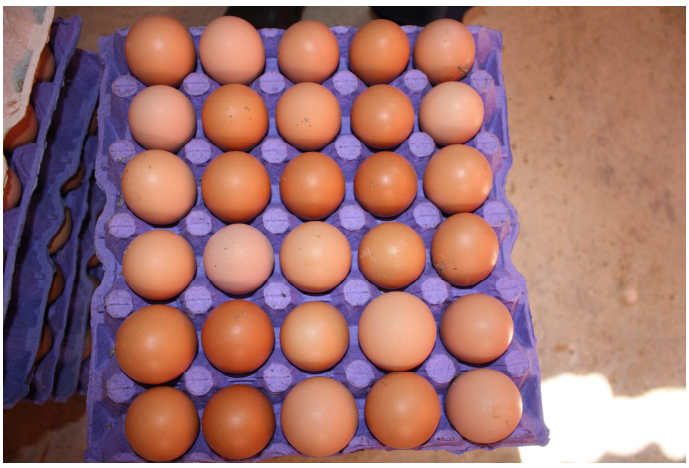
Bacterial contamination of eggshells in conventional cages and litter floor systems for laying hens in Jordan



by

Sophie Jenssen Söderström

Institutionen för husdjurens utfodring och vård

Swedish University of Agricultural Science
Department of Animal Nutrition and Management

Examensarbete 318
15 hp C-nivå

Uppsala 2010

Bacterial contamination of eggshells in conventional cages and litter floor systems for laying hens in Jordan

by

Sophie Jenssen Söderström

Handledare: Klas Elwinger och Hamza Al-Qadiri
Examinator: Ragnar Tauson

Nyckelord: Egg, laying hens, bacteria, *Salmonella, Campylobacter, Escherichia coli*, Jordan, housing system

Institutionen för husdjurens utfodring och vård

Examensarbete 318
15 hp C-nivå
Kurskod: EX0550

Swedish University of Agricultural Science
Department of Animal Nutrition and Management

Uppsala 2010

Nr	Titel och författare	År
309	Urea som kvävekälla till växande ungnöt Urea as a nitrogen source for growing cattle 15 hp C-nivå Sofia Åström	2010
310	Metoder för hullbedömning av hästar Methods for body condition assessment in horses 15 hp C-nivå Eva Andersson	2010
311	Tungmetaller i metabolismen hos värphöns och slaktkycklingar Metabolism of heavy metals in poultry 15 hp C-nivå Elin Svedberg	2010
312	Utfodringens betydelse för hästens hälsa The impact of feeding for the health in horses 15 hp C-nivå Cornelia Andersson	2010
313	Faktorer som påverkar magnesiumabsorptionen i våmmen hos kor Factors affecting ruminal magnesium absorption in cows 15 hp C-nivå Emily Wallström	2010
314	Samband mellan högt hull innan könsmognad och mjölkkors förmåga att producera mjölk Relationship between high body condition before onset of puberty and the dairy cow's ability to produce milk 15 hp C-nivå Lottie Björkegren	2010
315	Effekten av olika andelar grovfoder och kraftfoder i foderstaten på mjölkproduktion, välfärd och hälsa hos mjölkkor The effect of different forage and concentrate ratios on milk production, welfare and health in dairy cows 15 hp C-nivå Susanna Herlitz	2010
316	Effect of plant maturity at harvest of haylage on digestibility and faecal particle size in horses fed foragedominated diets 30 hp E-nivå Emelie Wickström	2010
317	Methane production of dairy cows fed cereals with or without protein supplement and high quality silage 30 hp E-nivå Christina Yunta Bernal	2010

I denna serie publiceras examensarbeten (motsvarande 15 eller 30 högskolepoäng) samt större enskilda arbeten (15-30 högskolepoäng) vid Institutionen för husdjurens utfodring och vård, Sveriges Lantbruksuniversitet. En förteckning över senast utgivna arbeten i denna serie återfinns sist i häftet. Dessa samt tidigare arbeten kan i mån av tillgång erhållas från institutionen.

DISTRIBUTION:
Sveriges Lantbruksuniversitet
Institutionen för husdjurens utfodring och vård
Box 7024
750 07 UPPSALA
Tel. 018-67 28 17

Abstract

In the country of Jordan, egg production is very important economically, but several bacteria are involved in food borne diseases transmitted from eggs or other poultry products. The most important are *Salmonella* spp., *Campylobacter jejuni* and *Escherichia coli*. In Jordan two different housing systems are used for hens in large scale egg production; conventional cages and litter floor systems. Eggs from four different farms were analysed and compared to see if a difference could be found regarding the presence of pathogenic bacteria on the egg surfaces between these two systems. Total number of aerobic bacteria was analysed, the presence of *Salmonella* spp., *C. jejuni* and *E. coli*, as well as the number of cracked eggs on the farms. Also air samples were taken in all systems and egg storage rooms. Our results showed significantly more pathogenic bacteria on eggs from the floor systems, both *Salmonella* spp. and *E. coli*. On the other hand, no significant difference could be detected in the amount of *Campylobacter jejuni*. The air samples showed more bacteria in the air of floor systems. Our conclusion is that eggs from hens in Jordanian floor systems are more contaminated with pathogenic bacteria than eggs from cage systems.

Keywords: egg, laying hens, bacteria, *Salmonella*, *Campylobacter*, *Escherichia coli*, Jordan, housing systems

Sammanfattning

Äggproduktion är Jordaniens femte största jordbruksprodukt, räknat i ekonomiskt produktionsvärde, och är därför mycket viktig. Värphönsen i den intensiva äggproduktionen hålls i två typer av system, i burar ämnade för 4 hönor eller i golvsystem på ströbädd. Det finns för- och nackdelar med båda systemen. Nackdelen med burar är att de inte tillåter fåglarna att röra sig mycket och utföra sina naturliga beteenden. Fördelarna är att de är rena och att sjukdomskontrollen underlättas. I golvsystemen har man däremot problem med fjäderplockning och kannibalism, något som man i Jordanien löser genom att näbbtrimma hönorna. Detta ingrepp kan vara förenat med smärta i olika grad beroende på när det utförs och med vilken metod och är därför förbjudet i bl.a. Sverige. Golvsystemet är det vanligaste, ca 80 % av hönsen hålls i denna typ av system. Fördelarna är att värphönsen här kan röra sig fritt och kan utföra flera av de naturliga beteenden som de inte kan i konventionella burar.

Då äggen ska bli livsmedel är hygien en viktig aspekt inom äggproduktionen. Flera bakterier som är inblandade i allvarligare matförgiftningar kan finnas på ägg, bl.a. *Echerichia coli*, *Salmonella* spp. och *Camylobacter jejuni*. Målet var att jämföra om förekomsten av dessa på äggen skiljer sig mellan de två inhysningssystemen.

Prover togs från 4 olika gårdar i Jordanien; en gård med båda golv- och bursystem, 2 gårdar med enbart golvsystem och en gård med enbart bursystem. Från varje system togs 3 prover med 4 ägg i varje. Prover togs även från gårdarnas förvaringsrum - 3 prover från varje. Dessa sammansatta prover bereddes sedan för isolering och identifiering av det totala antalet aeroba bakterier, *E. coli*, *Salmonella* spp. och *C. jejuni*. Som komplement till äggproverna togs även svabbtest på ytor i redena i golvsystemen, på äggrännan i bursystemen och på ytor i förvaringsrummen. Detta var huvuddelen i studien, men dessutom togs luftprover på samtliga gårdar och för identifiering av eventuella sprickor i skalen lystes 100 ägg på varje gård. Bakterier som växte på selektivt medium för respektive bakterie benämndes som presumtiva och bakterier som gav positivt svar på konfirmerande tester kan betraktas som bakterien i fråga.

Resultaten från de statistiska analyserna visade att det var signifikant fler bakterier inklusive presumtiva *E. coli* och *Salmonella* spp. på äggen från golvsystemen. Ingen signifikant skillnad fanns mellan systemen när det gäller presumtiva *C. jejuni*. Konfirmerande *E. coli*, *Salmonella* spp. och *C. jejuni* återfanns dessutom enbart på ägg ifrån golvsystemen eller i förvaringsrum där dessa ägg förvarades, de var dock för få för att göra statistiska analyser på.

Vi kom fram till att ägg som kommer från golvsystemen tycks vara smutsigare och ha mer patogena bakterier på ytan än ägg som producerats i bursystem.

Table of Content

1 Introduction

Egg production is the fifth primary agricultural commodity in the country of Jordan, ranked by value. Only olives, tomatoes, sheep milk and cow milk have higher turnover. This Middle East country with its population of about 6.4 million people, produced in 2007; 38 764 tons of eggs and had at the same time 1.86 million laying hens in production (FAOSTAT, 2007). The housing systems used for these hens in intensive egg production, are either deep litter floor systems or different battery cage systems. The latter are exclusively conventional cages without nest, dust bath and perch, and are designed for a maximum of 4 birds (Zakaria, personal communication 2010). This type of cage is not allowed in the European Union from the beginning of year 2012 mainly because of the limitations for the birds to behave naturally (Rådets direktiv, 1999). No similar law against the use of conventional cages is suggested to be legislated in Jordan in the foreseeable future.

The floor systems are the most common way of keeping laying hens in Jordan; about 80 % of the hens are housed in litter floor systems (Zakaria, personal communication 2010). The advantages of the floor systems are many. Primarily the development of this system has enabled the production to reach its high profitability with its low production costs (Appleby *et al*, 2004). Also the hens are able to perform several comfort behaviours like dust bathing and foraging and show an increased activity compared to those in cages. Because of this, hens in the floor systems have shown to have stronger and better skeletons (Appleby *et al*, 2004). But there are disadvantages as well, mainly due to the high number of layers that are housed together. Because of the large flock size, the hens are not able to keep a natural peck order, often resulting in unwanted behaviour like cannibalism and feather pecking (Appleby *et al*, 2004). In Jordan and many other countries this is solved by beak trimming the birds at a young age, mainly when they are day old. This is meant to prevent the birds from hurting each other without effecting there ability to eat. The shortcoming of this solution is that the procedure of beak trimming is very painful for the chicks and can cause long lasting ache even after the

immediate pain is over (Perry, 2004a). Although the problem with cannibalism is bigger in floor systems, all hens in production in Jordan are beak trimmed, even those used in cages. Finally, the floor system may also show a high number of dirty eggs and a higher occurrence of disease in the birds compared to cage housing (Fossum *et al,* 2009). The control of disease is much better in cage systems. An infected bird is easier to detect and isolate and the disease is not spread to as many birds (Appleby *et al,* 2004).

Because of the risk of spreading of diseases, the aspect of food quality and hygiene of animal products for human consumption is an important aspect of egg production. Zoonoses are diseases that can be transmitted from animals to humans, either when humans come in direct contact with the infected animal or when consuming or handling the animal food products (Willey *et al,* 2009). Campylobacteriosis and salmonellosis are two zoonoses that can be transmitted to humans by contact with either the poultry itself or their eggs (Willey *et al,* 2009). The animals can serve as reservoirs without showing any symptoms of these diseases but still transmitting it to humans. Eggs can be contaminated by coming in contact with contaminants like dust or droppings in the nest or on the litter floor (Perry, 2004b) but in fact, much of the salmonellosis originates from a feed ingredient.

Campylobacteriosis is caused by *Campylobacter fetus* or *Campylobacter jejuni* and symptoms include severe diarrhoea. *C. jejuni* is considered the prime cause of acute bacterial gastroenteritis in humans. This pathogen is often transmitted by consumption of raw or under cooked chicken or poultry products. Infectious dose is as few as 10 viable bacteria (Willey *et al,* 2009). Salmonellosis is caused by several *Salmonella* serovars, but mainly by *Salmonella typhimurium* and *Salmonella enteritidis*. These bacteria are often ingested by consumption of eggs and other food products and also cause severe gastroenteritis (Willey *et al,* 2009). Infective dose of *Salmonella* spp. could be as low as 15-20 bacterial cells depending on the age and health of the host (Ministry of Health, Jordan).

Escherichia coli are found naturally in the gastrointestinal tract of all warm blooded animals. It is part of the non taxonomic group of coliform bacteria, these are gram-negative bacteria which is characterised by the fact that they within 48 hours produce gas and acid when fermenting lactose (BAM, 2002). Most strains of *E. coli* are not considered as pathogenic, but a few are and can cause gastrointestinal illness in humans. For instance serotype EHEC has a low infective dose and can cause watery and bloody diarrhoea to those infected. Because of the easy detection of *E. coli*, these bacteria are commonly used as an indicator of faecal contamination of food products (Willey *et al,* 2009).

Cracks in the egg shells are another quality concern. For example, eggs with cracks spoil faster than intact eggs (Gietema, 2005). If not infected during formation, an egg with intact shell is almost completely free of bacteria inside. If there are damages and cracks on the other hand, pathogenic bacteria can more easily invade (Rose, 1997). Cracks are not always seen with the bare eye, the finest "hairline cracks" can only be detected when shining a bright light trough it, so called candling (Solomon, 1991).

According to Jordanian standards, eggs for human consumption should be collected under hygienic conditions from healthy birds. The farm should also be clean and free from any, for human, pathogenic microorganism. Personal hygiene of the workmen should be applied. Before selling the eggs they should bee free from faecal contamination and dirt (Jordanian Standards, 1988). Although these standards exist in writing, Jordan has no developed program of checking the implications of these directives on farms, implying that many farmers do not consider these rules in there production. Moreover, there is no practise of cleaning the eggs in Jordan before selling (Zakaria, personal communication 2010).

The objective of this study was to evaluate the hygienic conditions of Jordanian egg production and further to compare the bacterial micro flora on eggs from hens housed in floor systems with eggs from hens housed in cage systems. Especially we would like to see if there is a difference in the amount of *Salmonella*, *Campylobacter* and *E. coli* present in the two systems. Because of the high risk of food borne illness linked to eggs this was an important study for public health reasons but also for the aspect of animal welfare because of the different features of the two systems compared microbiologically. This is the first study looking at the microflora of eggs in Jordan and could highlight the need for further and more detailed studies in this subject.

2 Materials and methods

Four different farms were selected according to what type of housing system they used. A summary of the practises on the farms is shown in table 1. All data in the table originates from information from the farmers. Therefore the information could be biased.

Table 1. Summary of facts on the 4 farms included in this study

	Farm 1		Farm 2	Farm 3	Farm 4
Housing system	Cage	Floor	Floor	Floor	Cage
Age of hens (weeks)	20	50	48	28	23
Breed	Mixed breeds	Hy-line W36 White	Hy-line W36 White	Hisex Brown + Bovans Black	Hisex White
Collecting of eggs (per day)	2	2	5	2	1
Hens per m^2 ground area resp. hens per cage	4 hens/ cage	10 hens/ m^2	5,2 hens/ m^2	7-8 hens/ m^2	5 hens/ cage
Hens per nest	-	10	5	5	-
Use of antibiotics (in water or feed)	Yes	Yes	If needed	If needed	No
Vaccination against	No info.	No info.	Salmonella, New Castle and others	Salmonella, New Castle and others	No info.
Eggs laid out of nest (% per day)	-	2,2	1	8	-
Time between batches	No info.	No info.	No info.	1-3 weeks	8 weeks
Age of buildings (years)	5	5	17	6	12
Cleaning of interiors between rounds	Water + antibacterial agent	Water + antibacterial agent	Water	Water + antibacterial agent	Water + antibacterial agent

In addition to the information in Table 1, the hens at all farms were beak trimmed to prevent cannibalism and feather pecking. Also, according to the farm-

ers, all farms had less than 1-2 dead birds per day. All farmers mixed their feed on the farm, using both imported and Jordanian feed ingredients. There was no heat treatment of feed, it was an all mash feed. Feeding was automatic by chain feeder and water provided in automatic water bells in the floor systems. In the cage system of farm 1, the birds were fed manually and had water in nipples. At farm 4 feeding were automatic with chain feeders and water in water troughs. All farms were applying all-in all-out of birds in their production and cleaned the houses with water and antibacterial agents between rounds. The exception was farm 2 which only used water when cleaning the stable, no antibacterial agent. Finally all farms collected the eggs manually by hand and stored the eggs in storage rooms without cooling systems.

2.1 Sampling

Samples were taken from the 4 different farms at 4 separate occasions. The first farm had cage- and floor system in separate buildings, the following two farms had the hens in free range floor systems (figure 1), and the last farm had cage systems (figure 2). In total samples were taken from 3 floor systems and 2 cage systems. If the farms had several stables, sampling was only carried out in one of them. All farms were selected by the university, but should be representative of the egg production in Jordan.

Egg samples were gathered from the storage rooms and from the production site at all farms. In the case of floor systems the eggs were collected from the nests and if possible one of the samples was collected from the floor. In the cage systems eggs were taken from the egg cradle. Each egg sample was made up by 4 eggs taken randomly from the same location. At all 4 farms 3 samples were taken from each location. The exception was one of the floor systems where only 2 samples could be collected; due to the farmer's request.

In addition to the egg samples, swabs were taken in the storage rooms, the nests of the floor systems and on the egg cradle in the cage systems. These swabs were meant to give information on the general hygienic conditions in the farms. The swabs were not used to compare the amount of bacteria in the different swabbing sites since they were not done with standardized methods. Rather the swabs were used to complement the egg samples collected from the same location.

To make out the risk that bacteria could get in to the interior of the egg, 100 eggs per farm were candled for detection of cracks on the shell. The eggs were selected randomly from the storage room and from ten different egg trays. The

eggs were examined for holes, hair cracks and star cracks. In all farms, eggs with large holes or broken eggs had previously been sorted out by the farmer before taken to the storage room. Candling was not performed from farm 2. Instead 100 eggs from a separate floor system in farm 4 were candled.

In addition, air samples were taken in farm 2, 3 and 4, performed by placing tryptose soy agar plates (TSA) in the storage rooms and next to the cages or the nests in the housing systems. The plates were left with open lid for 5 minutes.

Figur 1. Litter floor system on farm 2. Photo: Sophie J. Söderström.

Figur 2. Hens held in conventional stair stepped cages in farm 4. Photo: Jenni Nordenskjöld

2.2 Identification and confirmation

Starting the laboratory work each sample with 4 eggs was washed for 1 minute in 100 ml buffered peptone water in a stomacher bag. Meanwhile the eggs were gently rubbed with fingers to make all the dirt come off. The egg was then taken out of the solution which was subsequently Stomachered for 1 minute to dissolve any bigger materials in the fluid. After making a dilution series in saline, 1 µl from two different dilutions were spread on tryptose soy agar (TSA) for total viable count. The remaining solution in the bag was then incubated for 3 hours in 37 ^{o}C. The incubation was done to resuscitate possible *Salmonella* spp. in the solution. After incubation 1 µl of the homogenized solution was spread on different agar plates, either directly from the solution or after diluting it in saline to get a suitable number of bacteria on the plates. The agar plates that were used were deoxy cholate citrate agar (DCA) for identification of *Salmonella* spp., violet red bile agar (VRBA) for identification of coliform bacteria, and campylobacter Agar (Camp) for identification of *Campylobacter* spp. All agars used contain selective agents for the wanted bacteria.

In addition each egg was weighed and an average egg weight per sample was calculated. Using the equation below the average area of the egg shell surface could then be calculated in cm^2.

$$S = P^{(2/3)} \times 4,68$$

S is for egg surface in cm^2 and P is for egg weight in grams (Bonnet and Mongin, 1965).

Each plate for total viable count was incubated in 37 ^{O}C for 24 h, this included the air sample plates. Plates for identification of *Salmonella* spp. and coliform bacteria were also incubated in 37 ^{o}C but for 24-48 h. The campylobacter agar plates were kept in 42 ^{o}C for up to 72 h under micro aerofilic conditions.

2.2.1 Bacterial counts

After incubation, all colony forming units (CFU's) were counted, and the number of bacteria per cm^2 on the egg shell was calculated.

If there was growth on two different dilutions, with between 25 and 300 CFU's, both were used to calculate the number of bacteria by using the following equation:

$$(CFU1 + CFU2) / (dilution1 + dilution2)$$

All plates with overgrowth were excluded, otherwise standard methods for counting were followed (Ministry of Health, Jordan).

2.2.2 *Salmonella* spp.

From each DCA plate showing growth after incubation, one or two colonies were transferred to triple sugar iron agar (TSI) slants for confirmation of *Salmonella* spp. The slants were then incubated for 24 h in 37 ^{o}C. A positive result showed growth and turned the agar yellow.

2.2.3 Campylobacter jejuni

Growing colonies on camp agar was transferred to TSA for further testing using the thirteen spread method. The bacteria on the TSA plates were incubated for 24 h under same conditions as previously on the campylobacter plates. After incubation oxidas-, katalase- and hippurate tests were done on all plates. Colonies with positive results in all three tests were confirmed *C. jejuni.*

2.2.4 Coliforms and *Escherichia coli*

One colony from each violet red bile agar plate, showing growth after incubation, was transferred to test tubes with LST broth to confirm that the colonies were coliform bacteria. The test tubes had previously been prepared with Durham tubes to check for gas formation in the broth. The tubes were then incubated for 24 h in 37 ^{o}C. A positive result would show growth and gas formation.

The tubes with positive result were tested further to confirm presens of the coliform, *E. coli*. 1 ml of the LST-broth was transferred to EC-MUG with Durham tube and incubated for 24 h in 42 ^{o}C. Test tubes that showed gas production after incubation were inoculated with UV-light. If the tube is fluorescent the bacteria are confirmed *E. coli*. Confirmatory tests for *E. coli* were done according to the Jordanian Ministry of Health manual for food analysis (Ministry of Health, Jordan).

2.3 Statistical analyses

Statistical analyses of data were performed using SAS software. Total count between farms was analysed and all presumptive *Salmonella* spp., *C. jejuni* and *E. coli.* Concerning the floor system, the results from the production site of farm 1, 2 and 3 plus the results from the storage rooms of farm 2 and 3 were included. For the cage system the production sites of farm 1 and 4 plus the storage room of farm

4 were included. The difference between these two systems were analysed statistically.

The confirmed bacteria were only noted as present or not present on each farm and were not analysed statistically.

3 Results

3.1 Bacterial results analysed statisticly

The statistical analyses showed that it was significantly more presumptive *Salmonella* spp. and *Escherichia coli* on eggs from floor systems than from cage systems. It was also significantly more total bacteria on eggs from floor systems. No significant differences could be found regarding presumptive *Campylobacter jejuni* between the two systems. The results are summarised in table 2.

*Table 2. Result from statistical analyses of data. Cage- and floor system were compared regarding the number Salmonella, Campylobacter and E. coli. Analyses were done on the results from both storage room and nest/egg cradle on the different farms. The different bacteria were only presumptive. Significant difference is ** when p<0.01 and *** when p<0.001. There is a more significant difference when p is smaller*

	Total aerobe bacteria	*Salmonella*	*Campylobacter*	Coliform- *E. coli*
Cage system compared to floor system	** significant difference	*** significant difference	No significant differences found	** significant difference

3.2 Confirmed bacteria

3.2.1 Egg samples

All eggs from floor systems had confirmed *E. coli* on them; two of these farms also had *E. coli* on eggs in the storage room. No *E. coli* was confirmed on eggs from cage systems.

Confirmed *Salmonella* spp. was only found on eggs from the storage rooms on farm 2 and 3, and on eggs from the floor system on farm 1.

Finally *C. jejuni* was found on eggs from floor system on farm 2 and 3 and also on eggs from the storage room in farm 1 which had mixed eggs from cage- and floor systems.

The confirmed *Salmonella* spp., *C. jejuni* and *E. coli* on all farms are summarised in table 3.

Table 3. Confirmed bacteria, present on eggs from the storage rooms and the cage- and floor system of each farm

	Cage system	Floor system	Storage room
Farm 1	None	*Escherichia coli* *Salmonella* spp.	*Campylobacter jejuni*
Farm 2	-	*Escherichia coli* *Campylobacter jejuni*	*Escherichia coli* *Salmonella* spp.
Farm 3	-	*Escherichia coli* *Campylobacter jejuni*	*Escherichia coli* *Salmonella* spp.
Farm 4	None	-	None

3.2.2 Swabs

The swabs were positive for all 3 bacteria. *E. coli* was found in the nests in the floor systems of farm 2 and 3. Confirmed *C. jejuni* was identified in both storage room and in the housing system of farm 3 and 4, consequently the bacterium was found in both farms with cage and in a farm with floor system. *Salmonella* spp. could only be found on the egg cradle of farm 4.

Table 4. Confirmed Escherichia Coli, Campylobacter jejuni and Salmonella spp. from swabs in the storage rooms, the nests in the floor system and on the egg cradle in the cage systems

	Cage system	Floor system	Storage room
Farm 1	None	None	None
Farm 2	-	*Escherichia coli*	None
Farm 3	-	*Campylobacter jejuni* *Escherichia coli*	*Campylobacter jejuni*
Farm 4	*Campylobacter jejuni* *Salmonella* spp.	-	*Campylobacter jejuni*

3.3 Air samples

The results of TSA plates placed in storage rooms and housing systems in farm 2, 3 and 4 are shown in table 5. There seems to be more bacteria in the air of floor systems than in the air of the stable with cage system, but no difference in number of air bacteria in the storage rooms.

Table 5. Total count in CFU's (colony forming units) of viable bacteria in the air of the storage rooms and various housing systems

	Cage system	Floor system	Storage room
Farm 2	-	Overgrown	90
Farm 3	-	Overgrown	Overgrown
Farm 4	165	-	122

3.4 Candling

All farms show a low number of cracked eggs. Between 94-100% of the eggs are intact when stored in the storage room. A little bit more eggs with cracks were found on farm 1, but no trend could be seen.

Table 6. Results from candling of eggs from farm 1, 3 and 4. Because of missing values from farm 2 candling was done on eggs from a separate floor system on farm 4 as well

	Intact	Hair crack	Star crack	Pin hole
Farm 1 cage + floor	94 %	2 %	4 %	0 %
Farm 3 floor	98 %	0 %	2 %	0 %
Farm 4 cage	100 %	0 %	0 %	0 %
Farm 4 floor	99 %	0 %	0 %	1 %

4 Discussion

The results from the statistical analysis suggest that there are significantly more aerobic bacteria in the floor systems than in the cage systems. This result gives information about the over all hygienic conditions in these housing systems. A high number of bacteria indicates a less clean environment for the hens and for the workmen and also, it is more likely to find pathogenic bacteria. The air samples verified this result, as floor systems had much more bacteria in the air then the cage systems. One explanation for the high number of bacteria in the air of the litter floor houses is that the hens move around more, which causes dust from the litter to stir up. Because we had to enter the stable to be able to lay out the plates for sampling, the hens became somewhat frightened and moved around more than they normally would when permanent workmen entered. Even though, visits and work amongst the animals is part of the general function of the systems.

The floor system also showed a significantly higher number of presumptive *E. coli* compared to the cage systems. When examining just the confirmed bacteria, *E. coli* was found on eggs from all floor systems, and from 2 out of three storage rooms with floor system housing. No *E. coli* was confirmed on farm 4, neither in the cages or the storage room. These results suggest that the eggs from the floor systems probably had more faecal contaminations on them, which was expected. The hens walk around in their faeces and then enter the nest to lay their eggs. Also some of the eggs are laid outside the nest boxes, directly in the litter floor where the droppings are. On the other hand, eggs laid in conventional cages come in little or no contact with faeces as they role out into a clean egg cradle whilst the droppings fall trough the net floor of the cages. The swabs verified this as they only showed confirmed *E. coli* in the nests of the floor systems, on farm 2 and 3, and nowhere else. Since no further testing was performed on the confirmed *E. coli* there was no way to find out whether these bacteria were pathogenic or not.

Furthermore, the eggs from the floor system also showed a significantly higher number of presumptive *Salmonella* spp. although these bacteria could actually be some other bacteria, because they did not test positive for *Salmonella* on the TSI slants. However they did grow on selective media for *Salmonella* (DCA) hence, we regarded them as presumptive. The same was true for both *E. coli* and *Campylobacter*. Confirmed *Salmonella* spp. could be detected on eggs from the floor system of farm 1 and from the storage room of farm 2 and 3. We could see a trend in that these bacteria only could be found on eggs from a floor system, either directly from the floor system or from the storage room. With the swabs, on the other hand, confirmed *Salmonella* was found in the cage system of farm 4. Because of the low infectious dose it is not important how much *Salmonella* we found quantitatively but rather their presence qualitatively (Willey *et al*, 2009). Of course, the aim should be to keep the environment free from *Salmonella*. However, in this study we found *Salmonella* in all the farms indicating an insufficient hygienic standard. And because no effort is done to keep the contaminated eggs away from the consumers, there is a very high risk for food born illness.

No significant difference in the number of *Campylobacter* could be determined between the two systems. The reason was that presumptive *Campylobacter* was not found in a sufficient amount in any of the systems and therefore did not give enough data to analyse. Because campylobacter are facultative anaerobic bacteria, it is unlikely that they grow on the eggs at all. The eggs were stored under aerobic conditions which would not be optimal for them to grow, which could explain the very low bacterial number we got. Another explanation could be the difficulty to cultivate these bacteria. This was supported by the fact that when growth was obtained, it was in very small numbers. Confirmed *C. jejuni* on eggs were only found in farm 2 and 3 in the floor systems, and on farm 1 on eggs from the storage room. The eggs in the storage room of farm 1 that had confirmed *C. jejuni* could either have come from the floor system of that farm, or the cage system. Eggs from the two systems were stored together without differentiation. If it came from the floor system, this would mean that *C. jejuni* could not be found in any cage system.

In conclusion, there is a strong indication that the hygienic conditions of the floor systems is not as good as in the cage systems in Jordan. The total bacterial number is higher in the floor systems, but also, both *Salmonella* and *E. coli* were found in a higher numbers.

The results from the candling were not significant. There were a bit more cracked eggs on farm 1, but not a lot more. Since the farmers had sorted out the eggs with visible defects prior to us candling them, the small deviations could just

monitor how thorough the different farmers were sorting out eggs before storage. Also the different age of the birds on the farms makes it even more difficult to draw any conclusions about shell quality in the different systems. This is because egg shell strength deteriorates a lot when the birds laying period increases (Rose, 1997).

Because the 4 farms were not uniform in their production system, several factors should be discussed that could have affected the result. The breed of the hens and their age are two such factors. For example, if one of the breeds is predisposed to lay egg in the litter, this could result in more dirty eggs, but no such evidence has been reported in these breeds. Rather; Wall *et al* (2008) have shown that in layers held in furnished and conventional cages, genotype did not affect the bacterial contamination of the eggs. The age of the hens, on the other hand, could affect the microflora on the surface of the eggs. Kretzschmar-McCluskey *et al* (2009) have found that the surface microflora increases at 32 weeks of age. Two of the farms had hens above this age; both in floor systems (farm 1 and 2). All hens in cages were under this age. This fact could therefore have affected our results in the number of total bacteria on the eggs. On the other hand Wall *et al.* (2008) found that the age of hens did not affect the total count or the presence of *Enterococcus* on the eggs, so instead, the increased microflora could just be an accumulation of bacteria because the birds have been in the house for a longer time. Because of linguistic difficulties and the lack of record keeping, it was very difficult to obtain full information about all the farms. Because of this and the lack of information on use of antibiotics for example, full evaluation could not be performed here.

From a food safety point of view the conventional cage would be the best housing alternative of the two. But as discussed in the introduction there are welfare problems with the conventional cage. To get the benefits of small group sizes, hygienic control and to give the hens' opportunity for optimal comfort behaviours, alternative system like the furnished cage could be considered as in the EU. As in the case of the conventional cage, the furnished cage has shown to exhibit reduced levels of bacteria then the floor housing systems (Nimmermark *et al*, 2009). But since Jordan is still a developing country with limited resources to introduce new housing systems, they are likely to give priority to high production efficiency rather than animal welfare.

Ultimately we can see a great need for better awareness about animal welfare in relation to bacterial contamination of eggs and how to prevent this. Even though Jordan has standards for production and handling of eggs, there is no control of the implementation (Zakaria, personal communication 2010). The Jordanian standard

for poultry and poultry products are therefore in many cases not applied on the farms. Also because such regulation was written as early as 1988, it is probably in need of revision. In addition, more research in Jordan on alternative housing system is recommended as well as research on contamination factors of the eggs, something that up to now has been paid very little attention to.

References

Appleby, MC., Mench, JA. & Hughes, BO. 2004. *Poultry behaviour and Welfare.* CABI Publishing, UK. p.194-208. p.130-142.

Bonnet, Y. & Mongin, P. 1965. Mesure de la surface de l'œuf. Ann Zootech. 14: 311-317.

Fossum, O., Jansson, D.S., Etterlin, P.E. & Vågsholm, I. 2009. *Causes of mortality in laying hens in different housing systems in 2001 to 2004*. Acta Vet Scand. 2009; 51(1): 3.

Gietema, B. 2005. *The Basics of Chicken Farming (in the tropics).* Agromisa Foundation, Wageningen. p.18-44.

Jordanian standards for *Chicken and chicken products – Eggs – Fresh chicken table eggs* (JS 574:1988)

Kretzshmar-McCluskey, V. *et al.* 2009. *Influence of hen age and strain on eggshell exterior, eggshell interior with membranes, and egg contents of microflora, and on Salmonella incidence during a single production cycle.* Poultry Science Association, Inc.

Ministry of Health, Jordan. *Manual of Microbiological Food Analysis for Food Laboratories*. Ministry of Health, Jordan.

Nimmermark, S., Lund, V., Gustafsson, G. & Eduard, W. 2009. *Ammonia, dust and bacteria in welfare-oriented systems for laying hens.* Ann Agric Environ Med. 2009 Jun;16(1):103-13.

Perry, GC. (eds) 2004a. *Welfare of the Laying Hen.* CAB International, Oxfordshire. p.166-169.

Perry, GC. (eds) 2004b. *Welfare of the Laying Hen.* CAB International, Oxfordshire. p.53.

Rose, SP. 1997. *Principles of Poultry Science.* CAB International, Wallingford. p.22-30.

Solomon, SE. 1991. *Egg and Eggshell Quality*. Wolfe Publishing Ltd, Aylesbury. p. 111

Wall, H., Tauson, R. & Sorgjerd, S. 2008. *Bacterial Contamination of Eggshells in Furnished and Conventional Cages*. Poultry Science Association, Inc.

Willey, JM., Sherwood, LM. & Woolverton, CJ. 2009. *Prescott's Principals of Microbiology*. The McGraw Hill Companies, Inc., New York. p.787-808.

Online sources

BAM, Bacterial Analytical Manual Online. 2002. *Enumeration of Escherichia coli and the Coliform Bacteria*. U.S. Food and Drug Administration.

FAOSTAT, 2007. *Food and Agriculture Organization of the United Nations*. http://faostat.fao.org/site/339/default.aspx. 2010-05-16.

Rådets direktiv 1999/74/EG av den 19 juli 1999 om att fastställa miniminormer för skyddet av värphöns. *Europeiska gemenskapernas officiella tidning.* http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1999:203:0053:0057:SV:PDF

Personal communication

Zakaria, AHH. Personal communication, 2010- 04-29. Assistant professor in Poultry nutrition, University of Jordan.

Acknowledgements

This study could not have been possible without the help and support from Dr. Hamza Al-Qadiri, Laboratory supervisor Rana and research assistant Nadia at the University of Jordan and from our Swedish supervisor Klas Elwinger. I also would like to thank the Faculty of Agriculture and the University of Jordan for welcoming us and allowing us to do our study and laboratory work in their facilities. For all the help with the microbiology a thank goes out to Dr. Hans Jonsson. I would also like to thank Nivin and Dr. Ayed Abdullat for all their kindness and help with everyday life in Amman. At last I would like to thank my friends Åsa Carlsson and Jenni Nordenskjöld whom I have done this project with and Per Santesson for all his help.