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Effect of rearing techniques and feed composition on productive traits, bird welfare and quality of poultry products

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CONTENTS

CHAPTER 1

General overview on animal welfare

1.1 - GENERAL INTRODUCTION

Awareness about animal welfare seems to have increased since the early 1990s. An important awakening factor has been the various scandals involving the meat sector and debates in the mass media, followed up by an increasing amount of legislation to improve the welfare of farm animals within the EU.

Across Europe, a large majority of consumers say that farm animal welfare is important. This ranged from 69% of respondents in the Netherlands, 73% in the UK, 75% in France to 83% in Hungary and Sweden. Norway and Italy scored the highest, with 84% and 87% respectively. This situation has naturally affected the political interest and attitude accordingly.

Recently, the European Commission adopted a new Action Plan on the protection and welfare of animals. It outlines concrete measures to improve the protection and welfare of animals over the next five years. For the period 2006–2010, five main areas of action have been set out to meet this objective: upgrading minimum standards for animal welfare; promoting research and alternative approaches to animal testing; introducing standardized animal welfare indicators; better informing animal handlers and the general public on animal welfare issues; and supporting international initiatives for the protection of animals. The Action Plan, which was called for by the European Parliament and the Council, aims to clarify existing EU legislation on animal welfare while suggesting proposals for areas currently lacking of sufficient actions. The Plan proposes that current minimum standards for animal welfare be upgraded across the EU, in line with the latest scientific information and public demands. It suggests expanding these minimum standards to include species currently not covered by EU provisions.

Moreover, consumers' concern and the apparent demand for information on animal welfare was the starting point of an EU funded project born on 2004 - Welfare Quality® (Blockhuis, 2007). It is an EU funded project designed to integrate farm animal welfare into the food chain by addressing such societal expectations and market demands, and developing reliable on-farm welfare assessment systems. The Welfare Quality® project therefore set out to develop scientifically based tools to measure animal welfare and to convert these measures into accessible and understandable information.

From the farmer's point of view, increased animal welfare standards in Europe have resulted in costs linked to changes in production systems and other investments. Estimations of the costs have been produced for new animal welfare proposal such as egg production, broiler meat and sows. It is clear that the economical consequences have to be calculated or estimated carefully to be able to manage the professional and political debate (Husu-Kallio, 2008).

The research work on animal welfare is more demanding in many ways compared to animal health or to most of the food safety questions. It is easy to understand the need for more research on animal welfare. Without studies and scientific arguments it is very easy to postpone any good initiatives for new legislation.

The objectives of this thesis was as initial step to investigate the chicken rearing condition adopted in our country to assess the Italian situation in relation of the European Directive "Laying down minimum rules for the protection of chickens kept for meat production" (Commission of the European Communities, 2005), with the aim to check the conditions of chicken production, considering both the performance traits and the carcass lesions. Secondly, the same evaluations have been carried out in an experimental facility to compare the effects of 2 litter types (wheat straw and wood shaving) and 2 different rearing conditions (Welfare and Standard) on broiler welfare indicators, performances, carcass and meat quality, both in winter and summer seasons. As third step of the poultry welfare investigation, laying hen enriched diets have been administered to evaluate whatever is possible to improve the products characteristics as well as the welfare status of the bird with the nutrition.

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CHAPTER 2

Italian chicken rearing conditions: effects of litter quality and stocking density on productivity, foot dermatitis and carcass injuries

2.1 - INTRODUCTION

Intensive rearing of broilers in large flocks is highly criticised by animal-welfare associations and is frequently questioned by consumers. With increasing consumer concern for the welfare of reared animals and also for the quality of the food, the need for assessment systems of welfare, applicable to commercial situations, is becoming more evident. Therefore the European Commission has decided to propose a specific Council Directive “Laying down minimum rules for the protection of chickens kept for meat production” (Commission of the European Communities, 2005) after a report about broilers rearing conditions published by the Scientific Committee on Animal Health and Animal Welfare (2000). The directive proposal of the Commission aims “to introduce animal welfare improvements in the intensive farming of chickens by means of technical and management requirements for the establishments, including enhanced monitoring on the farms and an increased flow of information between the producer, competent authorities and the slaughterhouse based on a welfare monitoring program of the chicken carcasses after slaughtering”.

An important parameter taken into account by the European Commission in the proposal is the stocking density of chickens kept in the farm which does not exceed at any time 32 kg live weight and ensure that the average of the maximum stocking density of the last 3 crops is not greater than 30 kg per m^2 to ensure good welfare conditions to the chickens. As for lighting, all building shall have light with an intensity of at least 20 lux during the light periods measured at the bird eye and illuminating the whole of the floor area, the light must follow a 24-hour rhythm and include periods of darkness lasting at least 8 hours in total with the exception of the first and last 3 days of life. Moreover other specifications regarding drinking, feeding and litter management as well as ventilation, heating, cleaning and inspection procedures are mentioned. The competent Authority may provide that chickens be kept at a maximum stocking density which does not at any time exceed 40 kg per m^2 and ensure that the average of the maximum stocking density of the last 3 crops is not greater than 38 kg per m^2 provided that the owner complies with several requirements. The main needs are the following: CO_2 and NH_3 have not to exceed 3,000 ppm and 20 ppm respectively at the level of chicken heads; the inside temperature, when the outside temperature exceeds 30°C, has not to exceed the outside temperature by more than 3°C; the inside relative humidity, when the outside temperature is below 10°C, has not to exceed 70%. Moreover low mortality rate, low foot pad score, frequent inspections of the poultry house are requested; in particular mortality rate must be lower than 1%+ 0.06% multiplied by the slaughter age of the flock in days. The foot pad dermatitis score has not to exceed 50 points calculated on a 200 feet sample per flock. The foot score is established according to the following procedure: 0= no lesions, 1= mild lesions and 2= severe lesions. The number of feet from group 0 is not taken into account. The number of feet from group 1 is multiplied by 0.5, the number of feet from group 2 by 2. Both results are added, then the total is divided by the sample size and multiplied by 100. On the 28th of June 2007, the Council of the European Union approved the Directive 2007/43 EC. From the original draft, the maximum density was fixed at 33 kg liveweight/m^2, instead of 32, with the possibility to increase the limit to 39 and 42 kg liveweight/m^2, instead of 38, if some of the above mentioned requirements are fulfilled. The period of darkness was reduced to 6 h a day instead of 8 h. As for the foot pad dermatitis score, even if it is considered from a scientific point of view a good indicator of broiler welfare, it was elicited from the Directive. Member states shall bring into force the laws, regulations and administrative provision necessary to comply with the directive by 30 June 2010 at the latest (European Commission, 2007).

The European Commission decision to limit stocking density is in conflict with the point of view of the producers who want to increase the number of birds housed to improve their profit. Stocking density seems to be a critical problem linked to the increase of mortality, to the worsening of litter conditions, and to the increase of health problems like leg disorders and contact dermatitis, in particular food pad dermatitis, hock and breast burn. Footpad dermatitis is a contact dermatitis affecting the plantar region of the feet (Greene *et al*., 1985; Martland, 1985; Ekstrand *et al*., 1997; Martrenchar *et al*., 2002; Mayne, 2005). The lesions can develop in less than a week, first accompanied by a discoloration of the skin, later the erosions can develop into ulcerations with inflammatory reactions of the subcutaneous tissue and hyperkeratosis of the surface area diagnosed by the observation of brown-black lesions on the feet. Crusts, formed by exudates, litter and faecal material, often cover the ulcerations. Foot pad dermatitis can lead to a painful condition of the broiler which means a decrease on walking ability and growth rate but also can cause a meat hygiene problem, as these lesions may be a gateway for bacteria. Several factors can affect the foot pad lesion incidence, such as broiler genotype, body weight, sex, diet composition, management parameters and type of poultry facilities and equipments. High stocking density increased the incidence of food pad dermatitis, hock and breast lesions (McIlroy *et al*., 1987; Dozier *et al.*, 2005) as well as bird growth, particularly with densities exceeding 30 kg live weight/m^2 (Bessei, 2006); however increased ventilation rates can alleviate these negative effects (Grashorn and Kutritz, 1991). On the contrary, Martrenchar *et al.* (2002) did not find any influence of stocking density on the prevalence of foot pad dermatitis. Moreover poor litter quality, with particular regard to its moisture content, and climate conditions, in particular humidity (McIlory *et al.*, 1987), exhibit a negative effect on the incidence of contact dermatitis (Berg, 1998; Algers and Berg, 2001; Dozier *et al.*, 2005). As for the amount of litter, a thinner layer should be better than a thick layer since chickens, scratching and turning litter material, are able to maintain it in dryer conditions (Ekstrand *et al.*, 1997). Among the dietary factors, deficiency of biotin and excess of crude protein have been well documented for turkeys (Clark *et al*., 2002). Feed efficiency, broiler mortality and carcass quality are not influenced by the stocking density (Feddes *et al.*, 2002) while feed intake and body weight gain decrease as stocking density increases because physical access to feed and water is impeded (Shanawany, 1988; Dozier *et al*., 2005).

Considering the lack of published reports concerning the overall Italian rearing conditions of broiler chickens, a survey was carried out to assess the welfare conditions of broiler reared in the most important poultry companies in Italy to verify if they are in accordance with the advices given in the European proposal COM (2005) 221 final.

2.2 - MATERIALS AND METHODS

2.2.1 - Experimental design

Five integrated Italian poultry production companies (here named A, B, C, D and E), which represent about 70% of the domestic broiler production, accepted to be part of this survey. Each farm was chosen on the basis of its production indexes but also of the poultry house and equipments characteristics that had to be representative of those prevailing in the integrated group. The farms were geographically spread in the north of

Italy, where the majority of the domestic poultry production is concentrated. The survey was carried out in 10 flocks, 5 in winter and 5 in summer, on a total of 279,640 birds. All broilers were reared indoors in controlled environment houses: 3 houses were equipped with tunnel ventilation and 2 with cross ventilation. Ross and Cobb males and females were reared respecting the current use of the two strains in Italy where around 80% of birds are Ross. Commercial diets and water were provided *ad libitum*. Chopped wheat straw and rice hulls made up the litter, deal out in different amounts according to the season. The management procedures of the farms were subjective statements made by the companies.

2.2.2 - Rearing measurements

Management and husbandry data were collected from each farm. These information, included commercial strain, sex as well as slaughtering age of birds. According to the usual Italian management procedures, about 20% of birds was removed 1 or 2 times during their life (thinning) in order to have the following classes of weight: light-size (1.6-1.7 kg), medium-size (2.4-2.5 kg) and heavy-size (3.2-3.8 kg). Stocking densities were calculated as bird number per square meter considering the housed birds or the live birds remained after thinning. Moreover stocking densities were recorded also as kg of live weight per m^2 for each slaughtering. Mortality was daily recorded and expressed both as a percentage on the basis of the whole rearing cycle and as percentage calculated with the formula appearing on the European proposal COM (2005) 221 final (1% added to 0.06% multiplied for the age of the birds at each slaughtering). Feed intake was measured and feed efficiency was calculated on the basis of whole feed consumed throughout the cycle as well as on the total weight of slaughtered birds. For litter condition evaluations, an area of 0.4 m^2 of litter was collected from the main representative areas of the floor: under the feeding troughs, under the drinkers and in the intermediate lanes. In total 20 samples per each poultry house were collected. Every sample was weighed on site immediately after collection and litter moisture was determined by drying the samples in an oven at 100° C for 12 hours. Litter pH was measured with a pH-meter on 2 sub-samples obtained from the 20 samples collected.

2.2.3 - Slaughtering measurements

Birds of the 3 classes of weight (light, medium and heavy size) were observed on the slaughterhouse chain and carcass data were collected. Samples of 200 birds/market class/farm/season for a total of 4,800 chickens were controlled to check the incidence of carcass injuries such as skin lesions, bruises and bone fractures. Only bruises and haematomas attributable to the husbandry period were considered on the basis of presence or absence of the lesion.

Furthermore for each flock, class of weight and season, 200 feet were systematically collected for macroscopically examination and scored in three classes of foot pad dermatitis (FPD) as follows: 0= no lesions, 1= mild lesions and 2= severe lesions according to the classification reported by Ekstrand *et al*. (1997). The feet number of each class was multiplied according to the formula reported in the EU proposal COM (2005) 221 final. The number of feet from class 0 did not contribute to the score. The number of feet from class 1 was multiplied by 0.5, the feet from class 2 were multiplied by 2 and those scores were added. Then the total was divided by the sample size and multiplied by 100. Moreover on the same feet the incidence of hock burn was evaluated on the basis of the presence or absence of the lesion.

2.2.4 - Statistical analysis

The data concerning litter characteristic and foot dermatitis have been submitted to one way ANOVA considering as main effects season and farms and means were separated by using the Student Newman Keuls test. Moreover the Pearson's correlation coefficients for stocking density, litter pH, moisture, foot pad dermatitis, hock burns were calculated, (Statsoft Inc., 2001).

2.3 - RESULTS

2.3.1 - Rearing conditions and productive traits

In Table 1 and 2 are summarised the housing conditions and productive traits recorded during winter and summer respectively. Although poultry house characteristics were very similar, the farmers kept the birds at different densities according to their own procedures. In winter the stocking density at housing ranged from 15 to 18 birds/m^2 whereas the maximum density ranged from 24 to 32 kg of liveweight/m^2. In summer, stocking density at housing ranged from 14 to 17 bird/m^2 and the maximum density ranged from 24 to 31 kg of liveweight/m^2.

Feed conversion rate (FCR), calculated on the whole cycle, ranged from 1.8 to 2.1 kg without any consistent differences between the seasons.

Mortality values ranged from 2.9 up to 9.3% in winter and from 2.6 to 6.7% in summer. Data were very different among the farms and were lower than the mortality score of the EU proposal COM (2005) 221 final with the exception of one farm both in winter and in summer.

2.3.2 - Carcass quality

In Table 3 and 4 the carcass evaluations carried out in winter and summer on the slaughterhouse chain are summarized. The incidence of skin lesions was very low for all the flocks except for flock E in which the value reached 17.5%. Moreover a high incidence of bruises, reaching 24 and 26% was detected in medium and heavy classes of weight (Table 3). However the occurrence of bruises recorded for farm E is mainly due to an unsuitable handling of broilers during catching and transport. This outcome is also supported by the high incidence of bone fractures both in wings and legs.

2.3.3 - Foot pad dermatitis

The incidence of foot pad lesions and hock burns recorded in winter and summer are reported in Table 5 and 6. In winter, the FPD scores ranged from 24 to 185, exceeding in all the flocks, with the exception of flock D, the value of 50 points considered by the EU proposal COM (2005) 221 final the threshold that must not be exceeded with stocking densities higher than 30-32 kg of live weight/m^2. In summer too the variation between flocks of the FPD score was very large (from 3 to 168) but only flock E exceeded 50 points. From the statistical analysis of data, emerged that foot pad dermatitis score was significantly higher in flock E than that of farm D ($P < 0.01$, Figure

1). Moreover in winter the FPD score was significantly higher than that recorded in summer ($P < 0.01$, Figure 2).

Concerning the hock burn we observed higher values in winter but, due to the high variability among farms, the differences did not appear significant (Figure 2). Moreover in farm B and E the values were significantly higher ($P < 0.01$) than those recorded in other farms (Figure 3).

The litter characteristics are summarized in Table 7. The most used material in Italy is chopped wheat straw whereas wood shavings and rice hulls are less used. The moisture content was consistently higher ($P < 0.05$) in winter with values close to 40% in comparison with summer (27%). The litter pH ranged between 6.9 to 8.4 without significant differences neither for season nor for farm. From the statistical analysis of data concerning some litter traits (moisture and pH) and foot lesions, positive correlations between litter humidity and foot pad dermatitis score ($r = 0.87$; $P < 0.05$) as well as hock burn incidence ($r = 0.75$: $P < 0.05$) were detected. Moreover the two latter traits were also significantly correlated each other ($r = 0.76$; $P<0.05$) (Table 8). An important outcome is that stocking density is not correlated neither with litter moisture and pH nor with foot dermatitis.

2.4 - DISCUSSION

The stocking density ranged between 24-32 kg liveweight per m^2 in winter cycles and 24-31 in summer. The densities recorded in summer were similar to those observed in winter in spite of the advice given by the breeding companies which recommend to keep the birds at a lower density during the hot season to make easy the heat dissipation from the bird body, alleviating thus the negative effects of high environmental temperature. The stocking density adopted during summer can be explained as a response to the increasing demands of chicken meat from the domestic tourist industry. This different choice of Italian farmers relied on the efficient cooling and ventilation equipments and on the application of innovative strategies for increasing air speed at the broilers levels (use of curtains). The densities currently used in Italy are in accordance with the European proposal COM (2005) 221 final which suggests to keep broilers at a density lower than 30-32 kg live weight/m^2 and to not exceed 38-40 kg live weight/m^2. From a welfare point of view, high stocking densities may create various problems such as the increase of air ammonia and heat produced from the birds which can lead to stressful conditions and cause the death of chickens. The effect of stocking density is still controversial: Elwinger (1995) and Feddes *et al.* (2002) reported that stocking density significantly affected broiler performance, in particular growth rate, carcass traits and uniformity. On the contrary Blokhuis and Van der Haar (1990), studying the behaviour of chicks, found that the percentage of birds drinking and eating was not significantly influenced by the housing density.

The mortality rates in summer and winter agree with the mortality score calculated following the formula reported in the EU Proposal COM (2005) 221 final except for farm B. It is interesting observe that the highest mortality has been recorded for both the seasons in that farm in which stocking density was the lowest. Several Authors had found no relationship between stocking rate and mortality (Thomsen, 1994; Feddes *et al.*, 2002). Similarly Proudfoot *et al.* (1979) and Puron *et al*. (1995) supported that an increase of stocking density resulted in a significant reduction in body weight but mortality and feed conversion were not significantly affected. Heier *et al*. (2002) too, investigated the factors associated with mortality identifying housing factors and management as the main reasons. Furthermore, the above mentioned Authors found in their experiment that mortality during the first week was the lowest in flocks with the highest stocking density, probably because when chick density is high, the heat loss is reduced and also because in a dense flock it is easier for the untrained chicks to find the way for food and water. On the contrary, Hall (2001) affirmed that mortality is affected by stocking density when birds are reared at 40 kg of liveweight/m^2 compared to birds kept at 34 kg liveweight/m^2.

The incidence of damaged carcasses was very low and did not seem related to the stocking density, at least with density lower than 30 kg live weight/m^2 as those adopted in our survey. Hall (2001) found that increasing the stocking density from 34 to 40 kg of live weight/m^2, increased the percentage of birds exhibiting wing and leg bruises. Feddes *et al*. (2002) found that stocking densities has little effects on grading or removal of carcasses from the processing line due to condemnation.

In this survey the FPD scores were generally above the maximum limit advised by the EU proposal COM (2005) 221 final, although the stocking densities were lower than 30-32 kg live weight per m^2. Some Authors claim that stocking density alters foot pad score particularly with values over 40 kg of live weight/m^2 (Dozier *et al.*, 2005; Jones *et al.*, 2005). Our results does not show any significant correlations between stoking density and foot dermatitis probably because the stocking rate was lower than 32 kg liveweight/m^2.Our data are in accordance with previous findings of Algers and Svedberg, (1989) who found that the incidence of dermatitis varies with humidity of the litter and ammonia concentration but not with stocking density as such. Therefore the results seem to support the concept that stocking density per se is less important to bird welfare than litter characteristics.

Moreover the incidence of FPD lesion was significantly higher in flocks reared in winter in comparison with those kept in summer since the reduced ventilation rate, as normally happens in winter for economical reasons, does not remove the excess of humidity from the air and litter. Several experiments showed that the prevalence of contact dermatitis in broilers is related to multiple factors such as biotin deficiency (Harms and Simpson, 1975; Harms *et al.*, 1977; Whitehead and Bannister, 1981), high protein dietary level (Jensen *et al*., 1970; Clark *et al.*, 2002), sex and age of the birds, stocking density and drinkers design (Bray and Lynn, 1986), but the main factor seems to be the litter condition. Meluzzi *et al.* (2004) observed that a high incidence of foot pad lesions in birds kept at a stocking density of 35 kg/m^2 is associated to a high litter nitrogen content which leads to a lower litter pH. The litter status is a result of different factors such as type of material, depth, friability and moisture as well as housing conditions, technical equipments and management. When the litter is wet, sticky and compact, dermatitis are commonly seen (Greene *et al*., 1985; Martland, 1985; McIlory *et al*., 1987; Ekstrand *et al*., 1998; Dawkins *et al*., 2004; Haslam *et al*., 2006). Several Authors identified the litter conditions, in particular the friability and the moisture, as the main factors responsible of the onset of hock burns (Bray and Lynn, 1986; Tucker and Walker, 1992; Dawkins *et al*., 2004). As the chickens aged, they spend more and more time lying down on the litter due to their increased live weight (Kjaer *et al.*, 2006). It is assumed that walking ability decrease and the permanent contact with a damp litter probably leads to the development of hock burn lesions. Dawkins *et al*. (2004), Jones *et al*. (2005) and Haslam *et al*. (2006) reported that higher levels of litter moisture and ammonia are positively correlated with more dirty pads, more leg score as angle-out and fewer birds with unblemished hocks. Similar results were observed in our survey in which the higher the content of litter moisture the higher the values of foot pad dermatitis score and hock burn incidence.

In summary our survey suggests that in Italy the broiler production system do adopt stocking density not exceeding 30-32 kg of liveweight/m^2 is following the advice of the EU proposal COM final (2005) 221. Season markedly influence the prevalence of contact dermatitis that is an important broiler welfare indicator. In addition, the score of foot pad dermatitis in winter overcomes the threshold of 50 points set up by the proposal for stocking density higher than 30 kg of liveweight/m^2. Moreover with similar stocking densities the differences of management and capability of farmers seem to play a higher impact on broiler welfare. In conclusion it can be stated that the control of the environmental conditions, particularly litter quality, appears a key issue to control the onset of foot dermatitis.

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Table 1. Productive traits of broiler chickens recorded in five flocks during winter.

Farm	A			B		C		D		E		
Classes of weight	light	medium	heavy	light	heavy	light	medium	light	heavy	light	medium	heavy
Age at slaughter (days)	37	53	58	42	60	42	53	38	57	37	49	55
Live weight (kg)	1.756	2.891	3.824	1.775	3.228	1.757	2.508	1.688	3.530	1.773	2.566	3.470
Stocking density (birds/m^2)	17	11	8	15	8	16	10	18	9	16	12	8
Stocking density (kg live weight/m^2)	28.89	30.58	30.18	24.10	26.43	27.79	23.73	29.24	32.12	28.18	30.07	28.72
FCR (kg/kg)		1.97		2.00		1.82		1.86			1.86	
Mortality (%)		4.64		9.33		2.90		3.44			4.20	
Mortality score (EU proposal) (%) [1]		4.72		4.66		4.18		4.42			4.36	

[1] Mortality score= 1%+ (0.06%* slaughter age of the flock in days)

Table 2. Productive traits of broiler chickens recorded in five flocks during summer.

Farm	A			B		C		D		E		
Classes of weight	light	medium	heavy	light	heavy	light	medium	light	heavy	light	medium	heavy
Age at slaughter (days)	39	49	56	39	56	38	52	38	56	41	57	62
Live weight (kg)	1.827	2.471	3.494	1.796	3.334	1.676	2.516	1.670	3.462	1.662	2.766	3.284
Stocking density (birds/m2)	17	12	8	14	8	16	10	17	9	16	11	9
Stocking density (kg live weight/m2)	30.00	29.51	27.52	24.27	23.87	25.77	26.25	27.77	30.92	26.91	29.02	29.51
FCR (kg/kg)	1.95			2.06		1.78		1.84		2.13		
Mortality (%)	4.32			6.75		4.01		2.56		3.07		
Mortality score (EU proposal) (%)[1]	4.36			4.54		4.12		4.36		4.72		

[1] Mortality score= 1%+ (0.06%* slaughter age of the flock in days)

Table 3. Carcass downgrades in broiler chickens observed in five flocks during winter.

Farm	A			B		C		D		E		
Classes of weight	light	medium	heavy	light	heavy	light	medium	light	heavy	light	medium	heavy
Skin lesions (%)	0	0	0	0.5	0	0.5	0.5	0	8	17.5	16.5	7
Bruises (%)	6	5.5	1.5	2.5	6	2	6	2.5	3.5	4	24	26
Fractures (%)	7.5	9	9.5	3	4.5	3.5	1.5	3	2	3.5	13.5	11.5

Table 4. Carcass downgrades in broiler chickens observed in five flocks during summer.

Farm	A			B		C		D		E		
Classes of weight	light	medium	heavy	light	heavy	light	medium	light	heavy	light	medium	heavy
Skin lesions (%)	0	1	1	0.5	2	0.5	0	0.5	1	0	0	3.5
Bruises (%)	8.5	11.5	5	12	7	3.5	5.5	1	2	2.5	4.5	4
Fractures (%)	0.5	4	10.5	2.5	5	1.5	2	0	2	4.5	5	3

Table 5. Incidence and score of food pad dermatitis (FPD) observed in light, medium and heavy sized broilers of five flocks during winter.

Farm	A			B		C		D		E		
Classes of weight	light	medium	heavy	light	heavy	light	medium	light	heavy	light	medium	heavy
FPD Class 0 (%)	0	8	19	1	1	4.5	3	52	31.5	0	0.5	2.5
FPD Class 1 (%)	55	88.5	80	17	9	38	40	48	64	48.5	32	28
FPD Class 2 (%)	45	3.5	1	82	90	57.5	57	0	4.5	51.5	67.5	69.5
FPD score*	118	51	42	173	185	134	134	24	41	127	151	153
Hock burns (%)	0	34	20	56	55	42	37	4	49.5	62.5	74	87

Foot pad dermatitis classes: 0 no lesions; 1 mild lesions; 2 severe lesions.

* FPD score= (n. lesion class 1x0.05 + n. lesion class 2x2) x 100) / 200

Table 6. Incidence and score of food pad dermatitis (FPD) observed in light, medium and heavy sized broilers of five flocks during summer.

Farm		A		B		C		D		E		
Classes of weight	light	medium	heavy	light	heavy	light	Medium	light	heavy	light	medium	heavy
FPD Class 0 (%)	53	46.5	52	44	68.5	94.5	92.5	59	84	0.5	1	0.5
FPD Class 1 (%)	46.5	52.5	48	56	31.5	5.5	7.5	41	16	55.5	51	21
FPD Class 2 (%)	0.5	1	0	0	0	0	0	0	0	44	48	78.5
FPD score*	24	28	24	28	16	3	4	21	8	116	122	168
Hock burns (%)	2.5	5.5	10	37.5	48.5	0	6.5	18	8	78.5	56	73.3

Foot pad dermatitis classes: 0 no lesions; 1 mild lesions; 2 severe lesions.

* FPD score= (n. lesion class 1x0.05 + n. lesion class 2x2) x 100) / 200

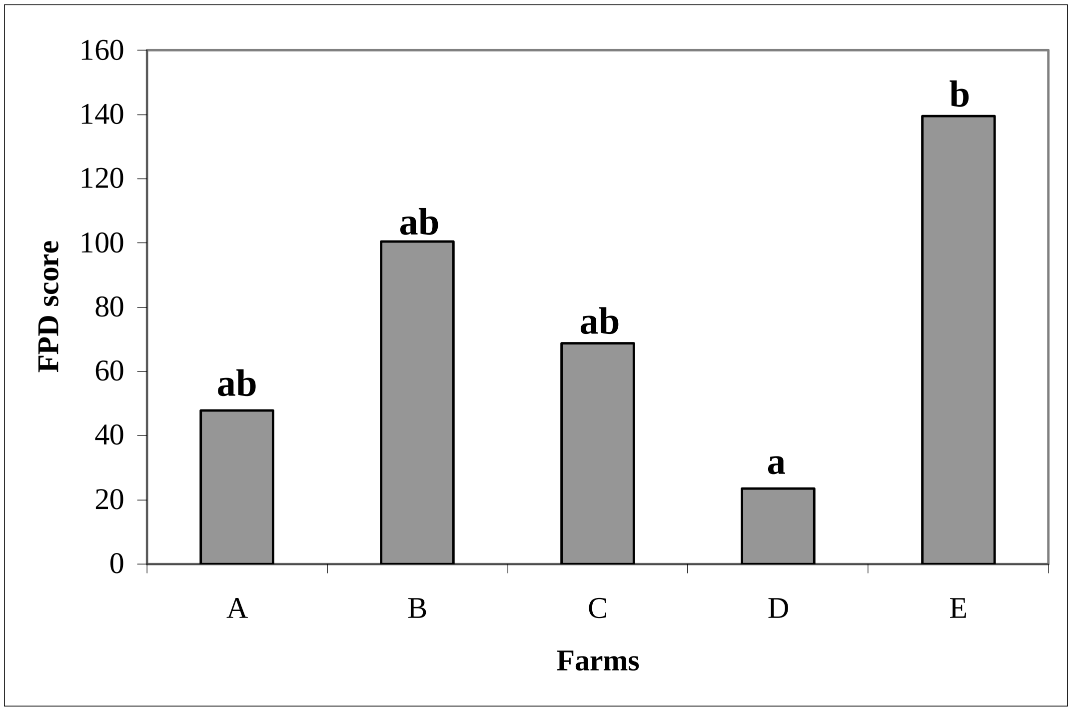
Table 7. Characteristics of the litter collected at the end of the rearing period in winter and summer.

Farms	A		B		C		D		E	
Litter type	Chopped wheat straw		Chopped wheat straw		Rice hulls		Chopped wheat straw		Chopped wheat straw	
Season	winter	summer	winter	summer	winter	summer	winter	summer	winter	summer
Litter moisture (%)	37.9	27.1	44.5	24.8	38.7	25.3	32.1	21.9	50.7	39
Litter pH	8.41	6.9	7.68	8.3	8.37	8.4	6.95	8.30	7.62	7.80

Table 8. Pearson's correlation coefficients among stocking density, litter characteristics and foot lesions (* P < 0.05).

	Stocking density	Litter moisture	Litter pH	Food pad dermatitis
Stocking density	-	-	-	-
Litter moisture	0.02	-	-	-
Litter pH	-0.37	-0.15	-	-
Food pad dermatitis	-0.11	**0.87***	-0.12	-
Hock burns	0.03	**0.75***	-0.25	**0.76***

Figure 1. Foot pad dermatitis score evaluated in the different farms.



Bars with different letters are statistically different for $P < 0.01$.

Figure 2. Foot pad dermatitis (FPD) score and percentage of hock burn (HB) according to the seasons (** P < 0.01).

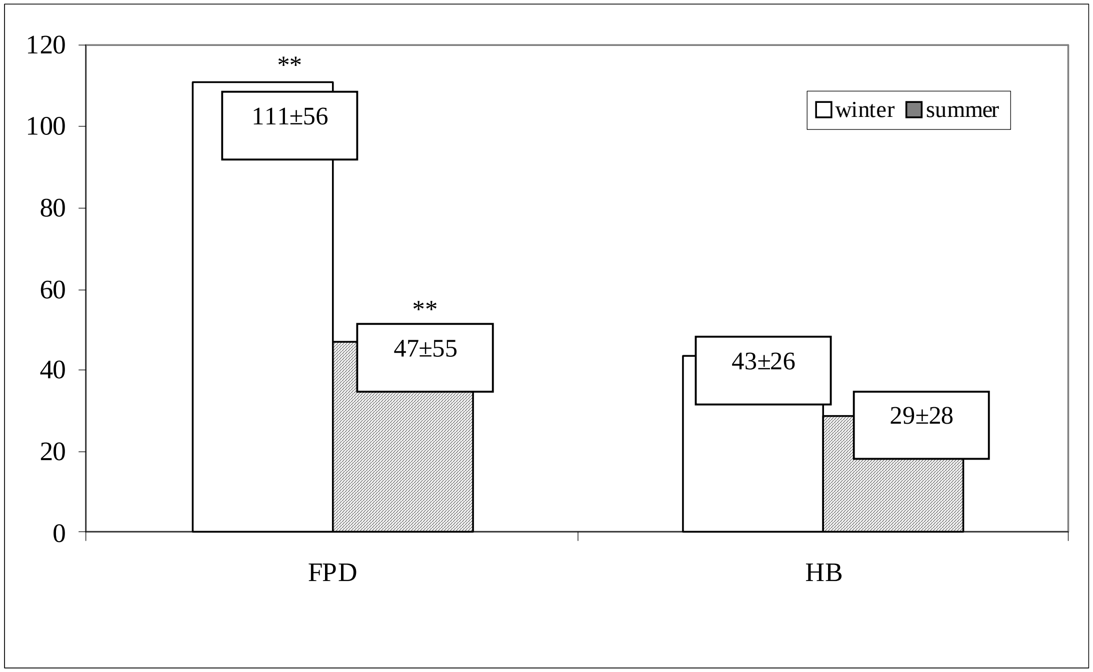
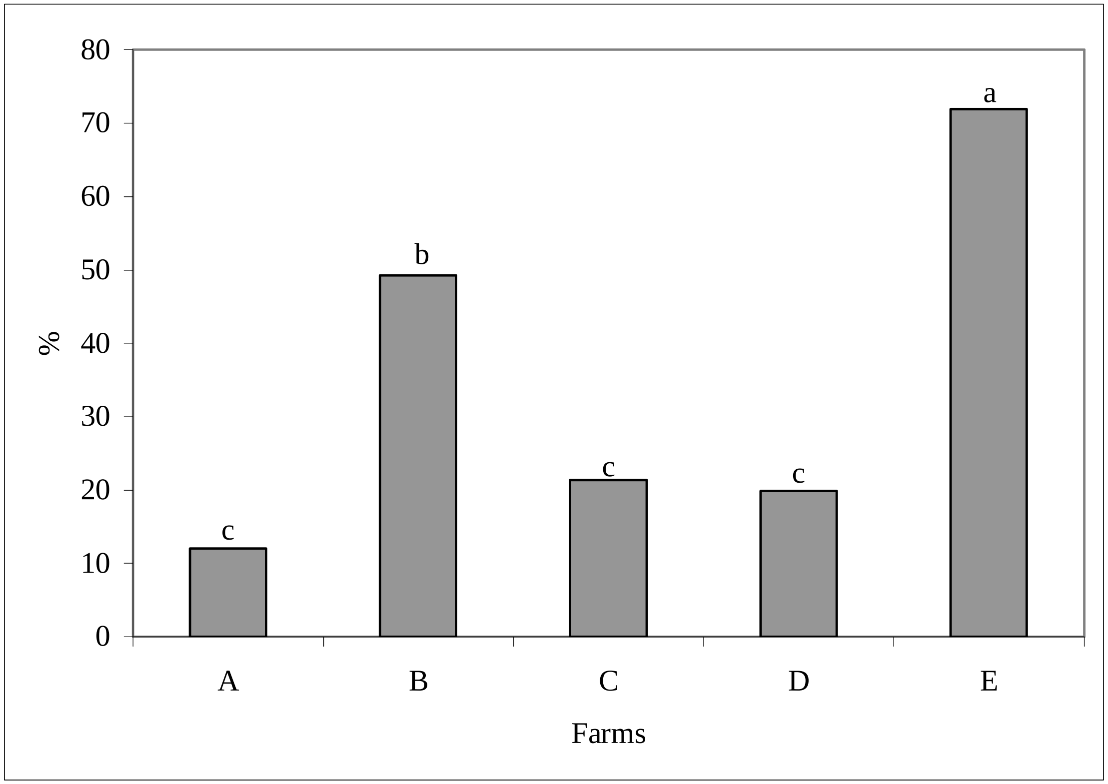


Figure 3. Hock burn percentages in the different farms.



Bars with different letters are statistically different for $P < 0.01$.

CHAPTER 3

Effect of improved rearing conditions on carcass injuries, meat quality, broiler performance and litter characteristics during winter season

3.1 - INTRODUCTION

Issues like animal welfare, food quality, safety and the environment protection have assumed much greater importance for the public, especially for consumers. The production of broiler chickens is the most uniform form of animal rearing, in terms of genotypes, husbandry, housing and handling techniques adopted, thus, it is perceived by the public as one of the most intensive rearing system. Generally, criticism on modern production systems does not focus on the exploitation of animals as such, but on the minimal living space allowed per animal, on the inadequate light duration and intensity, the barren environment in which the animals are kept, the high meat yields and production levels reached and the adverse effects of these on the behaviour and welfare of the chickens. It is stated that by generating appropriate and adequate responses about farm management and rearing techniques this will lead to ongoing improvements in welfare status without overlooking the economical parameters.

The European Union is currently in the process of developing proposals to introduce minimum standards for broiler chicken welfare, which would include legislation on stocking density for birds kept for meat production. The proposal Directive COM 221 (2005) states that the stocking density of chickens should not exceed 30 kilograms of live weight/m^2. Derogation is available for establishments to use stocking densities of up to a maximum of 38 kg/m^2 subjected to more rigorous requirements for documentation relating to production, environmental quality, and record keeping. The permitted stocking density is determined also by the incidence and severity of food pad dermatitis (FPD) measured at the processing plant.

FPD is a type of contact dermatitis affecting the plantar region of the bird's feet, caused by a combination of moisture and chemical irritants in the litter. At an early stage, discoloration of the skin is observed. Hyperkeratosis and necrosis of the epidermis can develop, and in severe cases, these changes are followed by ulcerations with inflammatory reactions of the subcutaneous tissue (Greene *et al.*, 1985; Martland, 1985) Dozier *et al*. (2005) found that stocking density alters foot pad scores due to an increase of caked litter. Dawkins *et al*. (2004) and Haslam *et al*. (2006) too, underlying the key role of litter conditions rather then stocking density *per se,* found a relation between litter quality, ammonia concentration and FPD.

Podo-dermatitis can be caused by several factors, the most important being the condition of the litter in the broiler house since broilers spend the majority of their time lying (Bessei, 2006). Litter quality can be affected by the ventilation in the house (Ekstrand *et al*., 1998; Feddes *et al*., 2002), stocking density (Sørensen *et al*., 2000), type, depth and moisture content of the litter (Su *et al*., 2000; Bradshaw *et al*., 2002; Dawkins *et al*., 2004; Meluzzi *et al*., 2004), behaviour of the birds, diet composition, number and design of the drinkers in the house (Lynn and Elson, 1990; Jones *et al*., 2005).

The objective of the work was to compare the effects of 2 litter types (wheat straw and wood shaving) and 2 different rearing conditions (Welfare and Standard) on broiler welfare indicators, performances, carcass and meat quality in winter season.

3.2 - MATERIALS AND METHODS

3.2.1 - Experimental design

A trial was carried out in an experimental housing complex involving a total of 2,400 broiler chickens randomly distributed into 32 pens of 6 m^2 each located in 2 identical blocks.

Chickens were split up in 2 groups: Welfare (W) and Standard (S). In Welfare groups 1,056 Ross 508 male broilers were housed in 16 pens and kept at a concentration (11 birds/m^2) lower than the usual adopted on the commercial farms. The birds were maintained to a daily photoperiod of 16 hours light and 8 hours dark, that is similar to the natural one occurring in temperate zones, and on high litter amounts. In Standard groups 1,344 male broilers Ross 508 were housed in 16 pens and kept at high stocking density (14 birds/m^2), with a photoperiod of 23 hours light and 1 hour dark, and on low litter amounts.

In Welfare groups broilers were housed in 8 pens littered with 3 kg/m^2 of wheat straw and in others 8 pens bedded with 4.5 kg/m^2 of wood shaving whereas in Standard groups 8 pens were covered by 2.3 kg/m^2 and 8 pens by 3 kg/m^2 of wheat straw and wood shaving respectively.

At 43 days of age birds were depopulated and 18 birds/pen (3 per m^2) from Welfare groups and 24 birds/pen (4 per m^2) from Standard groups were slaughtered.

Each pen was equipped with circular feeders and nipple drinker system. Feed and water were made available *ab libitum* for all groups. A 4-phase feeding program was adopted (pre-starter diet: from 1 to 9 d, starter: from 10 to 19 d, grower: from 20 to 39 d and finisher from 40 d to slaughtering).

Birds were slaughtered either at 2.7 kg of live weight at 43 days or at 3.2 kg of live weight at 49 days. At depopulation and at the end of the experiment, birds were weighted and their feed consumption recorded as well as daily mortality.

3.2.2 - Slaughtering measurements

Bones fractures, skin lesions, bruises and hock burn dermatitis occurred during the rearing cycle were checked both at depopulation and at the end of the trial. Hock burns were assessed on the basis of their presence or absence and the incidence of lesions was expressed as a percentage of the total number of carcasses examined. The occurrence of food pad dermatitis (FPD) was checked by collecting one foot for each bird slaughtered. The prevalence and severity of FPD was assessed on the processing line using the method reported by Ekstrand *et al*. (1998) thus, feet showing no lesion were classified as 0, feet showing mild lesions were classified as 1 and feet showing severe lesions were classified as 2. The lesion score was calculated applying the formula reported in the EU proposal COM (2005) 221 final. The number of feet from class 0 did not contribute to the score. The number of feet from class 1 was multiplied by 0.5, the feet from class 2 were multiplied by 2 and those scores were added. The total was divided by the sample size and multiplied by 100.

3.2.3 - Litter evaluation

Litter conditions were evaluated at the end of the trial. All the litter of each pen was thoroughly mixed on site immediately after collection, weighted and one sample of about 1 kg was prepared for subsequent analysis. Total solids were determined by drying the samples in an oven at 100°C for 12 hours, volatile solids were obtained by calcination of samples previously submitted to total solids determination. Total nitrogen content of litter was analysed following the Kjeldhal method (APHA, 2005). Ammonia was determined using the Kjeldhal procedure pre-treating the sample (10 g) with 100 ml of KCl 1N instead of the mineralization with H_2SO_4 (96%). Litter pH was measured by using a pH-meter YS I60 on 2 samples per each pen of a blend of 50 g of fresh litter and 250 g of distilled water.

3.2.4 - Carcass evaluation and meat composition

At the processing plant, after chilling, 15 representatives carcasses per treatment were collected and used for subsequent meat quality evaluations. At 24 h post mortem, the colour (L*, a*, b*) of the breast (P. major) meat was measured using the Minolta Chroma Meter CR-300. The pH was determined using the pH meter HI98240 equipped with electrode FC230. The two fillets from each whole breast were separated and used for the determination of drip and cooking loss. Drip loss was carried out on one intact fillet kept suspended in a sealed glass box for 48 h at 2-4°C and expressed as percentage of weight loss during storage. Cook loss was measured on the other fillet by cooking the samples in a convection oven on aluminum trays at 180°C until 80°C at core sample.

The fillets were then allowed to equilibrate to room temperature, reweighed, and cook loss determined as percentage of weight loss. Furthermore AK-shear values of cooked meat was measured using an TA HDi Heavy Duty texture analyzer equipped with an Allo-Kramer shear cell using the procedure described by Papinaho and Fletcher (1996). Finally, P. minor muscles were frozen for subsequent determination of lipid, crude protein, ash and moisture contents. Proteins were determined using a standard Kjeldahl copper catalyst method (AOAC, 1990). Total lipids were measured using the chloroform:methanol procedure described by Folch *et al*. (1957) modified. Ashes and moisture were determined using the procedure described by the AOAC (1990).

3.2.5 - Statistical analysis

The data were analysed by two-way ANOVA with interaction using the general linear model procedure of SAS software (SAS Institute, 1989) with the main factors being rearing conditions (Welfare and Standard) and litter materials (wheat straw and wood shaving). Means were separated by the Student Newman Keuls test. Mortality data were subjected to arc sine transformation before ANOVA. Differences were considered significant at $P < 0.05$ and $P < 0.01$ levels.

3.3 - RESULTS

3.3.1 - Productive traits

At depopulation (43 d) chickens of Welfare group reached a significantly higher body weight (2,792 *vs* 2,713 g; $P < 0.01$), ate significantly higher amount of feed (110.2 *vs* 105.2 g/bird/d; $P < 0.01$) but showed almost the same feed conversion rate in comparison with Standard birds (Table 1). Moreover the mortality was significantly lower in Welfare groups (1.61 *vs* 3.49; $P < 0.05$).

The density achieved at depopulation was 30.2 kg of live weight/m^2 in Welfare groups and 36.6 kg of live weight/m^2 in Standard groups. Litter type did not significantly influence the productive traits at 43 d. Indeed, birds reached respectively 33.4 kg of live weight/m^2 on wheat straw litter and 33.5 kg of live weight/m^2 on wood shaving litter (Table 1).

At the end of the trial (49 d) any statistical difference concerning the final body weight between the chickens of Welfare groups (3,248 g) and Standard group (3,218 g) emerged. Welfare groups had the worst FCR (1.84 *vs* 1.79; $P < 0.01$) and a slightly lower mortality in comparison with Standard groups (2.18 vs 3.79%). Litter type exerted neglecting effects on broiler performance (Table 2).

The stocking density reached at the end of the trial was 25.4 kg of live weight/m^2 in Welfare groups and 31.0 kg of live weight/m^2 in Standard ones, without any important differences among litter materials being 28.0 kg of live weight/m^2 for wheat straw and 28.4 kg of live weight/m^2 for wood shaving.

3.3.2 - Carcass quality, foot dermatitis and litter characteristics

At 43 d carcass lesions were evaluated on 288 birds for Welfare groups and on 384 birds for the Standard groups at the processing line. Carcass downgrades are presented in Figure 1. Hock burn prevalence were lower in Welfare groups than in Standard ones (27.5 *vs.* 75.5%) and in birds kept on wood shaving than those reared on wheat straw (40.5 *vs.* 62.5%). No skin lesions were recorded in the different groups whereas either bruises and bone fractures percentages were not affected neither by rearing condition nor by litter type.

At the end of the trial lower incidences of both bruises and bone fractures emerged in all groups in comparison with those recorded at depopulation. The ranking for hock burns was similar to that recorded at 43 days but the incidence in birds kept on wheat straw was considerably lower than the previous observations (Figure 2).

FPD score in Welfare groups ranged from 21.4 in chickens of Welfare group to 63.6 in birds reared in Standard conditions exceeding the threshold of 50 points reported by the European proposal (COM 221, 2005). The incidence of FPD of birds kept on wheat straw was 20 points higher than those kept on wood shaving (Figure 3).

Litter moisture content, total nitrogen, calculated on the dry matter, ammonia released by litter, both expressed as percentage of total nitrogen, as well as on fresh litter, were significantly lower in Welfare groups, those birds were kept at a lower density, compared to Standard groups. The volatile solid content of litter was significantly higher in Standard groups, 83.7 *vs.* 80.8% of total solid (Table 3).

For the effect of litter type, a higher content of volatile solids over pens bedded with wood shaving was observed (462 *vs.* 413 g/kg of litter, corresponding to 83.7 *vs.* 80.8% of total solids). The total nitrogen content is similar in both types of litter on fresh litter basis but it is lower in wood shavings if it is calculated on the basis of its dry matter. pH was insensitive both to the rearing condition and litter type (Table 3).

3.3.3 - Meat quality

Meat quality traits are given in Table 4. Breast meat of Welfare birds showed different colour with higher values of yellowness and redness. Moreover they had lower cook loss and shear values that means a better meat tenderness than that of Standard birds. Litter type did not exert any important effect on meat quality. The proximate analysis of meat (Table 5) did not show any differences among groups and values are within the range of the standards reported for commercial chickens.

3.4 - DISCUSSION

This study was undertaken to compare 2 litter types (wheat straw and wood shaving) and 2 rearing conditions, Welfare and Standard. In Welfare groups the rearing conditions were improved providing a photoperiod close to the natural one, a lower stocking density and a greater amount of litter material. In Standard groups the conventional intensive conditions were adopted. For each rearing condition and litter type, the effects of the litter material (wheat straw and wood shaving) on production and on the incidence of broiler lesions as indicators of animal welfare were investigated.

Even though 2 different stocking densities were established in this study, the performances achieved from the chickens were almost identical among groups.

The FCR was significantly better in Standard conditions contrarily to birds reared in Welfare conditions with lower stocking density, more litter material and with a light program of 16 hours light and 8 hours dark. In a previous trial Meluzzi *et al*. (2004) found that feed intake and efficiency were insensitive to the improvement of the rearing conditions in agreement also with Feddes *et al*. (2002). On the contrary Dozier *et al*. (2005) found that increasing the broiler density beyond 30 kg/m^2 elicited some negative effects on live performance of heavy broilers. The data collected suggest that a higher stocking density did not depress the growth of the broilers. The chickens of Welfare groups could benefit of a wider feeder space but the adoption of a photoschedule of 8 hours dark has limited the access to them, therefore the performances data were similar in both treatments. As found by Meluzzi *et al*. (2003) in previous investigations, more exploitable space led the broilers of Welfare groups, reared in a lower concentration, to increase their locomotor activity, to eat more feed and consequently to worsen their feed efficiency. Bessei (2006) stated that a photoperiod of 16 hours light and 8 hours dark had a positive effect on chicken behaviour since they can develop a circadian activity pattern improving thus their leg conditions and walking ability.

A key role is also played by the litter material, the wood shaving is more absorbent than chopped wheat straw because of its porosity. This type of litter determines a better welfare status of the birds because of its absorbent capacity and less moisture content of the exhausted litter. Indeed the wood shaving is more easily turned by the chickens, if compared to wheat straw, favouring the moisture evaporation. On the contrary wheat straw determines a strong crust formation which preserves the gas emission and increases the moisture in contact with the bird foot leading to the development of contact dermatitis (Dozier *et al*., 2005). In our trial, in Standard groups we observed a higher content of moisture, nitrogen and ammonia released from the litter. Therefore it can be assumed that the environmental characteristics have been positively changed by the improvements of the rearing conditions adopted for Welfare groups. This is witnessed either by the lower percentage of ammonia released by the litter in Standard treatments (22.6 *vs.* 18%) or lower incidence of contact dermatitis of Welfare groups.

In Welfare groups other than stocking density and photoperiod, also the amount of litter was changed. Indeed we used a greater amount of litter both wood shaving and wheat straw since in Italy it is believed that the higher the litter layer the better the living conditions for the chickens even if in literature it is reported that birds reared on a thin layer of litter (< 5 cm) had a lower prevalence of FPD (Ekstrand *et al*. 1997). From our experimental design it is not possible to highlight the effect of the litter amount itself since it is combined with low stocking density and short photoperiod in Welfare groups.

Anyway in Welfare groups the exhausted litters of the pens were dryer and broilers showed a lower occurrence of FPD. In recent years the prevalence of FPD has been used to characterize the health and welfare of broiler flocks. Our findings agree with those of Ekstrand *et al*. (1998) who reported that the main factors in connection with a high prevalence of dermatitis have been shortcomings in the management of ventilation, heating systems and litter quality. Dawkins *et al*. (2004) too claimed that litter moisture and ammonia were related to bird health and that higher levels of both were correlated with more dirty pads.

Closely related to FPD are hock burn lesions, in which the skin of the hock becomes dark brown. Welfare groups have shown a low hock burn incidence at depopulation and slightly more marked at 49 days whereas it was always high in Standard especially for chickens reared on wheat straw. The prevalence of hock burn lesions, like FPD, is high with poor litter quality conditions in agreement with Dawkins *et al*. (2004). Besides

Sørensen *et al*. (2000), Broom and Reefmann (2005) and Kjaer *et al.* (2006) found that the incidence of hock burns is affected by body weight.

The incidence of carcass injuries such as skin lesions, bruises and bone fractures was almost low in all groups. Bessei (2004) claims that at high stocking density birds step on their pen-mates and thus injures the skin especially in the area of the back and legs. We can support that the different stocking densities adopted in this trial had no effect on the grade of the carcass and on the percentage of birds downgraded in agreement with Feddes *et al.* (2002).

In our trial emerged that breast meat of Standard birds lost more water during cooking and had a lower tenderness than that of Welfare birds. In literature there are not data regarding the effect of improved rearing conditions on the quality traits of meat. Lambooij, 1999; Warriss *et al.*, 1999 found in birds submitted to stress prior to slaughter some changes in the post mortem metabolism of muscle and subsequent meat quality characteristics such as colour, water holding capacity and texture. Further investigations are needed to better understand if different rearing conditions may act as stressors thus affecting the quality of meat.

In conclusion, the combined effect of a lower stocking density, a greater amount of litter material and a photoperiod similar to the natural one, have positively influenced the chickens welfare status, as a matter of the fact that the occurrence of FPD in Welfare groups was the lowest keeping the score under the European threshold. Moreover, improved rearing conditions led to a faster growth associated to a worse feed efficiency without a clear effect on meat quality traits.

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Table 1. Productive traits at 43 d.

		Rearing Conditions (RC)		Litter type (L)					
		Welfare	Standard	Wheat straw	Wood shaving	SE	RC	L	RC*L
Bird housed	n/m^2	11	14	11-14	11-14	-	-	-	-
Body live weight 0 d	g	45.2	45.4	45.3	45.3	0.01	n.s.	n.s.	n.s.
Body live weight 43 d	g	2,792 A	2,713 B	2,747	2,756	6,782	0.01	n.s.	n.s.
[1]DWG	g/bird/d	63.7 A	61.5B	62.4	62.7	3.53	0.01	n.s.	n.s.
Feed intake	g/bird/d	110.2 A	105.2 B	107.5	107.8	2.23	0.01	n.s.	n.s.
[2]FCR	kg/kg	1.73	1.71	1.72	1.71	0.002	n.s.	n.s.	n.s.
Mortality	%	1.61	3.20	2.62	2.19	0.009	n.s.	n.s.	n.s.

[1]DWG: daily weight gain;

[2]FCR: feed conversion rate;

A, B: P<0.01.

Table 2. Productive traits at 49 d.

		Rearing Conditions (RC)		Litter type (L)					
		Welfare	Standard	Wheat straw	Wood shaving	SE	RC	L	RC*L
Body live weight	G	3,248	3,218	3,226	3,240	10,043	n.s.	n.s.	n.s.
DWG[1] (0-49d)	g/bird/d	65.1	64.1	64.6	64.9	4.29	n.s.	n.s.	n.s.
Feed intake (0-49 d)	g/bird/d	119 A	113 B	115 b	117 a	3,874	0.01	0.05	n.s.
FCR[2] (0-49 d)	kg/kg	1.84 A	1.79 B	1.81	1.82	0.002	0.01	n.s.	n.s.
Mortality (0-49 d)	%	2.18	3.79	3.40	2.58	0.006	n.s.	n.s.	n.s.

[1]DWG: daily weight gain

[2]FCR: feed conversion rate

a, b: P<0.05; A, B: P<0.01.

Table 3. Litter characteristics assessed at the end of the trial.

		Rearing Conditions (RC)		Litter type (L)					
		Welfare	Standard	Wheat straw	Wood shaving	SEM	RC	L	RC*L
Total solids	g/kg	576 A	490 B	512	551	3500	0.01	n.s.	n.s.
Moisture	g/kg	424 B	510 A	488	449	3500	0.01	n.s.	n.s.
Volatile solids	g/kg	482 A	396 B	413 b	462 a	2525	0.01	0.05	n.s.
Total nitrogen	g/kg	26.3	25.4	26.3	25.4	10.18	n.s.	n.s.	n.s.
Total nitrogen/total solids	%	4.57 B	5.22 A	5.17 A	4.65 B	0.13	0.01	0.01	n.s.
Ammonia	g/kg	4.65 B	5.69 A	5.46	4.92	80.21	0.01	n.s.	n.s.
Ammonia/total nitrogen	%	17.86 B	22.58 A	20.97	19.57	15.1	0.01	n.s.	n.s.
pH		7.74	7.72	7.68	7.78	0.15	n.s.	n.s.	0.05

a, b: P<0.05; A, B: P<0.01.

Figure 1. Incidence of carcass injuries of birds slaughtered at depopulation (43 d).

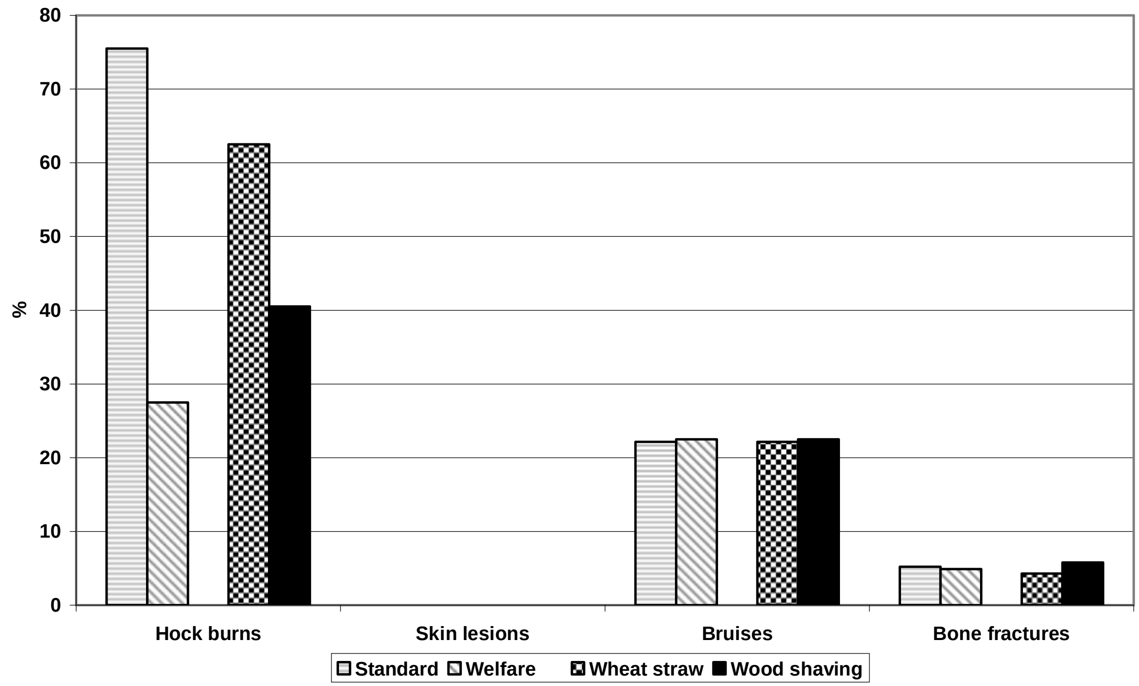

Figure 2. Incidence of carcass injuries of birds slaughtered at 49 d.

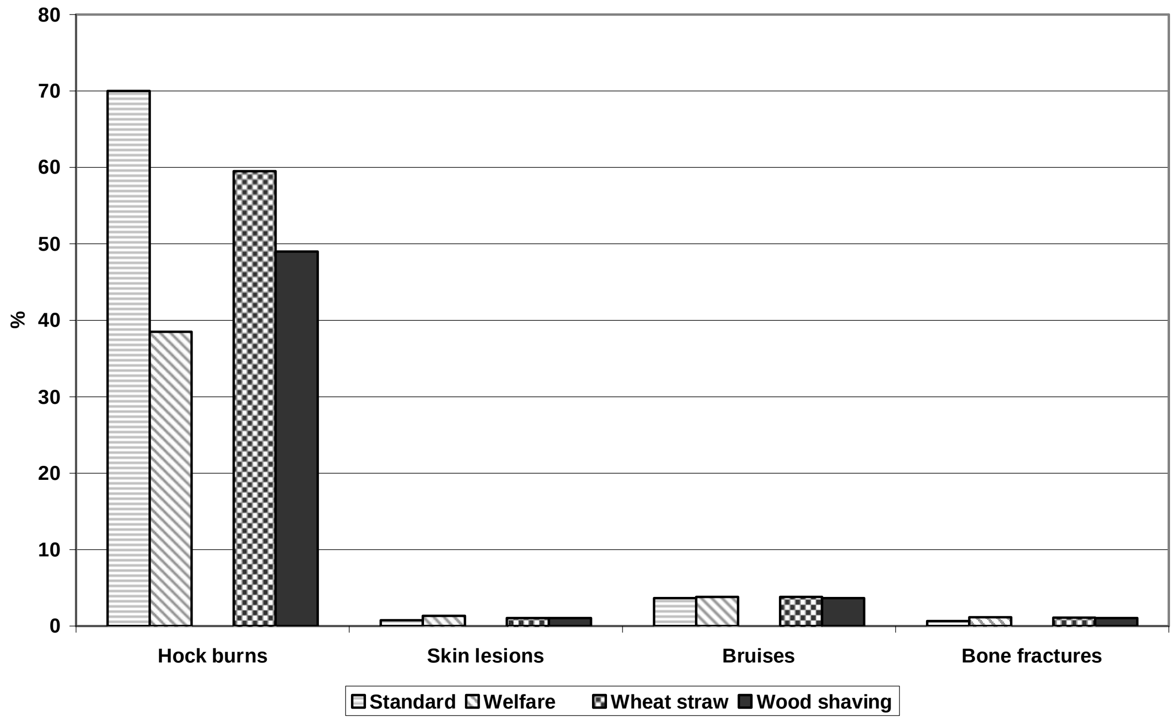

Figure 3. Food pad dermatitis score of birds slaughtered at 49 d.

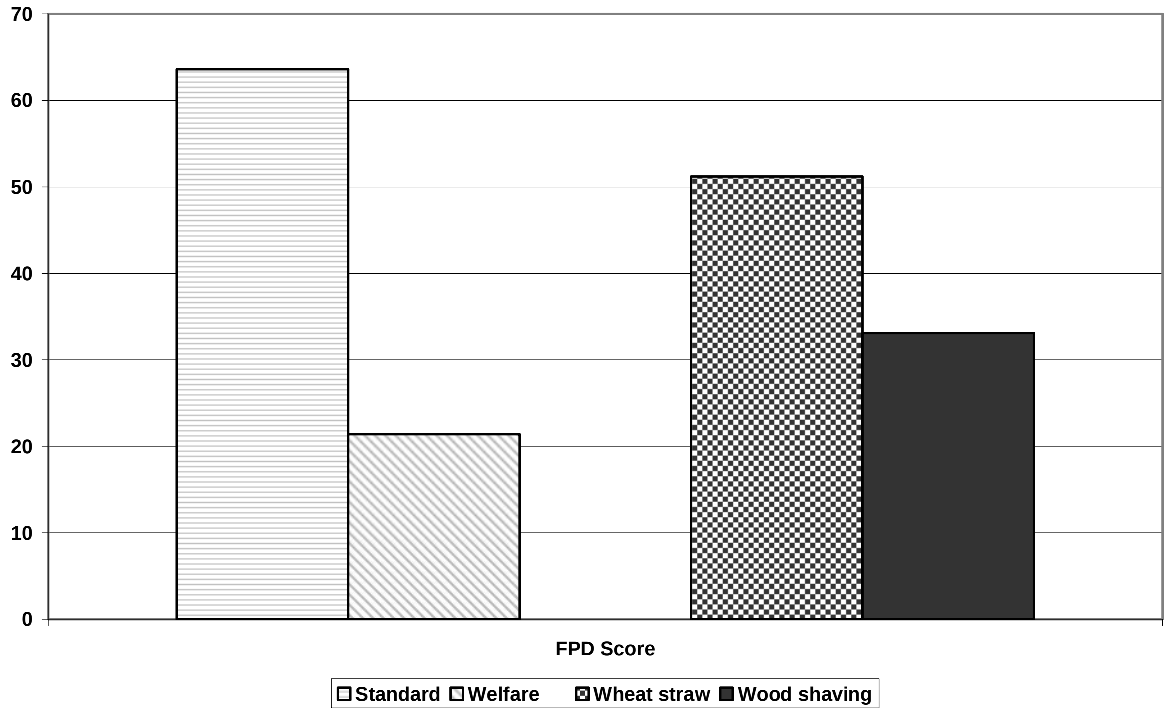

Table 4. Meat quality of bird slaughtered at 49 days.

		Rearing Conditions (RC)		Litter type(L)					
		Welfare	Standard	Wheat straw	Wood shaving	SEM	RC	L	RC*L
pH		5.91	5.88	5.92	5.87	0.01	n.s.	n.s.	n.s.
L* -meat		50.97	51.05	51.14	50.88	6.59	n.s.	n.s.	n.s.
a* -meat		3.57 b	4.36 a	3.89	4.04	1.78	0.05	n.s.	n.s.
b* -meat		2.90 B	3.82 A	3.54	3.19	1.42	0.01	n.s.	n.s.
Drip loss	%	1.24	1.15	1.31 a	1.08 b	0.17	n.s.	0.05	0.05
Cooking loss	%	16.45 B	19.42 A	18.53	17.33	6.71	0.01	n.s.	n.s.
Shear value	kg/g	2.74 B	3.53 A	3.01	3.26	0.46	0.01	n.s.	n.s.

a, b: P<0.05; A, B: P<0.01.

Table 5. Meat chemical composition of birds slaughtered at 49 d.

		Rearing Conditions (RC)		Litter (L)					
		Welfare	Standard	Wheat straw	Wood shaving	SEM	RC	L	RC*L
Dry matter	%	24.92	24.89	24.81	24.99	0.51	n.s.	n.s.	n.s.
Ash	%	1.31	1.34	1.30	1.35	0.11	n.s.	n.s.	0.04
Crude protein	%	22.61	22.97	22.79	22.78	0.38	n.s.	n.s.	0.0004
Total lipids	%	1.34	1.23	1.34	1.24	0.13	n.s.	n.s.	n.s.

CHAPTER 4

Effect of improved rearing conditions on carcass injuries, meat quality, broiler performance and litter characteristics during summer season

4.1 -INTRODUCTION

Animal welfare issues are considered controversial because it is generally assumed that any improvements in the area of animal welfare will have a negative impact on farm profitability.

At the European level, the welfare related issue is considered a priority and currently a specific proposal for a Council Directive has been preparing with the aim of protecting the chickens kept for meat production. An important parameter taken into account by the European Commission in the proposal is the stocking density since it seems related to the increase of mortality, to the worsening of litter conditions, and to the increase of health problems like leg disorders and contact dermatitis, in particular food pad dermatitis, hock and breast burn.

The purpose of the research was to study the effect of high or low stocking density of broiler chickens, different types of litter and the adoption of short or long lighting regimen on broiler welfare through the evaluation of their productivity and incidence of foot pad dermatitis during the hot season.

4.2 - MATERIALS and METHODS

4.2.1 - Experimental design

A trial was carried out in an experimental facility involving 2,400 males Ross 508 randomly divided into 8 groups of four replicates each and reared in 32 pens (6 m^2 each) located in 2 identical rooms of the same poultry house.

A 3 factors experimental design of two levels each was set up: low (LD) or high (HD) stocking density (11 and 14 birds/m^2 for LD and HD respectively), chopped straw (CS) or wood shaving (WS) litter and short (SL) or long (LL) light regimen (16 h light and 8 h dark or 23 h light and 1 h dark respectively for SL and LL). The trial was run from the mid of May to the end of June. At 42 days of age the broiler were slaughtered when the theoretic values of 30 and 35 kg of live weight per m^2 were reached for low and high stocking density respectively. Feed intake, body weight and mortality were recorded and feed conversion ratio was calculated.

4.2.2 - Slaughtering measurements

At the slaughterhouse, chickens were controlled long the processing chain for the incidence of carcass injuries such as skin and hock burn lesions, bruises and bone fractures. Moreover, chicken feet were collected for macroscopic dermatitis examination and scored in 3 classes: 0= no lesions, 1= mild lesions and 2= severe lesions (Ekstrand *et al.*, 1997). According to the formula reported in the EU proposal COM (2005) 221 final, the foot pad dermatitis score was calculated multiplying the number of feet from class 1 by 0.5, the feet from class 2 by 2 and the scores were added, then divided by the sample size and multiplied by 100.

4.2.3 - Carcass evaluation, meat quality and composition

At the processing plant, after chilling, 10 representative's carcasses per treatment were collected and used for subsequent meat quality evaluations. At 24 h post mortem, the colour (L*, a*, b*) of the breast (P. major) meat was measured using the Minolta Chroma Meter CR-300. The pH was determined using the pH meter HI98240 equipped with electrode FC230. The two fillets from each whole breast were separated and used for the determination of drip and cooking loss. Drip loss was carried out on one intact fillet kept suspended in a sealed glass box for 48 h at 2-4°C and expressed as percentage of weight loss during storage. Cook loss was measured on the other fillet by cooking the samples in a convection oven on aluminum trays at 180°C until 80°C at core sample. The fillets were then allowed to equilibrate to room temperature, reweighed, and cook loss determined as percentage of weight loss. Furthermore AK-shear values of cooked meat was measured using an TA HDi Heavy Duty texture analyzer equipped with an Allo-Kramer shear cell using the procedure described by Papinaho and Fletcher (1996). Finally, P. minor muscles were frozen for subsequent determination of lipid, crude protein, ash and moisture contents. Proteins were determined using a standard Kjeldahl copper catalyst method (AOAC, 1990). Total lipids were measured using the chloroform:methanol procedure described by Folch *et al*. (1957) modified. Ashes and moisture were determined using the procedure described by the AOAC (1990).

4.2.4 - Statistical analysis

Data were submitted to a 3 ways ANOVA with interactions, considering as main effects stocking density, litter type and light regimen and means separated by the Student Newman Keuls test (SAS, 1989).

4.3 - RESULTS

4.3.1 - Productive traits

Chickens of group LD have grown faster than HD reaching a body weight of 2,511 g *vs* 2,404 and a maximum stocking density of 27.3 *vs* 32.9 kg/m^2 (Table 1). Both LD and HD birds had the same daily feed intake (90.7 g/bird/day) and considering their different live weights the feed efficiency was better for the LD than for HD broilers. The light regimen significantly ($P < 0.01$) affected both feed intake and efficiency.

The feed conversion rate was significantly better in SL birds (1.55 kg *vs*. 1.62 respectively in SL and LL, $P < 0,01$) since they reached the same live body weight (Table 1).

The most used litter materials, chopped straw and wood shaving, at the commercial level did not significantly influence, as expected, productive performances of broilers.

Light regimen, stocking density and litter type did not statistically affect mortality, but in HD group the value was 1% greater than that of LD one.

4.3.2 - Carcass quality and foot dermatitis

The incidence of foot pad lesions was generally low and no severe lesions of score 2 were observed. Chickens reared on wood shaving litter showed a lower incidence of foot disorders ($P<0.05$), 7.7 *vs*. 12.0 respectively for WS and CS groups (Table 1). Either stocking density or light regimen did not exert a clear influence on the onset of foot lesions. Slightly higher values have been detected in HD and SL treatments.

Hock burns, skin lesions, bruises and bone fractures were not affected by the different factors considered in the experimental design (data not shown).

4.3.3 - Meat quality and composition

The color of breast meat from chickens of group SL was significantly ($P < 0.05$) paler (L*, 57.60 *vs*. 56.03) and more yellow (2.93 *vs*. 2.73). The yelloness values resulted statistically influenced ($P < 0.01$) also by stocking density, 3.05 *vs*. 2.62 respectively in HD and LD.

Cooking loss values was influenced from the litter type ($P < 0.01$), the higher percentage has been determined in bird reared on chopped straw, 19.29 *vs* 18.94% respectively for CS and WS groups.

There was no difference among treatments concerning the breast meat composition; values are within the range of standards reported for commercial chickens (data not shown).

4.4 - DISCUSSION

Both LD and HD birds had the same daily feed intake (90.7 g/bird/day) and considering their different live weights the feed efficiency was better for the LD than for HD broilers. These data are consistent with those reported by Meluzzi *et al.* (2003) and Bessei (2006) who observed a reduction of feed intake and body weight with increasing stocking density.

With a short light regimen the broilers have had less time available to feed themselves and therefore consumed a significant lower amount of feed.

The appearance of foot pad dermatitis was not influenced by stocking density. In France too, Martrenchar *et al.* (2002) conducted an epidemiology study of over 50 commercial flocks and reported a very low incidence of foot pad dermatitis and no detectable effects of density. However they indicated that a poor fan ventilation system was a significant risk factor for incidence of foot lesions. These results are in line with the findings of 2 more recent studies (Dawkins *et al*., 2004; Jones *et al*., 2005) that found few effects of density per se on the health and welfare of broiler chickens in commercial flocks in the United Kingdom. Results indicated that for a range of densities from 30 to 46 kg/m^2 (0.073 to 0.047 m^2/bird), broiler health and welfare was to a great extent determined by the quality of the environment provided by producers (Dawkins *et al*., 2004), and particularly relevant to welfare was the proportion of time that relative humidity in the facilities was maintained within the recommended guidelines (Jones *et al*., 2005).

They concluded that control over environment conditions and in particular a good ventilation system and management practices geared toward the maintenance of adequate relative humidity are critical to improve broiler welfare (Jones *et al*., 2005).

Dawkins *et al*. (2004) also pointed out that limiting stocking densities without adequate control over the environment will not lead to the expected improvements in welfare.

The foot examination revealed that the lesions occurred more in birds maintained on chopped wheat straw than on wood shaving. These results confirmed the previous data obtained during the winter trial, in fact the FPD score obtained is probably due to the different material characteristics of the two types of litter considered. The wood shaving is more porous, so more absorbent and it is more easily turned by the chickens allowing moisture evaporation. On the contrary the chopped straw tend to form a strong crust of faeces on the litter surface preserving the gas emission and increasing the moisture in contact with the chicken foot leading to develop a dermatitis lesion (Dozier *et al*., 2005).

In conclusion, stocking density lower than 30 kg of live weight per m^2 leads to a better growth rate and feed efficiency. The adoptions of a short light regimen similar to that occurring in nature during summer reduces the feed intake without modify the growth rate thus improving the feed efficiency. Foot pad lesion were not affected neither by stocking densities nor by light regimens whereas wood shavings exerted a favourable effect in preserving foot pad in good condition.

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Table 1. Productive traits and foot pad dermatitis score of broilers reared at different light regimens, stocking densities and litter types.

		Stocking density (SD)		Light regimen (LR)		Litter type (LT)		P value for main effects							
		High	Low	Long	Short	Chopped straw	Wood shaving	SD	LR	LT	LRxSD	LRxLT	SDxLT	LRxSDxLT	SEM
Live weight	g	2,404[B]	2,511[A]	2,457	2,458	2,434	2,480	0.01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	26.91
Feed intake	g/bird/d	90.7	90.7	92.2[A]	88.5[B]	90.5	90.8	n.s	0.01	n.s.	n.s.	n.s.	n.s.	n.s.	0.78
FCR	kg/kg	1.62[A]	1.54[B]	1.62[A]	1.55[B]	1.59	1.57	0.01	0.01	n.s.	n.s.	n.s.	n.s.	n.s.	0.02
Mortality	%	2.18	1.32	1.52	1.98	1.93	1.56	n.s	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.02
FPD score	-	10.4	8.9	8.4	10.9	12.0[a]	7.7[b]	n.s	n.s.	0.05	n.s.	n.s.	n.s.	n.s.	1.3

a, b: P<0,05; A, B: P<0,01; n.s.: not significant

Table 2. Meat quality of bird slaughtered at 42 d.

		Stocking density (SD)		Light regimen (LR)		Litter type (LT)		P value for main effects							
		High	Low	Long	Short	Chopped straw	Wood shaving	SD	LR	LT	LRxSD	LRxLT	SDxLT	LRxSDxLT	SE
pH		5.86	5.84	5.85	5.85	5.84	5.86	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.02
L*		57.10	56.57	56.03[b]	57.60[a]	56.91	56.76	n.s.	0.02	n.s.	n.s.	n.s.	n.s.	n.s.	0.35
a*		3.25	3.37	3.11	3.48	3.26	3.37	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.12
b*		3.05[A]	2.61[B]	2.73[b]	2.93[a]	2.89	2.75	0.01	0.05	n.s.	0.02	n.s.	0.02	n.s.	0.12
Drip loss	%	1.14	1.02	1.09	1.07	1.08	1.09	n.s.	n.s.	n.s.	0.02	n.s.	0.02	n.s.	0.04
Cooking loss	%	19.00	18.72	18.79	18.94	19.29[A]	18.26[B]	n.s.	n.s.	0.01	n.s.	0.01	n.s.	n.s.	0.19
Shear value	kg/g	3.18	3.18	3.10	3.25	3.19	3.17	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.09

a, b: P<0,05; A, B: P<0,01; n.s.: not significant

CHAPTER 5

Laying hens welfare, eggs characteristics and bone tissue status

5.1 - INTRODUCTION

5.1.1 - Laying Hen Welfare Regulation

In 2005 and 2006, two EUROBAROMETER surveys on consumers' attitudes to the welfare of animals were carried out in the EU. They show that animal welfare is an issue that citizens rank highly giving it 8 out of 10 on average in terms of importance. Poultry is a priority area of action for animal welfare in the views of citizens. The conditions under which laying hens are kept remain a major animal welfare concern. It is one of the most intensive forms of animal production and the number of animals involved is very high. Widespread public opinion has stimulated the call for more animal friendly and alternative systems to prohibit conventional cages in which laying hens were usually kept.

Welfare of farmed poultry and in particular of laying hens represents a core value for EU citizens. By Council Directive "Laying down minimum standards for the protection of laying hens" (Commission of the European Communities, 1999/74), Member States agreed on various measures, with a staged implementation of some provisions over a time period extending to 2012 taking into account the economic impact of the measure.

Directive 1999/74/EC on minimum standards for the protection of laying hens states that, from 1st January 2012, the rearing of laying hens in conventional cages (known as "unenriched") will be prohibited in the EU. From that date, laying hens will be reared in enriched or furnished cages (750 cm^2 of cage area per hen) or using non-cage system, called aviary system, with or without free-range facilities, with nests, adequate perches and where the stocking density does not exceed 9 laying hens per m^2 usable area. Hens kept in these systems must also have litter to allow pecking and scratching and unrestricted access to a feed.

Article 10 of the Directive (1999/74/EC) requires the Commission to submit to the Council a report, drawn up on the basis of a scientific opinion, regarding the various systems of keeping laying hens. The scientific opinion should take account of pathological, zootechnical, physiological and ethological aspects related to this issue. The Commission required the European Food Safety Authority (EFSA) to issue a scientific opinion on the welfare aspects of systems of rearing laying hens. The Authority was asked in particular to address housing infrastructure such as feeders, drinkers, perches, nests, provision of litter, access to open runs as well as space allowances per hen. The opinion also had to make reference to the implications of these systems towards obtaining safe eggs for consumers. Several panels with vary rules provided to give a scientific opinion building up a report. The Scientific Panel on Animal Health and Welfare (AHAW) provided to a report based on the laying hens welfare and health, while the Biological Hazards (BIOHAZ) and Contaminants in the Food Chain (CONTAM) panels, provided to a report on food safety and microbiological aspects. The scientific opinion of the AHAW panel was adopted the 10th November 2004 (EFSA Scientific Report, 2005) and stated that keeping laying hens in unenriched cages increased the risk of disease, bone breakage, harmful pecking, behavioral problems and mortality.

Article 10 of the European Directive (1999/74/EC) provides that the Commission shall submit to the Council a report on the various systems of rearing laying hens. The Commission adopted this report on 8 January 2008 (COM (2007) 865 final). The report stated that there is scientific and economic support for the ban of conventional battery cages, the deadline fixed on 2012 will be maintained.

Broken and weak bones of laying hens are major welfare concerns in the table egg industry. The incidence of broken and weak bones at the end of lay is a serious problem in the table egg industry. The increasing number of broken bones of layers dramatically affects the collecting procedure of the hens at the end of rearing cycle and during the slaughtering.

5.1.2 - Laying hen bone structure and metabolism in relation to bird welfare

Bone is a complex tissue that is continuously undergoing changes throughout the life of an animal due to the processes of bone formation and bone resorption.

Bone consists of living cells and of an intracellular matrix that is impregnated with mineral salts. It is formed approximately by 70% mineral, 20% organic matter and 10% water. Collagen is the major organic matrix that confers tensile strength to the bone, whereas hydroxyapatite provides compression strength (Rath *et al*., 2000). Bone is a vascularised supporting tissue which is deposited by osteoblasts and by osteocytes, and removed, and hence remodelled, by osteoclasts.

It serves as structural support for the muscles and also as a reservoir of minerals, primarily calcium and phosphorous.

Metabolically bone provides a labile pool of calcium and phosphorous that can be accessed during disturbances in mineral homeostasis (Norman, 1979).

Several factors affect the bone calcification process including age, hormones, dietary calcium and vitamin D.

Parathyroid hormone (PTH) is a polypeptide, which is synthesised by parathyroid glands (Price and Russell, 1992) and plays a major role in bone calcification. The direct effect of PTH on bone is to stimulate bone resorption (Price & Russell, 1992). The decrease in calcium intake stimulates the secretion of PTH (Moreki, 2005). The increased level of PTH in the blood acts on cells in the kidney to stimulate formation of an active form of vitamin D, which in turn acts on cells in the intestine to increase calcium-binding capacity (Moreki, 2005).

Sex hormones also play an important role in calcification. The appearance of sex hormones at puberty hastens bone calcification and the hardening of the area of bone growth called ephyseal junction.

There are two primary types of bone formation: intramembranous ossification and endochondral ossification. All the long bones are formed by endochondral ossification. Endochondral ossification can also be defined as cartilage that is subsequently eroded by vascular and marrow elements (Caplan, 1988). In this process, hyaline cartilage is deposited in the shape of the required bone and is subsequently transformed into bone by mineralisation (Ali, 1992). On the other hand, intramembranous ossification results in the formation of flat bones, and contributes to cortical bone shafts of long bones (Stevens and Lowe, 1992).

Structurally, three types of avian bone have been found: compact bone or cortical bone, cancellous bone or trabecular bone and medullary bone (Hodges, 1974). The hard compact cortical bone is largely found in the shafts of long bones, which surround the marrow cavities. Cancellous or spongy bone is made of a network of fine interlacing partitions, the trabeculae, enclosing cavities that contain either red or fatty marrow (Stevens and Lowe, 1992). Cancellous bone is a type of structural bone that gives internal support (Whitehead *et al*., 1998). It has a large surface area and a greater blood supply than cortical bone, thus making the cancellous bone more responsive to changes in circulating hormones such oestrogen, parathyroid hormone, calcitonin and testosterone, which can affect bone metabolism than cortical bone (Kenney, 2000).

At the hen's onset of sexual maturity, the function of osteoblasts changes from forming lamellar cortical bone to producing a woven bone called medullary bone, which is unique to laying hens. This bone is laid down on the surfaces of structural bone and in spicules within the medullary cavities, especially in leg bones (Whitehead, 2004). Medullary bone is a woven bone that acts as a labile and dynamic source of calcium to supplement dietary calcium for eggshell formation (Whitehead, 2004). Osteoclastic resorption of structural bone continues with the result that structural bone content of the hen declines. Loss of cortical bone, as judged by cortical bone thickness, is a gradual process. This progressive loss of structural bone during the laying period is characteristic of osteoporosis and results in weakening of the skeleton and increased the incidence of fractures. The general net effect of the replacement of structural bone, with medullary bone is to weaken the overall strength of the hen's skeleton and thus to increase fracture risk (Whitehead, 2004). In the absence of structural bone formation, continued osteoclastic resorption would be expected to result in a net depletion of structural bone, leading ultimately to osteoporosis.

The processes described above are reversed when the hen goes out of lay (Whitehead, 2004). Medullary bone gradually disappears and structural bone formation recommences. This can be demonstrated by the appearance of a new layer of structural bone being laid down on top of the layer of medullary bone that previously coated the structural bone surface. This cycle of structural bone loss during egg laying followed by regeneration is normal in a hen laying eggs in clutches followed by incubation and allows the hen to maintain good bone quality over its lifetime. However, selection of the modern hen to remain in a continuously reproductive condition over a prolonged period makes it highly susceptible to osteoporosis.

The mechanism behind these changes is driven by oestrogens. Oestrogens stimulate osteblast function and have an inhibitory effect on osteoclast function. It appears that in hens, the considerable rise in circulating oestrogen at the onset of maturity has a stimulatory effect on osteoblasts, causing them to produce medullary bone instead of structural bone. This is reversed when hen goes out of lay and estrogen levels decline (Whitehead and Fleming, 2000). There is an increased demand of calcium for shell formation during the period the egg spends in the shell gland. Because this usually occurs during the night, when supply of calcium from the digestive system is low, a high proportion of shell calcium comes from resorbed medullary bone.

Causes of osteoporosis are not well defined. It has been suggested that the problem is largely genetic in origin, resulting from the breeding of light weight, energetically efficient birds that maintain a high rate of lay over a prolonged period (Whitehead and Wilson, 1992). It has frequently been assumed that the predisposition to osteoporosis of modern laying strains is a direct consequence of selection for high egg production, and that is the high output of calcium in shells that depletes bone. Osteoporosis increases risk of bone fracture in laying hens, and one might presume that bone fracture is painful, moreover has a number of implications for the welfare of commercial laying hens.

A more specific problem can arise from loss of bone from the spine. This can lead to fracture or collapse of vertebrae, often the free thoracic vertebra, which may result in damage to the spinal cord causing the condition known as cage layer fatigue or layer paralysis.

5.1.3 - Role of Vitamin D_3

Vitamin D is a complex of secosteroids that must undergo metabolic alterations to reach optimal biological activity. Circulating vitamin D is derived from different sources, from the precursor 7-dehydrocholesterol in skin exposed to ultraviolet irradiation, or from the diet in the form of cholecalciferol (D_3) or from the plant sterol, ergocalciferol (D_2). The previtamin D_3 is then converted to vitamin D_3 by a temperature-dependent isomerisation. The dependence on ultraviolet irradiations limits cholecalciferol synthesis to areas of skin with no feather cover. In addition to be produced in the skin, vitamin D compounds (cholecalciferol, ergocalciferol and their hydroxylated metabolites) can be absorbed from the diet in the intestinal tract.

The first metabolite of vitamin D_3 is the 25-hydroxycholecalciferol (Blunt *et al*., 1968). The 25 hydroxylation of vitamin D_3 is catalyzed by 25-hydroxylase and occurs in the liver from where the product is transported by specific binding proteins (DBP) (DeLuca *et al*., 1988). Then in the kidney the 25-hydroxycholecalciferol will be further hydroxylated in 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) and 24,25-dihydroxycholecalciferol ($24,25(OH)_2D_3$).

The $1,25(OH)_2D_3$ stimulates calcium resorption from bone and re-absorption from glomerular filtrate, induces the intestinal epithelium to synthesize calcium binding proteins (CaBP) and increases the calcium absorption from the guts (Bar and Hurwitz, 1973). This CaBP has also been identified in the uterus of laying hens (Fuller *et al*., 1976) and it is responsible for calcium deposition in egg shell at the onset of egg production (Bar and Hurwitz, 1973).

Summarizing, vitamin D is a crucial companion nutrient to calcium and phosphorous and it plays an essential role in the utilization of the dietary calcium to maintain or regulate calcium homeostasis. In the normal young chicken, about 70% of calcium absorption is vitamin D dependent (Hurwitz, 1992).

In fowls, vitamin D is responsible for normal growth, egg production, shell quality, and reproduction. The essentiality of vitamin D in hen feed is particularly significant for profitable egg and meat production in the modern poultry industry where the birds are raised indoor.

It has been suggested that the efficiency of the hydroxylation reactions necessary to convert cholecalciferol into its metabolically active form i.e. $1,25(OH)_2D_3$ may be reduced in aged hens (Frost *et al.*, 1990; Elaroussi *et al.*, 1994)). Adequate level of $1,25(OH)_2D_3$ is required for the regulation of calcium absorption and excretion and for the mobilization of calcium from the bone to provide adequate calcium amount required for egg shell formation. Investigations into the ability of the older hens to metabolize or respond to vitamin D_3 have shown that shell quality and bone strength deteriorates more rapidly (Bar and Hurwitz, 1987). However, supplementation of vitamin D_3 to the deficient diets alleviated the decline in productivity and shell quality (Newman and Leeson, 1997).

The present study was carried out to investigate more widely the possible role of 25-hydroxycholecalciferol supplemented in the diet of a laying hen commercial strain (Lohmann brown) in comparison of diets supplemented with D_3 or with D_3 + 25-hydroxycholecalciferol. Egg traits during a productive cycle as well as the bone characteristics of the layers have been as well evaluated to determine if there the vitamin D_3 may enhance the welfare status of the birds.

5.2 - MATERIALS ANS METHODS

5.2.1 - Birds and housing

63.000 Lohmann Brown pullets, 16 wk of age, were used. Birds were divided in 3 different groups of 21.000 hens each one. Each group was split into 2 subgroups and housed in 2 farms (2 replicates per group). Hens were reared till 73 wk of age in windowless poultry houses with litter-covered floor, nesting boxes and perches at a density of 9 hens/m^2.

5.2.3 - Experimental design and treatments

Three feeding treatment were performed. Group D3 (control) received a commercial standard diet containing 3,000 IU of vitamin D_3/kg of feed. Group D3+25D3 received the basal diet containing 3,000 IU of D_3 per kg of feed till 39 wk of age and from 40 to 73 wk the standard diet containing 1,500 IU of D_3 per kg of feed and 37.5 µg of 25-hydroxycholecalciferol (Hy•D®, DSM trade name product) that is the equivalent of 1,500 IU of D_3. Group 25D3 received the standard diet containing 1,500 IU of D_3 per kg of feed plus 37.5 µg of 25-hydroxycholecalciferol (Hy•D®, DSM trade name product) from 18 wk until 73 wk.

5.2.4 - Procedures

At 30, 50 and 71 wk of age, 300 eggs were collected per each group.

After collection, the eggs were taken to the laboratory, and stored at room temperature for one day before measurements.

Eggs were individually weighed and eggshell quality was determined by measuring the eggshell breaking strength with “Egg Shell Force Gauge – Robotmation Co. Ltd.”.

Yolks were weighed immediately after breakage, whereas shells were weighed after drying overnight in an oven at 100°C. Albumen weight was calculated as the difference of whole egg weight minus yolk and shell weight.

At the same sampling time 16 birds were randomly selected from each group and killed by cervical dislocation. From each hen both tibiae were removed and bones were cleaned of soft tissue and stored at 0-4°C for max 4 days prior to bone quality analysis.

Tibiae have been radiographed in a Faxitron 405 soft X ray apparatus using Kodak MRE-1 high resolution mammography film in Min-R2 cassettes with a single Min-R intensifying screen. Exposure was for 15 seconds at 30 Kv. Each exposed plate included a 16 step aluminium step-wedge, with 0.25 mm increments, for calibration purposes. The films were digitized via a Panasonic WVBL600 monochrome video camera connected to an Apple Macintosh-based image analysis system running the public domain software package, NIH Image 1.60 (Universal Resource Locator: <http://rsb.info.nih.gov/nihimage/>). Each bone was delineated from the background and the mean radiographic density (pre-calibrated in mm of aluminium equivalent) of the bone was measured.

After bone mineral density was completed, breaking strength was determined. This test was carried out by 3-point bending using an Instron Universal Machine. The centre of each bone was aligned with the breaking probe (10 mm diameter) which approached at 30 mm/min. The supports for each bone were 30 mm apart. The breaking strength was determined from the failure point (peak) of each loading curve.

The individual bones were dried for 24 h at 100°C and weighed, followed by ashing at 600°C overnight. Percentage of ash was calculated on a dry bone basis.

5.2.5 - Statistical analysis

The data were analysed by one-way ANOVA using the general linear model procedure of SAS software (SAS Institute, 1989), the main factor was the vitamin supplementation. Means were separated by the Student Newman Keuls test. The data corresponding to eggshell/egg weight were subjected to arc sine transformation before ANOVA. Differences were considered significant at $P < 0.05$ and $P < 0.01$ levels.

5.3 - RESULTS

5.3.1 - Eggs characteristics

The first sampling has been done at 30 weeks collecting samples of eggs and bones only from group D3 and group 25D3. Samples of group D3+25D3 have been collected only in the middle and at the end of the production cycle, since at 30 weeks the diet and the farm conditions of this group were identical to the group D3.

The results obtained from the replicates of each group were analyzed together and considered as only one farm because similar data were found.

At 30 weeks, hens of group 25D3, which received the vitamin D3 and 25-hydroxycholecalciferol for the whole laying cycle, laid eggs significantly ($P < 0.01$) heavier than those of group D3 (63.29 *vs*. 59.41 g). To a greater mass of the egg corresponded as well a greater weight of the egg components, albumen and yolk, as well as the eggshell (6.45 g *vs* 6.17 g, $P < 0.01$). The eggshell/egg weight ratio resulted higher in D3 group ($P < 0.05$), whereas the eggshell breaking strength did not result significant even if the values of group D3 were slightly higher.

During the second egg sampling, at 50 weeks, also the eggs characteristics of group D3+25D3 have been evaluated. The eggs of group 25D3 were statistically the heaviest, confirming the results obtained during the former sampling. Eggs laid by the hens of group D3+25D3 registered the lowest weight. Eggs from group D3 appeared more resistant to the breakage, in fact the eggshell breaking strength resulted significantly higher ($P < 0.01$) compared to the resistance of the other groups (Table 2).

In occasion of the last sampling, at 71 weeks, the difference among the eggs of the three groups considerably changed. Indeed the eggs produced by the hens that have received the 25-hydroxycholecalciferol from 40 wk of age onwards, exhibited the highest weight of whole egg and of its parts ($P < 0.01$). Moreover, surprisingly, also the eggshell/egg weight ratio as well as the eggshell breaking strength of these eggs appeared significantly higher ($P < 0.01$) than both D3 and 25D3 eggs (3.550 *vs* 3.335 and 3.336 kg respectively) (Table 3).

5.3.2 - Tibia characteristics

Tibiae were collected in occasion of the egg collection (30, 50 and 71 wk of age). As for egg sampling at 30 weeks, only hens from group D3 and 25D3 were sacrified. The data coming from the replicates of the same groups were elaborate together because of the data equality.

At 30 wk any difference between groups emerged as for radiographic density, breaking strength and ash content of bones (Table 4).

At 50 weeks, when also the tibiae of birds of group D3+25D3 were collected, a greater difference among the dietary treatments was observed. The treatment with 25-hydroxycholecalciferol appeared to affect breaking strength. Indeed 352.71 N were necessary for the breakage of bones of group D3+25D3, 348.30 and 303.22 N respectively for tibiae of groups 25D3 and D3 (Table 5). These differences approached statistical significance for $P < 0.05$.

In the last sampling, at 71 weeks, the better condition of bones of group receiving 25-hydroxycholecalciferol observed in the previous sampling did not appear and the data concerning the tibiae characteristics resulted rather similar in all groups (Table 6).

5.4 - DISCUSSION

The objective of the present investigation was to analyze the effect of a standard diet for laying hens supplemented with 25-hydroxycholecalciferol, instead of a normal supplementation of vitamin D3, on egg quality traits and tibia status of hen for evaluating the bird welfare conditions.

Egg weight is one relevant productive trait in poultry, is highly heritable and its economic implications are obvious (Di Masso *et al*., 1998). Under the experimental conditions employed emerged that the weight of the egg and of its components is often greater in hens fed a diet enriched with 25-hydroxycholecalciferol. The major differences can be appreciated at the beginning of the production cycle where the eggs of the 25D3 group were 3 g heavier than those of the D3 group. In literature there are no data able to explain the effect of 25-hydroxycholecalciferol on egg size.

Our results do not agree with those of Keshavarz (2003) who did not find any beneficial effect of 25-hydroxycholecalciferol, when administered to hens from 50 to 66 wk of age, on shell quality and egg mass. However it must be considered that the author did his experiment only in the last period of laying hen cycle. Indeed according to Korver & Saunders-Blades (DSM pre-symposium, WPSA European Poultry Symposium, Verona 2006) the efficacy of 25-hydroxycholecalciferol on bone tissue and shell quality is more evident if the vitamin metabolite is administered early particularly from the first stage of laying cycle, from 12 to 64 weeks. In their trial, the Authors observed that hen fed 25-hydroxycholecalciferol had equivalent cortical bone reserves than hens fed vitamin D_3 but a decreased amount of medullary bone. This suggests that hens fed 25-hydroxycholecalciferol had preferentially used the medullary bone to support the formation of a greater number of well-shelled eggs, rather than using excessive cortical bone.

Since the calcium binding protein (CaBP) activity depends on the vitamin D_3 for calcium absorption and transport, it can be reasonable to think that the 25-hydroxycholecalciferol enhances the mineral absorption, thus eggshell should be more resistant to breakage.

In our trial we did not find a clear effect of the metabolite on eggshell strength: it might argue that the metabolite supplementation favors the calcium absorption but since eggs of treated groups are heavier and a larger amount of shell is needed, a direct effect on shell strength is observed. Our results are consistent with those of De Ketelaere *et al.* (2002) who stated that there is very strong evidence that egg weight is not related to the breaking force. However at 71 wk of age to a greater egg weight corresponded the highest value of breaking strength when 25-hydroxycholecalciferol is supplied from 40 to 72 weeks.

Nearly to the end of the production cycle, birds are stressed because of the farm conditions and the highly production performances. This physiological situation could compromise the status of the liver and the enzymes activities, like the 25-hydroxylase which converts vitamin D_3 into 25-hydroxycholecalciferol. Bar *et al.* (1999) observed an apparent defect in vitamin D metabolism in older hens.

Since the 25-hydroxycholecalciferol is the first hydroxylated form of vitamin D_3, feeding hens directly this metabolite, it is possible to by-pass the hydroxylation step in the liver.

25-hydroxycholecalciferol is absorbed easily than vitamin D_3 because of the increased polarity of the molecule (and increased water solubility) that probably minimizes its dependence on micelle formation for absorption (Applegate and Angel, 2002; Ward, 2004). This condition allows the birds to use the vitamin D_3 metabolite directly and presumably they have a better response in term of shell calcification. Grobas *et al.* (1999) and Al-Batshan *et al.* (1994) indicated that aged hens are less efficient in absorbing calcium than younger ones; this physiological situation could be another reason that can explain the results detected in the study carried out. During the last phase of the egg production also the level of estrogens decreased and since estrogens are required for activation of 25-hydroxylase liver enzyme (Tanaka *et al.*, 1978) it might be less production of vitamin D_3 metabolites.

Since in aged hens the metabolism of vitamin D and, by consequence the calcium transport and absorption, are less efficient, it can be hypothetically assumed that the feeding of 25-hydroxycholecalciferol in addition to vitamin D_3 in a commercial laying hen diet, can improve both calcium and vitamin D metabolisms with beneficial effects on hen performances and egg traits.

At 30 and at 50 wk of age hens fed 25-hydroxycholecalciferol exhibited greater values of bone breaking force (near to the statistical significance only for 50 wks) than the control birds. This result might suggest that the metabolite has some impact on bone characteristics, although in this study the effect declined over time. Apart from the diet supplementation with the vitamin D_3 metabolite, also the differences in laying hen bone tissue from growing poultry, in particular concerning the medullary bone formation have to be considered.

In laying hens other than structural bone (cortical and trabecular bones) there is the medullary bone, a unique bone structure for mature laying hens that does not exist in growing poultry. The difference between immature and mature birds in bone structure may create different relationships between bone parameters (Zhang B. and Coon C. 1997), like bone strength, densitometry and ash percentage. During the period of egg production, the content of medullary bone increases at the expense of cortical bone, loading to a progressive structural bone loss (Whitehead and Fleming, 2000).

At 71 weeks of age the effect of metabolite on bone strength disappeared and data concerning bone characteristics of three groups were quite similar. Fleming *et al.* (1996) assessed that medullary bone may contribute to strength and that bone breaking strength is largely a combination of the density and area of cortical bone. The lack of difference between treated and control groups might be due to an increased amount of medullary bone.

Radiographic density values obtained in the trial carried out are always higher in hens fed with 25-hydroxycholecalciferol of both treatments: supplemented for the whole laying cycle (25D3) or from 40 weeks of age onward (D3+25D3). At 71 weeks the greater bone density corresponds to the lower breaking strength. Since the differences of tibia breaking force are not particularly important among groups, and the density values are higher in the treated groups, it can be suppose that the cortical areas of tibia of these hens are slightly well preserved than in the control ones which received only vitamin D_3.

The ash values are very similar during each sampling. The amount of ashes is negatively correlated to the presence of medullary bone (Clark *et al*., 2008).

In the trial carried out we observed a more evident effect of the 25-hydroxycholecalciferol on the egg size when the vitamin metabolite is supplemented from 18 weeks of hen's age. Due to the extra egg size the shell breaking strength determined is lower in the treated groups than in the control one. On the contrary, a beneficial effect of the 25-hydroxycholecalciferol is found in the last phase of the production cycle on eggshell strength. Birds fed 25-hydroxycholecalciferol from 40 weeks of age laid bigger and stronger eggs.

Tibia breaking strength resulted largely improved in hens receiving a diet supplemented with vitamin D_3 and 25-hydroxycholecalciferol. This improvement declined over time and at the end of the laying cycle the data obtained among groups are very similar.

Further researches are needed to better explain the relation between the feed administration of the 25-hydroxycholecalciferol on egg characteristics such as egg size and shell strength. It would be also interesting to deepen the effect of the metabolite on bone development over the physiological changes of the tissue during the phases of the laying production.

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Table 1. Eggs characteristic determined at 30 weeks of age of D3 (Control) hens fed a basal diet containing 3,000 IU of vitamin D_3/kg of feed and in group 25D3 receiving the basal diet containing 1,500 IU of D_3 per kg of feed plus 37.5 µg of 25-hydroxycholecalciferol (Hy•D®, DSM trade name product), corresponding to 1,500 IU of D_3 from 18 wk until 73 wk.

		D3	25D3	SE	P
Egg weight	g	59.41B	63.29A	0.30	0.01
Yolk weight	g	13.29B	14.25A	0.06	0.01
Albumen weight	g	39.94B	42.63A	0.24	0.01
Eggshell weight	g	6.17B	6.45A	0.03	0.01
Eggshell/egg weight	g	0.104a	0.102b	0.00	0.05
Eggshell breaking strength	kg	4.056	4.032	0.39	n.s.

a, b: P<0.05; A, B: P<0.01.

Table 2. Eggs characteristic determined at 50 weeks of age either of D3 (Control) hens fed a basal diet containing 3,000 IU of vitamin D_3/kg of feed, or D3+25D3 hens receiving the basal diet containing 3,000 IU of D_3 per kg of feed till 39 wk of age and from 40 to 73 wk the basal diet containing 1,500 IU of D_3 per kg of feed plus 37.5 µg of 25-hydroxycholecalciferol (Hy•D®, DSM trade name product) that is the same as 1,500 IU of D_3. Group 25D3 received the basal diet containing 1,500 IU of D_3 per kg of feed plus 37.5 µg 25-hydroxycholecalciferol (Hy•D®, DSM trade name product) from 18 wk until 73 wk.

		D3	D3+25D3	25D3	SE	P
Egg weight	g	65.68AB	64.79B	66.23A	0.29	0.01
Yolk weight	g	17.21A	16.87B	16.99AB	0.08	0.01
Albumen weight	g	41.96AB	41.48B	42.70A	0.24	0.01
Eggshell weight	g	6.50	6.44	6.51	0.03	n.s
Eggshell/egg weight	g	0.099	0.096	0.99	0.00	n.s.
Eggshell breaking strength	kg	3.769A	3.568B	3.519B	0.04	0.01

a, b: $P<0.05$; A, B: $P<0.01$.

Table 3. Eggs characteristic determined at 71 weeks of age either of D3 (Control) hens fed a basal diet containing 3,000 IU of vitamin D_3/kg of feed, or D3+25D3 hens receiving the basal diet containing 3,000 IU of D_3 per kg of feed till 39 wk of age and from 40 to 73 wk the basal diet containing 1,500 IU of D_3 per kg of feed plus 37.5 µg of 25-hydroxycholecalciferol (Hy•D®, DSM trade name product) that is the same as 1,500 IU of D_3. Group 25D3 received the basal diet containing 1,500 IU of D_3 per kg of feed plus 37.5 µg 25-hydroxycholecalciferol (Hy•D®, DSM trade name product) from 18 wk until 73 wk.

		D3	D3+25D3	25D3	SE	P
Egg weight	g	67.80ab	68.57a	67.42b	0.32	0.05
Yolk weight	g	17.79B	18.27A	17.44C	0.09	0.01
Albumen weight	g	43.60	43.68	43.57	0.27	n.s.
Eggshell weight	g	6.48B	6.67A	6.46B	0.04	0.01
Eggshell/egg weight	g	0.095B	0.098A	0.096AB	0.00	0.01
Eggshell breaking strength	kg	3.335B	3.550A	3.336B	0.04	0.01

a, b: $P<0.05$; A, B: $P<0.01$.

Table 4. Tibia characteristics determined at 30 weeks of age of D3 (Control) hens fed a basal diet containing 3,000 IU of vitamin D_3/kg of feed and in group 25D3 receiving the basal diet containing 1,500 IU of D_3 per kg of feed plus 37.5 µg of 25-hydroxycholecalciferol (Hy•D®, DSM trade name product), corresponding to 1,500 IU of D_3 from 18 wk until 73 wk.

		D3	25D3	SE	P
Tibia weight	g	11.78	11.50	0.24	n.s.
Tibia radiographic density	mm Al eq.	1.90	1.92	0.02	n.s.
Bone breaking strength	N	315.89	344.02	16.25	n.s.
Ash	g/dry matter	0.28	0.28	0.00	n.s.

Table 5. Tibia characteristics determined at 50 weeks of age either of D3 (Control) hens fed a basal diet containing 3,000 IU of vitamin D_3/kg of feed, or D3+25D3 hens receiving the basal diet containing 3,000 IU of D_3 per kg of feed till 39 wk of age and from 40 to 73 wk the basal diet containing 1,500 IU of D_3 per kg of feed plus 37.5 µg of 25-hydroxycholecalciferol (Hy•D®, DSM trade name product) that is the same as 1,500 IU of D_3. Group 25D3 received the basal diet containing 1,500 IU of D_3 per kg of feed plus 37.5 µg 25-hydroxycholecalciferol (Hy•D®, DSM trade name product) from 18 wk until 73 wk.

		D3	D3+25D3	25D3	SE	P
Tibia weight	g	11.47	11.31	11.60	0.27	n.s.
Tibia radiographic density	mm Al eq.	2.04	2.09	2.10	0.03	n.s.
Bone breaking strength	N	303.22	352.71	348.30	15.26	n.s.
Ash	g/dry matter	0.28	0.29	0.29	0.00	n.s.

Table 6. Tibia characteristics determined at 71 weeks of age either of D3 (Control) hens fed a basal diet containing 3,000 IU of vitamin D_3/kg of feed, or D3+25D3 hens receiving the basal diet containing 3,000 IU of D_3 per kg of feed till 39 wk of age and from 40 to 73 wk the basal diet containing 1,500 IU of D_3 per kg of feed plus 37.5 µg of 25-hydroxycholecalciferol (Hy•D®, DSM trade name product) that is the same as 1,500 IU of D_3. Group 25D3 received the basal diet containing 1,500 IU of D_3 per kg of feed plus 37.5 µg 25-hydroxycholecalciferol (Hy•D®, DSM trade name product) from 18 wk until 73 wk.

		D3	D3+25D3	25D3	SE	P
Tibia weight	g	11.44	11.23	11.21	0.23	n.s.
Tibia radiographic density	mm Al eq.	2.10	2.14	2.13	0.03	n.s.
Bone breaking strength	N	332.67	322.82	328.55	13.09	n.s.
Ash	g/dry matter	0.30	0.31	0.31	0.00	n.s.

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E. Folegatti, F. Sirri, A. Meluzzi, T. Toscani (2006) – Prevalence of foot pad lesions and carcass injuries as indicators of broiler welfare conditions in Italy. World's Poultry Sci. J., 62 (suppl. 1): 578-579.

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