rotating rod, and these durations were associated the gait score. We conclude that the rotarod test provides an objective alternative method for assessing walking ability in chickens without the need for intense observer training or the risk of observer biases and propose that this novel methodology has the potential to function as a precise, objective indicator of animal welfare.

Introduction

Reduced walking ability and leg weakness are major welfare problems in modern strains of meat chickens (*Gallus gallus domesticus*) (Bessei, 2006; Caplen et al., 2013) and can cause pain (Weeks et al., 2000). In fast-growing broilers (daily growth > 44 g), more than 30 % of the birds can show gait abnormalities, whereas the walking ability of slow-growing broilers - under 5 % of birds - (Keppler et al., 2009), is comparable to that of laying hens (Knowles et al., 2008). Clearly, the prevalence of leg weakness is related to the growth rate and live weight of broiler chickens (Kestin et al., 2001).

Walking ability can be assessed using a gait score system as described by Kestin et al. (1992). This method is often proposed for welfare assessment protocols (e.g. Welfare Quality® 2009). In the gait score system, walking ability is assessed in six categories ranging from 0 (fluent locomotion, no detectable abnormality) to 5 (unable to walk). In order to achieve sufficient intra-observer reliability, assessors are required to train and evaluate the criteria of the scoring scheme. Subjectivity, as regards score assessment, not to mention different training protocols in laboratories and countries around the world has meant that results from different observers are often difficult to compare, ie interobserver reliability is often low (Butterworth et al., 2007). Furthermore, the observer position (ie, from the front, rear or side of the assessed chicken) can directly affect assessment results (Garner et al., 2002). It has thus been suggested that broiler gait score should be assessed by two observers (Martrenchar et al., 1999), which is often not possible in on-farm assessments due to limited personnel resources.

Several attempts have been made to increase the interobserver reliability in assessment of walking ability via alternative methodological approaches. For example, pedobarography or force plates have been assessed as possible barometers of walking ability based on foot pressure and torque characteristics (Corr et al., 1998; Sandilands et al., 2011). Other studies have used an automatic treadmill with or without weight relief to measure proxies of the gait (Djukic, 2007; Reiter and Bessei, 2009), three dimensional kinematic techniques (Caplen et al., 2012), or automated monitoring systems based on activity levels (Aydin et al., 2010). Furthermore, tests such as the latency-to-lie tests use the chickens' avoidance of sitting in water to measure leg weakness (Berg and Sanotra, 2003). However, many of these methods are difficult to implement commercially due to their technical requirements or expenditure of time.

Health problems such as skeletal disorders from high muscle mass growth are widespread among broilers (Su et al., 1999) and can cause a cranial shift in their body's center (Duggan et al., 2017). This can have a negative effect on balance (Cavagna et al., 1977; Corr et al., 2003), and thus on walking ability. LeBlanc et al. (2016) investigated the maintenance of balance in adult laying hens of laying Shaver White Leghorn hens. Balance skills were tested on a static perch or on a round-edge, square rod with altered swaying speed. Here, the number of drops and jumps from the perch were recorded, and the latencies to leave the rod measured. Laying hens showed no significant differences in the latency to leave the static or swaying perches (LeBlanc et al., 2016).

Another method to test balance or, more precisely, the physical ability to regulate body balance, i.e., the motor coordination, is the rotarod test. This is an established standardized paradigm for laboratory rodents (Hamm et al., 1994; Lalonde et al., 1995; Lynch and Mittelstadt, 2017). In general, the experimental setup of the rotarod test consists of a frame with a rotatable horizontal rod and assembly device to enable turning. Following a short period of habituation (e.g., 10 s in mice [Mus musculus]), rodents are encouraged to walk counter to the direction of the rod (Hamm et al., 1994). The rotating speed of the rod is set either at a fixed value or to accelerate over time. When a test animal falls from the rod, it is replaced up to five times. In rodent tests, the most commonly measured variables include latency to fall, duration on the rod for a single trial, total duration on the rod in a session, and number of falls or replacements back on the rod (Hamm et al., 1994; Monville et al., 2006; Shiotsuki et al., 2010).

In this study, the rotarod test was modified in order for use with chickens. For evaluation of this as a possible alternative technique for assessing the walking ability of rearing chickens, three strains differing in growth performance were tested. In order to validate the rotarod test results, the classic gait score of the same chickens was also assessed. It was our assumption that the latencies to leave the rotating rod would correlate with the gait scores of chickens.

Materials and methods

Ethical statement

All investigations were carried out with the approval of the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES, Oldenburg, Germany, file number 33.19-42502-04-16/2108). This study was performed in compliance with national regulations (TierSchNutztV as of 2006) at the research station of the Institute of Animal Welfare and Animal Husbandry (FLI, Celle, Germany). The chickens show no injuries after performing the rotarod test.

Study animals and housing

We used three different strains of male chickens that differed in terms of growth performances: Ross 308 (Ross, meat strain, fast-growing), Lohmann Dual (Dual, dual purpose strain, medium-growing), and Lohmann Brown Plus (LB, layer strain, slow-growing). In two successive trials, chickens of each strain were randomly assigned to four experimental pens in groups of 50 animals of the same strain (total number of pens: 24 = 2 trials x 3 strains x 4 pens). In both trials, six chickens were randomly selected for testing from three pens plus another five chickens from a further pen. This resulted in 46 test chickens from each of the three strains. The number of tested animals were calculated *a priori* using a power analysis based on data from preliminary tests.

The animals were obtained as day-old chickens from commercial hatcheries. The rearing period lasted five weeks for Ross (weight gain per week = (body weight at slaughter – weight of the day-old chicken) / total number of weeks of the rearing period = 345.4 ± 68 g*week^{-1}), and 10 weeks for both Dual (weight gain: 222.5 ± 19.4 g*week^{-1}) and LB (weight gain: 129.1 ± 19.4 g*week^{-1}).

A stable climate was maintained via an automatic ventilation and heating system (Equal pressure ventilation system, Pooch Klimatechnik GmbH, Willich, Germany) with an intermediate program and temperature started at 36°C on the first day, decreasing continuously to 18°C at 36 days. The artificial light regimen included dimming phases of 15 minutes and started with 24 L (light period): 0 D (dark period) for the first three days and reduced to 16 L: 8 D from day four onwards. Lighting was provided with at a minimum of 20 lux by flicker-free tube bulbs (Newlec cold white, HFT 18/840, REXEL Germany GmbH & Co. KG, Munich, Germany). Each pen (3 x 2 m; length × width) was covered with wood shavings as litter (in the case of wet litter, chopped straw with a length < 8 cm was added). One round water dispenser and two round feeding troughs were provided with feed offered as single-phase pellets at an energy content of 12.90 MJ ME kg^{-1} (21 % crude protein) which met the broiler and layer chickens' feed requirements. Feed and water were available *ad libitum*.

To potentially further increase variability in walking ability, we used chickens from three different housing conditions were utilised. Housing conditions varied with respect to enrichment, with elevated platforms and perches offered at different heights in the first trial (for a detailed description see Malchow et al 2018). In the second trial, half of the pens were furnished with grids at three different heights, and the chickens in the other pens were kept without additional structure. These housing conditions were equally distributed between chicken strains.

Experimental setup of the rotarod test

The experimental setup of the rotarod test consisted of two parts: a wooden framework within which the chicken was placed, and electronic control equipment (see Figure 1).

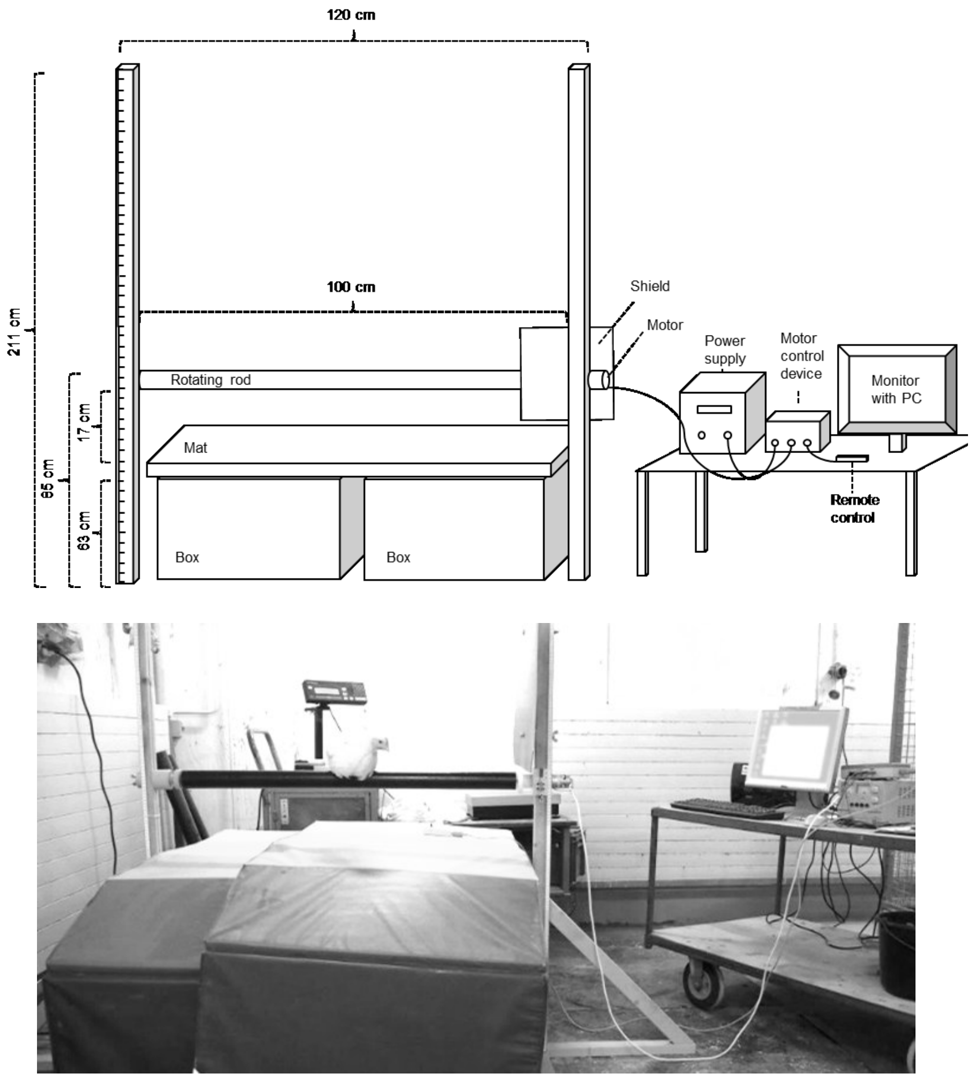

Figure 1 Schematic view (top) of the rotarod test set-up including the wooden frame with rotating rod, mats, boxes under the mats, visual shield and motor, and electronic control device consisting of a motor box, power supply, monitor with PC and remote control. Photograph (bottom) of the rotarod set-up with a Dual chicken placed on the rod.

The wooden framework consisted of a rod (50 × 1,000 mm [diameter × length]) and a frame (211 × 121 cm [width × height]). The rod was positioned between the two vertical pillars at a height of 85 cm and, for extra grip, the surface of the rod was covered with a thin layer of black rubber. Two soft foldable mats (185 × 78 cm [length × width]) were placed approximately 17 cm beneath the rod as padding for when chickens left the rod. A wooden shield (50 x 50 cm) was attached at one side of the frame to limit visual distraction and protect chickens from contact with the motor next to the rod.

The electronic control equipment consisted of a motor rotating the rod, a power supply, a motor control device, and a computer. A direct-current 24 V motor (Model DSMP 420-24-061; gear reduction: 61:1; Drive-System Europe Ltd., Werther (Westfalen), Germany) was driven with an accelerating speed controlled by self-customized software (the software can be provided upon request) written in Visual Studio C++ 2010 (Microsoft Corporation, Redmond, USA). The motor control device received a digital value command (hereafter referred to as diva) from 0 to 255 (8-bit) from the computer and controlled the motor with a 28-V pulse width modulation supply. When program began it counted the command value and the motor accelerated. The motor started with a diva of 22 because below this value, the motor power was insufficient to turn the rod. To accelerate from 0 to a maximal rotation within 360 s, every 1.545 s the diva was increased by one (from 22 to 255 diva; 360 s/ [255 diva – 22 diva] = 1.545 s). The maximum speed reached after 360 s was 2.1 RPS (revolutions per second) (Figure 2).

An accelerating speed of rotation has been suggested to be more suitable for the assessment of motor coordination than a fixed speed (Monville et al., 2006). The observer was able to start and stop the motor of the rotating rod via remote control. The animal number, time and latency until a chicken left the rotating rod was recorded with the software.

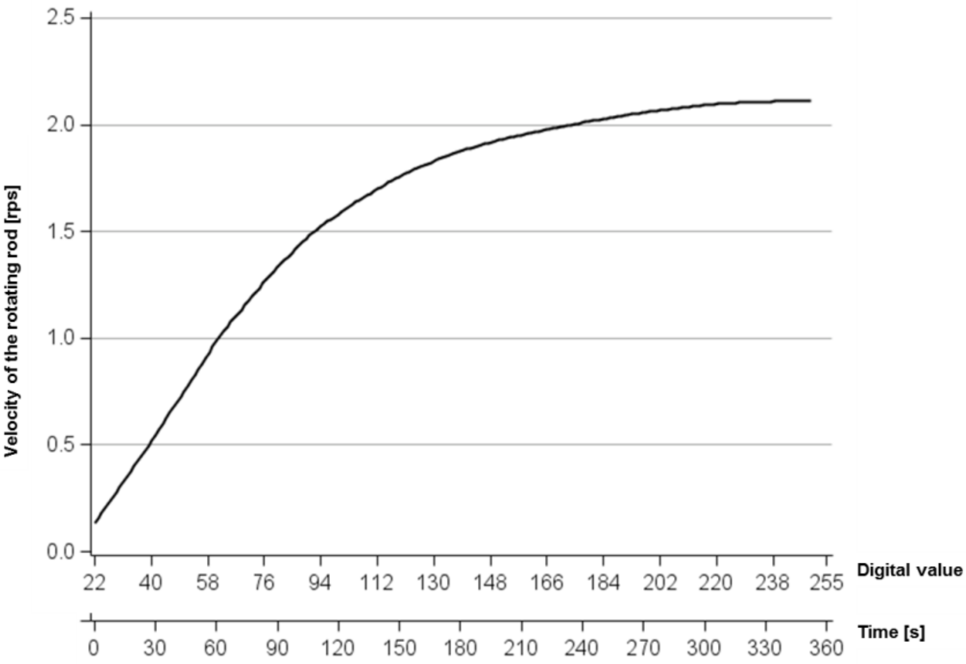

Figure 2 The velocity course of the rotated rod from 0 s to 360 s. Velocity was controlled automatically via software that sent an increasing digital value (diva) every 1.545 s to the motor. The maximum speed reached after 360 s was 2.1 rps.

Measurements

Assessment of walking ability

Walking ability was assessed using the gait score system developed by Kestin et al. (1992). Here, the chickens' gait is classified into one of six distinct categories: Gait score 0—fluent locomotion, no detectable abnormality, furled foot in the air; Gait score 1—slightly undefined defect in its gait; Gait score 2—easily detectable walking abnormality, no restriction in its movement, quick and short steps; Gait score 3—limited walking ability, visible defective locomotion, limp in one leg, quick steps; Gait score 4—able to walk for a brief period, strong detectable walking abnormality, quick squats down; Gait score 5—unable to walk, locomotion could be achieved with the assistance of wings.

The Ross chickens were tested on the two days prior to slaughter at five weeks of age, and the Dual and LB chickens were tested at ten weeks of age. Tests were performed in the morning (from 0800 to 1200h) by one observer (JM) in a separate test arena (2.5 × 0.5 m, length × width) located in the stable alley. The test chickens were randomly selected from all pens and gently transferred from their home pen to one end of the test arena. To encourage the chickens to walk, three non-tested conspecifics from the same pen were placed at the opposite end of the test arena. Chickens were given 1 minute to habituate and typically began to walk independently. If a chicken did not voluntarily start walking after 2 min, it was gently coerced into doing so forced. The observer assessed the chickens from a distance of at least 1 m via a vantage point on the long side of the test arena.

The assessment of walking ability with the gait score system was always carried out before the rotarod test. This was to ensure the observer avoided being biased from the results of the rotarod test. There was a possibility chickens may have had a short latency to leave the rotating bar, which was not necessarily related to a poor gait score. It was also possible that results could have been influenced by the order in which tests were carried out. However, our proposal was approved by the competent authority who would only sanction the minimum number of animals sufficient for statistical analysis. Thus, we decided only to use only one test order. A greater number of animals would have been needed in order to test the effect of test order. An additional reason was that the rotarod test probably demands greater muscle strength compared to walking in the gait score system, ie the effect of a rotarod test prior to a gait score test is likely to be more profound than *vice versa*.

Rotarod test procedure

After the individual gait score was assessed, each test chicken was taken to the rotarod apparatus and placed at the middle of the rod. The motor of the rotating rod was started when both of the chicken's feet were grasped around the rod and was stopped when the chicken actively or passively left the rotating rod. This was registered as the latency to leave the rotating rod (s). Following this, the chicken was weighed (nearest [± 10] g) and returned to its home pen. Additionally, each test was video-recorded (Model VAZ2S, AIPTEK International GmbH, Willich, Germany) in order to analyse the manner in which the chickens left the rod, ie active (jumping / flying down from the rotating rod) or passive (falling from the rotating rod.

Statistical analyses

To relate the latencies obtained from the rotarod test to the gait scores, we used a linear mixed effect model (LME). For the initial model, the latency to leave the rotating rod was the dependent variable; gait score (numerical factor), weight gain per week (numerical factor), manner of leaving the rod (categorical factor) and their respective two-way interactions were included as fixed factors. Compartment ID (24 pens) was considered a random factor nested within the random factor strain. Nonsignificant factors were stepwise excluded by backward selection of the respective least significant factor, while the three main factors remained in the final model, as they were the parameters of interest. Residual plots indicated no deviation from a normal distribution. In the case of significant effects of GS, a post hoc analysis was performed using post hoc pairwise t-tests with Bonferroni correction. All models were run in R. 3.2.5 (R Core Team 2016) using the nlme package (Pinheiro *et al* 2017).

Results

The three strains of chickens showed differing distributions of gait scores (hereafter GS, as shown in Table 1). Most Ross chickens were assigned to GS 2 and 3, the majority of Dual chickens to GS 1, and almost all LB chickens to GS 0. GS 5 was not assigned to any chicken.

Table 1 Number of chickens in the three strains assigned to different gait scores (n = 46 per strain).

	Strain			
Gait score	Dual	LB	Ross	Total
0	7	44	0	51
1	36	2	5	43
2	3	0	18	21
3	0	0	19	19
4	0	0	4	4

The latency to leave the rotating rod showed a significant association with the GS (LME, factor gait score, $F_{1,\ 109}$ = 9.83, P = 0.0022, Figure 3). Further post hoc tests revealed that chickens with GS 0 and GS 1 stayed significantly longer on the rotating rod compared to chickens with GS 2, 3, and 4 (Figure 3).

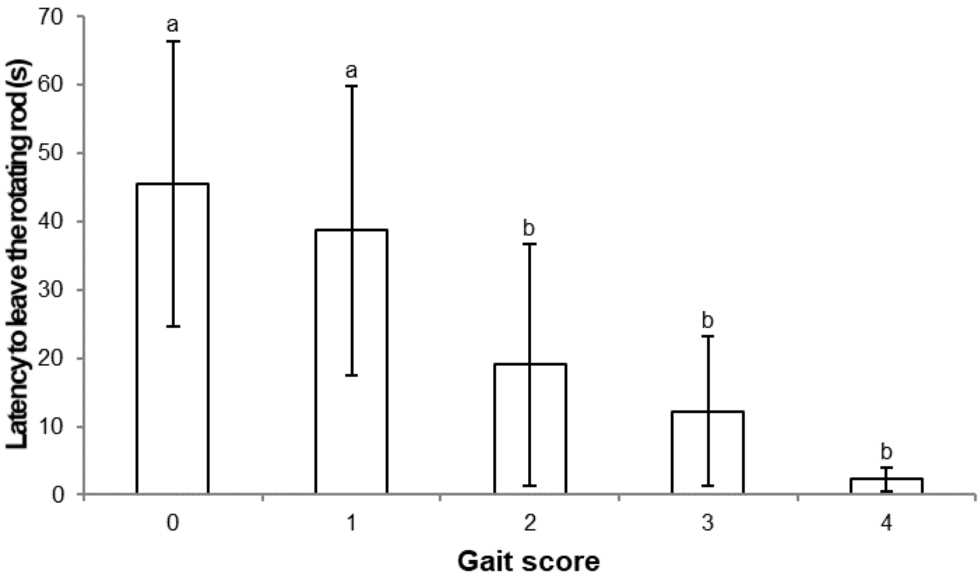

Figure 3 Effect of gait score on the latency to leave the rotating rod (means ± SD). Significant differences between gait scores are marked by different superscripts (P < 0.05).

The latency to leave the rotating rod was significantly affected by weight gain per week (LME, factor weight gain per week, $F_{1,\,109}$ = 11.34, P = 0.001; Figure 4).

The manner of leaving the rod (active or passive) did not affect the latency to leave (LME, factor manner of leaving the rod, $F_{1,\,109}$ = 0.06, P = 0.81).

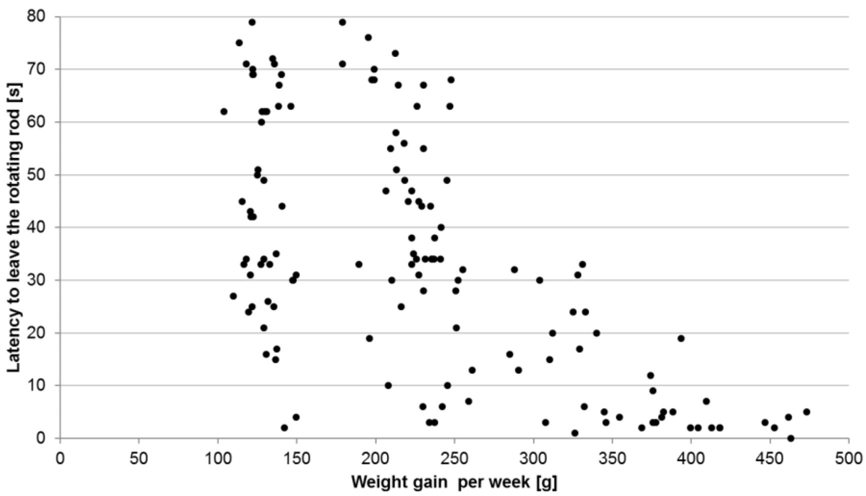

Figure 4 The relation between the latency to leave and weight gain per week.

Discussion

A modified rotarod test was used to assess the motor abilities of three strains of chickens differing in terms of weight gain per week. Latencies to leave the rotating rod were significantly related to walking ability as assessed by the gait score system. Thus, the rotarod test seems to be a valid method of measuring walking ability on the basis of motor skills of rearing chickens. In addition, this method provides a greater level of objectivity and avoids possible observer bias as has often been reported for the gait score system. Furthermore, a key advantage of the rotarod approach is that walking ability is measured in a more precise and continuous measurement than is seen with the categories of the gait score system.

Compared to the slow-growing chickens (eg Dual and LB), fast-growing chickens (e.g., Ross) displayed a poorer walking ability in both the rotarod test and the gait score system. This result was in accordance with the findings of Kestin *et al*. (2001) who compared GS in 13 broiler genotypes and found that the growth rate affected walking ability. Additionally, the distributions of GS 2 and GS 3 for Ross chickens were comparable to previous studies, which reported that more than 90 % of the chickens indicated lameness at slaughter date, and approximately 30 % were classified as GS worse than 2 (Kestin et al., 1992; Sanotra et al., 2001). The strains differed anatomically, in terms of breast muscle size, tibial length and maximal breaking force (Mueller et al., 2018). It is likely that these differences affect both the latency to leave the rotating rod and the gait score. However, in this study different strains from differing housing conditions were deliberately utilised in order to increase the variability of the data. Comparison different strains was not the aim of this study *per se*.

The chickens left the rod either actively or passively, ie either by jumping intentionally or flying from the rod or falling unintentionally. If chickens with good motor co-ordination had jumped off earlier this would have resulted in misleading latency to leave data. In such instances, a short latency to leave would not necessarily indicate a GS of 5 (worst walking ability). Interestingly, neither the manner of leaving the rod nor its interaction with either of the other factors significantly affected the latency to leave the rotating rod. Thus, the data obtained from the rotarod test seem to be robust in terms of how chickens left the rotating bar.

Conducting the rotarod experiments requires only the briefest of introduction to master the set-up and electronic control device. Training is unnecessary and the rotarod test easy to handle.

Conversely, use the gait score system demands practical training and clear instructions for the observer. In the studies by Garner et al. (2002) and Webster et al. (2008), the gait score system was modified from 6 to 3 categories, whereby both the interobserver and the retest reliability were better in the 3-point compared to the 6-point scale. An advantage of the gait scoring system is that the observer requires minimal equipment (simply a paper and pen) as only rough categorical measures are taken as opposed to the continuous measures needed for the rotarod test. Also, the rotarod test requires additional space for the set-up and an electrical power source. However, our study device was a first prototype and readily able to be reduced in size for on-farm use. For example, frame height could be reduced down to 120 cm or less, and a 50 cm long rod should offer ample space for the test chicken to manoeuvre along. In addition, the electronic devices can be miniaturised. When compared to other electronic equipment used to assess walking ability, such as video-based techniques (Aydin et al., 2010), the rotarod method seems less complex and arguably takes less time for preparation.

In our study, only one diameter size was utilised for the rod, and no adjustment were made to this to cater for the foot size of the different strains. Perhaps an association exists between foot size and rod diameter which affects the birds' ability to remain on the rod. So it would be advisable to adjust the diameter of the rod to reflect the respective foot size of birds to be tested. Here, we occasionally observed that Ross chickens required several attempts to claw around the rod and tilted forward. Thus, the optimum rod diameter may depend not only on the birds' foot size but also on their ability to manipulate and control their toes. However, this trait also affects walking ability, which is the crucial parameter tested by the rotarod test. Furthermore, for the rotarod test, chickens must mainly walk when the rod is rotating; however, by moving the feet, grasping and perching are also involved in maintaining balance. The specific surface and the anatomic properties of birds' feet are usually regarded as being adapted to allow precise positioning the feet and maintain balance (Galton and Shepherd, 2012; Sustaita et al., 2013). However, modern strains selected for different purposes such as for laying or for growth may differ in their ability to grasp due to different body traits. Thus, these properties should be investigated in more detail.

The aim of the rotarod test is to show different latencies between normal and detectable abnormalities in the walking ability of chickens. In future studies, it should be possible to refine the results and provide a clear link between a range of latencies to leave and categories of walking ability. In our study, each chicken was tested only once in the rotarod test to avoid possible effects of training or habituation. These effects are potentially more likely to be seen with the rotarod test as opposed to the GS test since, in the latter, an easier and often voluntary movement is assessed. A general problem with repeated testing in fast-growing chickens is that they must be performed within a few days to control for the effects of weight. Nevertheless, future studies should be performed to test the repeatability of the rotarod test within subjects. Furthermore, all tested chickens were assessed for a short time, 5 minutes maximum, in the rotarod test. The test situation was identical for all animals, all were separated visually from their pens yet they always had acoustic contact to conspecifics. However, it might be possible that social isolation affect strains or individuals differently. A previous study has shown differences in the social reactions between different layer strains (Dudde et al., 2018). Even although, we have used a standardized test protocol for the rotarod test, but we could not fully exclude any potential strain-specific effects of the brief visual separation. However, strain differences were beyond the scope of our study.

When applying the gait score system, the position of the observer can affect the assessment. For example, an observer from the side position could assess a limping based on sharp turns from a healthy chicken which could be lead to a wrong choice of score (Garner et al., 2002). The results of the rotarod test are independent of the position of the observer, as the observer has only to note when the chicken leaves the rod.

The latencies to leave the rotating rod are a continuous measure, whereas the GS are categorical data. It is possible to modify the GS data into continuous data (Tuyttens et al., 2014), but the method is still subjective and highly dependent on the observer. The great advantage of a continuous measure is that the latencies allow a more precise analysis of the walking /locomotor performance of the animals compared to the six GS; thus, more subtle but welfare-relevant differences in walking skills may be detected by the rotarod test. Moreover, continuous data can be subjected to particular types of statistical analyses, compared to categorical data, which provide greater statistical power.

Animal welfare implications and conclusion

The rotarod test provides an objective alternative method for assessing walking ability in chickens without intense observer training or the risk of observer biases. In contrast to the gait score system, the rotarod test is less dependent on observer experience for assessing gait. The results of the rotarod test showed a significant association with normal gait and detectable abnormalities in the gait of the chickens. In its current state, the rotarod test requires a degree of fine-tuning to align it to the anatomic differences between strains. This novel methodology of an objective assessment with a precise measure has to be potential to be implemented as an indicator of animal welfare.

Acknowledgement

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CHAPTER III

Based on:

Perches or grids? What do rearing chickens differing in growth performance prefer for roosting?

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Abstract

The domestic fowl (*Gallus gallus* f. dom.) is highly motivated to roost on elevated structures. Previous studies indicated that broiler chickens hardly use elevated perches but frequently use elevated platforms. However, it is unclear which height and type of elevated structures broilers prefer at various daytimes.

We investigated the use of elevated perches and grids varying in height (10, 30, and 50 cm above the floor) by chickens of 3 strains differing in growth performance. In 2 successive trials, male chickens of Ross 308 (Ross, fast-growing, n = 200), Lohmann Dual (Dual, medium-growing, n = 200), and Lohmann Brown Plus (LB, slow-growing, n = 200), were tested in 12 experimental compartments (each strain in 4 compartments), respectively. Usage of structures was recorded on video and analyzed with time-sampling observation for each week of age during dawn, the light period, dusk, and the dark period. In addition, behavioral activity was measured with an antenna-transponder-system.

The results showed that Ross and Dual chickens preferred grids to perches throughout the entire observation period. With increasing age, chickens of all strains increasingly used the elevated structures during all daytimes. In contrast to Dual and LB, Ross chickens reduced the use of structures from the 3rd to 4th week of age during the light but not the dark period. Additionally, during the dark period, chickens of all 3 strains at the end of fattening period used elevated structures at 50 cm height most often. The behavioral activity decreased significantly in all 3 strains but was much lower in Ross chickens compared with Dual and LB.

The results indicated that chickens are motivated to roost at nighttime on high-elevated and suitable structures such as grids. Further studies are needed to assess the space requirements for elevated structures that should be offered to chickens.

Introduction

Like its ancestor the jungle fowl (*Gallus gallus*), the domestic fowl (*Gallus gallus* f. dom.) roosts on elevated structures such as branches or perches (Wood-Gush and Duncan, 1976; Schrader and Müller, 2009). High-elevated structures likely offer fowl protection against predators (Newberry et al., 2001). Beginning at an early age, wild and domestic chickens explore their environment during daytime (McBride et al., 1969) up to heights of 10 m (Wood-Gush and Duncan, 1976; LeVan et al., 2000) with or without their mother hen (Roden and Wechsler, 1998). In addition, they begin roosting at nighttime at an age of 10 d (Riber et al., 2007).

Under commercial housing conditions, meat chickens are kept in large floor housings equipped with litter and lines of drinkers and feeders allowing them to eat, drink, forage, and perform comfort behaviors. However, elevated structures that would allow them to use vertical space and to roost are not offered. Although the EU Council Directive 1999/74/EC requires offering suitable perches for laying hens (EFSA, 2015), perches are not required for rearing chickens like pullets or meat chickens. However, if they have the opportunity, chickens from both layer and broiler strains use elevated structures during the rearing period (Faure and Jones, 1982; Newberry et al., 2001; Heikkilä et al., 2006; Hongchao et al., 2014). Offering broiler chickens elevated structures has been suggested to result in positive physical effects such as a reduction of leg problems (Reiter and Bessei, 2009), the support of bone strengthening by higher activity (Yan et al., 2014; Kaukonen et al., 2016), or better health of footpads through drying (Hongchao et al., 2014). In the case of layer chickens, offering elevated structures during the rearing phase can prepare them for using elevated structures and perches in the layer housing (Gunnarsson et al., 2000). In animal welfare labeling schemes for broiler chickens, elevated structures such as platforms, perches, or straw bales are already mandatory to improve the animals' welfare (e.g., Neuland, Germany; Beter Leven, Netherlands; RSPCA ASSURES, United Kingdom; Coop Naturafarm, Switzerland). If not properly designed, however, perches can result in breast blisters (Nielsen, 2004) and thus may negatively affect the welfare of broiler chickens.

In studies on the use of elevated structures by broilers, perches (Martrenchar et al., 2000; Su et al., 2000; Pettit-Riley and Estevez, 2001; Bokkers and Koene, 2003; Bailie and O'Connell, 2015), platforms (Bailie and O'Connell, 2014), or both (Oester et al., 2005; de Jong and van Wijhe-Kiezebrink, 2014; Kaukonen et al., 2016; Norring et al., 2016) were offered at varying heights from

8 cm up to 75 cm. De Jong and van Wijhe-Kiezebrink (2014) offered lucerne bales, perches, and platforms at different locations within the same compartments and at the same time, and locations were changed every 2 weeks. Although in none of these studies different types and heights of elevated structures were simultaneously tested in the same groups, their results suggest that broiler chickens use elevated grids more than perches and prefer high structures to low structures. In laying hens, there is also strong evidence that they prefer higher structures for roosting compared to lower ones and, in addition, that the height of roosting site seems to be more important compared to its structure (i.e. grid or perch) (Schrader and Müller, 2009).

The use of elevated structures can considerably differ between daytimes (Nielsen, 2004). In the few studies that compared perching behavior of growing chickens during the light and dark periods (Heikkilä et al., 2006; Norring et al., 2016), chickens perched more often during the light compared with the dark period. In studies on perching during dawn and dusk, no effects of a progressive or an instantaneous dimming were found (Martrenchar et al., 2000), and until 7 weeks of age, chickens used perches during dawn and dusk to the same degree as during the night (Nielsen, 2004). In addition, the growth performance of chickens seems to affect the use of elevated structures. Slow-growing broiler chickens used elevated structures more frequently than did fast-growing broiler chickens (Bokkers and Koene, 2003; Oester et al., 2005; Wallenbeck et al., 2016), possibly due to a reduced walking ability in fast-growing broilers (Martrenchar et al., 2000).

Here, we used chickens of 3 strains and tested the use of perches and grids offered in parallel at 3 heights. The chickens were males from a fast-growing broiler line, a medium-growing dual-purpose line, and a slow-growing layer line. In addition to the use of elevated structures, we recorded the general locomotor activity of the birds. We expected that 1) with increasing age of the birds, the use of elevated structures will increase, and the increase in usage will be lower in fast-growing compared with slow- and medium-growing chickens; 2) chickens of all strains will prefer grids to perches and will perch most often on the highest level of the elevated structures. Additionally, we hypothesized that chickens will show decreased activity with increasing age and growth performance and will use the elevated structures at different frequencies during different daytime periods.

Materials and Methods

Birds and Housing

The experiment was conducted in a stable at the research station of the Institute of Animal Welfare and Animal Husbandry (FLI, Celle, Germany). In each of 2 successive trials, 200 male chickens of a fast-growing strain (Ross 308, hereafter Ross), 200 male chickens of a medium-growing dual-purpose strain (Lohmann Dual, hereafter Dual), and 200 male chickens of a slow-growing layer strain (Lohmann Brown Plus, hereafter LB) were used. This study was of the project "Integhof" which is focused on preventing the killing of day-old male chicks by using them for meat production. For this reason, we used only male chickens.

In both trials, the birds were kept in groups of 50 birds of the same strain in 12 experimental compartments (floor space: 2 × 3 m; height: 2 m; stocking density at the end of the fattening period for Ross and Dual: 18.5 kg/m^2, for LB: 11.7 kg/m^2). Groups were randomly assigned to the compartments resulting in a total of 8 repetitions per strain (2 trials × 4 groups per strain). All chickens were delivered by commercial hatcheries as day-old chickens and had an average weight ± standard deviation of 44.7 ± 1.0 g (Ross), 40.4 ± 2.6 g (Dual), and 38.3 ± 0.8 g (LB). Due to their fast growth, Ross chickens were kept for 5 weeks (body weight at slaughter date 2,099 ± 583 g), whereas Dual (body weight at slaughter date 2,234 ± 283 g) and LB (body weight at slaughter date 1,411 ± 121 g) chickens were kept for 10 weeks in the experimental compartments until slaughter. The mortality rate of Ross was higher compared to Dual and LB but within the ranges of commercial farms (Table 1).

Table 1 Overview on the mortality for the entire observation period

Strain	Ross		Dual		LB	
Trial	1	2	1	2	1	2
1st week [%]	0.0	0.0	0.0	0.5	0.5	0.0
Total [%]	3.0	2.4	0.5	1.0	1.0	1.5
Mean of both trials [%]	2.7		0.7		1.5	

Climate conditions in the stable were automatically controlled by a ventilation and heating system, and an intermediate temperature program was run to meet the different temperature demands of broilers and layer chickens, with a temperature of 36 °C at an age of 1 d continuously decreasing to 18 °C at an age of 36 d. The stable was artificially lighted for 24 h during the first 3 d of age and afterwards until slaughter for 15 h from 4:45 AM to 7:45 PM at a light intensity of at least 20 lx achieved by flicker-free tube bulbs. The light program included 2 dimming phases of about 30 min each at the onset and end of the light period. Each compartment was equipped with wood shavings as litter, 2 round feeding troughs, and 1 round water dispenser with 8 drinking nipples (Figure 1).

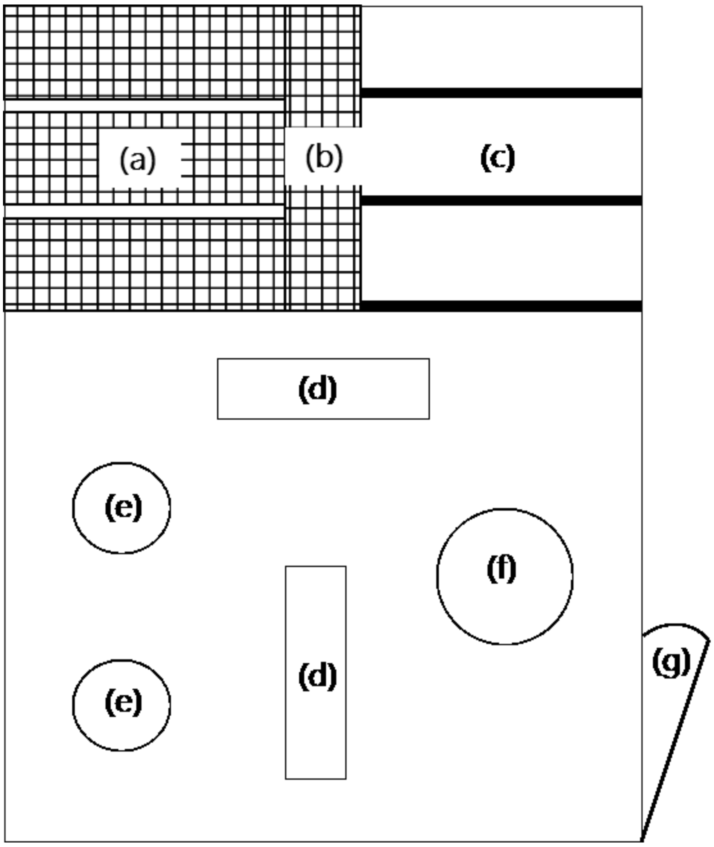

Figure 1 Schematic view of a compartment: (a) grids), (b) ramp, (c) perches, (d) antennas, (e) feeder troughs, (f) water dispenser, (g) door

For Ross chickens the existing litter was supplemented by chopped straw [length <8 cm] from the 4th week of age in case of wet litter. In Dual and LB chickens this was not necessary as the litter remained dry and friable throughout the fattening period. Single-phase pelletized feed (21 %

crude protein, 12.90 MJ ME/kg) with energy content between the requirements for broiler and layer chickens and drinking water were available ad libitum.

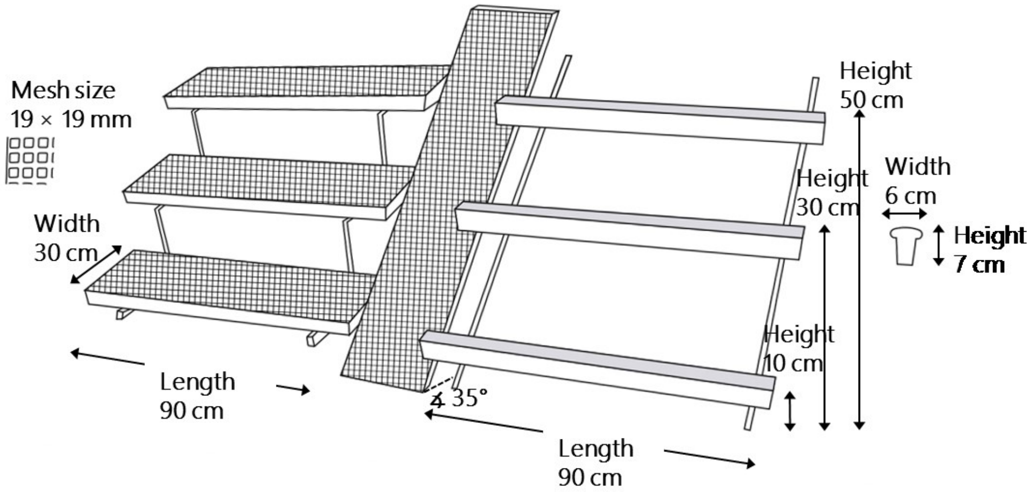

Figure 2 Schematic view of the elevated platforms (plastic grids) and perches (plastic mushroom perches) with a ramp. Both structures were installed at the same 3 different heights.

Each compartment was additionally equipped with perches and grids at 3 different heights (Figures 1 and 2). Perches and grids were randomly positioned to each other and placed at the narrow sides of the compartments. To facilitate access to the perches and grids, an inclined ramp of the same material as the grids was installed between the perches and the grids (Figures 1 and 2). The total grid area was 8,100 cm^2, and the total perch length was 270 cm. Based on the area of 514 cm^2 covered by a male broiler with an average weight of 2,468 g at the end of the fattening period (6 weeks) (Bokkers et al., 2011) and a perch length of 15 cm occupied by a broiler, we estimated that perches and grids each offered space for about 30 % of the chickens in each compartment at the end of the fattening period. This percentage corresponds to the use of perches and grids found by Nielsen (2004) and de Jong and van Wijhe-Kiezebrink (2014).

The plastic grids (30 × 90 cm; Big Dutchman International GmbH, Vechta, Germany) had a mesh size of 19 × 19 mm and a slat width of 10 mm. The plastic perches (LUBING Maschinenfabrik Ludwig Bening GmbH & Co. KG, Barnstorf, Germany) had a length of 90 cm, were mushroom shaped, and had a width of 60 mm at the top. The vertical gap of 20 cm between the perches and between the grids (see Figure 2) allowed the chickens to use the litter area below the elevated structures. For recordings of chickens' activity in each compartment 2 antennas were located on the floor (see Figure 1 and text below describing the measurement of locomotor activity).

Measurements

Use of Structure

In each compartment, the area of the perches and grids was recorded by an infrared video camera (Model VTC-E220IRP, color camera for corner mount with IR-LEDs; SANTEC BW AG, Ahrensburg, Germany) connected to standard computers with a self-customized recording software, and recordings were saved on hard disks. For each week of age (week), the recordings of 2 d (usually Saturday and Sunday) were analyzed by counting the number of chickens on the 6 different elevated structures and on the ramp from 3:00 AM to 10:00 PM in 30 min intervals (time-sampling observation, total number of scans per d: 39).

Locomotor Activity

The behavioral activity was recorded with an antenna-transponder-system (PLB SPEED Antenna and Chip Glastag HITAGS 3.15 × 13 × 3 mm; Gantner Pigeon Systems GmbH, Schruns, Austria) connected to a standard computer with self-customized recording software. In each compartment, 2 antennas (length: 90 cm, width: 30 cm, height: 3 cm; Figure 1) were put on the floor in the littered area. On the 12th d of age, 25 randomly selected chickens per compartment received a transponder that was attached to the leg by using a plastic case (height: 23 mm, width: 4 mm, weight: 2.5 g) and cable straps (Figure 3). The cable straps were regularly checked for fitting, and their width was adjusted if necessary.

Figure 3 Image of Dual, LB and Ross (12th day of age) with transponder attached at the leg with a cable strap.

The transponders were registered at a distance of less than 20 cm by an antenna at a frequency of 2.5 s. The tag number of the respective antenna, the number of the transponder, the time, and the date were recorded from each transponder when within the range of an antenna. From 4:00 AM until 8:00 PM, activity was counted whenever a transponder was registered by one and subsequently by the other antenna, i.e., the change between antennas was counted as locomotor activity and calculated with SAS® 9.4 (SAS Inst. Inc., Cary, NC, USA).

Statistical Analysis

Use of Structures

All statistics were done using SAS® Enterprise Guide Version 6.1. For statistical analysis, each day was divided in 4 periods: dawn (values at 4:30 AM), light period (values from 5:00 AM to 7:00 PM), dusk (values at 7:30 PM), and dark period (values from 3:00 AM to 4:00 AM and from 8:00 PM to 10:00 PM). According to our hypotheses, we used different general linear mixed (GLM) models. In case of multiple comparisons, *P*-values were Bonferroni corrected.

For testing the effect of age, the mean proportion of chickens using either of the elevated structures within each week of age was used for an intra-strain comparison in separate models, i.e., effect of age was tested for Ross from the 1st to the 5th week of age and for Dual and LB from the 1st to the 10th week of age. Week of age was included as fixed factor and compartment number as random factor. For post hoc comparisons, we only considered consecutive weeks.

For testing differences between the 3 strains, the mean proportions of birds using either of the elevated structures from the 1st to the 5th week of age were used. Here, strain, week of age, and the interaction strain with week of age were used as fixed factors and compartment number as random factor.

To test the preferences for grid or perch for each strain, the mean proportions of birds observed on either the perches or the grids were calculated across the entire observation period (i.e., Ross from the 1st to 5th week, Dual and LB from the 1st to 10th week). The number of birds observed on the ramp was not included in this analysis. Structure (perch, grid) was included as fixed factor and compartment number of compartments as random factor.

For analyzing the effect of height, we applied the mean proportion of the use of the 3 heights (10, 30, 50 cm) irrespective of the type of elevated structure, and we excluded the ramp. For each strain, separate models were calculated. Week of age, height, and their interaction were used as fixed factors and compartment number as random factor.

The effects of the factors mentioned above were tested separately for the 4 time periods (dawn, light period, dusk, dark period). To test the effect of daytime for each strain, we calculated the mean proportion of birds observed during the 4 time periods throughout the entire observation period. These data were subjected to a GLM model with daytime as fixed factor and compartment number as random factor.

Locomotor Activity and Growth Performance

For testing the effect of age on the locomotor activity of birds recorded by the transponder-antenna-system, the mean activity of each bird was calculated for each day of age, and data were averaged per week per compartment. Due to the differences in age at slaughter, we calculated separate GLM models for each strain (Ross with 5 weeks, Dual and LB with 10 weeks) with week of age as fixed factor and compartment number as random factor. Comparisons of consecutive weeks of age were done by a multiple comparison between all pairs of LSMEANS with Bonferroni correction. In a second GLM model, we included all 3 strains but only data from the 1st to the 5th week of age. This model included strains, week of age, and their interaction as fixed factors and the compartment number as random factor.

The different growth performances of the strains were calculated as the average weight gain (= [slaughter weight − day-old weight] / week of age) and analyzed by a Kruskall–Wallis test.

Results

Use of Structures

Effect of Age, Strain, and Daytime. Chickens of all 3 strains increasingly used the elevated structures with increasing age during each of the 4 daytimes (all $P \leq 0.0001$; Figure 4). In all 3 strains, this increase was particularly evident from the 1st to the 2nd week of age at dawn and during the light period (Ross, Dual, and LB: $P < 0.0001$; Figure 4a, b). There was an additional increase in the use of elevated structures from the 2nd to the 3rd week for Ross during the light period ($P < 0.0001$; Figure 4b) and for Dual at dusk ($P = 0.0018$; Figure 4c) and during the dark period ($P = 0.0283$; Figure 4d). From the 3rd to the 4th week, Ross chickens showed a significant decrease in the use of the elevated structures at dawn ($P = 0.02$) but, in contrast, a significant increase during the dark period ($P = 0.0056$). During the light period, Ross chickens used the elevated structures less often in the 5th compared with the 4th week of age ($P = 0.001$). The Dual chickens showed a significant increase in use from the 5th to the 6th week during the light period ($P = 0.0017$) and at dusk ($P = 0.016$). From the 6th week onwards, the use of elevated structures did not change significantly between successive weeks during any of the 4 daytimes.

The highest proportion of chickens on the elevated structures was found at dawn with 26.9 ± 1.6 % in the 3rd week for Ross, 40.4 ± 2.9 % in the 8th week for Dual, and 48.8 ± 3.1 % in the 2nd week for LB.

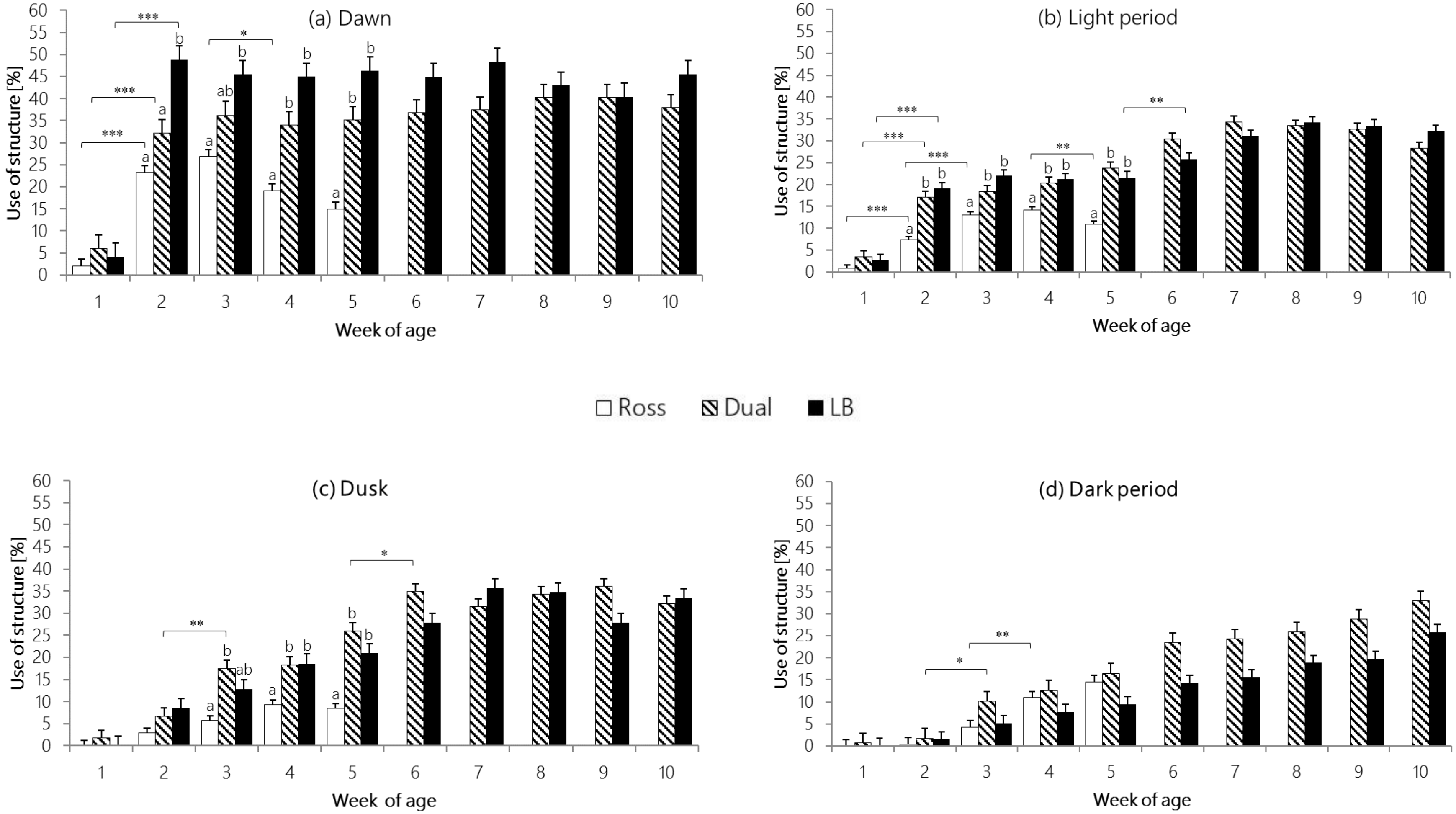

Figure 4 Age effect on use of structure (least square mean): (a) at dawn, (b) during the light period, (c) at dusk, and (d) during the dark period. Significant differences between consecutive weeks of age within strains are marked by asterisks: * P = 0.05, ** P = 0.01, *** P < 0.001. Significant differences within the first 5 weeks of age are marked by different letters (P < 0.05); (n = 8). Bars indicated the standard error.

Strain significantly affected the use of elevated structures at dawn, during the light period, and at dusk (all $P < 0.0001$) but not during the dark period ($P = 0.1179$). Ross chickens used the elevated structures to a significantly lesser degree compared with Dual and LB chickens (Figure 4). Exceptions were the 1st week at dawn and during the light period and the 2nd week at dusk. During the dark period, the use of elevated structures did not differ between the strains in any of the first 5 weeks of age. At dawn in the 2nd week LB chickens used the elevated structures significantly more in comparison with Dual.

For each strain, the use of elevated structures differed significantly between the dark period and dawn (all $P < 0.0001$), between dawn and dusk (all $P < 0.0001$), and between dawn and the light period (all $P < 0.0001$). In addition, Dual and LB chickens used the elevated structures more at dusk compared with the dark period (Dual: $P = 0.0065$; LB: $P < 0.0001$) and more often during the light compared with the dark period (Dual: $P = 0.0058$; LB: $P < 0.0001$).

Effect of Structure. Ross and Dual chickens preferred the grids to the perches at all daytimes (Ross: dawn: $P < 0.0001$, light period: $P < 0.0001$, dusk: $P = 0.0001$, dark period: $P = 0.0022$; Dual: dawn: $P = 0.0001$, light period: $P = 0.0035$, dusk: $P = 0.033$, dark period: $P = 0.0024$; Figure 5). LB chickens showed a higher use of the grids compared with the perches only at dawn ($P < 0.0001$) and during the light period ($P = 0.0016$; Figure 5).

Effect of Height. The height significantly affected the use of elevated structures in all 3 strains at all 4 daytimes ($P < 0.05$). Chickens of all strains preferred structures of 50 cm height compared with 10 and 30 cm during the dark period at an age close to slaughter (Figure 6b, d, f). During the light period, preferences for height were not consistent and only shown in single weeks (Figure 6a, c, e).

At dawn, the chickens showed preferences for 50 cm height in some weeks (all $P \leq 0.05$; Ross: weeks 2 to 4; Dual: weeks 2 to 5; LB: weeks 2 to 8 and 10). Only Ross chickens showed a clear preference for 10 cm at dusk in the 5th week ($P < 0.05$).

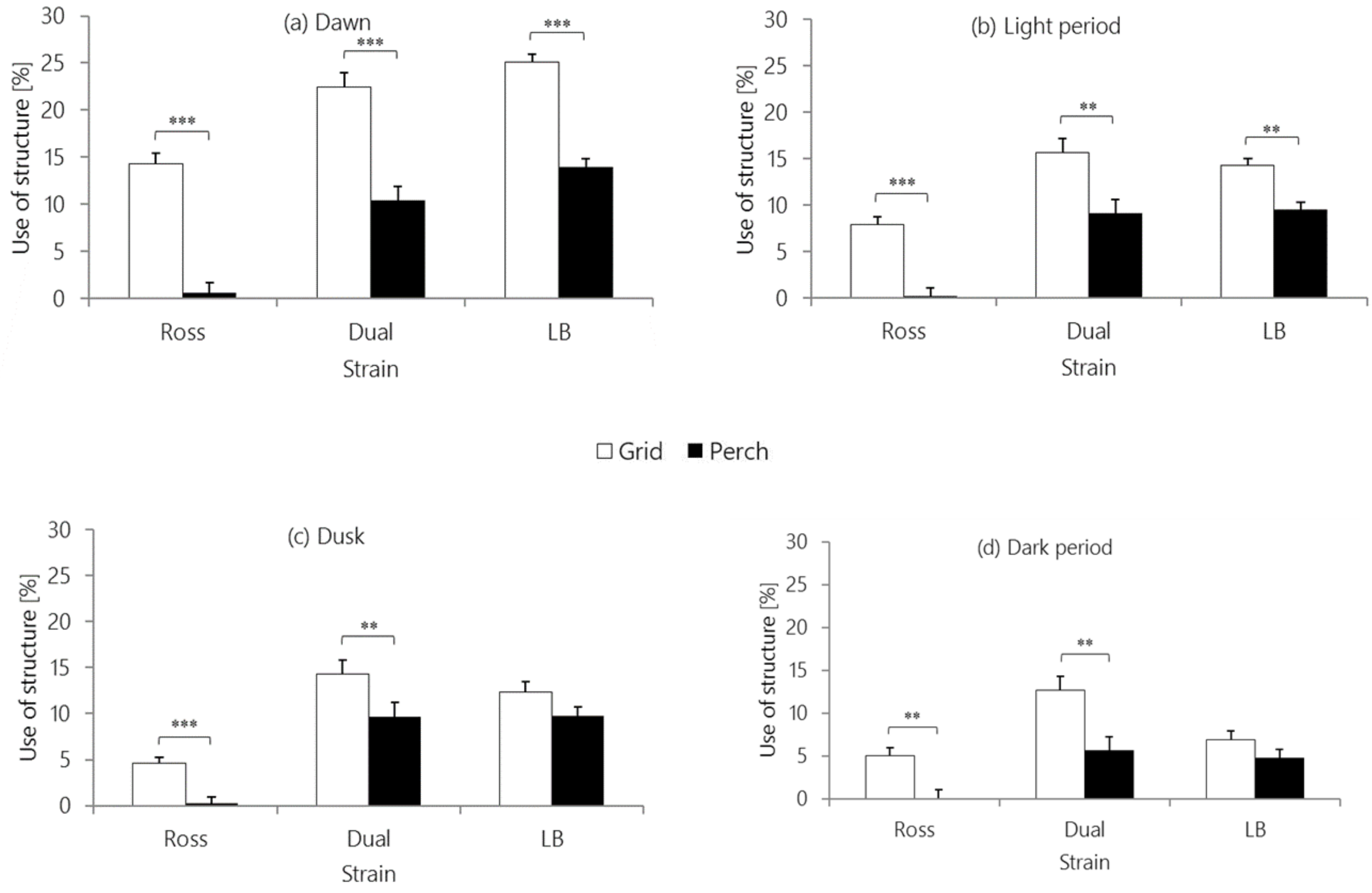

Figure 5 Structure effect over the entire observation time (5 weeks of age) for all 3 strains: (a) at dawn, (b) during the light period, (c) at dawn, and (d) during the dark period. Significant differences between grid and perch are marked by asterisk: * P = 0.05, ** P = 0.01, *** $P \geq 0.0001$. Bars indicated the standard error.

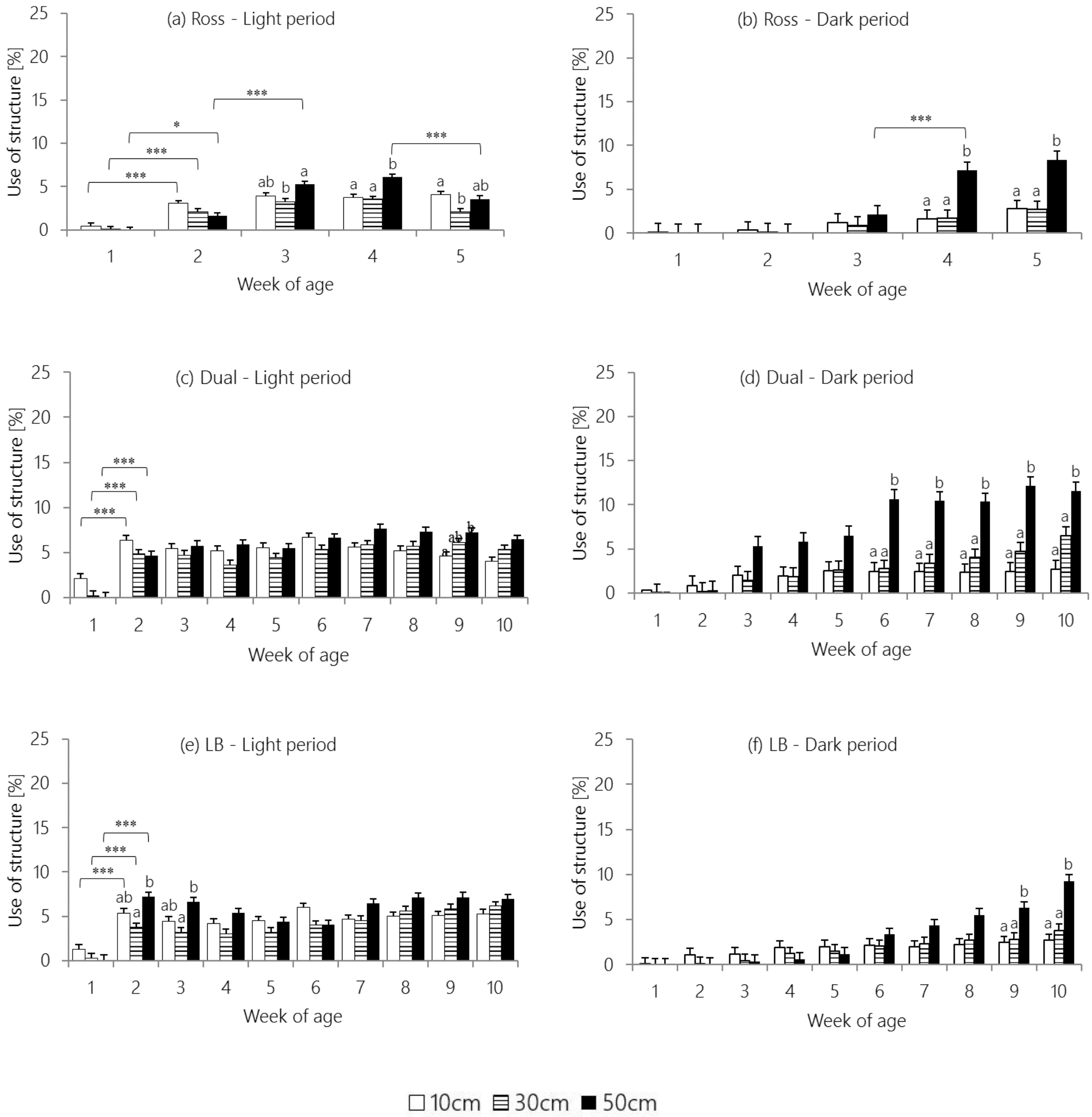

Figure 6 Height effect on use of structure during the light period (a, c, e) and the dark period (b, d, f) for each strain (Ross, Dual, LB). Significant differences between consecutive weeks of age within heights are marked by asterisk: * *P* = 0.05, ** *P* = 0.01, *** *P* ≥ 0.001. Significant differences between heights within the first 5 weeks of age are marked by different letters (*P* < 0.05); (n = 8). Bars indicate the standard error.

Locomotor Activity and Growth Performance

Chickens of all 3 strains showed a significantly decreasing locomotor activity throughout the observation period (Figure 7). This decrease with increasing age was significant between almost all successive weeks (Table 2). Ross chickens showed a comparable locomotor activity between the 3rd and the 4th but a decrease to the 5th week of age. Locomotor activity of LB chickens did not change from weeks 7 to 9. Ross chickens were significantly less active in the 4th and the 5th week of age compared with Dual and LB chickens (all $P < 0.01$; Figure 7).

The strains significantly differed in weight gain ($P < 0.0001$). The average weight gain was 326 g/week in Ross (5 weeks), 219 g/week in Dual (10 weeks), and 142 g/week in LB (10 weeks).

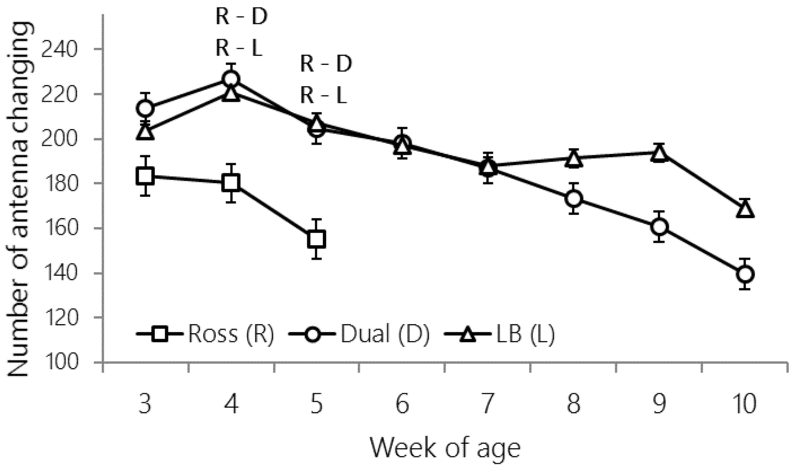

Figure 7 Locomotor activity within consecutive weeks of age. Differences between Ross and Dual (R-D) and between ross and LB (R-L) are significant: $P < 0.05$. Bars indicated the standard error.

Table 2 Locomotor activity (additionally to Figure 7): *P*-values between consecutive weeks of age within strains.

Intra	Ross	Dual	LB
Weeks		*P*-value	
3 to 4	0.8543	<0.0001	<0.0001
4 to 5	<.0001	<0.0001	<0.0001
5 to 6	n/a	0.0341	0.0021
6 to 7	n/a	<0.0001	0.0064
7 to 8	n/a	<0.0001	1.00
8 to 9	n/a	<0.0001	1.00
9 to 10	n/a	<0.0001	<0.0001

n/a = not applicable

Discussion

Our results showed that elevated structures are used by all breeds, differing in growth performance. The use of elevated structures started at an age of 2 weeks and continued until slaughter. Chickens of all strains preferred elevated grids to elevated perches. Slow- and medium-growing chickens showed a higher usage of elevated structures compared with fast-growing chickens.

Effect of Age, Strain and Daytime

In particular during the light period and at dawn, chickens of all 3 strains increasingly used the elevated structures from the 1st to the 2nd week of age. This finding corresponds with those of Heikkilä et al. (2006) and Riber et al. (2007), who also found that chickens, especially of layer strains, start to perch from the 10th day of age. Presumably, in the first week of age, the chickens are hardly able to climb on the elevated structures.

Interestingly, the use of elevated structures was highest at dawn, followed by the light period and dusk, and lowest during the dark period. A possible explanation for the intensive use of elevated structures at dawn and dusk might be the general increase in activity of chickens, which has been observed 1 h before sunrise and sunset (Gerken et al., 1988; Olsson and Keeling, 2000). Laying hens are known to actively search for elevated structures during dusk but they stay there for nighttime roosting (EFSA, 2015) . In meat chickens Wedin (2016) studied disturbances during the dark period. She found that male chickens showed more disturbance activity than female chickens. Thus, an additional explanation for our finding on higher number of birds at dawn compared to dark period might be that chickens are falling down or are pushed from elevated structures during dark due to disturbances and a high density of birds on the elevated structures. However, also at dawn there was empty space on the elevated structures. Moreover, we did not observe a high degree of disturbances on the elevated structures although this was not systematically recorded in our study. Thus, the high use of the elevated structures in particular at dawn is remarkable but remains to be explained.

The fast-growing Ross chickens showed a lower use of elevated structures compared with Dual and LB chickens. In addition, their use of elevated structures decreased from the 3rd to the 4th week of age at dawn and from the 4th to the 5th week of age during the light period. A decrease in the use of elevated structures during the light period by fast-growing broiler chickens has also been observed by Zhao et al. (2012), Hongchao et al. (2014), and Norring et al. (2016) (but see Martrenchar et al. (2000)). This decrease in the use of elevated structures by Ross chickens can be explained by their comparably low and additionally declining locomotor activity due to their fast growth. Fast growth often is associated with impaired walking ability and leg problems (Stojcic and Bessei, 2009; Hongchao et al., 2014), which additionally may have hampered the broilers in using the elevated structures with increasing age and weight.

In contrast, during the dark period, the Ross chickens increasingly used the elevated structures until slaughter, and the Dual and LB chickens tended to use the structures more frequently with increasing age (cf. Figure 4). This increase in the usage of elevated structures may indicate an increasing motivation the perch to protect from predators with increasing age. When observed under natural conditions, the domestic fowl starts to roost on elevated structures at about 7 to 8 weeks of age, and nighttime roosting is guided by the mother hen (Wood-Gush and Duncan, 1976). It seems that the chickens in our study started to use the elevated structures earlier for nighttime roosting compared with chickens observed in natural conditions. Interestingly, domesticated chickens with a brooded hen do not start to perch earlier than do chicks without a mother hen (Riber et al., 2007).

We also found that the proportion of chickens that used the elevated structures at nighttime was quite low during the first weeks of age. Nonetheless, our results suggest that despite a decreasing activity and a possible decreasing walking ability, chickens selected for fast growth such as Ross broilers are motivated to roost on elevated structures.

Effect of Structures

During all 4 examined daytime periods, Ross and Dual chickens preferred the grids to the perches. This finding is in line with other studies that compared the use of grids and perches in broiler chickens (Oester et al., 2005; de Jong and van Wijhe-Kiezebrink, 2014; Norring et al., 2016). The chickens from the layer line (LB) preferred the grids only at dawn and during the light period but did not show a significant preference at dusk and during the dark period. This finding may indicate that slow-growing layer chickens prefer a perch rather than a grid for nighttime roosting as has been shown for laying hens (Schrader and Müller, 2009). However, for fast-growing chickens, grids seem to be more suitable. A possible explanation could be that due to their weight, it is more difficult for heavy chickens to find a balanced resting position on a perch. Alternatively, the design of the perches used in our study might have been less suitable for use by fast-growing chickens.

Effect of Height

During the dark period, chickens of all 3 strains preferred the height of 50 cm by the end of the fattening period when their body weight exceeded 1,400 g. A study by Martrenchar et al. (2000) also found that broiler chickens close to slaughter increasingly used higher level perches. This finding corresponds to the preference of adult fowl to rest at heights up to 10 m (Wood-Gush and Duncan, 1976).

For each structure and height, we offered an area on which approximately 5 chickens could rest simultaneously at the end of the fattening period (10 % of the birds housed per compartment). We supposed an average grid space of 514 cm^2 per bird (Bokkers et al., 2011) or perch length of 15 cm per bird for a high-density (45.7 to 47.9 kg/m^2) occurrence on the elevated structures. Thus, our study was not designed as a free choice experiment and it was not possible for each chicken to choose each structure at each height in this setting. Consequently, we cannot exclude that we observed a second or even third best choice of chickens in case the preferred site of structures was already occupied. We decided on our design based on the results of other studies on structure usage by chicken and due to the limited space in our experimental facilities.

Ross broilers showed a maximum use of 8 % during the dark period. It might be the case that they need more space for sleeping than we expected. If we assume a grid space of 636 cm^2 per bird (Bokkers et al., 2011) for a low-density (37.8 to 38.7 kg/m^2) occurrence, the maximum occurrence on one level would be 8 %. Thus, we can presume that the highest level (50 cm height) was fully occupied and other chickens had to choose alternative sleeping places. In addition, the Dual chickens showed a usage of 12 % on the 50 cm level. Although they did not need less space than did the Ross chickens, they have a slower metabolism and produce less heat compared with Ross chickens. Thus, in Dual chickens, heat dissipation may be easier so they can rest more closely together (Sandercock et al., 2006).

During the light period, chickens of all 3 strains did not show a clear preference for a specific height. This may result from a higher activity of chickens during light period. Higher activity during daytime may result in a higher frequency of changes between the different levels and a performance of different behaviors on the elevated structures compared to nighttime during which chickens predominantly rest.

Our results demonstrated that during rearing slow-, medium-, and even fast-growing chickens are motivated to use elevated structures with suitable furnishing. In particular for fast-growing broiler chickens, grids seem to be more appropriate than perches probably due to the birds' hampered physical abilities. Furthermore, for nighttime roosting, the height of structures matters for meat as well as for laying chickens.

Our results imply that elevated structures should be offered depending on the chickens' growth rate, in order to increase their welfare by better meeting their behavioral priorities. However, further studies should address more specifically whether space requirements on elevated structures differ between strains of divergent growth performance due to differences in the birds' preference for using such structures and in body size. Differences between sexes also should be addressed here. In addition, effects on behavior such as locomotor activity, leg health, and thermoregulation should be tested.

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CHAPTER IV

Based on:

Effect of Elevated Grids on Growing Male Chickens Differing in Growth Performance

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Abstract

Pullets, i.e. chickens of layer lines are often raised in housings equipped with perches. In contrast, broiler chickens most often are raised in a barren environment that lacks any three-dimensional structures, even though broilers also are motivated to use elevated structures. In addition, environmental enrichment may improve welfare problems in broiler chickens, such as skeletal disorders or contact dermatitis. Due to ethical reasons, currently there are attempts to fatten the male chickens of layer strains or to use dual purpose strains. However, there is only limited knowledge on the behavior of these chickens until now. The aim of this study was to test the use of elevated grids and their effect on animal-based indicators (e.g. physical condition). In 2 successive trials, we kept a total of 1,217 male chickens from three strains (Lohmann Dual, Lohmann Brown Plus, Ross 308) that show differences in growth performance in 24 pens (2 trials x 3 strains x 8 pens). In half of the pens, grids were offered at three different heights (enriched groups); in the other half of the pens, no elevated structures were installed (control groups). We recorded the number of birds using the grids at the different heights as well as locomotor activity, walking ability, plumage cleanliness, and the footpad health of chickens. Chickens with low and medium growth performance preferred the highest grids during both the light and dark periods. In contrast, fast-growing chickens used the lowest grid more frequently. Fast-growing chickens kept in the enriched pens tended to have a higher level of locomotor activity and reduced chest cleanliness. Chickens from the medium growth performance strain showed better walking ability when kept in the enriched pens. Enrichment did not affect any of the welfare measures in the slow-growing chickens. These findings suggest that elevated structures may improve chicken welfare, particularly for medium growing chickens. For fast-growing chickens we found evidence for an improvement of animal-based indicators although they used the elevated structures less. However, regardless of growth performance, elevated grids offer the birds an opportunity to rest in a species-specific manner.

Introduction

Broiler chickens commercially are kept in a barren environment, equipped only with littered floors, feeders, and drinkers. Growing chickens are motivated to use and explore elevated structures (McBride et al., 1969) and such structures can increase their level of activity and improve health of broilers (Reiter and Bessei, 2009). In addition, elevated places may offer chickens shelter in case of fear-eliciting situations (Jones, 1996), as suggested by the antipredator hypothesis (Newberry et al., 2001). Despite these advantages, they are rarely offered in commercial broiler chicken farms.

In previous studies, different elevated structures, such as perches, platforms, straw bales, or bars, have been provided (de Jong and van Wijhe-Kiezebrink, 2014; Bailie and O'Connell, 2015; Kaukonen et al., 2016). It is known that fast-growing broilers prefer platforms compared to perches (Norring et al., 2016; Malchow et al., 2018). In studies on height preferences, perches were most often installed at higher levels, whereas grids were installed only at heights up to 30 cm. In a recent study (Malchow et al., 2018), chickens from three strains that differ in growth performance preferred the highest structures, at 50 cm, during the dark period.

In addition to the behavioral restrictions in barren environments, skeletal disorders and contact dermatitis (Bessei, 2006) are common welfare problems in broiler chickens. High growth rate and the associated muscle growth, including that of large breast muscles, lead to cranial shifts in body balance (Duggan et al., 2017) and skeletal disorders (Su et al., 2000). Often, fast-growing chickens show impaired walking ability (Kestin et al., 2001), and increased time of rest (e.g., sit/lie) in the litter (Bessei, 2006). At the end of the rearing period, the litter is often moist or wet, a result of the high feed intake, metabolism and excretion of the broilers (Ekstrand et al., 1997). High resting duration combined with being in contact with moist litter can cause different types of contact dermatitis, including footpad dermatitis, hock burns and breast blisters (Ekstrand et al., 1997).

An enriched environment, such as one with elevated structures, can improve the broiler chickens' locomotor activity and can lead to better walking ability (Bailie et al., 2013). Moreover, broiler chickens that are more mobile and reduce their time resting in contact with litter may show a lowered prevalence of contact dermatitis (de Jong and van Harn, 2012). Furthermore, it has been suggested that offering an elevated structure can result in a cleaner state of the chickens' plumage due to less contact of the keel with the litter (Zhao et al., 2012).

In contrast to fast-growing broiler chickens, chickens of layer strains often are raised with environmental enrichments (Gunnarsson, 1999; Jung and Knierim, 2018). The main reason is, that pullets shall be prepared for the housing systems in which they will be kept as laying hens, for example in aviary systems with elevated tiers and perches (Campbell et al., 2018). Currently, there are attempts to also fatten the male chickens from layer strains although their weight gain is significantly lower compared the broiler strains. This is done in order to avoid killing of male day-old laying chickens (Koenig et al., 2012) which has increasingly become an ethical issue. A further approach is to use dual-purpose chickens where the female birds are used for egg production and the male chickens for meat production. However, there is still a lack of knowledge on the behaviour and in particular on the use of elevated structures by male chickens of both layer and dual strains (Lambertz et al., 2018).

In our study, we offered plastic grids at three different heights with a ramp in between to enable easy access. We used three strains to assess possible differences in the use of elevated structures between fast-, medium- and, slow-growing male chickens. To evaluate the effects of elevated grids, locomotor activity, walking ability, weight, plumage cleanliness, and footpad dermatitis were assessed and compared between unenriched (control groups) and enriched pens.

We predicted there would be a preference for the highest level of the offered grids in all three strains at the end of observation period, at least during dark periods. We expected an improvement in animal-based indicators in the group with access to the elevated grids compared to the control group. In particular, we hypothesized that chickens from enriched pens would show: (a) increased activity; (b) better walking ability; (c) same or higher weight; (d) better scores for total plumage and chest cleanliness, and a worse score for back cleanliness as well as (e) better footpad health.

Materials and Methods

Birds and Housing

A total of 1217 one-day-old male chickens from three different strains were randomly allocated to 12 pens in a stable at the research station of the Institute of Animal Welfare and Animal Husbandry (FLI, Celle, Germany). In 2 successive trials, 412 Ross 308 (fast-growing, commercial meat strain; hereafter, Ross; first trial included 200 chickens, and second trial included 212 chickens), 400 Lohmann Dual (medium-growing, dual-purpose strain; hereafter, Dual; first trial

200 included chickens, and second trial included 200 chickens), and 405 Lohmann Brown Classic (slow-growing, commercial layer strain; hereafter, LB; first trial included 200 chickens, and second trial included 205 chickens) were used for this study.

All chickens were reared in groups of 50 to 53 animals (depending on the total number of animals delivered) in experimental pens (floor space: 2 x 3 m; height: 2 m). Chickens of each strain were randomly assigned to four pens (2 trials x 4 groups per strain). The Ross chickens were kept for 5 weeks (body weight at hatch: 44.6 ± 0.4 g; body weight at slaughter date: 2307.45 ± 306.95 g; mortality: 2.2%), whereas Dual (body weight at hatch: 39.6 ± 2.0 g; body weight at slaughter date: 2265.0 ± 269,75 g; mortality: 1.5%) and LB (body weight at hatch: 37.7 ± 1.7 g; body weight at slaughter date: 1372.0 ± 122.75 g; mortality: 1.7%) were kept for 10 weeks (all body weight data: average weight ± standard deviation).

Air temperature and ventilation were automatically controlled with an intermediate program to meet the climate demands of broiler and layer chickens (temperature: 36°C at the first day continuously decreasing to 18°C until 36th day). For the first three days, the artificial light program started with a 24 h light period and changed to an 8 h dark period and a 16 h light period (04:00 am to 08:00 pm) at a light intensity of at least 20 lx, including 15 min dimming phases achieved by flicker-free tube-bulbs for the entire experimental periods of both trials.

Floors of all pens were littered with wood shavings. At the 4th week of age, the litter of Ross chickens was supplemented with chopped straw to keep the litter dry. Two round feeding troughs and one round water dispenser with eight drinking nipples were provided per pen (Fig. 1a, b). All chickens were fed ad libitum with a single-phase pelletized feed (21% crude protein, 12.90 MJ ME/kg) that met the nutritional needs of both broiler and layer chickens.

In both trials, half of the pens of each strain were equipped with elevated grids at 3 different heights (10 cm, 30 cm, 50 cm) but with the same shape (length x width: 90 cm x 30 cm). A ramp (width: 20 cm, inclination angle: 35°) was installed in between the grids to provide easy access (Fig. 1b). Both the grids and ramps were made of the same material (plastic: PP (Polypropylene)) and had a mesh size of 19 x 19 mm and a slat width of 10 mm.

Measurements & Statistical Analysis

All statistical analyses were done using SAS® Enterprise Guide Version 6.1. To test the effects of factors and their interactions, we used adapted generalized linear mixed models (GLMM). For examining significant effects, we used *post hoc* tests (Bonferroni) for testing pairwise differences based on our hypotheses. In each calculation, pen ID was included as a random factor nested within the random factor trial.

Use of Structures

In the enriched pens, the elevated structures were recorded using infrared video cameras (Model VTC-E220IRP, color camera for corner mount with IR-LEDs; SANTEC BW AG, Ahrensburg, Germany) connected to a commercial PC with memory function. From these recordings, the numbers of chickens on the grids were counted for each height using time sampling in 20-min intervals for each week of age throughout two successive days (usually Saturday and Sunday) from 03:00 am to 10:00 pm.

To test preferences for the height of elevated structures (10 cm, 30 cm, 50 cm) in the enriched pens ($n = 12$), each observation day was divided into a light period (from 04:00 am to 07:40 pm) and a dark period (from 03:00 am to 03:40 am and from 08:00 pm to 10:00 pm). As the dependent variable, we calculated the mean proportion of chickens at each height, week and strain separately for light and dark periods, excluding the number of chickens on the ramp. Height, week of age, strain, period, and their interactions were used as fixed factors.

Locomotor Activity

The locomotor activity was automatically recorded with a transponder-antenna system (PLB SPEED Antenna and Chip Glastag HITAGS 3.15 x 13 x 3 mm; Gantner Pigeon Systems GmbH, Schruns, Austria). In each pen, two antennas (length: 90 cm, width: 30 cm, height: 3 cm) were placed on the floor in the litter (Fig. 1a, b). At an age of 14 days, half of the chickens in each pen had a transponder attached (height: 23 mm, width: 4 mm, weight: 2.5 g) with a cable strap to their legs. In addition, the chickens received a chicken tag with an individual number at the wing and, thus, in case a chicken lost the transponder, a new one could be assigned.

The antennas recorded the transponders at a distance of less than 20 cm and a connected computer registered the number of the antenna and the transponder tag, as well as the date and time. As a proxy for the locomotor activity for each transponder (chicken), we calculated the mean number of changes between the two antennas per week for each chicken transponder using SAS® 9.4 (SAS Inst. Inc., Cary, NC) during the light period (04:00 am to 08:00 pm).

For testing the effects of treatment (enriched/ control) on the locomotor activity measured by the frequency of changes between antennae, we used treatment, strain and week of age as fixed factors.

(A)  (B) 

Figure 1 Pens without (A, control) and with elevated structures (B, enriched). All pens were equipped with wood shavings, two antennas in the litter, two round feeding troughs and one water dispenser.

Walking Ability

Walking ability was assessed with the rotarod test two days before slaughter. The data of the rotarod test covary with the results obtained by the gait score system (Kestin et al., 1992) and, thus, offer a more objective assessment of walking ability. In short, a chicken was placed in the middle of a rod. After both feet grasped around the rod, the motor that rotates the rod was started. The test stopped when the chicken actively or passively left the rotating rod. The details of this test are described in Malchow et al. (2019).

Chickens with a transponder were chosen in a random order from each pen. For each strain, we tested 46 birds from both trials (6 chickens from three pens and 5 chickens from one pen). The number of animals based on preliminary tests.

To test the effect of treatment (enriched/ control) on walking ability, we measured the latency to leave the rotating rod. Treatment, strain and their interaction were used as fixed factors.

Weighing & Assessment of Plumage Cleanliness and Footpad Health

All chickens that were equipped with a transponder were weighed (nearest ± 10 g), and their plumage cleanliness and level of footpad health were assessed at the end of the rearing period.

Plumage cleanliness and footpad health were assessed with the Welfare Quality protocol for poultry (Welfare Quality®, 2009). The scoring system for the plumage was classified in four categories: 0 – no contamination; 1 – light contamination; 2 – moderate contamination; and 3 – high contamination with litter glued to feathers. The categories were assessed on 5 different parts of the chickens: head/neck, back, tail, wings, and chest. To evaluate footpad health, we used a 5-scale system: 0 – no changes (no evidence of footpad dermatitis); 1 – light changes of the footpad (slightly evidence of footpad dermatitis); 2 – moderate changes of the footpad (minimal evidence of footpad dermatitis); 3 – entire footpad shows changes; and 4 – changes of the entire central footpad and also of the plantar toes (evidence of footpad dermatitis) (Welfare Quality®, 2009).For testing the differences between the weights of chickens from enriched and control groups, we used strain, treatment and the interaction between strain and treatment as fixed factors.

The total plumage cleanliness scores for three (head, tails, wings) body parts for each pen were added up to a total score ranging from 0 to 9. The lower the total score, the better the plumage cleanliness was. Effects on the total score for cleanliness were tested by including strain, treatment, and their interaction as fixed factors. With respect to plumage cleanliness, we expected differences between cleanliness particularly for the back and the chest plumage. Thus, for these two body parts and foot pad health, we did a separate analysis using a Mann Whitney U test as data were not normally distributed and statistics were done for each strain.

Results

Use of Structures

The use of structures was significantly affected by two-fold interactions between strain and period ($F_{(13,\ 528)}$ = 8.92, P = 0.0002), strain and height ($F_{(4,\ 528)}$ = 7.16, P < 0.0001), week and period ($F_{9\ ,528}$ = 2.7, P = 0.0044), week and height ($F_{(18,\ 528)}$ = 10.63, P < 0.0001), and period and height ($F_{(2,\ 528)}$ = 23.46, P < 0.0001). LB and Dual chickens used the elevated platforms more with increasing age both during the daytime and at night (Dual: dark period: $F_{(9,\ 87)}$ = 33.02, P = < 0.0001, light period: $F_{(9,\ 87)}$ = 58.12, P < 0.0001; LB: dark period: $F_{(9,\ 87)}$ = 3.47, P = 0.0011, light period: $F_{(9,\ 87)}$ = 38.80, P <

0.0001). Ross chickens showed a very low use compared with the chickens from the slower growing strains and usage of structure was only affected by the week of age during light period in Ross chickens (dark period: $F_{(4, 42)}$ = 1.94, P = 0.122; light period: $F_{(4, 42)}$ = 35.54, P < 0.0001, Fig. 2).

The maximum proportion of animals (LSM ± SE) on the elevated grids varied from 5 ± 0.44% (Ross) in the light period to 26 ± 3.75% (LB) and to 36 ± 1.83% (Dual) except in Ross during the dark period. The height significantly affected the use of the grids (see Tab. 1). In general, Dual and LB chickens primarily preferred the highest grids both during the light and dark periods in the middle and at the end of the observation period (Fig. 2). In contrast, in the light period, Ross chickens preferred the lowest level of elevated platforms. During the dark period, Ross birds showed no preference for any of the three heights.

Table 1 Interaction between the heights of the structure and the week of age on the frequency of structure usage within strains and daytime (GLMM, factor height*week of age).

Strain	Daytime	numDF	denDF	*F* statistic	p-value
LB	Light period	18	87	3.47	< 0.0001
	Dark period	18	87	2.18	0.0087
Dual	Light period	18	87	3.49	< 0.0001
	Dark period	18	87	18.50	< 0.0001
Ross	Light period	8	87	3.0	0.0094
	Dark period	8	87	0.8	0.6031

Locomotor Activity

Regardless of environmental enrichment, all three strains showed decreasing activity with increasing age (LB: $F_{(7, 42)}$ = 23.81, P < 0.0001; Dual: $F_{(7, 41)}$ = 40.72, P < 0.0001; Ross: $F_{(2, 12)}$ = 67.44, P < 0.0001 Fig. 3). There was a significant interaction between strain and week of age ($F_{(9, 95)}$ = 3.13, P < 0.0001), and between treatment and week of age ($F_{(9, 95)}$ = 3.08, P = 0.0057).

In LB chickens, the treatment showed no effect on activity. Dual ($F_{(1, 41)}$ = 3.42, P = 0.072) and Ross chickens ($F_{(1, 12)}$ = 3.87, P = 0.073) tended to show higher activities in the enriched group compared to the respective control group.

Walking Ability

Walking ability was significantly affected by the interaction between treatment and strain ($F_{(2, 114)}$ = 3.12, *P* = 0.0478). LB and Dual chickens showed a comparable latency to leave the rotating rod (*P* = 0.2397). Ross chickens showed a worse walking ability than Dual (*P* < 0.0001) and LB (*P* < 0.0001) chickens. In Dual chickens, birds from the enriched groups showed a significantly longer latency to leave the rotating rod than chickens from the control groups (*P* = 0.0346, Fig. 4). Ross and LB had no differences between the treatments.

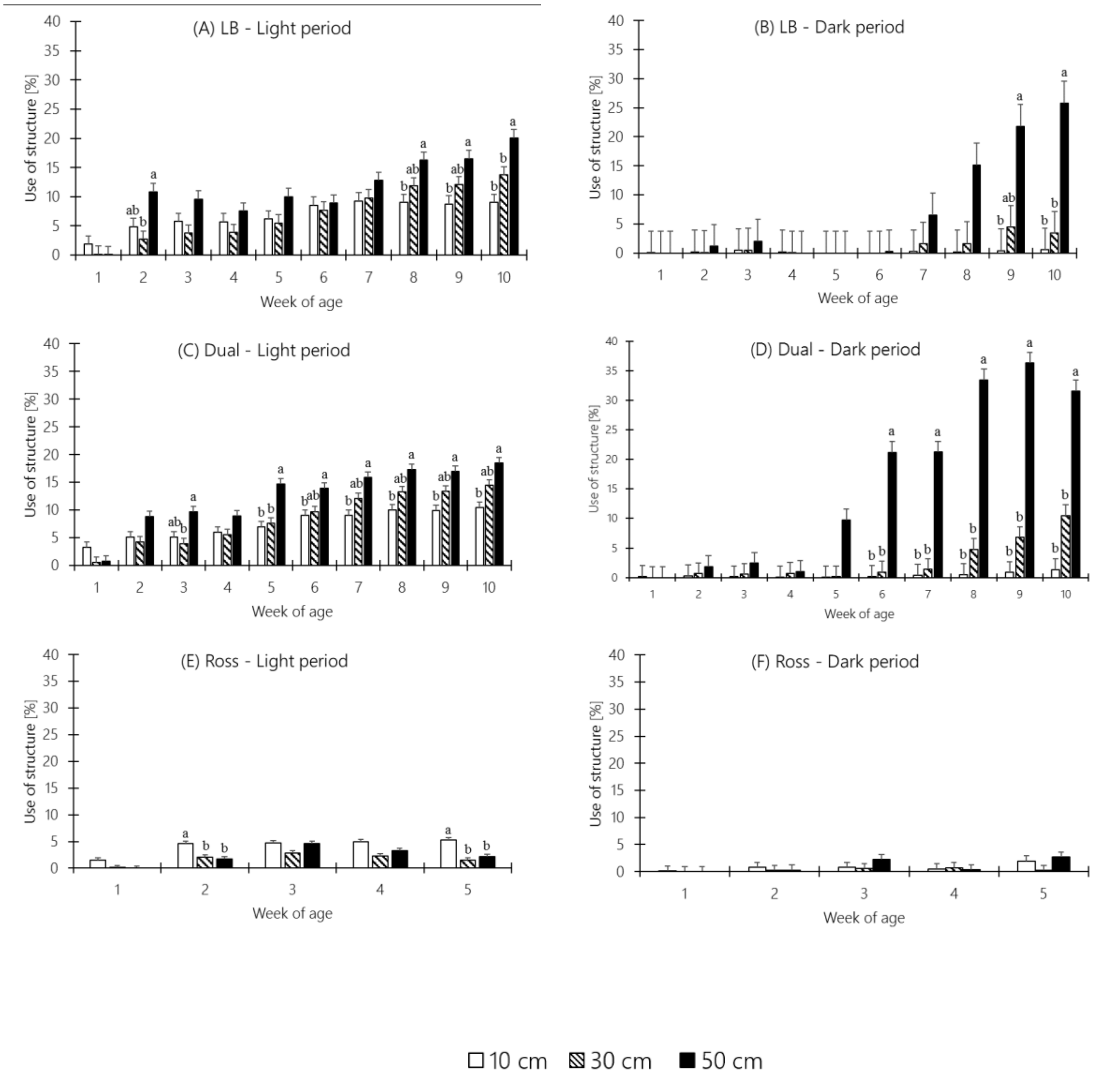

Figure 2 Proportion of the use of structures of different heights (LSM ± SEM) by chickens from three strains (LB, Dual, Ross) during the light and dark periods. Significant differences between heights within week of age are marked by different letters (*P* < 0.05).

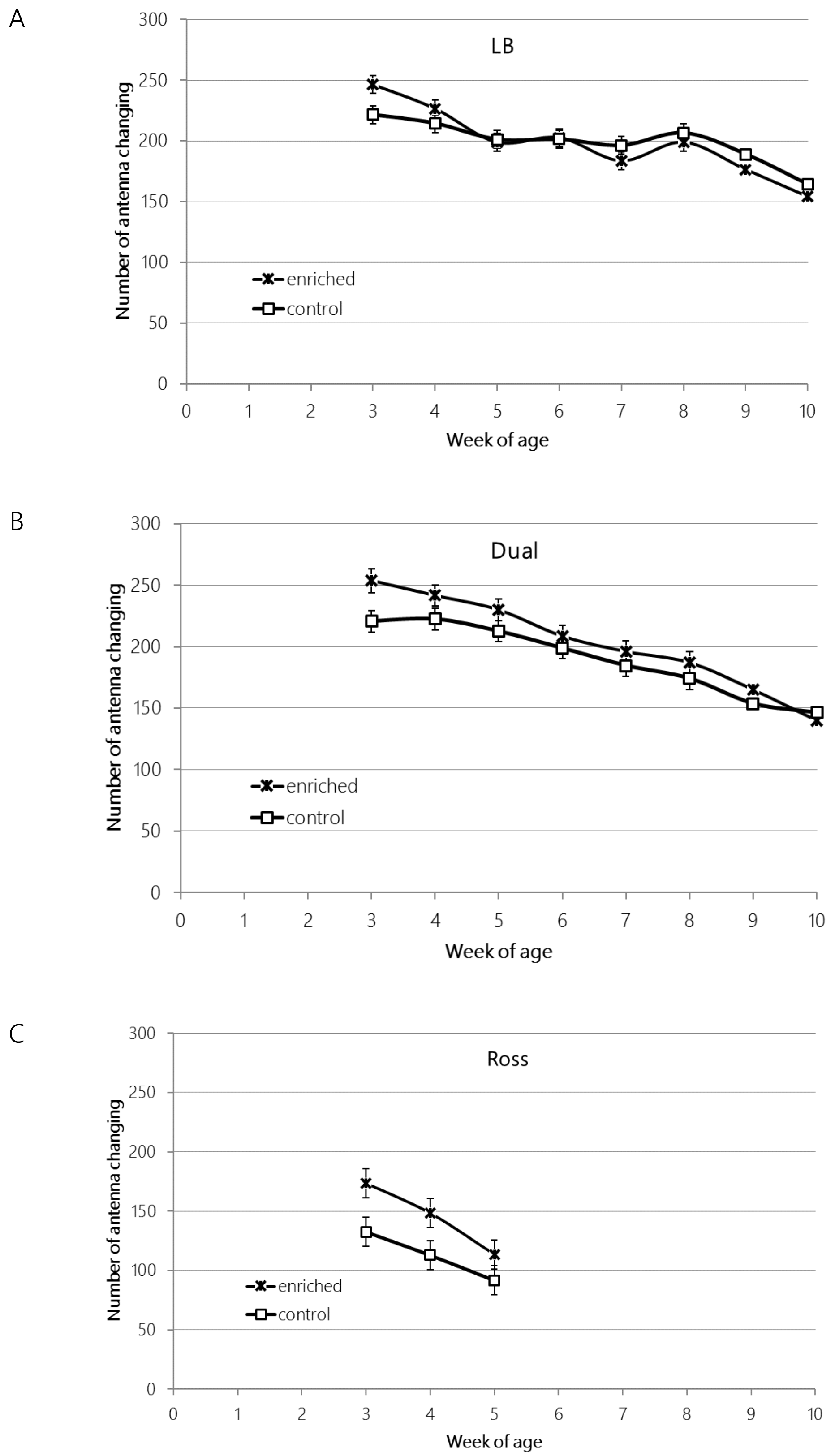

Figure 3 Locomotor activity (LSM ± SEM) of chickens from three strains [(A) LB, (B) Dual, and (C) Ross] with (enriched) and without (control) elevated structures. The locomotor activity was measured in means of the number of changes between two floor antennas.

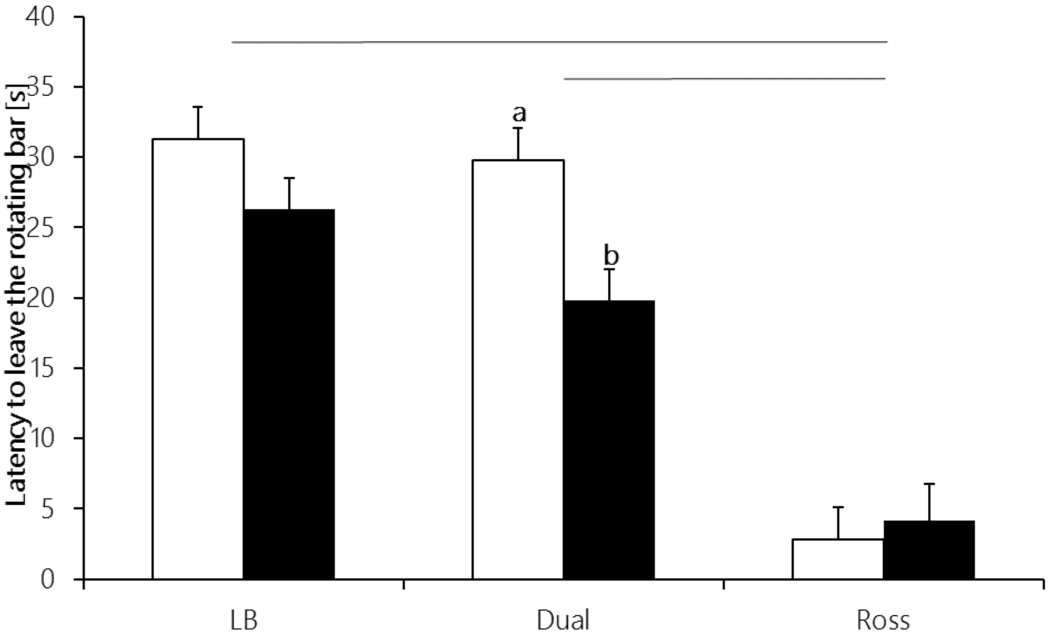

Figure 4 Latency to leave the rotating bar (LSM ± SE) in the rotarod test for LB, Dual, and Ross chickens housed with (enriched, white bars) and without (control, black bars) elevated structures. Significant differences between enriched and control pens are marked by different letters (*P* < 0.05). Significant differences between strains and treatments are marked by lines (*P* < 0.05).

Weight, Plumage Cleanliness, and Footpad Health

The treatment did not affect the weight of chickens ($F_{(1, 18)}$ = 0.14, P = 0.87).

We found differences in the total cleanliness score of the plumage between strains ($F_{(2, 597)}$ = 24.33, *P* < 0.0001), but no differences between the treatments (total cleanliness score: $F_{(1, 597)}$ = 0.15, *P* = 0.7). In general, Ross chickens were dirtier than Dual (*P* < 0.0001) and LB (*P* < 0.0001), and Dual more than LB (*P* = 0.0414). Only Ross chickens showed differences between the treatments in back and chest cleanliness. Both body parts were dirtier in the enriched groups than in control groups (back: Z = 36.43, *P* = 0.0563; chest: Z = 200.39, *P* < 0.0001, Fig. 5).

Footpad health was only affected by treatment in Dual chickens (Z = 6.1019, *P* = 0.0135). Dual chickens from the control groups showed worse footpad health compared to chickens from the enriched groups. Footpad health significantly differed between strains in the control groups (Ross > Dual > LB, Z = 143.87, *P* < 0.001) and in the enriched groups (Ross > Dual = LB, Z = 149.44, *P* < 0.0001). In sum, Ross showed worse footpad health in comparison to Dual and LB.

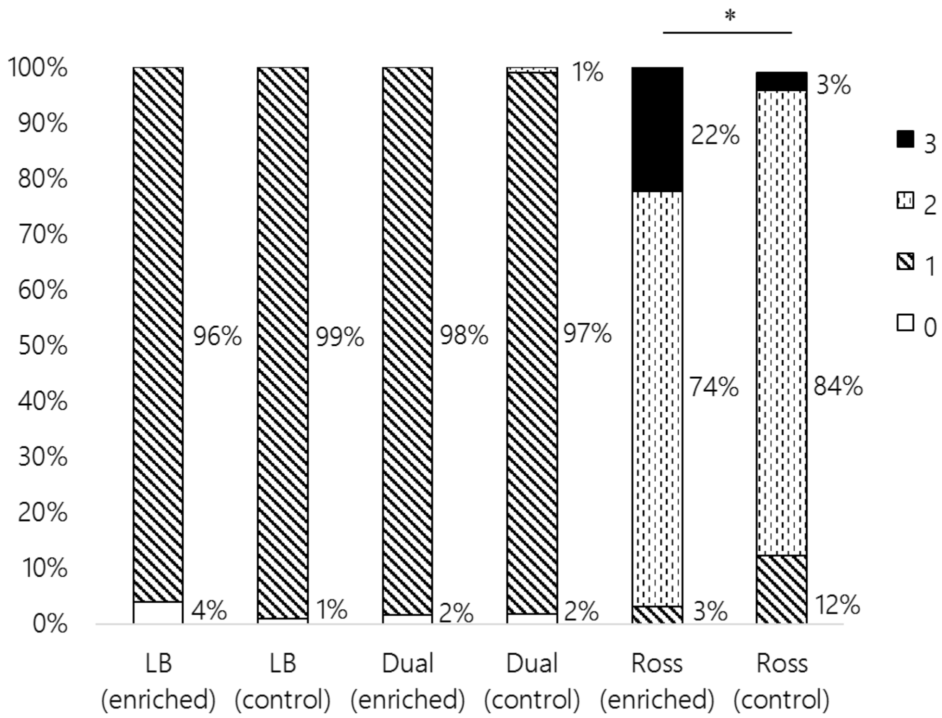

Figure 5 Chest cleanliness scores (percentage of animals assessed for each score) of the three strains (LB, Dual, Ross) for the two treatments (enriched and control pens). Significant differences between treatments within strains are marked by an asterisk, $P < 0.05$.

Discussion

In general, chickens of all three strains showed increasing use of elevated structures with increasing age during both the light and dark periods. This result confirms the outcomes of other studies conducted with fast-growing chickens (Bailie and O'Connell, 2015; Norring et al., 2016). In general, growing chickens' use of elevated structures may result from the motivation to rest, sleep and explore on high levels (McBride et al., 1969). Another explanation could be that they may use the structures to avoid agonistic interactions with dominant conspecifics, as observed in laying hens (Cordiner and Savory, 2001).

In our study, chickens with slower growth (LB and Dual) additionally showed mostly a preference for the highest grids both during the day and night time. This preference corresponds to the preference for layers for high perches (Schrader and Müller, 2009; Brendler et al., 2014; Brendler and Schrader, 2016; Malchow et al., 2018) and can be explained by the antipredator hypothesis (Newberry et al., 2001) suggesting that chickens experience better protection from predators if they stay on elevated structures. Although a preference for high resting areas is particularly pronounced in adult fowl for night roosting (Schrader and Müller, 2009; Brendler et al., 2014; Campbell et al., 2016b), already growing chickens show a high motivation to stay at high levels overnight (Martrenchar et al., 2000; Malchow et al., 2018), which indicates that elevated areas may offer shelter for growing chickens, as well. In contrast to the slow-growing chickens, most of the fast-growing chickens were observed on the lowest level during the light period. This result correlates with the outcomes of the studies of Estevez et al. (2002) and of Norring et al. (2016) on the use of perches offered to fast-growing broilers at different heights. This outcome likely results from the rapid growth and the resulting reduced locomotor activity of these chickens. At the end of the rearing period, their balance is impaired, which is caused by the high mass of breast muscle that leads to a cranial shift of the body centre (Duggan et al., 2017). To facilitate access to the elevated grids, we included a ramp that was most often used in particular by the fast-growing chickens (personal observation). However, the design of the ramp used in our study seemed to be less suitable for use by the heavy broilers. The ramp might have been too steep, the ramp might have been unsteady, or the width of the ramp might have been too small for the fast-growing chickens (only one bird could use the ramp at a time). Climbing up a ramp requires particular force from the chickens because they need a higher force to take a step and to balance on one foot while climbing against the ascent of the ramp (LeBlanc et al., 2018). In a previous study (Malchow et al., 2018) we used the same type of ramp and the chickens, especially the fast-growing chickens (Ross), showed higher usage (14% vs. 4%) during both the light and dark periods. However, the chickens of the first study were lighter (2,099 ± 583g) than the chickens of the present study (2,307 ± 307g). This heavier weight might have reduced the chickens' ability to balance and climb up the ramp in the present study.

Chickens of all three strains showed decreasing locomotor activity with increasing age. Furthermore, the fast-growing chickens reflected a lower locomotor activity than the medium- and slow-growing chickens. Similar to previous studies (Bizeray et al., 2002a; Bailie et al., 2013), our findings suggest that in chickens with a faster growth, environmental enrichment, i.e., elevated structure, had an effect on chickens' locomotor activity. Compared to the slower-growing LB chickens, the fast-growing Ross chickens and the medium-growing chicken Dual tended to higher activity in week 3 of observation in the enriched pens compared to chickens in the control pens. Ventura et al. (2010) observed a higher activity level when broilers had to cross perches as a barrier in their pens. This larger effect of enrichment in the fast-growing Ross chickens is particularly interesting because they used the elevated grids to a lower degree compared to the slower growing chickens. Thus, this result suggests that in chickens with faster growth, elevated grids seem to increase their activity, even if this type of enrichment is used infrequently. However, although activity is closely associated with walking ability in chickens (Weeks et al., 2000), the fast-growing chickens from enriched pens did not differ from those in control pens in their walking ability, as indicated by the results of the rotarod test. In this test, only Dual chickens were affected by the enrichment, i.e., Dual chickens from enriched pens showed a longer latency to leave the rotating rod compared to the Dual chickens from the control pens. Thus, the enrichment may have only trained the motor abilities of the medium-growing, but not that of the slow- or fast-growing chickens. For fast-growing chickens, this result corresponds to the low use of the elevated structures and their low activity level in both the enriched and control groups. Other studies with fast-growing chickens found either positive (Kaukonen et al., 2016; Yildirim and Taskin, 2017) or no (Su et al., 2000; Hongchao et al., 2014) effects of enrichment on walking ability. The slow-growing chickens (LB) showed a good walking ability with long latencies to leave the rotating bar but did not differ between enrichment treatment. These chickens are from a layer line that shows more mobile and active phenotypes in general. Hence, in LB, the elevated structures used in our study may not further improve their already well-developed motor skills. In comparison, the dual-purpose breed (Dual) used in this study is a crossbreed from a slow- and a fast-growing chicken line (Urselmans and Damme, 2014). Therefore, enrichment with elevated grids seems to have an effect on walking ability only in the medium type of growth performance.

In our study, we did not find differences in weight between chickens from enriched and control groups, which confirmed the results of Bizeray et al. (2002a) and Simsek et al. (2009). Thus, enrichment by elevated structures had no detrimental effect on production efficiency.

We also did not find an effect of elevated structures on the total plumage cleanliness or on the cleanliness of the back. In contrast, the fast-growing chickens showed poorer (higher score) cleanliness of the chest in the enriched groups compared to the control groups. A possible explanation may be that Ross chickens in enriched pens did not use the area under the elevated structures (personal observation), and at the same time, they used the elevated grids a small amount. This outcome may have resulted in a higher density of chickens in the litter (in front of the elevated structures) compared to the control pens in which the respective area was freely accessible for the chickens. Thus, the feces were concentrated within a smaller area of the enriched pens of the fast-growing chickens, which may have resulted in their poorer chest cleanliness scores.

According to our expectation, the elevated grids affected footpad health, but only in Dual chickens. We had expected that by using the grids, the feet of chickens may be healthier because footpad lesions can result from the contact of footpads with moist litter (Bessei, 2006). However, footpad health in our study was quite good compared with footpad health observed in commercial housings (prevalence of 42%, Sanotra et al. (2001). This may explain that we did not find differences in the layer (LB) and the meat (Ross) strain in our study.

In conclusion, chickens from all three strains differing in growth rate used the elevated grids, although strains differed in the usage frequency. In particular, at night, the slower growing strains LB and Dual preferred the highest grids, and even young chickens were motivated to rest and roost on elevated structures. The usage of the elevated grids should to be adapted to the respective strain, as indicated by the results for the fast-growing Ross chickens. The elevated grids used in our study did not have a negative effect on the growth performance of chickens. In contrast, some of the animal-based indicators were improved by the elevated grids, such as locomotor activity and walking ability. However, these positive effects on the chickens' welfare depended on the strain, i.e., the effects interacted with the growth rate of the chickens. Thus, elevated grids seem to better fulfil the behavioral demands of growing chickens but have to be adapted to their skills and abilities, particularly for fast-growing chickens, in order to improve their welfare.

Data Availability

All datasets generated for this study are included in the manuscript and/or the supplementary files.

Ethics Statement

This study was performed in compliance with national regulations (TierSchNutztV as of 2006) at the research station of the Institute of Animal Welfare and Animal Husbandry (FLI, Celle, Germany). All investigations were carried out with the approval of the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES, Oldenburg, Germany, file number 33.19-42502-04-16/2108).

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CHAPTER V

General discussion

One objective of this thesis was to validate an objective method suitable to assess the walking ability in chickens. We used a rotarod test from the pharmacological area for rodents to monitor the ability of motor coordination in order to assess the walking ability of meat chickens in an objective manner. The validation was carried out using the gait score system by Kestin et al. (1992) as this system is widely used and applied in practice. It has been shown that changes in the gait patterns of meat chickens can be assessed using the rotarod test. In addition, the different gait scores are reflected by the results of the rotarod test. With the rotarod test, it is possible to measure continuous data, which have the advantage of finer scaling compared to categorical data. Furthermore, with this easily applicable objective method subjective assessments and observer bias can be avoided.

Another objective was to provide appropriate elevated structures to support species-specific behaviour patterns and to investigate their effects on the behaviour of chickens differing in growth performances. Suitable elevated structures in form of plastic grids were offered to all three strains, which supported the perching. Positive effects such as increased activity and improved footpad health were also found in certain strains.

In the first part of the general discussion, strengths, weaknesses and further development opportunities for the rotarod test practice will be discussed. The second part will address the offer of elevated structures as environmental enrichment and their design. In addition, suggestions for further investigations to optimize the elevated structures and their implementation for chickens of different strains in commercial husbandry systems are discussed.

The rotarod test – An assessing tool for walking ability

Lameness of meat chickens is an major animal welfare issue animal-based welfare indicator (Bessei, 2006) and the walking ability can be used as animal-related indicator to assess animal welfare of chickens. In the last three decades, the leg health of meat chickens (Kapell et al., 2012) had been improved by breeding, but lameness of chickens remains a major problem due to the existing and increasing growth rate (de Jong et al., 2012). There are several ways to evaluate the walking ability of meat chickens (Kestin et al., 1992; Garner et al., 2002; Berg and Sanotra, 2003;

Djukic, 2007; Nääs et al., 2009; Caplen et al., 2012; Aydin et al., 2015; Aydin, 2017). The most commonly used practical method for assessing walking ability is the gait score system developed by Kestin et al. in 1992. The gait score system corresponds to fast practicability, if the appropriate training units have been carried out and the score was adjusted beforehand. Garner et al. (2002) modified the 6-scale-system of Kestin et al. (1992) into a 3-scale-system, which could achieve similar or even better values in the observer reliability. In the present thesis (see Chapter II), it was difficult to evaluate the walking ability of the three different strains. Fast growing chickens showed different gaits compared to the moderate and slow growing chickens due to the phenotypical differences. This means that a slight change in gait in slow-growing chickens is evaluated with gait score 1, while a comparable change in fast-growing chickens is classified with gait score 2. It is also known that the growth rate strongly correlates with walking ability (Kestin et al., 2001), which makes the comparison of meat chickens with different growth performances using the gait score system even more difficult. Therefore, the assessment of the housing condition on this aspect of animal welfare is also difficult.

Other methods for the assessment of the walking ability require more equipment or a test arena, but their application is easier to learn and provide therefore evaluations that are more objective. Modern technology such as kinematic cameras (Caplen et al., 2012) or pressure measurements with plates (Corr et al., 1998) provide a more objective assessment of the chickens' lameness. However, the high degree of specialisation makes it difficult to use these methods under practical conditions, as both the available space and the mobility of the test equipment is limited in the stable. The approaches to evaluate the walking ability mostly refer to the pressure exerted by the feet (Sandilands et al., 2011), the stride lengths (Duggan et al., 2016) as well as the walking speed (Nääs et al., 2009). The changed balance, especially in fast growing chickens, is not considered that much (LeBlanc et al., 2016). LeBlanc et al. (2016) showed that the ability to maintain balance is highly dependent on the physical condition of chickens. To determine the balance of chickens they placed chickens on a swinging pole (LeBlanc et al., 2016). Especially for the fast growing chickens, the displacement of the equilibrium centre leads to a mechanical challenge for the locomotion (Paxton et al., 2014). As a result, the motor coordination of the musculoskeletal system is also altered (Redfern et al., 2017).

A rotarod test can be used to test motor coordination in animals (Lalonde et al., 1995). The test is used in rodents considering different applications of drugs and their influence on the cerebellum

(Hamm et al., 1994), which is responsible for controlling motor coordination (Sokolov et al., 2017). In the present study (Chapter II), we used a modified rotarod test to assess the walking ability in meat chickens objectively and validated this test by the 6-scale gait score system by Kestin et al. (1992). We have proven that the rotarod test can be used to mirror changes in the gait pattern. In addition, the differences in the walking ability between the three different strains could be shown using the test. However, only differences between categories 0 and 1 to 2, 3, and 4 reached statistical significance but the latency to leave indicated a continuous course from score 0 to 4. The results of the rotarod test in relation to the gait score showed the differences in the chickens' walking ability. The rotarod test provides clear, finely defined continuous data that can be used to better assess the walking ability between chickens.

It must be noted that the walking ability can only qualify the leg health in chickens to a limited extent. The leg health can be determined by several factors that are obviously visible or are only pathologically recognizable. In Bradshaw et al. (2002) a distinction is made between three pathological causes: infectious (I), developmental (II) and degenerative (III). The most common diseases in meat chickens are bacterial chondronecrosis with osteomyelitis (BCO; I), varus valgus disease (VVD, II), tibial dyschondroplasia (TD, II), osteochondrosis (III) and dermatitis (III) (Bradshaw et al., 2002). Individual diseases not only occur on its own but also interact. TD, ossification disorder or rather cartilage retardation (Leach and Nesheim, 1965) is one of the causes of lameness in fast-growing meat chickens (Farquharson and Jefferies, 2000). In studies a low association between gait score and pathological aspects was shown (Paxton et al., 2013). Sandilands et al. (2011) found a significant relationship between abnormalities and the gait score. But the correlation between these parameters was weak, as animals with a gait score better than 3 had pathological findings compared to chickens with no abnormalities and a gait score worse than 3 (Sandilands et al., 2011).

In addition, differences in stride length, stride frequency and limb angle to the body between different chickens strains (Paxton et al., 2013) must be taken into account. Fast growing meat chickens show a longer stride length and a much higher frequency of leg changes, but they walk much more cautiously and slowly than slow-growing chickens (Paxton et al., 2013) to control their stability (Kuo, 1999). The reason for this is among other things their lateral movements (Corr et al., 2003) and the displacement of the body centre in the dorso-cranial direction (Duggan et al., 2017). The validation of the rotarod test did not cover certain aspects such as characteristics of the bone (density or thickness; Reiter and Bessei (2009)) or deformities of limb (Paxton et al., 2014).

Furthermore, with the rotarod test it is possible to make a more objective comparison between different chicken strains. Further research could identify additional factors for differences in walking ability, considering such as age, strain, management (litter quality), pathological causes that may influence the walking ability.

In comparison to other technical assessments of walking ability, the rotarod test used in this thesis and its apparatus can also be adapted to respective practical needs. For example, the wooden frame and the technical equipment can be reduced in size. Furthermore, an increasing speed for the rotating rod was used for the validation. The speed can also be adapted as a fix speed (with a short initial phase from zero to a steady velocity) in order to allow the chickens to adapt to the test routine. This gives the opportunity to detect changes in walking ability at different ages, because it must be noted that animals have different behavioural abilities depending on their age or strains (Keer-Keer et al., 1996; Bokkers and Koene, 2003). It must also be kept in mind that walking velocity can be a limiting factor in the performance of the rotarod test. Fast growing chickens have a slower walking velocity than slow-growing chickens (Paxton et al., 2013) due to their altered mechanical lateral limb movement (Corr et al., 2003). Perhaps a slower, more constant velocity of the rotating rod could also help to detect other aspects of leg health.

The approach of the rotarod test is based on the individual animal level. No group level can be included, which could make the assessment even more automated and time-saving. There are studies to measure the walking ability using the activity of the animals (Dawkins et al., 2013; Aydin et al., 2015). However, expensive camera systems are needed for this approach. Furthermore, it cannot be distinguished to which extent the walking ability has changed. In dairy farms, it is easier to monitor lameness or body condition (Song et al., 2008; Grégoire et al., 2013), as there is a daily routine of milking and passing through a corridor for the each individual cow. Especially in meat poultry, this daily process does not exist, since just a daily inspection of the flock twice a day is required (TierSchNutztV, 2006). The number of chickens per commercial stable is generally about 10,000 chickens up to approx. 40.000 (Thobe, 2018) so that an individual control is hardly possible and a single chicken, which is in a bad state of health, can be overlooked. Daily inspection requires clearly defined behaviour patterns that show whether a group is in a poor state of health (Miller et al., 2019). Recommendation protocols for practical application on farms to assess animal-related parameters also indicate that animals should be measured at individual level in a sample size (Welfare Quality®, 2009; Knierim et al., 2016) in order to obtain a good assessment of the animal welfare status. It needs to be evaluate a representative sample size to mirror the state of animal health.

Nevertheless, the leg health cannot be fully predicted and some further investigations are needed in order to make statements about other causes that may contribute to changes in the walking ability.

Suitable elevated structures for growing chickens

Environmental enrichment through additional species-adapted structures can affect the chickens' behavioural patterns (Newberry, 1995) like perching (Heikkilä et al., 2006), locomotor activity (Bailie and O'Connell, 2015), foraging, or dustbathing (Baxter et al., 2017). A practicable solution to improve natural behaviour of chickens are elevated structures. It is already known that older chickens use elevated structures to roost at night (Wood-Gush, 1971; Giersberg et al., 2015) and show a high motivation to perform this behaviour (Olsson and Keeling, 2000). Nevertheless, even in the first weeks of their lives, chickens are looking for three-dimensional structures for exploration, resting or roosting overnight (McBride et al., 1969; Newberry et al., 2001; Riber et al., 2007).

Previous studies have shown that slow-growing meat chickens use elevated structures more than fast-growing chickens (Bokkers and Koene, 2003; Yngvesson et al., 2016). There are more studies that investigated the effect of offering elevated structures to fast-growing broilers (Su et al., 1999; Martrenchar et al., 2000; Sandilands et al., 2011; Norring et al., 2016) than to slow-growing (Nielsen, 2004; Lee and Chen, 2007) or to both in comparison (Bokkers and Koene, 2003; Yngvesson et al., 2016). In most studies, perches were offered (Pettit-Riley and Estevez, 2001; Bokkers and Koene, 2003; Bailie and O'Connell, 2015). Only in a few studies platforms in form of grids (Pedersen et al., 2017), straw bales (Bailie and O'Connell, 2014) or wide slats (Martrenchar et al., 2000) were offered as elevated structures. Only in a study by de Jong and van Wijhe-Kiezebrink (2014) perches and elevated platforms were offered at the same time in the same room or compartment. In other studies dealing with perches and elevated platforms, the elevated structures were offered in different compartments or rooms or not at the same time (Oester et al., 2005; Kaukonen et al., 2016; Norring et al., 2016). In one study of this thesis, described in Chapter III, three strains differing in growth performance (slow-, moderate-, and fast-growing) were offered perches and elevated grids parallel in three different heights with an intermediate ramp in each compartment. It was not a real free choice experiment, because each structure type did not offer space for 100 % of the chickens in the compartment at all heights. The available space (10 % of the chickens) was calculated on the basis of previous studies, in which only studies with fast-growing meat chickens were included (Bokkers et al., 2011; Giersberg et al., 2016). The results of my study do not show the utilization of the individual elevated structures but only the proportion of chickens that used them in total. It is possible that there was a 100 % occupancy of the highest level. Other chickens, which could not get a place on the highest level needed to find other places. Furthermore, it is possible that more chickens found space on the grids than on perches, as the perch offers a very limited area. On the grids, chickens can find place in a row of two or three at a young and older age. In addition, the different growth rates and thus the respective phenotypical body characteristics must be taken into account in relation to the elevated structures. There are differences between strains in body cover area with the same weight. For example is the mean area cover of laying hens with a weight of 1,000 g is 347 cm^2 (Spindler et al., 2013), whereas the mean cover area of fast-growing chickens with the same weight is 224 cm^2 (Giersberg et al., 2016). There are no planimetric data for dual-purpose chickens yet, but due to the large framing, they are more likely to fall within the area coverage of laying hens. With regard to perches, the chest width, which is expressed according to the genetic predisposition (Lambertz et al., 2018), must be taken into account for different growth performances in meat chickens. In laying hen husbandry it is required to offer the chickens at least 15 cm perch length (TierSchNutztV, 2006). As the chest muscles of fast-growing broilers are more pronounced than those of laying hens, more than 15 cm should be offered per chicken. At present, the German Animal Welfare label "Deutscher Tierschutzbund" prescribes 1 cm per chicken (DTB, 2017). However, in this programme there should also be a place on the perch for each chicken that is motivated to use a perch. According to our and also earlier knowledge (de Jong and van Wijhe-Kiezebrink, 2014) it is not necessary to offer elevated structures for all chickens of a group. Even if all rearing chickens use elevated structures, not all do so at the same time.

When interpreting the results about the suitability of the elevated structures, it should also be considered that the shape of the perches could perhaps have been better adapted to the strains specific morphology of the chickens, so that more animals would have used the perches. In laying hen systems, there are countless forms of perches which can differ in shape, width, length, surface, material or surface temperature (EFSA, 2015). In the present study (Chapter III), mushroom-shaped plastic perches were offered, which are very suitable for laying hens. Although the semi-circular surface was slightly roughened, young chicks may still have felt insecure due to the slippery surface. For example, wooden perches are preferred to plastic and steel seat bars (Scott and MacAngus, 2004). A further aspect could be the width of the perch: Pickel et al. (2010) found out that the balance of laying hens becomes worse with increasing diameter of the perch compared to a smaller one. Interestingly, this point coincides with the results for fast-growing chickens of the present study. The birds usually show a changed or bad balance (Kuo, 1999; Paxton et al., 2013), so that they could not have a good movement or resting on the offered perches. Therefore, it could be concluded that the fast-growing chickens avoided the perches. Furthermore, the foot size of the chickens and the choice of the perch plays an important role. The offered perch is used for adult laying hens. Pickel et al. (2011b) observed that heavy laying hens have larger feet than lighter strains. All three strains in the present study differ not only in the growth rate, but also body part growth, whereby the feet have different sizes. Furthermore, the feet are not fully grown and the chickens cannot fully grasp the offered perch because of the width, as is usual in chickens due to the digital tendon locking mechanism (Sustaita et al., 2013).

All three strains showed a higher preference for the grids compared to the perches at light and dark period, except slow-growing chickens at night. These results correspond to other studies such as those conducted by Oester et al. (2005), Kaukonen et al. (2016) or Norring et al. (2016) which found out that especially fast-growing chickens prefer platforms compared to perches. In fast-growing chickens, the preference of the grids can be explained by the shifted equilibrium and thus by the more stable support of the grids. In moderate- and slow-growing chickens, balance may not be a problem, but exploration behaviour could play a major role (Newberry, 1999), as grids provide a flat and safe ground to hop on and off (Newberry et al., 2001).

Especially at a young age, the activity is higher than in mature chickens and the exploration behaviour is more performed (Nicol, 2015). In laying hens considerably fewer birds visited elevated levels during light period compared to the night period (Campbell et al., 2016b). However, a comparison of a hybrid hen to a jungle fowl showed that the wild chickens perch more during the day than the domesticated birds (Eklund and Jensen, 2011). The chickens showed an increasing use of elevated structures with increasing age (Chapter III). Still, the usage at night until the end of the fattening period is less than during the light period. Nevertheless, there is a tendency that with increasing age, the use of elevated structures during the day remains the same after the seventh week of age, while at the dark period, the chickens showed a continuous increase in the use of elevated structures. In fast-growing chickens the usage of elevated structures during the light period decreased up to fifth week of age, whereas at night there is no descent. Although the fast-growing chickens hardly entered the elevated structures at the end of the fattening period, they showed the motivation to climb up especially at night comparable to a very high percentage of laying hens (Schrader and Müller, 2009; Brendler et al., 2014). A possible explanation are different motivation of using elevated structures for the night and the day: At the night chickens roost on high levels to protect themselves for ground predators (Newberry et al., 2001). To rest without disturbance from conspecifics (Martrenchar et al., 1999) or explore the environment (McBride et al., 1969), chickens used elevated structures during the light period.

In laying hens it is known that perches not only affect natural behavioural patterns but can also lead to skeletal damages or dermatitis and reduce the animals' welfare (EFSA, 2015). Especially the keel bones are heavily loaded when chickens sitting on the perch (Pickel et al., 2011b), which can lead to deformation or fractures, particularly in combination with collisions with other stable elements (Stratmann et al., 2015). The prevalence of the keel bone injuries in a flock can increase up to 97 % (Rodenburg et al., 2008). Bumble foot can also occur due to wooden surface (Oester, 1994). Deformations in keel bone were also found in meat chickens: Slow-growing chickens indicated a higher prevalence (19.1 %) than fast-growing chickens (2.4 %) in the presence of perches (Bokkers and Koene, 2003). In slow-growing chickens, skin irritations such as breast blisters were associated with the higher usage of perches (Nielsen, 2004). However, there is not study, to date, comparing grids with perches regarding to the prevalence of breast blisters.

In this thesis, no investigations on skeletal damages were carried out, too. In the third study (Chapter IV), we have investigated the effect of elevated structures using animal-related indicators, which will be discussed in the following chapter.

Effects of elevated structures on aspects of animal welfare

In order to investigate the effects of environmental enrichment on behaviour and health of the meat chickens, a comparison must be made between standard practices and enriched housing conditions in order to be able to predict the effects later. Considering these aspects, the results from the previous study were used (Chapter III). The grid preference of the chickens for perching was used to define the conditions of a study that compares the enriched with practical barren housing conditions using animal-related indicators. In addition, the grids were offered in three different heights (10 cm, 30 cm, and 50 cm) for deriving conclusions about the height preference of rearing chickens.

If the proportions of chickens using the grids in the three heights are added together per week, all three strains showed a similar pattern of usage for the elevated structures as in Chapter III. The moderate and slow-growing chickens showed an increase in use with increasing age at both daytimes. When only providing grids, a higher average of chickens (10%) could recorded. The elevated grids could support the motivation to use three-dimensional structures and give more chickens this opportunity to use them. At the light period, the fast-growing chickens also showed an increase in the use of elevated structures, but only up to the third week of age (descriptive analysis, study from Chapter III to the fourth week of age). During the dark period, there was no steady usage course with a clear increase, but the highest usage was in the last week of fattening period like in Chapter III. The fast-growing chickens tended to show a low use in the presence of elevated grids. This result is controversial as it could be shown that grids should be better suited for meat chickens (Oester et al., 2005; Kaukonen et al., 2016). The expectation was that the fast-growing chickens would show a higher frequency of use than in the previous study as considerable more grid area was offered. With regard to activity and weight at the end of the fattening period, the fast-growing chickens of the first study were more active (number of antenna changing at fifth week of age: 155 to 113) and about 160 g lighter than the chickens of the study from Chapter IV. In view of the small differences, it should be noticed that each group can behave differently. Chickens often behave synchronously, for example when feeding (Collins and Sumpter, 2006), there may have been a low motivation to use the elevated structures. This fact also shows that some behaviour patterns such as perching of domesticated chickens are less practiced than those of their ancestor the Red Junglefowl (Eklund and Jensen, 2011).

In order to make the elevated grids and their presentation more attractive for meat chickens especially for fast-growing chickens, a more stable and wider ramp have to be offered. In the studies of Chapter III and IV, there are indications that fast-growing chickens are motivated to use elevated structures but depending on the weight or the shape. There should be enough space for at least two birds so they can pass side by side to avoid agonistic interaction or fear situation and frustration. The angle of the ramp should also be taken into account: LeBlanc et al. (2017) showed that the needed force to make the first step from the ground to the ramp increased with rising angle. With regard to the bad walking ability of the fast-growing chickens, the ramp should not have an angle below 35°. Furthermore, the grid area should be extended, as only a few birds had space on a grid in one height. The chickens could hardly pass each other at the end of the rearing period.

Previous studies have suggested that for chickens, especially at night, not the shape but the height of the elevated level plays an important role (Lambe et al., 1997; Schrader and Müller, 2009). Based on the anti-predator thesis, it is shown that even domesticated chickens prefer the highest level for night roosting (Newberry et al., 2001). In the study of height preference (Chapter IV), the rearing chickens showed already after a few weeks a preference for the highest grids offered. Also in the second study of this thesis, the preference of the highest level of meat chickens was confirmed (Chapter III). Nevertheless, fast-growing chickens behaved exceptionally, they used to a large extent the grid level of 50 cm, but also the grid level of 10 cm (Norring et al., 2016). This could lead to the conclusion that although the birds are motivated to perch, they cannot physically afford (Bessei, 2006) to climb to the highest level.

In order to assess the influence of elevated structures and thus aspects of animal welfare of the chickens, animal-related indicators were measured. As stated in the Welfare Quality protocol for poultry (Welfare Quality®, 2009) walking ability, weight, plumage cleanliness and footpad dermatitis were assessed. In addition, a comparison of locomotor activity between enriched and barren (control) compartments was made. Environmental enrichment can affect the locomotor activity and result in better walking ability as demonstrated by Reiter and Bessei (2009) or by Bailie et al. (2013). On the one hand, in the comparison of the two experimental groups and the three different strains, a higher locomotor activity in supply of elevated structures in fast-growing chickens could be found. On the other hand, the walking ability was improved only in dual-purpose chickens by the enrichment. The walking ability of the fast-growing chickens was at a low but almost at the same level in both experimental groups. Bailie and O'Connell (2014) was also able to find a similar effect: walking ability was not improved by the offered straw bales. Norring et al. (2016) found that elevated platforms had no effect on locomotor activity, which is comparable to the results in slow- and moderate-growing chickens in the study in Chapter IV. However, the presence of grids led to an improvement of walking ability of chickens housed in commercial farms (Kaukonen et al., 2016). The dual-purpose chickens showed a better walking ability in contrast to the fast-growing chickens. The walking ability was not assessed with the common gait score system (Kestin et al., 1992), but with the objective method, the rotarod test (described in Chapter II). Interestingly, a difference could be found between the experimental groups due to the fine scaling of the rotarod test. Furthermore, the very high use of the elevated grids may positively affected the leg health (Kaukonen et al., 2016), skeletal growth and physical exercise (Yan et al., 2014). A possible explanation for the higher activity of fast-growing chickens with the offered elevated structures could be the use of the floor in combination with the arrangement of the antennas. The area under the elevated grids was accessible to the chickens by overcoming the 10 cm platform. The chickens might not have been able to reach this area, or it would have been too much physical effort, as facilities would then be further away. For this reason, the stocking density was increased by reducing the area in front of the structures and less use of elevated structures. The chickens increasingly crossed the antennas, which were located between feed and water through. In the control groups, all areas could be reached without crossing a barrier, so the antenna frequency could have been lower. However, there is contradictory observations, showing that chickens with offered barriers has an increased activity (Ventura et al., 2012).

For the farmers and their revenue, an important point is the weight of the chicken at the end of the fattening period. With regard to the predicted increased activity and better walking ability, it could be expected that the chickens will have a lower daily gain with elevated structures compared to chickens from control conditions. However, in this study (Chapter IV) there was no final body weight differences between the experimental groups with or without elevated structures in neither of the three strains. Other studies like Bizeray et al. (2002b), Bailie and O'Connell (2015) and Aksit et al. (2017) came to the same conclusion, except Martrenchar et al. (2000), who found that chickens fattened in compartments with elevated structures had a lower final body weight.

One could expect that in terms of the total plumage cleanliness, chickens with elevated structures would be less dirty than chickens kept under common housing conditions, because the birds had less contact with the litter and could dry their feathers. However, there were no significant differences in the total cleanliness score. Only in the case of chest cleanliness, a higher degree of dirt with elevated grids could be found. In a previous section, the reduction of the compartment area due to the difficult access to the area under the grids was already described. This resulted in a possible higher stocking density in the remaining area, and the distribution of excrement was more concentrated on a smaller area. This result should be further investigated, because in previous studies there were hardly any or no differences between experimental groups (Martrenchar et al., 2000; Zhao et al., 2012). Back soiling did not differ between the experimental groups in neither of the three strains (Chapter IV). In Martrenchar et al. (2000) a slightly higher level of plumage dirtiness was found in chickens kept in compartments with perches, probably because the chickens which sat on the perches defecated on chickens sitting underneath the perches.

Elevated levels can also be counted as additional allocable floor area for the calculation of the stocking density, as the example from Switzerland shows (BLV, 2018). In Germany, there are still no governmental regulations for elevated structures in the housing of meat chickens. In order to offer elevated levels as an additional allocable floor area for laying hens elevated levels must be designed in such a way that no faeces may fall through this level to the lower level (§13 Abs. 5 Nr. 1; TierSchNutztV (2006)). However, the elevated level should primarily been provided as a resting area in order to structure the housing environment in meat chickens. In the case of laying hens, all chickens must have access at all times to the required perch as resting area (§13 Abs. 5 Nr. 6; TierSchNutztV (2006)). If the requirements for additional allocable floor area and resting areas (perches) of laying hens are related to elevated grids for meat chickens, these regulations need to be adapted. It must be taken into account that meat chickens are not kept for one or more years and in aviary system with several levels. In addition, no feeding or watering facilities should be offered, as is the case with elevated areas for laying hen systems.

Furthermore, even slow-growing chickens do not use the elevated level to 100 % until slaughter. Therefore, it can be concluded that on the one hand elevated platforms do not have to be offered for all chickens in the group. On the other hand, a manure belt is not necessary, as the plumage cleanliness is good when structural elements are presented (Martrenchar et al., 2000). Further, it has to be examined to which extent the area under the elevated level is used by chickens. There are indications that young birds are using protected covers for resting (Newberry and Shackleton, 1997; Newberry, 1999). This could lead to an increased stocking density and thus to a higher pressure in the form of moist bedding (Shepherd and Fairchild, 2010). The concentrated accumulation of excrements from elevated grids and chicken in litter can contribute to poor footpad health (Ekstrand et al., 1997).

It must be considered that the expected effects of drying the bedding material and footpads of the chickens by largely using the elevated structures did not occur with the fast-growing chickens. Interestingly, worse footpads could be found in fast-growing chickens in contrast to the moderate and slow-growing chickens. However, differences in footpad health were found in moderate-growing chickens between the treatments in Chapter IV. The elevated grids had a positive effect on the footpad health compared to control group. In the literature, only an improvement in footpad health in meat chickens in terms of perch use could be found (Ventura et al., 2010; Zhao et al., 2012). Elevated platforms have not yet any effect on skin irritations (Kaukonen et al., 2016). However, it should be noted that very intensive animal care was carried out in the experimental stables used in this study and appropriate measures to improve the dryness of litter, e.g. supplementing of chop straw, were taken. For this reason, all three strains already had very good litter conditions, which reduces the probability of footpad dermatitis or hock burns.

Dual-purpose chicken strains as an alternative to meat strains and "Laying Hen Brothers"

In all three presented studies, the dual-purpose male chickens play an intermediate role in comparison with the other rearing chickens, the slow-and fast-growing strains. The dual-purpose chicken belongs with its lower growth rate to the slow-growing chickens and reaches in 9 to 10 weeks a similar living weight of approx. 2,200 g as a fast-growing chicken. In contrast, in 10 weeks slow-growing chickens (layer-type) only reach a weight of approx 1,500 g.

However, there is a renewed focus on alternatives to avoid killing male day-old chicks. The dual-purpose strain "Lohmann Dual" has not yet been intensively investigated with regard to its behaviour. In comparison to other genotypes, some behavioural demands of individual and group parameters could be clarified. It turned out that the dual-purpose chickens had a high locomotor activity similar to the slow-growing chickens. The course of the activity was alike for all three strains: At the beginning the chickens showed a higher activity than at the end of the observation period (Chapter III & IV). Furthermore, these chickens showed an increased use of the offered elevated structures which could support the walking ability of these birds.

Problems such as skeletal disorder, high body weight, muscle disorder, contact dermatitis, ascites or sudden death syndrome (de Jong et al., 2012) caused by the high growth rate of commercial broilers have so far played a smaller role in dual-purpose strain, although the dual-purpose chicken reaches similarly high weights as for example the strain Ross 308 of 2,200 g in the double time (Aviagen, 2014; Damme et al., 2015). Due to the slower growth rate, less of the above mentioned consequences may occur. In the present study, only locomotor activity, walking ability and footpad health were examined. The mortality rate (range between 1 to 3.5 %) was similar for both strains.

Despite the clear higher final body weight, compared to laying hybrid chickens the dual-purpose chickens showed in terms of activity, walking ability and use of the elevated structures similar (activity, footpad health, plumage cleanliness, walking ability) or even better usage of three-dimensional levels in terms of activity, walking ability and use of the elevated structures. With regard to the tested parameters, improvements through the use of elevated structures could be found in the dual-purpose strain (Chapter IV). The offering of elevated structures did not affect animal-related indicators in laying hybrids.

Possible further advantages of elevated structures

The results of the studies from Chapter III and IV show that rearing meat chickens use elevated structures with increasing age during the light period, except for fast-growing chickens, as well as during dark period. Usage of elevated structures may affect animal-related indicators such as locomotor activity, walking ability or plumage cleanliness. Elevated structures, as an element for enriching the housing environment (Riber et al., 2018), may also influence further biological and physiological mechanisms which have not been examined in this thesis like thermal comfort, emotional states or immune system.

Support of thermoregulation, coping and resilience

In recent years in Europe and Mediterranean regions climate changes have led to more frequent heat waves in summer time (Pasqui and Di Giuseppe, 2019). From the 7th day after hatching on, the chicken belongs to the homoeothermic group (Freeman, 1965) and needs to keep the body core temperature constant regardless of the ambient temperature. To regulate the body temperature, chickens dissipated heat for example by lifting the wings or by panting (Gerken et al., 2006). If the thermoneutral zone of chickens, especially in the last fattening period (35°C at the 4th week of age; Teeter and Belay (1996)), is exceeded, a stress reaction, the heat stress, occurs. Heat stress can lead to an activation of the hypothalamic-pituitary-adrenal-axis/stress axis (Quinteiro-Filho et al., 2012). A change of behaviour, e.g. reduced activity (Mack et al., 2013), a reduced feed intake (Mitchell and Carlisle, 1992) leading to a reduced living body weight (Mashaly et al., 2004), a reduction of the immune function (Quinteiro-Filho et al., 2010) or an increasing mortality (McDougald and McQuistion, 1980) can be the consequence. Especially in meat chickens, which have a lower heat tolerance than laying hens (Sandercock et al., 2006), heat stress is reinforced by a high stocking density, a high growth rate with a resulting high metabolism (Simmons et al., 1997). Due to the high stocking density and resulting coverage of almost the entire littered floor by chickens, the litter cannot be dried due to the lack of empty areas (Bessei, 2006). This complicates dissipation and thermoregulation (Gerken et al., 2006). In addition, the body core temperature of chickens, which is increased by rapid growth (Deeb and Cahaner, 2002), leads to an increase in the ambient air temperature of the environment. There are different approaches to support chickens in thermoregulation: nutritionally, genetically and by management based factors (Teeter and Belay, 1996; Deeb et al., 2002; Syafwan et al., 2011). Stable climate and husbandry conditions play an important role in the support of chickens' thermoregulation (Teeter and Belay, 1996). In terms of supporting biological functions (Newberry, 1995), in this case influencing thermoregulatory mechanisms, the use of elevated structures could strongly support the thermoregulation of chickens. The use of three-dimensional structures reduces the stocking density in the litter area (Martrenchar et al., 1999) and the dissipation of body heat to the ambient is supported (Estevez, 2006). As a result, heat stress can be reduced and a contribution to the welfare of the chickens can be afforded.

In previous studies, cooled perches were offered for laying hens (Pickel et al., 2011a; Gates et al., 2014) as well as for meat chickens (Estevez et al., 2002; Zhao et al., 2012), which showed good acceptance. However, from this thesis and another studies, it is known that perches are not particularly suitable for fast-growing chickens and that platforms are more likely to be offered. Platforms in form of grids can also be prepared with cooling systems, for example ventilation hoses, in order to support the cooling of the chickens.

In order to improve animal welfare, possible aversive stimuli in the husbandry environment which initiate distress have to be reduced. Aversive stimuli can cause negative emotional states like fear in animals. Fearfulness is a predisposition state whose resulting reactions serve to protect from possible dangers (Boissy, 1995). Fearful reactions such as the escape from a novel stimulus, for example when stockperson enters the compartment, can lead to pilling, scratches and consequently to the crushing of individuals and an increased mortality. Studies in farm animals have shown that fear can be reduced by enriching the environment in meat chickens (Campbell et al., 2016a; Tahamtani et al., 2018) or in pigs (Day et al., 2002; Puppe et al., 2007). The use of elevated structures can support the exploration behaviour, so that the growing chickens can learn to deal with their environment. Accordingly, elevated grids can reduce fear and protect against potential injuries and stress reactions.

High practical effort to support animal welfare

It cannot be denied that environmental enrichment entails additional costs. However, it must be taken into account whether the additional elements can only be used once or several times, and how high the respective costs are. For example, straw bales as well as plastic grids have proven positive effects on chicken behaviour and animal welfare-related indicators but differ considerably in the cost-benefit calculation (Ventura et al., 2010; Ventura et al., 2012; Zhao et al., 2012; Bailie et al., 2013; Bailie and O'Connell, 2014; Kaukonen et al., 2016; Aksit et al., 2017). Straw bales can only be used once, but they are beneficial to footpad health and litter quality, but pose an increased risk to biosafety as this element cannot be extra disinfected. In contrast to this plastic grids are purchased once, can be disinfected without problems, are height adjustable and offer additional retreat under the grids. However, it is questionable whether animal control is more difficult and whether cleaning the entire stable will require more labour depending on the position and construction of the elevated grids.

Environmental enrichment means that more costs are incurred, but also the animal welfare and animal health can be increased, which is reflected in the animal performance. At that time, there are less cost-benefits calculations yet. But one example showed that offering panels has increased egg production in broiler breeders and thus increased profits (Leone and Estevez, 2008). This resulted in a win-win situation for the chickens and the farmer (Leone and Estevez, 2008). However, a study by Kulke et al. (2014) showed that the costs are higher than the benefit for non-beak-trimmed tom turkeys with additional enrichment and a reduced stocking density of 10 %. Due to the higher mortality of turkeys without a shortened beak, a compensatory payment would have to be provided to reward the farmer for the additional efforts.

In addition, there is a trend towards “precision livestock farming” (Berckmans, 2006; Van Hertem et al., 2017) to detect automatically data such as stable data (e.g., water conversion (Kashiha et al., 2013)), behavioural data (e.g., activity , lameness (Silvera et al., 2017)) or animal health data (e.g., weight (Van Hertem et al., 2017)). In mammals such as cows or pigs there are already sensor-supported systems like kinematic 3-D-cameras that can assess the body condition score (Grégoire et al., 2013; Spoliansky et al., 2016). This type of technique can also be used to assess and evaluate the lameness in cows (Song et al., 2008; Van Hertem et al., 2014). Overall, the focus is the individual animal. Especially in dairy farms, it is easy to record a single animal separately during the production flow without additional handling.

By using the elevated structures and their course over the fattening period, animal-related parameters such as activity or weight, which could be measured automatically with appropriate built-in weighing devices, can be assessed. In addition, these data could be used to draw conclusions about animal health, for example, if there is a decrease in weights in a particular week, there may be a disease in the group. There are already automated weighing beams installed in the litter area of broiler husbandry, but they are demonstrably not used by heavy chickens (Chedad et al., 2003). The increased level could give a better overview of animal health of the group. With regard to the use of elevated structures of light and heavy chickens, no statement can yet be made. It is known that male chickens, which are usually heavier than female chickens (Aviagen, 2014), use the elevated structures less than their female conspecifics (Estevez et al., 2002). For these aspects, further investigations need to be carried out. Furthermore, it is not yet entirely clear how much area on an elevated grid the chickens occupy depending on the growth rate. This information and the proportion of chickens can be used to formulate recommendations for practical use.

Final conclusion

To date, meat chicken husbandry offers no structure in its environment to provide additional elements for acting out species-specific behaviour. At a young age chickens are already motivated to use elevated structures to explore their environment or to rest on higher level (McBride et al., 1969; LeVan et al., 2000). The shape and height of the structures and phenotypical body characteristics of the chickens were considered in existing studies in meat chickens, especially in fast-growing chickens. Elevated structures can affect animal-related indicators such as improving activity or walking ability resulting to influence animal welfare. In particular walking ability is mostly assessed with a subjective scoring system which is susceptible to reliability, leaves out different phenotypical characteristics or emotional states. This thesis presented an alternative objectively method to assess the walking ability continuously and with limited external failure. The continuous data sets were validated with the scoring systems by Kestin et al. (1992) and showed defined changes and differences between the three chicken strains and their gaits. There were evidences based on the different growing performances that the equipment should be adapt to different foot sizes and ages of the chickens, and the reliability of the rotarod test needs further clarification as well. Furthermore, possible options to provide elevated structures for meat chickens differing in growth performance were developed. Depending on the shape and height of the elevated structures, the genetic and age of the birds, and the daytime, chickens showed differences in utilisation of these structures. The most suitable shape for elevated structure were grids in slow-, medium- and fast-growing strains. There were hints that elevated structures improve species-specific behaviour and affect animal-related indicators. Nevertheless, the offered elevated structures need further research to optimize their suitability for fast-growing chickens, especially the ramp. This thesis provided approaches for objective assessment method for walking ability, a common animal welfare issue, and a structuring element in almost barren environment in meat chicken husbandry.

Summary

In commercial meat chicken housing systems, chickens are kept in barren environmental conditions only equipped with feeder and drinker lines and littered floors. It is known that growing chickens, like their ancestors and also adult chickens, already show a high motivation to use elevated structures. There are no legal requirements to provide three-dimensional levels for meat chickens like those for laying hens (Council Directive 1999/74/EC). Offering three-dimensional elements in a captive environment may improve the natural behaviour and thus to a better animal welfare. To support natural behaviour like locomotion, perching and roosting, rest and sleep, and exploration, elevated structures has to be chosen shape and height whose are in accordance with the phenotypical characteristics of growing chickens, and their effect on animal-related parameters has to be evaluated. Currently, male laying chickens are fattened increasingly in order to avoid the killing of male day-old chickens. Another alternative to solve this ethical issue is to use dual-purpose chicken strains. To date, for both strains there is a little knowledge about species-specific behaviour or needs. In this thesis these two strains as slow- (Lohmann Brown Plus/ Classic) and medium-growing (Lohmann Dual) meat chickens are compared with a fast-growing strain (Ross 308). Considering leg problems as a major common issue occurring in fast-growing meat chickens and the resulting reduced walking ability, the use of elevated structures may lead to increasing animal welfare through greater motivation for movement due to physical training. Assessment methodology of walking ability like the gait score system after Kestin et al. (1992) provide a fast but subjectively method that is depending on observer assessment and chickens' performance. The walking ability is closely linked with the motor coordination of the body system. The rotarod test, which is a common test for motor coordination in rodents, probably can be used to assess walking ability in meat chicken. The first aim of this thesis was to develop and validate an appropriate object method for assessing the walking ability, the rotarod test. The second aim was to further design and validate elevated structures as an environmental enrichment of meat chickens.

Objectively methods to assess the walking ability exists in different variants but require a high level of technical equipment. The rotarod test, an established pharmacological test in rodents, provides a simple apparatus to assess the motor coordination. The motor coordination is linked with the walking ability, which we used to assess the gait with a modified rotarod test for chickens (Chapter II). In order to validate the results of the rotarod test, we first assessed the walking ability

with the categorical gait score system. For the rotarod test, the same chickens were placed on a steady rod. Afterwards the test started with an increasing velocity of the rotating rod. The target value was the latency to leave the rotating bar. We found that the latency to leave was associated with the gait score and provides an alternative objectively method. Consequently, we applied this test in one of the successive studies.

To develop suitable elevated elements for meat chickens, we offered common types of structures such as perches and grids in the chickens' environment (Chapter III). For analysing the preferences at different daytimes, we used a time-sampling observation for each week of age. Additionally, we measured the activity to characterise the differences between the three different strains. All chickens showed a motivation to use the offered elevated structures, especially the grids were more frequented as the perches by the birds. The activity decreased during the observation period in all strains and can be associated with the different percentage of using the elevated structures. The grid preference was used in the subsequent study to assess the possible effects of environmental enrichment on the chickens' behaviour.

For suitable three-dimensional elements in meat chickens, not only the shape but also the height is essential for optimal elevated structures. We provided elevated grids in three different height in half of the disposable compartments. The other compartments were equipped only with common elements such as feeder and drinker as control group (Chapter IV). By these two groups, we assessed effects of the environmental enrichment on animal-related indicators like locomotor activity, walking ability, weight, plumage cleanliness and footpad health. The both slower growing strains showed a great preference for the highest level at the end of the observation period. The fast-growing chickens preferred the lowest grid level and showed a lesser usage of elevated structures than in the previous study. In regard to the animal-related indicators in comparison between enriched and control groups, we found an increased walking ability in medium-growing chickens and evidence of an improvement in locomotor activity in fast growing chickens. The slow-growing chickens were not affected by an environmental enrichment.

To conclude, a modified rotarod test for chickens affords an objective easy method for assessing walking ability in chickens and provide fine-scaled measure of an animal welfare indicator. High-level grids are suitable as elevated structures for slow- and medium-growing chickens. In fast-growing chickens, grids are preferred but the access need further research in terms of ramp, angle and space. Also, the effect on animal-related indicators of environmental enrichment should be subjected to further investigations. Providing elevated structures can enrich and improve the environmental structure of growing chickens of different growth performances. Natural behaviour like locomotor activity, perching and roosting or exploration of chickens can also be supported.

Zusammenfassung

In der kommerziellen Masthühnerhaltung werden Hühner in einer strukturarmen Haltungsumwelt gehalten, die nur mit Fütterungs- und Tränkvorrichtungen sowie einer eingestreuten Bodenfläche ausgestattet ist. Juvenile Hühner, ebenso wie ihre Vorfahren und adulte Tiere, sind motiviert, dreidimensionale Ebenen zu nutzen. Anders als bei Legehennen gibt es für die Haltungsbedingungen von Masthühnern keine gesetzlichen Vorschriften, die dreidimensionale Strukturen vorschreiben (Council Directive 1999/74/EC). Das Angebot von erhöhten Strukturen in der Haltungsumgebung kann die Ausübung von natürlichen Verhaltensweisen verbessern und folglich das Wohlbefinden steigern. Um die natürlichen Verhaltensweisen, wie beispielweise Lokomotion, Aufbaumen, Ruhen, Schlafen und Explorationsverhalten zu unterstützen, müssen unter Berücksichtigung der phänotypischen Körperkonditionen der juvenilen Hühner die Form und Höhe der erhöhten Strukturen ausgewählt und deren Effekte auf tierbezogene Parameter überprüft werden. Derzeit werden männliche Legehühner zum Vermeiden der Tötung der männlichen Eintagsküken als Alternative gemästet. Ein weiterer Ansatz ist die Nutzung des Zweinutzungshuhns. Derzeit ist noch wenig über das Verhalten und die Bedürfnisse dieser Linien bekannt. Aus diesem Grund werden in dieser Dissertationsschrift diese zwei als langsam wachsend (Lohmann Brown Plus/ Classic) und mittel langsam wachsend (Lohmann Dual) mit einer schnell wachsenden (Ross 308) Hühnerlinie verglichen. In Anbetracht des häufig auftretenden Problems der Beinschäden und daraus resultierenden reduzierten physischen Aktivität der Masthühner, speziell bei schnell wachsenden Hühnern, können angebotene erhöhte Strukturen zu einer gesteigerten Bewegungsmotivation und folglich physischen Training führen. Eine Methode um die Lauffähigkeit von Masthühnern zu bewerten ist das Gait Score System von Kestin et al. (1992), welches eine einfache aber subjektive Methode darstellt, die abhängig von der Beurteilung des Beobachters und der Laufpräsentation des Huhns ist. Die Lauffähigkeit selbst hängt eng mit der motorischen Koordination des Bewegungsapparates zusammen. Die motorische Koordination kann mit Hilfe des Rotarod-Tests, welcher bei pharmakologischen Untersuchungen bei Nagetieren Anwendung findet, erfasst werden. Dieser Test kann eine objektive Methode zur Bewertung der Lauffähigkeit bei Masthühnern darstellen. Das erste Ziel dieser Dissertationsschrift umfasste die Entwicklung sowie Validierung einer geeigneten objektiven Methode zur Beurteilung der Lauffähigkeit von Hühnern. Das zweite Ziel war es erhöhte Strukturen als zusätzliche Elemente in der Haltungsumgebung weiterzuentwickeln und zu validieren.

Objektive Methoden zur Beurteilung der Lauffähigkeit gibt es bereits zahlreiche, die allerdings einen hohen technischen Aufwand haben. Der Rotarod-Test, ein etablierter pharmazeutischer Test für Nagetiere, bietet einen einfachen Aufbau an, um die motorische Koordination zu untersuchen. Die motorische Koordination ist eng mit der Lauffähigkeit verbunden. Diesen Aspekt nutzten wir, um mit einem für Hühner angepassten Rotarod-Test die Lauffähigkeit zu beurteilen (Chapter II). Um die Ergebnisse des Rotarod-Tests validieren zu können, wurde zunächst die Lauffähigkeit der Hühner mit dem Gait Score System erfasst. Dieselben Tiere wurden dann auf eine zuerst ruhende und dann allmählich rotierende Stange gesetzt, deren Geschwindigkeit anstieg. Die Zielgröße war die Latenzzeit des Tieres bis zum Verlassen der rotierenden Stange. Wir fanden eine positive Korrelation zwischen dem Gait Score und der Latenzzeit auf der rotierenden Stange. Der Rotarod-Test bietet eine alternative objektive Methode zur Beurteilung der Lauffähigkeit. Aus diesem Grund wurde dieser Test in einer der folgenden Studie angewendet.

Um geeignete erhöhte Strukturen für Masthühner zu entwickeln, boten wir zwei gebräuchliche Formen wie Sitzstangen und Gitterroste in der Haltungsumgebung der Hühner an (Chapter III). Die Nutzung der zwei Elemente wurde für jede Lebenswoche und unterschiedlichen Tageszeiten mit einem time-sampling Verfahren analysiert. Zusätzlich wurde die lokomotorische Aktivität zur phänotypischen Charakterisierung der drei verschiedenen Linien erfasst. Alle Hühner nutzen die erhöhten Strukturen. Hierbei wurden besonders die Gitterroste präferiert. Die Aktivität sank über den Beobachtungszeitraum bei allen Linien und unterschied sich aufgrund der Wachstumsrate. Dieser Unterschied zeigte sich auch in der unterschiedlichen Nutzungsintensität der erhöhten Strukturen. Die bessere Eignung der Gitterroste als erhöhte Struktur wurde in der darauffolgenden Studie verwendet, um den Effekt durch eine Anreicherung der Haltungsumwelt auf tierbezogene Parameter zu untersuchen.

Nicht nur die Form, sondern auch die Höhe der angebotenen Struktur spielt eine wesentliche Rolle für die Anreicherung der Haltungsumwelt bei Hühnern. Wir boten die Gitterroste in drei verschiedenen Höhen in der Hälfte der verfügbaren Abteile an. Die andere Hälfte der Abteile wurde nur mit den üblichen Vorrichtungen wie Futtertrögen und Tränken ausgestattet (Chapter IV). Durch diese zwei Gruppen konnten wir den Einfluss von einer angereicherten Haltungsumgebung mit den tierbezogenen Indikatoren wie lokomotorische Aktivität, Lauffähigkeit, Gewicht, Gefiederverschmutzung und Fußballengesundheit beurteilen. Die beiden langsam wachsenden Linien präferierten die höchste Ebene der angebotenen Höhen am Ende des Beobachtungszeitraum. Die schnell wachsenden Hühner hingegen bevorzugten die niedrigste Höhe der Gitterroste, anders als in der Studie zuvor. Im Vergleich der zwei unterschiedlichen Haltungsumgebungen, fanden wir eine erhöhte Lauffähigkeit bei den mittel langsam wachsenden Hühnern und einen Hinweis zur Verbesserung bei den schnell wachsenden Tieren in Bezug auf die lokomotorische Aktivität. Die erhöhten Strukturen hatten keinen wesentlichen Einfluss auf die langsam wachsenden Hühner.

Es kann zusammenfassend gesagt werden, dass der für Hühner modifizierte Rotarod-Test eine objektive Methode zur Beurteilung der Lauffähigkeit bietet und als eine feinskalierte Messung für einen Tierschutzindikator genutzt werden kann. Für langsam wachsende Hühnerlinien eignen sich erhöhte Gitterroste zur Anreicherung der Haltungsumwelt. Bei schnell wachsenden Hühnern sind zwar die Gitterroste ebenfalls angemessen, allerdings müssen hier noch weitere Untersuchungen erfolgen, um den Zugang, beispielweise mit der Anpassung der Rampe, zu optimieren. Erhöhte Strukturen können als Anreicherung beziehungsweise Strukturierung der Haltungsbedingungen bei Masthühnern beitragen. Zusätzlich können diese, natürliche Verhaltensweisen wie die Lauffähigkeit, das Aufbaumen und Explorationsverhalten von Hühnervögeln unterstützen.

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Theses

Objectives of research

To date, meat chicken husbandry offers no structure in its environment to provide additional elements for acting out species-specific behaviour. At a young age chickens are already motivated to use elevated structures to explore their environment or to rest on higher level (McBride et al., 1969; LeVan et al., 2000). Offering three-dimensional elements in a captive environment may improve the natural behaviour and thus to a better animal welfare. To support natural behaviour like locomotion, perching and roosting, rest and sleep, and exploration, elevated structures has to be chosen shape and height whose are in accordance with the phenotypical characteristics of growing chickens and, thus, their ability to use environmental enrichment. In order to assess the effects of enrichment elements on animal behaviour and animal health, animal-related indicators such as walking ability and locomotor activity, also leg and footpad health and plumage cleanliness can be used. Especially walking ability is a welfare issue in fast-growing meat chickens. Assessment methods for the walking ability like the common gait score system indicated some weakness in objectivity as this system strongly depends on the observers' assessment and can lead to wrong conclusions. The walking ability is closely linked with the motor coordination of the body system. The rotarod test, which is a common test for motor coordination in rodents, probably can be used to assess walking ability in meat chicken. The first aim of my thesis was to develop and validate an appropriate objective method for assessing the walking ability. We developed a suitable rotarod apparatus for chickens and evaluate the relation to the gait score system. This new objectively methodology could be used in one of the studies in this thesis. The second aim of this thesis was to further design and validate elevated structures as environmental enrichment for chickens differing in growth rates. Firstly, the preference of the shape (perch or grid) and height were analysed depending on strain, age and daytime. Secondly, based on first study, in the successive study, only grids in three different heights were provided in half of the available compartments to deal with two objectives. This study focussed on the height preference and possible effects on animal behaviour and animal health. In respect of all aims, three different strains were used for the following studies (slow-growing: Lohmann Brown Plus/Classic; medium-growing: Lohmann Dual; fast-growing: Ross 308).

Main results

Modification of a rotarod test for chickens in order to assess objectively their walking ability (Published in: Animal Welfare, 2019)

- The walking ability of all three strains could be tested with the rotarod test
- Duration on the rotating rod correlated positively with the gait scores
- Provide an alternative objectively method for assessing the walking ability with fine-scaled measurements

Evaluation of suitable elevated structures that differ in shape and height in accordance to strain, age and daytime (Published in: Poultry Science, 2018)

- Use of elevated structures differed in growth performances – lower use in fast-growing chickens compared to slower growing chickens
- Strains differing in growth performance preferred grids in comparison to perches regardless of age and daytime

Evaluation of suitable elevated structures differing in heights with animal-related indictors in comparison to conventional housing systems (Published in: Frontiers in Veterinary Science, 2019)

- Slow- and medium-growing strains preferred higher level of elevated structures at the end of the observation period, fast-growing chickens used more the lowest provided level
- Tendency to higher activity through offered elevated structures in fast- and medium-growing chickens
- Improving of walking ability in medium-growing chickens with enriched compartments compared to control compartments

Conclusion

To conclude, a modified rotarod test for chickens afford an objective easy method for assessing walking ability in chickens and provide fine-scaled measure of an animal welfare indicator. High-level grids are suitable as elevated structures for slow- and medium-growing chickens. In fast-growing chickens, grids are preferred but the access need further research in terms of ramp, angle and space. Also the effect on animal-related indicators of environmental enrichment should be subjected to further investigations. Providing elevated structures can enrich and improve the environmental structure of growing chickens of different growth performances. Natural behaviour like locomotor activity, perching and roosting or exploration of chickens can also be supported.

Selbstständigkeitserklärung

Hiermit erkläre ich, dass ich die vorgelegte Arbeit selbstständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Ort, Datum, Unterschrift