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Occurrence and control of fatty liver haemorrhagic syndrome (FLHS) in caged hens

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by Shaniko Shini and Wayne L. Bryden

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Foreword

Fatty liver haemorrhagic syndrome (FLHS) is a metabolic condition occurring worldwide in caged layers and causes significant losses to the egg industry. The background to this study was a lack of data on the prevalence, and importance of this condition for the Australian Egg Industry. Given that some 74.3% of Australia's commercial layer flock is caged, this syndrome may be a major cause of mortality in laying hens.

FLHS is characterised by the accumulation of excess fat in the liver and liver haemorrhage and is associated with decreased production and high mortality in laying hens. Since the 1950s, there has been extensive research into the causes and prevention of this disease, especially in layers. However, the condition remains unresolved in laying hens. The objectives of this project were: (1) to determine the incidence of FLHS in caged layer flocks; (2) to ascertain factors that predispose hens to this condition; and (3) to understand the impact of this condition on hen health and performance.

This report confirms the presence of FLHS in caged laying flocks in Queensland, and suggests that age and housing conditions influence the incidence of FLHS in hens and cause producers significant economic loss. Studies conducted in this project showed that in addition to the metabolic state of the hen, inflammatory and immune responses appear to be involved in the pathogenesis of FLHS. Further studies are required to explore the interactions between metabolism, inflammation and endocrinology and explain why only some laying hens develop FLHS, while all have fatty livers. A greater understanding of the pathogenesis of FLHS will assist in developing diagnostic tools for early detection of the condition in the field.

This project was funded from industry revenue which is matched by funds provided by the Federal Government.

This report is an addition to AECL's range of research publications and forms part of our R&D program, which aims to support improved efficiency, sustainability, product quality, education and technology transfer in the Australian egg industry.

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Angus Crossan
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Abbreviations

AECL	Australian Egg Corporation Limited
ACTH	adrenocorticotropic hormone
AST	aspartate aminotransferase
ALT	alanine aminotransferase
ANOVA	analysis of variance
AP	alkaline-phosphatase
AST	aspartate aminotransferase
APPs	acute phase proteins
BW	Body Weight
CAL	control & ad libitum;
CHOL	Cholesterol
CRF	control & feed restricted
E_2	Oestradiol (β2 oestradiol)
EAL	oestrogen-treated & ad libitum
ERF	oestrogen-treated & feed restricted
Fig	Figure
FLHS	Fatty Liver Haemorrhagic Syndrome
GGT	gamma glutamyl transpeptidase
GLU	Glucose
GTP	Gamma Glutamyl Transpeptidase
HCT	Haematocrit
HDP	Hen Day Production
HGB	Haemoglobin
NAFLD	non-alcoholic fatty liver disease
OAL	oil-treated & ad libitum
ORF	oil-treated & restricted feed
R&D	Research and Development
RIA	Radioimmunoassay
RIR	Rhode Island Red
QEA	Queensland Egg Association
RBC	Red Blood Cells
TP	Total Protein
TRG	Triglycerides
WBC	White blood cells
Wk/s	Week/s
WL	White Leghorn

Executive Summary

Fatty liver haemorrhagic syndrome (FLHS) is a metabolic condition that occurs in caged hens, and is frequently the major cause of death. FLHS is characterised by excessive fat accumulation in the liver and liver haemorrhage following rupture. In an initial investigation in Queensland it was shown that 74% of mortalities in caged birds were due to FLHS.

- The aim of this project was to determine the incidence of FLHS in caged layer commercial flocks, and evaluate the impact of the condition on hen performance and mortality.

- In the first year of the project a questionnaire and an epidemiological survey were conducted. These were designed to identify farms that might have acute or sporadic outbreaks of FLHS, and ascertain factors that predispose hens to this syndrome.

- The questionnaire provided valuable data on hen management practices. However, it was difficult to draw any conclusion in relation to management practices and the incidence of FLHS, because of the small number of farms that participated.

It was apparent from the questionnaire that most producers were not aware of the condition or monitored hen body weight (BW) during the laying period.

- The presence of FLHS in caged commercial layers was confirmed in the epidemiological study. Post-mortem examination conducted in 3 farms with 7 flocks of different ages indicated that 36% to 42% (in naturally ventilated cages vs. environmentally controlled cages) of all mortalities were due to FLHS. Taking into consideration that the epidemiological monitoring was conducted for only 4 months, and that only 30-50% of birds that died during this period were necropsied, the incidence in the field might be higher.

The results of this study confirmed our previous observations that laying hens, in multi-tier cages and in controlled environment sheds, are most at risk of dying from FLHS.

- The results also indicated that heavier birds in a flock were more likely to die from the condition than lighter birds. The greater BW presumably reflects the lack of activity of caged birds. Moreover, birds maintained in a thermoneutral zone have lower energy requirements.

Both factors (lack of activity and controlled environmental temperature) contribute to increased BW and increased hepatic lipid deposition.

- It is likely that a significant number of birds within a flock may have predisposing conditions that result in FLHS but does not result in mortality.

The disease is a source of lost in egg production and demonstrates that FLHS is a neglected disease of significant economic importance.

- In the second year of the project FLHS was studied in an oestrogen-induced model of the condition. In addition, detailed monitoring of FLHS in a caged layer flock was undertaken.

- The induction of the disease in a laying hen model demonstrated that increased circulating oestradiol can precipitate FLHS. The reduction of feed intake in oestrogen-treated birds did not prevent the occurrence of FLHS, however it reduced the frequency significantly. Due to the negative effects on BW and egg production the restricted feeding strategy cannot be recommended to egg producers.

This study showed that manipulation of feed intake might disturb lipid synthesis, which is required for maintaining egg production.

- The experimental model of FLHS assisted in understanding the development of the disease, and revealed that an inflammatory response is involved in the pathogenesis of this metabolic disorder.

A better understanding of the metabolic, endocrine and inflammatory interactions during FLHS in hens will permit strategies to be developed that will prevent a fatty liver becoming a haemorrhagic fatty liver. Investigations of feed additives that may reduce the production of free radicals and regulate lipid metabolis, and/or protect the hepatocytes and endothelial cells and prevent the rupture of liver, should be undertaken.

- Detailed monitoring of a layer flock for 52 weeks (wks) provided accurate data on health status and performance of hens during different stages of laying period and helped to study FLHS as it progresses naturally in caged hens.

It was emphasised that post-mortem of dead hens is the only way to monitor the presence of the FLHS in a laying flock, while monitoring BW and blood parameters may help to detect the condition in a commercial laying flock.

- Diagnostic tools for regular monitoring of FLHS in commercial laying flocks are required and remain to be developed.

1 Introduction

Fatty liver haemorrhagic syndrome is a metabolic condition that occurs in commercial layers and is frequently the major cause of death in high producing laying flocks. FLHS is characterised by excessive fat in the liver and haemorrhage from a ruptured liver. The syndrome occurs in caged laying hens, primarily in birds that are in positive energy balance (Polin and Wolford, 1977), however other factors have also been implicated as potential contributory elements to the occurrence of FLHS (Thomson et al., 2003). The condition is easy to recognize at necropsy with hens having excess abdominal and liver fat, haemorrhages and haematomas of various size in the liver (Fig. 1-1 A and B), and in many cases large blood clots in the abdominal cavity (Fig. 1-1 C). Outbreaks occur sporadically in commercial flocks (Squires and Leeson, 1988), and 3-5% of the affected flocks die from the condition. Ugochukwu (1983), Weitzenburger et al. (2005) and Shini et al. (2006) have reported higher mortality (6-20%) due to FLHS. The decrease in egg production and increase in mortality associated with FLHS have implications for the welfare of hens and cause considerable economic losses to egg producers.

Since it was first observed in 1954 (Couch, 1956) numerous studies have explored the causes of FLHS. However, the aetiology of this syndrome is still poorly understood and the occurrence underappreciated. Since 1990s, there has been limited scientific information published on the occurrence of FLHS and no information for Australian flocks. The main factors that have been involved in the aetiology of the FLHS include:

1.1 (a) Nutritional factors (e.g. consumption of high energy diets)

Intake of high-energy diets that allows caged hens to consume energy in excess of the requirements for maintenance and egg production, results in a positive energy balance and increased hepatic fat deposition. The fact that FLHS can be experimentally induced through force-feeding and/or oestrogen administration indicates that the condition might be caused by a surfeit of energy rather than being specific to an excess of any nutrient such as fat or carbohydrate. Butler (1975) suggests that excess fat in the liver arises mainly from increased lipogenesis rather than from dietary lipids. Several studies have indicated that high energy maize or wheat diets produce higher incidences of FLHS (Pearson and Butler, 1978; Haghighi and Polin, 1982). Branton et al. (1995) observed a high incidence of FLHS in hens that consumed diets containing chelated minerals.

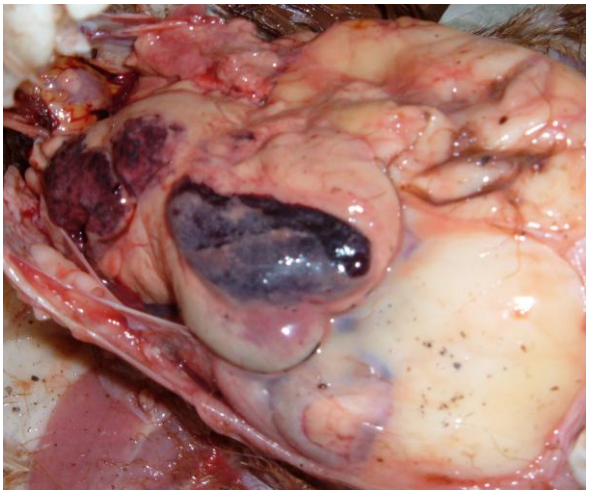
1.2 (b) Hormonal factors

Oestrogens influence the lipid synthesis which is required for yolk deposition. Polin and Wolford (1977) indicated that the liver haemorrhage score was markedly increased when excess energy intake was combined with exogenous oestrogen treatment. The possibility of a hormonal imbalance has been suggested by the observation of greatly elevated serum calcium and cholesterol in chickens from flocks with FLHS (Harms et al., 1972; Miles and Harms, 1981).

1.3 (c) Environmental temperatures (i.e. heat and cold stress)

Exposure to cold or heat induces stress and influences lipid metabolism in the fowl (Annison, 1983). The injection of adrenocorticotropic hormone (ACTH) also produces this response (Jaussi et al., 1962). However, most investigators have shown that increased lipogenesis may occur partly due to an excessive intake of carbohydrate during hot weather (Couch, 1956; Pearson and Butler, 1978). Jensen et al. (1976) observed more

FLHS in warmer vs. cooler regions of Georgia, despite feed intake is attenuated at high temperature, and increased in cold temperatures. Shini et al., 2006 found a significantly higher mortality due to FLHS in controlled environment cages (22-24°C) as compared with barn and free range systems (70% vs. 5 and 0% of all mortalities, respectively), although the strain (source), diet and other husbandry practices of birds were similar. It was also suggested that birds in battery cages and at temperatures of 22-24°C require less energy for exercise or maintain body temperature (Shini et al., 2007).



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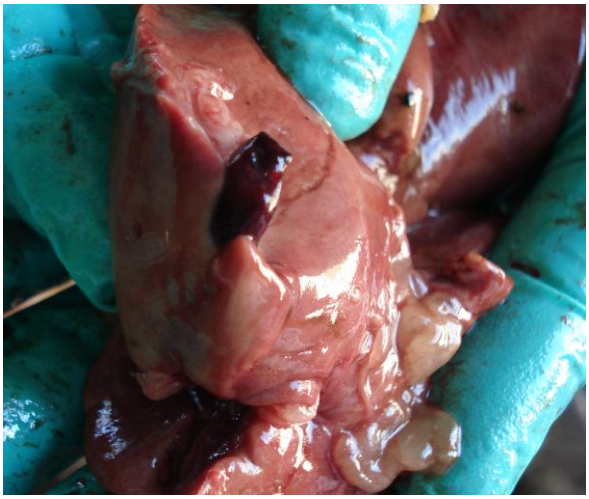

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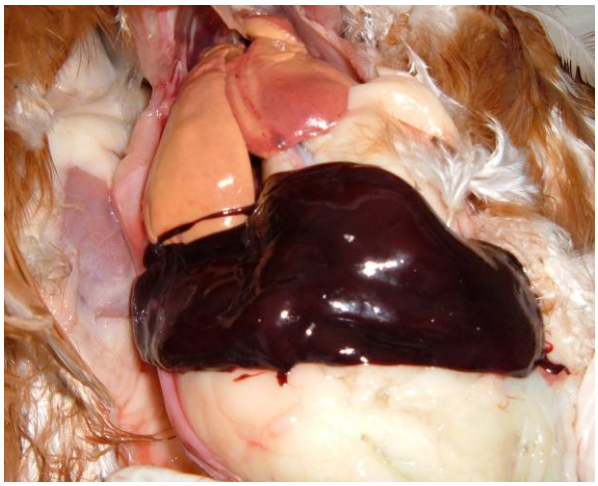


C

Figure 1-1 Pictures from necropsies with hens having excess abdominal and liver fat, and haemorrhages and haematomas of various sizes on the liver (A and B); in many cases large blood clots are found in the abdominal cavity (C) (©The University of Queensland 2006)

1.4 (d) Housing conditions

It has been demonstrated that caged laying hens are the most frequently affected by FLHS (Couch, 1956; Neill et al., 1975; Butler, 1976; Squires and Lesson, 1988; Riddel C. 1997; EFSA, 2005; Weitzenburger et al., 2005; Shini et al., 2006). Simonsen and Vestergaard (1978) and Squires and Leeson (1988) found that factors responsible for FLHS in commercial layers are stress and lack of exercise due to confinement and crowding.

1.5 (e) Genetics

It has been suggested that some strains of laying hens are more susceptible to FLHS (heavy and higher producing breeder hens). However there is little proof of this. A strain of single comb white leghorn laying hens (UCD-003) has been shown to be highly susceptible to FLHS (Abplanalp and Napolitano, 1987).

1.6 (f) Toxicological factors

Dietary factors other than excessive caloric intake, such as toxins and rapeseed products may stimulate lipogenesis (Pearson and Butler, 1978). There is evidence that mycotoxins (aflatoxin in particular) which may contaminate cereals will induce liver lipid accumulation (Bryden et al., 1979). Rapeseed meal in the diet increases the incidence of FLHS because erucic acid or other toxic metabolites affect the strength of the connective tissue in the liver (Bhatnagar et al., 1980; Martland et al., 1984).

To sum up, hens develop fatty livers under normal metabolic conditions of egg laying. With this underlying fatty liver that hens develop, additional predisposing factors are energy intake in excess of requirements and confinement. The additional effects of environmental factors including excess energy intake appear to alter liver function and lipid utilisation and therefore induce FLHS. The aetiology and pathogenesis of the FLHS have not been not fully established, making it an unresolved metabolic disease of laying chickens (Hansen and Walzem, 1993; Branton et al., 1995; Weitzenburger et al., 2005). Moreover, no definitive diagnosis criteria have been determined for live birds (Thomson et al., 2003). Recent evidence in humans indicates that the integration of metabolic, immune and inflammatory pathways is crucial, and dysfunction may underlie many chronic metabolic diseases, including non-alcoholic fatty liver disease (NAFLD) (Tilg and Moschen, 2008). As regard to laying hens, this could be a potential window through which to explore the link between metabolically triggered immune dysfunction and inflammation with the occurrence of FLHS.

In Australia, Neill et al. (1975) reported for the first time outbreaks of the disease in the egg industry of South Eastern Queensland. Since then there are no reports of the incidence of the disease in Australia. Enquires with industry and poultry researchers in Queensland and Australia suggest that FLHS continues to be a major cause of death in layer birds. Given that some 75% of Australia's commercial layer flock is caged, this syndrome will continue to be a major cause of mortality in laying hens in the Australian Egg Industry. Therefore, as a disease with economic and welfare significance FLHS requires further investigation.

2 Objectives

The objectives of this project were:

- to determine the incidence of FLHS in caged layer flocks;
- to ascertain important factors that predispose hens to the syndrome and understand its pathogenesis;
- to assess the impact of the condition on hen health and performance.

A long-term goal is to control and reduce FLHS in caged layer flocks in Australia.

3 Methodology

3.1 Experimental design

3.1.1 Part one of the project

The first part of the study involved a survey of all cage producers in Queensland. A draft of survey questionnaire was revised using a focus group, who represented the survey targets e.g. some members of Queensland Egg Association (QEA), a poultry epidemiologist, poultry extension officer and two poultry scientists. The questionnaire was modified and sent out to the producers. For the second part of the project an intensive epidemiological study was designed. From the farms that participated in the questionnaire and volunteered to participate, based on the age groups and breeds of laying flocks, three (containing 5 sheds and/or 7 flocks of different ages) were chosen to be involved in the epidemiological study. The farms were assigned according to a random selection process and location (distance from Gatton).

3.1.1.1 Questionnaire

The aim of the questionnaire (Appendix 7.1) was to collect data on bird management, health and productivity. The questionnaire covered questions on breed and flock age, feed source, mortality and egg production, vaccination, lighting program and routines on BW monitoring. Producers were also asked if they recognise the occurrence of FLHS in their flocks and, if so, could they estimate the percentage of mortalities in a flock. The questionnaire was sent to 20 registered cage layer operations in Queensland. The survey was conducted between September and November 2007 and the results from questionnaires were recorded immediately. Data from questionnaire were then used to randomly select farms for the epidemiological study.

3.1.1.2 Epidemiological survey

Three farms (each with 1-3 sheds) were chosen as units in the epidemiological study. Care was taken to ensure that farms included in the epidemiological survey had different backgrounds such as breed in use, size of flocks, age of flocks (start, middle, or end of lay), diet in use (commercial vs. farm-mixed feed) etc. Selection of farms was stratified across geographic location (east and west from Gatton). The farms were visited 3 times over a 4-month period (between January and April 2008) and closely monitored. Data on mortality and causes of mortalities, BW, production, haematological parameters and plasma metabolites were recorded. Frequency of FLHS was determined through necropsy. Approximately 597 birds were necropsied during the epidemiological study.

When designing epidemiological studies, sample size calculations should be performed in order to guarantee the design accuracy. It is recognised that required sample size is dependent on the prevalence of the syndrome studied. For this study sample size was based on a sufficient sample to describe prevalence at each level with a defined precision and confidence. It was expected that within shed sampling would allow a relatively large margin of error i.e. a sampling strategy that has a high probability of collecting effective samples but that involves a relatively smaller sample size and that produces prevalence or incidence estimates with a relatively wide confidence interval. For example, assuming an expected prevalence of 50%, a sample size of 20 birds per shed (assuming a shed total of 1000 birds) will allow estimation of prevalence with a precision of ~21% and a level of confidence of 95%. If the condition is present at a prevalence of ~50%, the sample will return prevalence estimates ranging from 30% to 70% with 95% confidence. This sample size (20 birds from a flock with 1,000 birds) will detect affected flocks with 100% confidence (Martin, 1987). Therefore, assuming that the FLHS condition could be present from 29%-71% of dead birds the number of birds to be necropsied from each flock should be more than 20 to detect affected flocks with 100% confidence.

The number of birds used for blood tests, was calculated as recommended by Birling Avian Laboratory and the University Melbourne, International Avian Health Laboratory, to sample at least 0.75% of the flock for a flock with less than 1,000 birds. Thus, for Farm 1 (with 5000 cage units per shed), birds in 40 cage units per shed or 0.80% of cage units were weighed; for Farm 2 (with 1096 cage units per shed) birds in 18 cage units (3 cages/row) or 1.64% of cage units per shed were weighed; for Farm 3 (with 1625 cage units per shed) birds in 27 cage units (3 cages/row) or 1.65% of cage units per shed were weighed. In all cases, 1 bird per cage unit was bleed. For the systematic observation of the flock kept at Gatton Facility, from a total of 1200 birds that were housed in the shed, 24 birds (2% of the flock) were sampled every 5 weeks for 52 weeks.

3.1.2 Part two of the project: experimental induction of FLHS

To study the disease under experimental conditions FLHS was induced in laying hens of 30 wks of age. The concept of the hormone-energy interrelationship in the induction of FLHS was previously explored in experiments using immature female and male chickens (Polin and Wolfort, 1977) and mature laying hens (Stake et al., 1981).

Polin and Wolford (1977) induced FLHS in immature male and female chickens, 11 weeks of age, of broiler and egg-laying breeds. Force-feeding three times a day for 21 days, amounts of feed equal to 125% and 150% of ad libitum intake, produced a gradient response in hepatic steatosis (measured by percentage of fat in the liver, and the ratio of fat to the fat-free dry weight), but not FLHS. Intramuscular (i.m.) injection of β-estradiol-17-dipropionate (E_2) at 2 mg/kg body weight, three times weekly for 21 days, produced a gradient response in hemorrhagic score and an increase in ad libitum feed intake. There was no significant difference between sex or breed in the score values used to evaluate FLHS, but females of both breeds accumulated significantly more fat in the liver than males.

Other reports have also shown that exogenous E_2 can induce fatty liver in immature birds (Campbell, 1959; Butler, 1976). However, from these studies it has been concluded that, the induction of FLHS in mature layers may prove to be more useful, since FLHS is commonly observed in high egg producing birds.

In 1981, Stake et al. used E_2 model to the further study of breed differences in FLHS susceptibility. They administered i.m. exogenous E_2 every 4 or 5 days (5.0 or 7.5 mg E_2/kg

body weight) to induce FLHS in 9 month old both Rhode Island Red (RIR), and White Leghorn (WL) hens. RIR hens exhibited ataxia and opisthotonus, and 30% died from hepatic haemorrhage within 14 days. No WL birds similarly treated for 32 days died or showed neurologic disorder, thereby indicating a major breed difference in response to exogenous E_2. Liver lipid, incidence of liver haemorrhage, and plasma volume increased as a direct result of E_2 injection.

In conclusion, all previous investigators that used chicken model of FLHS implicate oestrogen as a factor in the production of FLHS along with the necessity for the chicken to be in a positive energy balance creating sufficient hepatic fat for FLHS to occur.

We used these data to develop a model of FLHS. Our model was based on the observation that exogenous E_2 induces FLHS rapidly and reproducibly. Therefore, it was thought that the experimental model will help (1) to study the pathogenesis of the disease in a very short time (natural occurring of the FLHS is difficult to follow, as the disease happens sporadically and over an extended time); and (2) develop potential measures using the model. In addition, we evaluate the effect of the total energy intake (i.e. total feed intake) in the prevalence of FLHS. For this reason we controlled ad libitum feeding of hens by reducing their daily feed intake. For 3 weeks hens were fed 10% less feed the breeder recommended. It was thought that this would force birds to utilise the excessive fat which they had accumulated and/or could accumulate during treatments with oestrogen.

This investigation was carried out at the Gatton layer facility, University of Queensland. 96 Hy-Line laying hens were housed individually in stainless steel cages and kept in an environment controlled shed. The temperature of the shed ranged from 22ºC to 24ºC, and the photoperiod was controlled daily between 5 am and 9 pm. Hens were fed a commercial layer (wheat-sorghum-soy based) diet that contained CP - 17.5%; ME - 11.5 MJ/kg; Ca - 4.1%; Available P - 0.40%; Na - 0.18%; Lysine - 0.85%, Methionine+Cysteine 0.77%. FLHS was induced in 30 wks age Hi-sex laying hens by injecting exogenous E_2, 5mg/kg/body weight, every 4-5 days for 3 weeks. The treatment program and sampling was performed as indicated in Table 3-1. At each sampling point 6 hens/treatment were necropsied for liver macroscopic and microscopic evaluation.

Table 3-1 - Summary of the treatments and samplings for the experimental study[1]

Group[2]	Treatment & Time	Dose/Form	Samples & time of sampling	Feeding	Other records
EAL	E2 Day 1, 5, 10, 15, 21.	5mg/kg/BW, diluted in 0.5 ml oil, i.m.	Blood & Liver; 0 h, 24 h, 1 wk, 2 wks, 3 wks.	*Ad libitum*	Egg production & feed consumption (daily), BW (weekly), mortality
ERF	E_2 Day 1, 5, 10, 15, 21.	5mg/kg/BW, diluted in 0.5 ml oil, i.m.	Blood & Liver; 0 h, 24 h, 1 wk, 2 wks, 3 wks.	Restricted feed intake[3]	Egg production & feed consumption (daily), BW (weekly), mortality
OAL	Oil Day 1, 5, 10, 15, 21.	0.5 ml oil, i.m.	Blood & Liver; 0 h, 24 h, 1 wk, 2 wks, 3 wks.	*Ad libitum*	Egg production & feed consumption (daily), BW (weekly), mortality
ORF	Oil Day 1, 5, 10, 15, 21.	0.5 ml oil, i.m.	Blood & Liver; 0 h, 24 h, 1 wk, 2 wks, 3 wks.	Restricted feed intake	Egg production & feed consumption (daily), BW (weekly), mortality
CAL	Untreated	-	Blood & Liver; 0 h, 24 h, 1 wk, 2 wks, 3 wks.	*Ad libitum*	Egg production & feed consumption (daily), BW (weekly), mortality
CRF	Untreated	-	Blood & Liver; 0 h, 24 h, 1 wk, 2 wks, 3 wks.	Restricted feed intake	Egg production, & feed consumption (daily), BW (weekly), mortality

[1]Last treatment with exogenous E_2 was performed on day 21, but samples and records were taken also 1 week post-treatment on day 28.

[2]EAL = oestrogen-treated & ad libitum; ERF = oestrogen-treated & feed restricted; OAL = oil-treated & ad libitum; ORF = oil-treated & restricted feed; CAL = control & ad libitum; CRF = control & feed restricted

[3]Feed was restricted at 10% of the daily feed intake recommended by breeder for layers from 30 to 35 wks of age.

3.1.3 Part three of the project: systematic observation of a laying flock

Part 3 of the project was a systematic monitoring of an experimental laying flock for FLHS. The flock was maintained under commercial conditions for one laying cycle.

The layer flock, of 1200, 17 weeks old Hy-Line laying hens were housed in a controlled environment shed at Gatton and kept/monitored from 52 weeks. Table 3-2 presents the schedule of hen monitoring and numbers of hens sampled/recorded at each sampling point.

From 1200 hens, 24 hens (or 2% of the flock) were sampled every 5 weeks (for blood and liver examination); 12 hens (or 1% of the flock) were euthanized for liver macroscopic evaluation; egg production, feed consumption and mortality were recorded for all hens, daily. Body weight (BW) was recorded every 5 weeks (20% of all hens were weighed) and all dead hens were necropsied.

Table 3-2 - Plan of flock monitoring

Time/ weeks of age	Number (%) of total hens sampled	Samples and tests performed	Other records1
18 to 70	1200	NA	Egg production and mortality, daily
25	24	Blood (total RBC, total WBC and differentials, HCT, fibrinogen, oestradiol, TP, CHOL, TG, AST); Liver from sacrificed hens (gross pathology, weight, and histology).	BW, feed intake; and necropsy of hens that die.
30	24	The same	The same
35	24	»	»
40	24	»	»
45	24	»	»
50	24	»	»
55	24	»	»
60	24	»	»
65	24	»	»
70	24	»	»

[1]Feed intake and mortality was recorded daily. Mortality was calculated as cumulative starting from 18 weeks of age.

3.2 Measurements and records

3.2.1 Post-mortem examination of dead/sacrificed hens

All dead birds from the survey were collected and recorded. Only birds that died during first 10 days of each month (from January to April) were necropsied. This was due to insufficient storing space for dead birds on farm (i.e., freezers). Each farm was visited once a month for 3-5 days to conduct necropsies and to monitor BWs and collect blood samples. The necropsy included an examination of the overall condition, as well as external and internal observations. The abdominal cavity was examined for the presence of excess amount of fat and blood clots/coagulations. Livers of dead birds were carefully removed, checked for haemorrhages and haematomas, weighed, and stored for further analysis (total lipids and dry matter). At necropsy birds were also examined for internal ovulations, internal oviposition, ovarian regression and follicular atresia. Body weight of dead birds was also recorded.

3.2.2 Blood parameters

Blood samples were taken using individual vacuntainers and individual blood tubes from the wing vein. Each bird was appropriately restrained to ensure as little stress as possible on the bird. Lithium heparin whole blood was used to measure haematological parameters in an automated analyser (CELL-DYN® System 3700CS, Abbott Park, IL 60064). Results obtained from the haematology analyser were used for the total number of red blood cells (RBC), packed cell volume or haematocrit (HCT) and haemoglobin (HGB) concentration. It was thought that these parameters would identify the presence of haemorrhage/haematoma in the liver or abdominal cavity. The RBC, HCT and HBG are decreased in haemorrhages and haematomas. The HCT is one of the most precise methods of determining the degree of anaemia. HGB should be evaluated with HCT and RBC to determine anaemia and the type of anaemia.

Blood was also centrifuged (1500 rpm for 10 min) and plasma was stored at -20C for chemical analysis. In order to fully assess the condition of a liver, one must consider four groups of plasma metabolites: lipids, carbohydrates, proteins, and enzymes. Hence, plasma metabolites, such as, cholesterol (CHOL), triglycerides (TRG), total protein (TP), glucose (GLU) and gamma glutamyl transpeptidase (GGT) and aspartate aminotransferase (AST) were determined.

- Cholesterol is normally synthesized by the liver and is important as a constituent of cell membranes and a precursor to steroid hormones. Elevated levels of cholesterol are seen in a variety of metabolic disorders, including liver disease.
- Triglycerides are esters of glycerol and fatty acids. Since these esters and cholesterol travel in the blood stream together, they should be assessed together.
- Total protein represents the sum of the total albumin and total globulin in the plasma. Produced almost entirely in the liver they may reflect situations associated with various metabolic states and liver dysfunction.
- High levels of glucose in plasma can indicate stress in chickens, while low levels indicate liver disease.
- Many investigators recommend measurements of plasma enzymes to test for FLHS (Pearson and Butler, 1978; Walzem et al., 1993). The GGT test was used as it is a more sensitive and specific indicator of liver dysfunction than alkaline-phosphatase (AP) and in certain conditions than alanine aminotransferase (ALT) and AST. It is elevated in all common forms of liver dysfunction/disease. From studies in humans it has been shown that there is some correlation between GGT and TRG in patients

having steatohepatitis (fatty liver). AST levels are also often used to help determine fatty liver diseases.

Blood concentrations for plasma metabolites were determined using commercial kits and a chemistry system (VetTest chemistry analyser, IDEXX Laboratories, Inc. USA). In addition to these metabolites for part two of the study, measurement of plasma fibrinogen levels were conducted. Production-associated subclinical diseases are difficult to detect by blood analysis but might be diagnosed by the presence of changed levels of acute phase proteins (APPs). In chickens, fibrinogen is an acute-phase protein, and also plays a crucial role in the coagulation cascade. Plasma fibrinogen content was determined by the refractometer method.

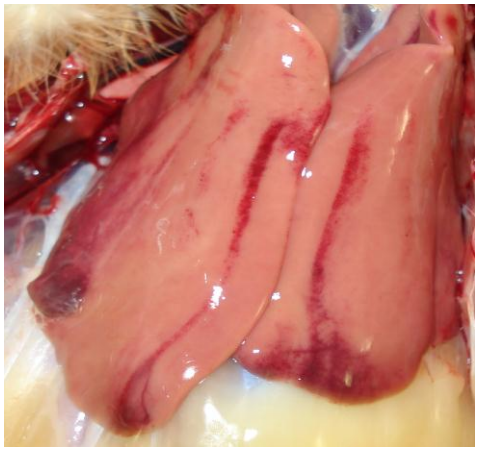
3.2.3 Plasma oestradiol determination

Serum oestradiol concentrations were determined by radioimmunoassay (RIA), using coated tube technology (Spectria) from Orion Diagnostics. The RIA was conducted according to the manufacturer's instructions using duplicate 200 µL samples in assays. Samples were counted on a Gamma Counter (Wallac 1470 Wizard Automatic). The tests were repeated twice. At 394 & 9520 pmol/L the inter-assay variations were 5.1 & 8 % respectively, and the intra-assay coefficient of variation was 4.33%. The assay sensitivity range was 30-15000 pmol/L. The antiserum was highly specific for 17-β oestradiol with a relatively low cross reactivity to other naturally occurring steroids in the plasma sample as stated by the manufacturer.

3.2.4 Liver tests

For the part one of the study, liver samples were taken for the determination of weight, dry matter and total fat content. The dry matter content was determined by oven-drying a pre-weighed sample as recommended by Association of Official Analytical Chemists (1984), and lipid content was determined by the method of Folch et al., 1956. Briefly, one gram of sample was weighed into a screw-capped test tube with 20 mL of chloroform/methanol (2:1, vol/vol), and homogenized with a polytron for 5 to 10 s at high speed. After an overnight incubation at 4°C, the homogenate was filtered through Whatman #1 filter paper into a 100-mL graduated cylinder, and 5 mL of 0.88% sodium chloride solution was added and mixed. After phase separation, the volume of lipid layer was recorded, and the top layer was completely siphoned off. Total lipids were determined gravimetrically after evaporating the solvent. The sample was then dried and weighed, and percentage of liver fat was calculated.

For the experimental model, the liver was removed, weighed, and individually examined for the presence of hemorrhagic lesions. Haemorrhages were counted on both the dorsal and ventral surfaces of the liver. Liver haemorrhages were graded on a scale from 0 to 5, with 0 indicating no haemorrhages; 1, up to 10 subcapsular petechial or ecchymotic haemorrhages; 2, more than 10 subcapsular petechial or ecchymotic haemorrhages (Fig. 3-1 A); and 3-5, large haematomas (Fig. 3-1 B) and massive liver haemorrhage (Fig. 3-1 C) accompanied by rupture of the Glisson's capsule (Diaz et al., 1999). A hemorrhagic score of three to five was considered highly characteristic of FLHS (Fig. 3-1). Two sections from the liver of each bird were dissected and used for histological tests.



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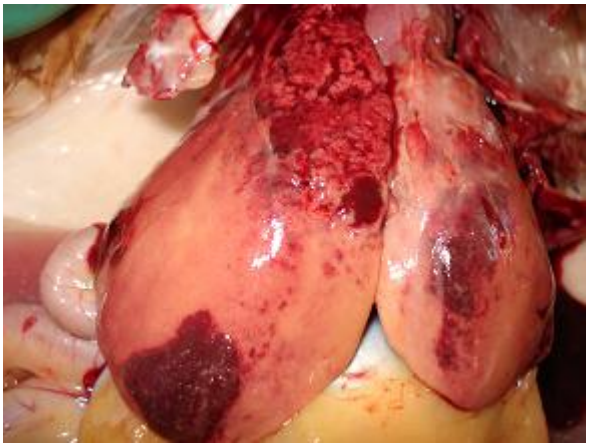

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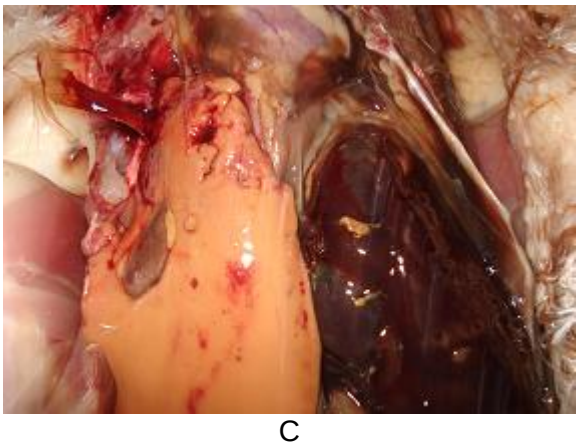


C

Figure 3-1 - Evaluation of liver haemorrhages

For liver histology, two slices of liver about 1 × 1 × 0.3 cm thick were taken from the right lobe of each hen, fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with haematoxylin and eosin stain prior to microscopic examination. For each section of liver, randomly located areas were assessed using a light microscopy (40x and 100x magnification). A digital camera connected to the microscope was used to take pictures. Fat content was assessed by evaluating the incidence of fat vacuoles inside and between hepatocytes. A fat vacuole was considered to be any nonstaining area of cytoplasm with a sharply defined border. Inflammation was determined as regard to the occurrence of focal infiltration with leukocytes, and haemorrhage was determined according to the dilatation of arteries and veins, including focal infiltration of liver tissue with RBC.

3.2.5 Performance parameters

Production and mortality report records from surveyed sheds were used to calculate:

- Egg production, expressed as eggs produced per hen per day (hen day production: HDP %);
- Mortality rates (%), expressed as cumulative from start of lay;
- Body weight (g), was recorded on a monthly basis; 18-40 cage units per shed were weighed and the result expressed as the average per bird;
- Feed consumption was recorded daily for 52 wks and the results are expressed as the average per bird, per week;
- Shed temperatures were also recorded by producers for the Part one of the study and given in this report only as an average of temperature measured during summer months (from January to March) for the purpose of the result discussion.

3.3 Statistical analysis

It is acknowledged that a direct comparison of the results from one farm with another is not valid due to the fact that there are many and different interacting variables associated with one management systems compared to another (part one of the project). For part two and three of the project, to test for age/time effect at each sampling point, recorded values were subjected to one-way analysis of variance ANOVA. All analyses were performed using the GLM procedure of SAS (SAS Institute, 1996). Significant differences among groups were determined using protected t-tests. Statements of significance were based on $P<0.05$.

4 Results

4.1 Part one of the project

4.1.1 Data from questionnaire

More than 50% of the cage egg producers in Queensland replied to the survey, and their location is shown in Figure 4-1. All of the farms had been operational for more than 20 years, 4 for more than 50 years. Table 4-1 shows some of the data recorded from the questionnaires. The average number of birds per producer per year ranged from less than 10,000 (2 producers); 20,000 to 190,000 (8 producers); and 900,000 birds (1 producer), with the number of sheds ranging from 1 to 16. Only two producers use controlled environment sheds, others have their sheds naturally controlled/ventilated. Eight of the producers use cages housing 5-6 birds/cage, and 4 use cages housing 3 birds/cage. All of producers use cages conforming to the welfare code. Five of producers use Hy-Line brown layers, 2 use Isa brown, 2 use HI-SEX birds and 1 uses both Hy-Line and Isa brown to operate farms. Seven of the producers used farm-mixed feed and only 4 used commercial feed. The mortality rate of flocks ranged from 2% to 11% and the average rate of production for laying cycle ranged from 70% to 89%. None of the producers know the causes of mortalities in their flocks, and only 3 use veterinary laboratories to determine causes of bird mortality. Six of producers monitor BW of their flocks, while all used lighting programs for laying flocks. Only 1 of the producers was aware of FLHS being sporadically observed in their flocks (dead birds).

Figure 4-1 - Location of farms that replied to the survey (questionnaire)

Table 4-1 - Summary of data recorded through questionnaire
*at the time of survey
** during the summer months

Farm ID	Breed	Age of flocks*	N of sheds/ N of birds*	System/ Cage type/ N of hens/cage*	Shed temp- (°C)**	Feed in use	Average mortality/ laying cycle	Cause of mortality/ who determines	Average production/ laying cycle	Weighing program
1	Hy-Line brown	Various	4/ 120,000	Controlled-environment/ Valli/6	23-25	Farm-mixed	4	Unknown/ manager	85	No
2	Hy-Line brown	28 wks	3/ 10,000	Natural/ Chainex/3	25-33	Farm-mixed	2	Unknown/ Manager (90%), Lab (10%)	70	Yes/ monthly
3	Isa brown	Various	1/ 9,500	Natural/ Single tier/3	Un known	Commercial Darwalla	7	Unknown/ owner	70	No
4	Hy-Line brown	47 wks	7/ 190,000	Natural/ Multi-tier/4&6	25	Farm-mixed	2	Unknown/ Manager (95%); Lab (5%)	85	Yes
5	Isa brown	Various	1/ 10,000	Natural/ old cages/3	Un known	Commercial	6	Unknown/ owner	80	No
6	HI-SEX brown	Various	1/ 8,000	Natural/ Tier-Sylvan/5	15-34	Commercial	8	Unknown/ owner	76	No
7	HI-SEX brown	Various	2/ 20,000	Natural/ multi-tier/ 6	25-30	Farm-mixed	5	Unknown/ manager	85	No
8	Hy-Line brown		2/ 18,000	Natural/square old colonial wire/3	28-30	Commercial-Riverina	11	Unknown/ manager	80	No
9	Hy-Line & Isa-brown	Various	16/ 900,000	Environmentally controlled/ Big Dutchman/6	21-28	Farm-mixed	2.1	Unknown/ manager	80	Yes/ monthly
10	Hy-Line brown	Various	2/ 74,000	Natural/ Tecno& HarrDison/5	Unknow n	Farm-mixed	10	Unknown/ manager	82	No
11	HI-SEX brown	Various	1/ 25,000	New cage system/6	22-23	Farm-mixed	2	Unknown/ Manager (50%), Lab (50%)	89	Yes/ three-monthly

4.1.2 Results from epidemiological study

4.1.2.1 Mortality and necropsy results

Table 4-2 presents general data on 3 farms monitored from January to April 2008. Table 5 shows results for mortalities (cumulative and during the study) and the number of necropsies conducted during the study. The mortality rate (cumulative) of flocks monitored by producers ranged from 0.8% (the youngest flock) to 11.6 % the oldest one. The mortality rates increased with age ($P<0.05$), although there were differences in mortalities between flocks of similar ages. Detailed data on mortality for farm 1 (shed/flock 1, 2, and 3) are presented in Fig. 4-2 (A, B, and C). The results indicate that for Farm 1 at the 29, 54 and 73 wks of age (end of April) the mortality rate was 2, 4.8 and 11.6%, respectively. At 72 wks, Farm 2 (shed/flock 1) mortality rate (cumulative) was 7.4% of the initial flock, and at 31, 49 and 64 wks of age the mortality rate (cumulative) for Farm 3 (shed 1, flocks 1, 2, and 3) was 0.8, 2.5 and 4.8%, respectively.

The number of dead birds recorded during the study is presented in the Table 4-3. As indicated in the methodology only 30-50% of dead birds were necropsied. The results indicate that 42% of birds necropsied from Farm 1 showed clinical signs of FLHS, while for Farm 2 only 28% of dead birds have had FLHS, and for Farm 3, ca. 34% died due to this condition. Interestingly, the results showed that of birds that died in Farm 1, between the ages 37 to 54 wks more than 50% demonstrated FLHS. The average BW of those dead birds was 2008±107 g. The average of BW of birds that died in Farm 2 and 3 was 1821±78 and 1954±92 g, respectively.

Table 4-2- Description of Queensland farms that participated in the epidemiological survey

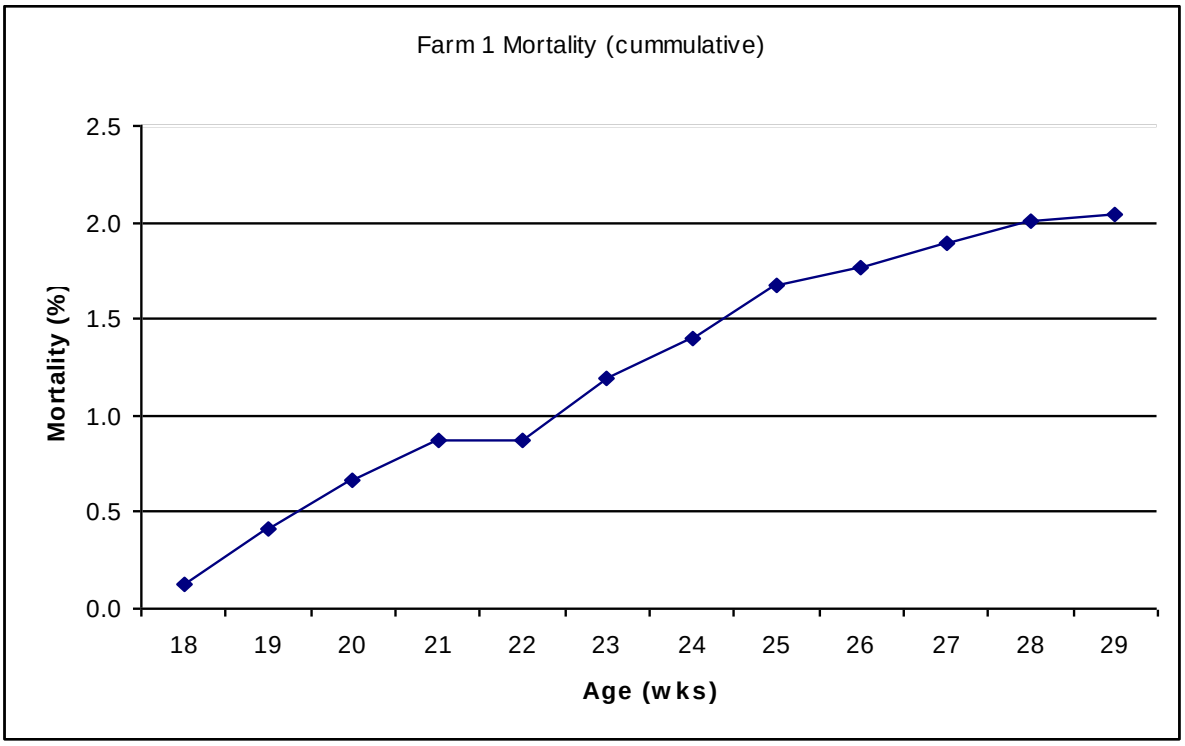
Farm ID	Location from Gatton	Breed/strain	Age (Number of flocks)[1]	Diet2	Lighting	Weighing	System/N Birds/space
Farm 1	South-west	Hy-Line brown	12 wks (1) 37 wks (1) 56 wks (1)	Farm-mixed	16 hrs	Yes-monthly	Controlled-environment; multi-tier; 6 birds/cage (conforming welfare code)
Farm 2	West	Hy-Line brown	56 wks (1)	Farm-mixed	16 hrs	Yes-monthly	Naturally controlled; single-tier, 3 birds at 675 cm^2
Farm 3	East	Isa brown	14 wks (1) 22 wks(1) 47 wks (1)	Commercial	17 hrs	No	Naturally controlled/ single-tier, 3 birds (conforming welfare code)

[1]At the start of monitoring (January 2008).
[2]For diet specifications see Appendix 7.2.

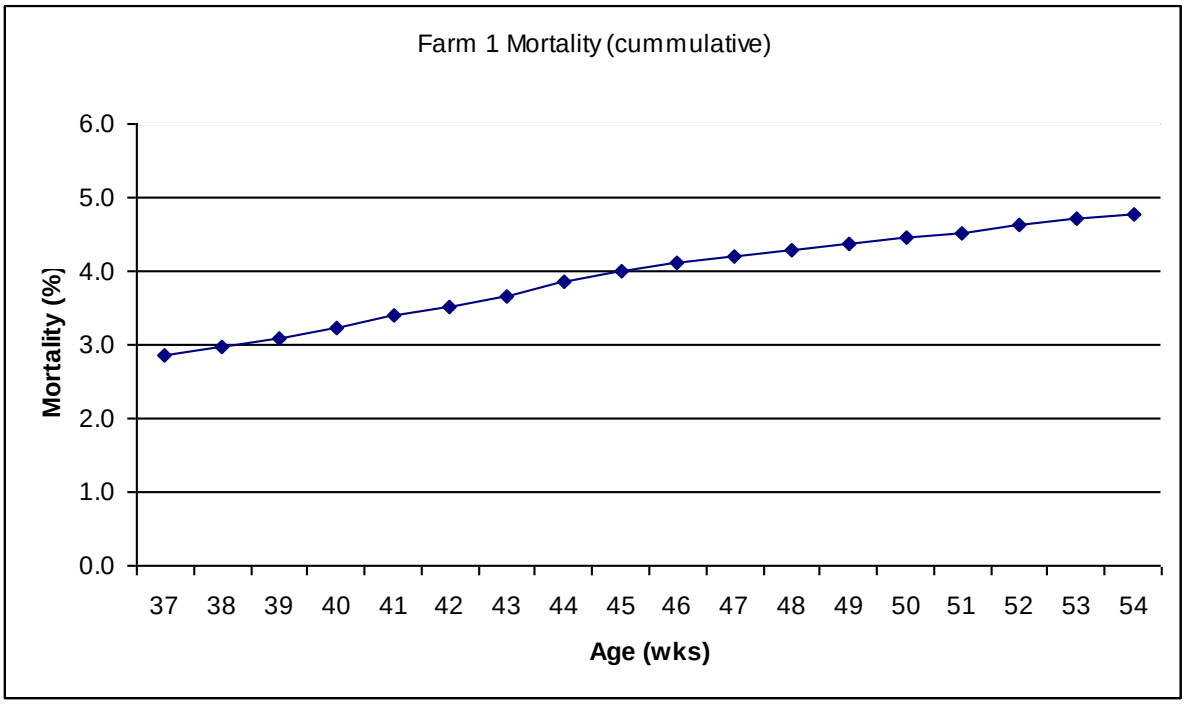
Table 4-3 - Mortality rates and post-mortems conducted for three farms in the epidemiological study

Farm/N of Sheds surveyed	Data on flock size and age		Mortality Cumulative from 18 wks (5)	Number of dead birds during study	Number of post-mortems conducted during the study	Incidence of FLHS (number of birds & %)
	N of rows N of birds/row	Age/wks				
1 (3)	6/5022 6/4776 6/4347 =14,145	29 54 73	2.0 4.8 11.6	619 579 407 =1605	166 182 134 =482 (ca. 30% of dead birds)	40 109 31 =180 (42 % of necropsied birds)
2 (1)	6/540 =540	72	7.4	121	64 (ca. 50% of dead birds)	18 (28 % of necropsied birds)
3 (3)	3/545 3/513 3/448 =1508	31 39 64	0.8 2.5 4.8	22 75 118 =215	18 22 30 =105 (ca. 50% of dead birds)	6 13 17 =36 (34 % of necropsied birds)

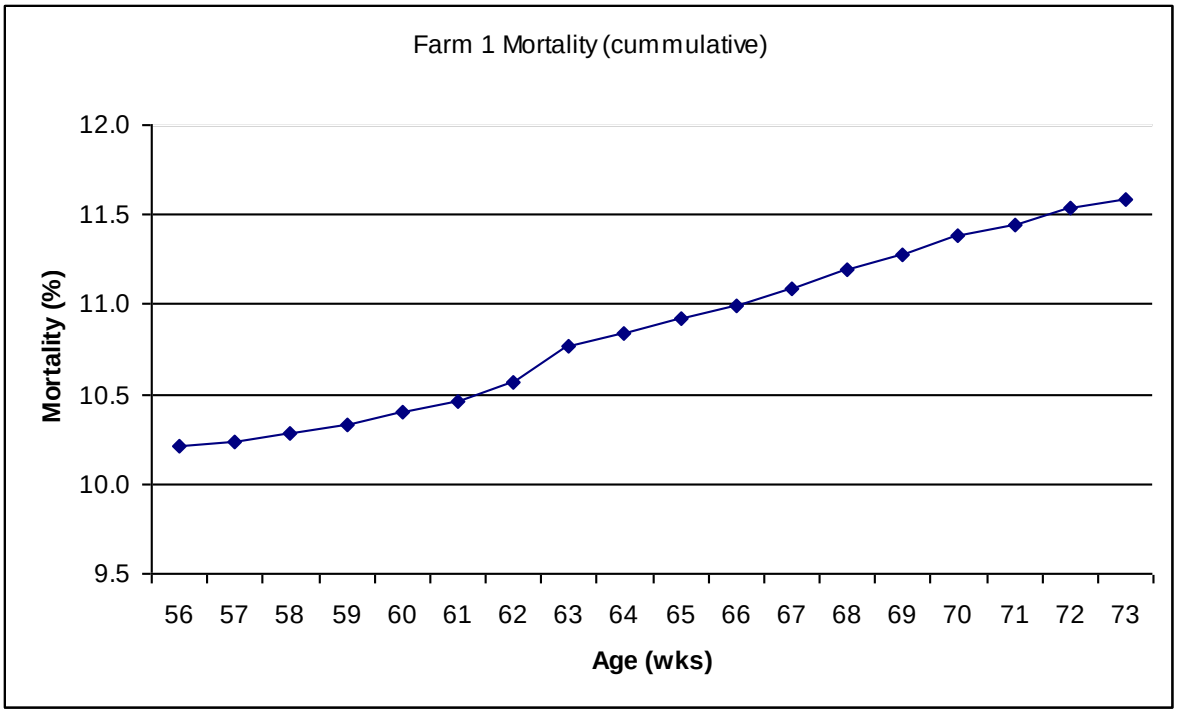
[1]Data on number of birds and age given for 30th April 2008 (end of survey)



A. Shed 1, flock 1(18-29 wks)



B. Shed 2, flock 2 (37-54 wks)



C. Shed 3, flock 3 (56-73 wks)

Figure 4-2 - Data on mortality for Farm 1, Shed 1 (A), Shed 2 (B) and Shed 3 (C), each contains 6 rows/replicates. Mortality is presented only for the period of study (January-April 2008) as a percentage (cumulative) of birds housed in the shed at start of lay.

Tables 4-4, 4-5 and 4-6 present data on blood cell profiles for Farms 1, 2 and 3 respectively. All blood tests (blood cell profile and plasma metabolites) for all farms were taken and analysed at the end of each month (February, March, and April 2008). Data presented here are calculated as an average of 40 birds per shed/age for Farm 1, 18 birds per shed/age for Farm 2, and 27 birds per shed/age for Farm 3 at each sampling point/time. Although, there was a slight increase of all parameters measured at 73 wks of age in Farm 1, this was no significant ($P>0.05$). No significant changes were found in blood cell profile (RBCs, HGB, and HCT) in hens at different flocks/ages at this Farm (Table 4-2). There were also no significant changes of blood cell profiles in hens from Farms 2 and 3 at all measurement points.

Table 4-4 - Blood cell profile (Farm 1)

Age (wk)	RBC ($x10^{6/L}$)	HGB (g/L)	HCT (%)
21	23.8	132	29.5
25	2.47	134	30.3
29	2.61	138	31.2
46	2.47	132	30.8
50	2.45	135	30.6
54	2.41	126	30.5
65	2.59	132	32.0
69	2.52	139	31.7
73	2.69	146	34.3

Table 4-5 - Blood cell profile Farm 2

Age (wk)	RBC ($x10^{6/L}$)	HGB (g/L)	HCT (%)
64	2.28	121	27
68	2.35	126	28
72	2.38	126	29

Table 4-6 - Blood cell profile Farm 3

Age (wk)	RBC ($x10^{6/L}$)	HGB (g/L)	HCT (%)
23	2.43	123	26
27	2.57	128	29
31	2.74	117	29
31	2.69	125	32
35	2.83	121	30
39	2.67	128	29
56	2.58	133	31
60	2.51	130	32
64	2.69	137	30

Tables 4-7, 4-8 and 4-9 present data on plasma metabolites of hens from Farms 1, 2 and 3, respectively (at three sampling points: February, March, and April). No significant changes were detected in plasma CHOL levels for birds of Farm 1, while birds of Farm 2 and 3 demonstrated higher plasma CHOL at a similar age. Blood metabolite analyses demonstrated a significant increase in plasma TRG over normal levels (at this age/level of production). An increased plasma TRG concentration was observed in birds of Farm 1 at over 40 wks of age, especially from 46 to 54 wks of age. At 64 to 72 wks birds of Farm 2, also showed high TRG levels in their plasma, and at 56 to 64 wks birds of Farm 3 have similarly increased plasma concentration of TRG than normally seen in laying hens at this age (Shini et al. 2007). GGT was significantly decreased at 31 to 39 and 56 to 64 in birds of Farm 3, probably reflecting the consequences of an increased of plasma TRG and CHOL in those birds. There were no significant differences in other plasma metabolites (GLU and TP) between birds of similar age from Farms 1, 2, and 3.

Table 4-7 - Blood metabolite profile (Farm 1)

Age (wk)	Cholesterol (Mmol/L)	Triglyceride (Mmol/L)	GGT (U/L)	Protein (g/L)	Glucose (Mmol/L)
21	2.5	12.3	33.7	43.7	12.1
25	2.7	11.4	32.7	50.7	14.8
29	2.3	11.1	35.0	48.7	15.1
46	2.4	19.9	32.3	56.7	12.9
50	2.8	23.5	31.7	58.3	14.8
54	2.5	19.9	34.7	55.0	15.9
65	2.5	20.8	41.7	53.7	12.7
69	2.6	23.3	43.7	55.3	14.5
73	2.3	24.6	46.0	54.0	15.1

Table 4-8 - Blood metabolite profile Farm 2

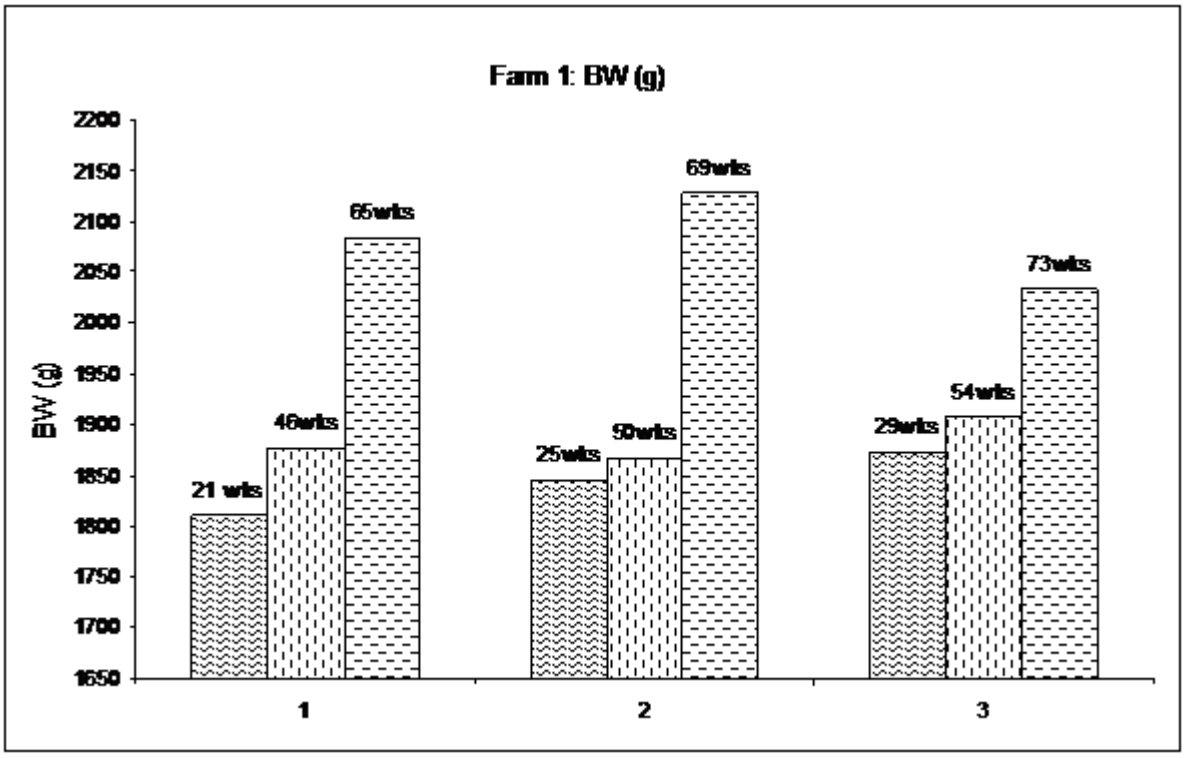
Age (wk)	Cholesterol (Mmol/L)	Triglyceride (Mmol/L)	GGT (U/L)	Protein (g/L)	Glucose (Mmol/L)
64	3.25	23.0	35.2	55.0	13.4
68	3.23	19.2	40.0	52.0	14.2
72	3.48	23.1	40.0	58.0	13.2

Table 4-9 - Blood metabolite profile Farm 3

Age (wk)	Cholesterol (Mmol/L)	Triglyceride (Mmol/L)	GGT (U/L)	Protein (g/L)	Glucose (Mmol/L)
23	2.4	12.4	34.5	48.4	12.8
27	2.7	15.6	37.2	43.5	14.2
31	2.8	15.2	40.0	49.0	14.6
31	3.1	17.7	29.0	47.7	12.9
35	3.8	17.8	27.3	53.0	12.8
39	3.3	15.2	29.0	52.7	12.9
56	3.4	25.1	27.3	54.8	13.0
60	3.2	26.1	26.7	54.3	13.0
64	3.5	24.1	25.0	40.3	13.3

4.1.2.2 Performance parameters

Figures 4-3, 4-4 and 4-5 present data on BW and egg production (HDP%) of hens from Farms 1, 2 and 3 at three sampling points: February, March, and April 2008. For breeder's recommendations at peak of production (32 wks) see Appendix 7.3. BW was increased with age. At 32 and 72 wks of age birds of Farm 1 and 2 had a BW comparable with that recommended by the breeder. At 31 and 64 wks of age, birds of Farm 3 had a significantly higher BW than that recommended for Isa brown.


Farm 1: BW (g)
BW (g)
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2150
2100
2050
2000
1950
1900
1850
1800
1750
1700
1650
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46wks
65wks
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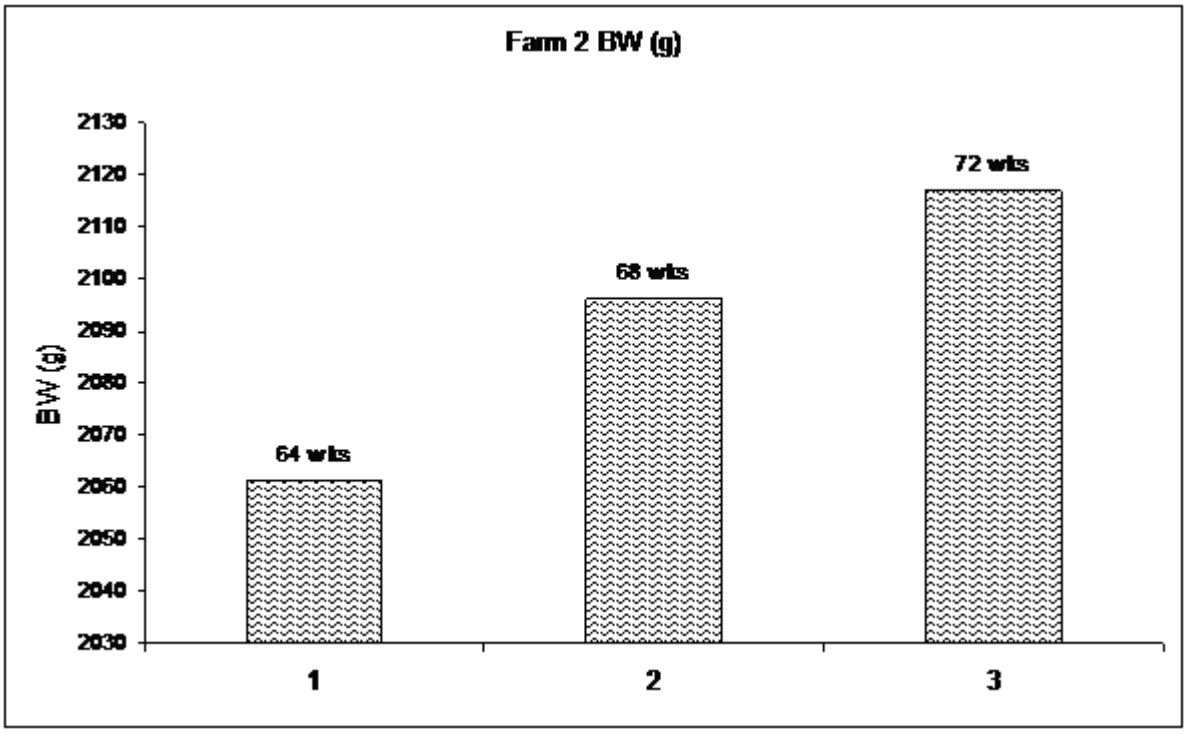
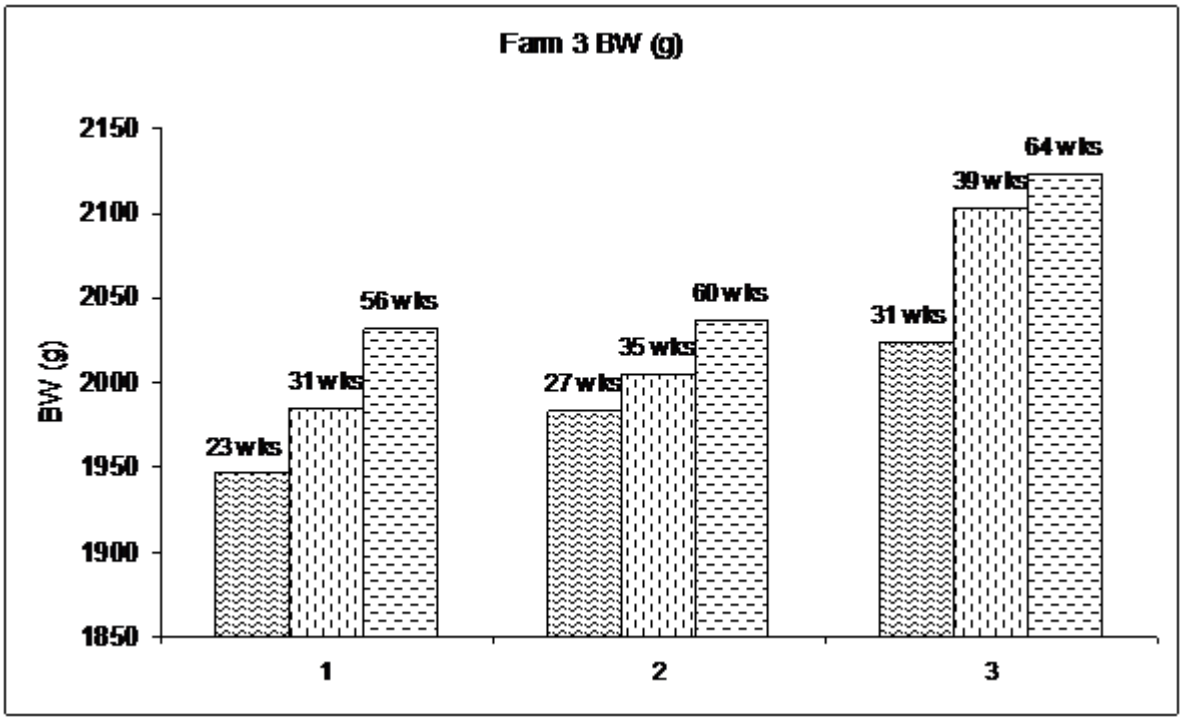
29wks
54wks
73wks
1
2
3

A


Farm 2 BW (g)
BW (g)
2130
2120
2110
2100
2090
2080
2070
2060
2050
2040
2030
64 wks
68 wks
72 wks
1
2
3

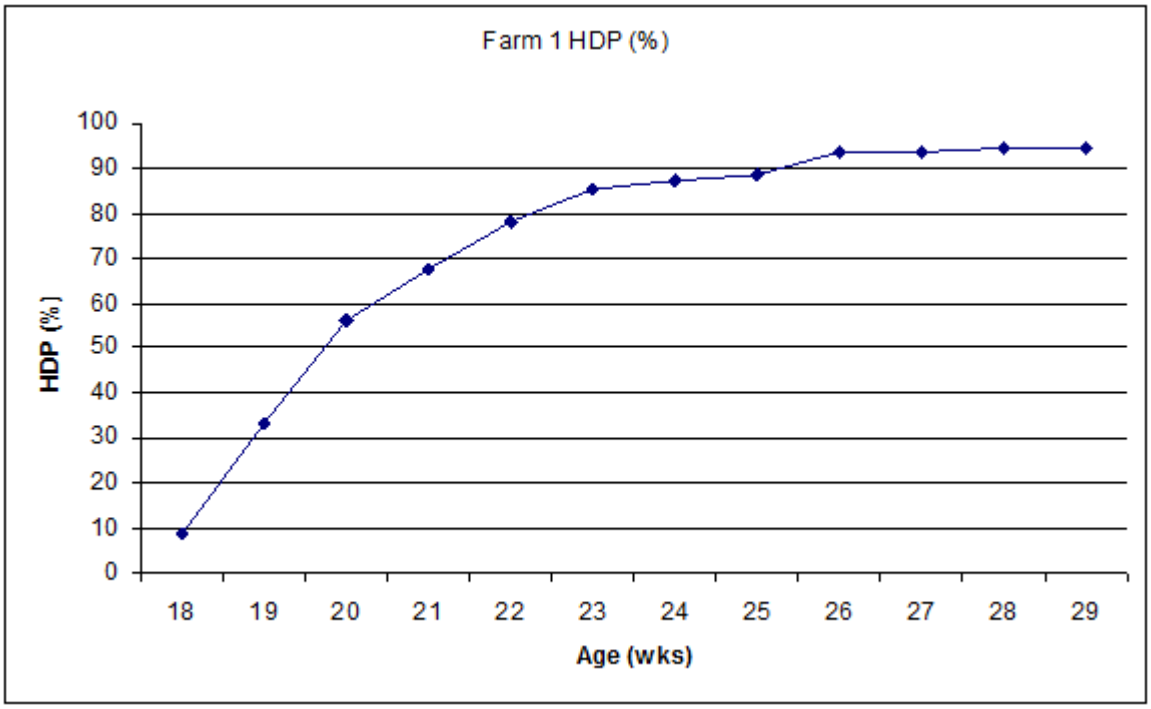
B



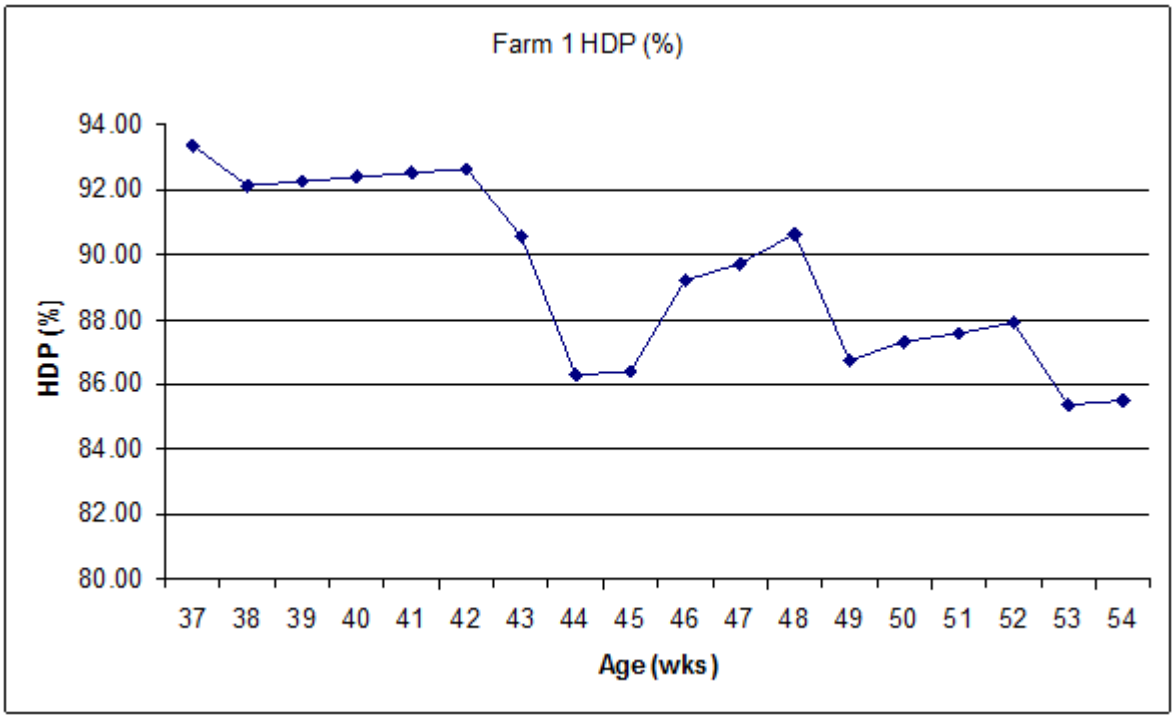
C

Figure 4-3 - BW measurements in Farms 1, 2, and 3 (3 consecutive measurement of the same cage units). At 32 and 72 wks of age birds of Farm 1 produce more than breeder's specification (Fig. 4-3, A, B, and C).

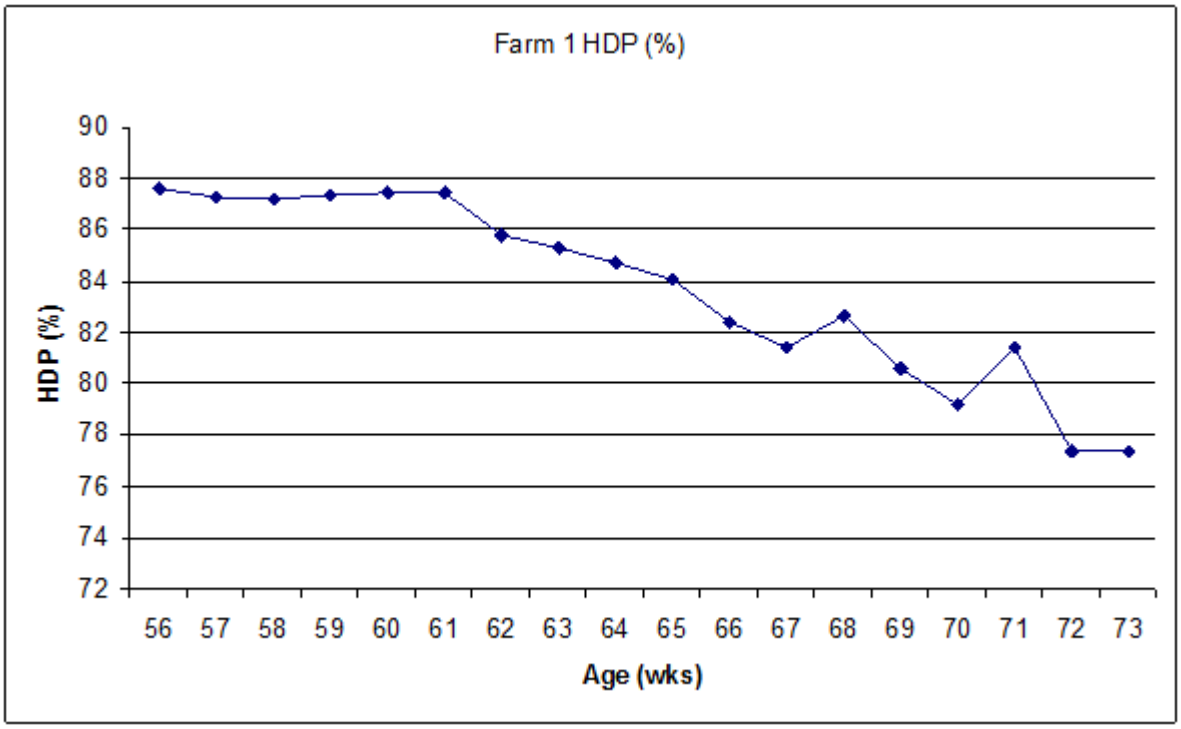
From start of lay until the peak HDP was increased with age; e.g. Farm 1 (Fig. 4-4 A, B) and 3 (Fig. 4-5 B) had a HDP over 92% and this continued to stay at this level until 37-39 weeks of age. At 64 weeks of age HDP was recorded between 82-85% for all farms. In general, HDP was comparable with the breeder's recommendations, although at 69 wks of age birds of Farm 3 have a higher production (HDP) than that recommended by the breeder at this age (Fig. 4-5 B).



A. Shed 1, flock 1(18-29 wks)

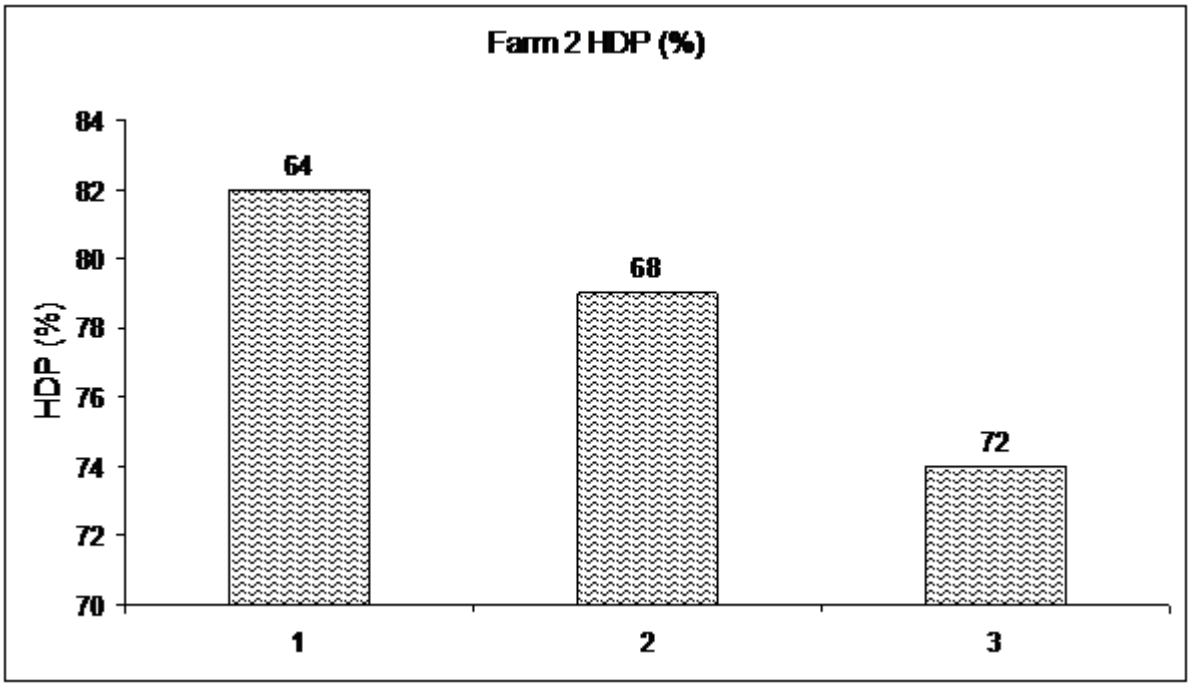


B. Shed 2, flock 2 (37-54 wks)

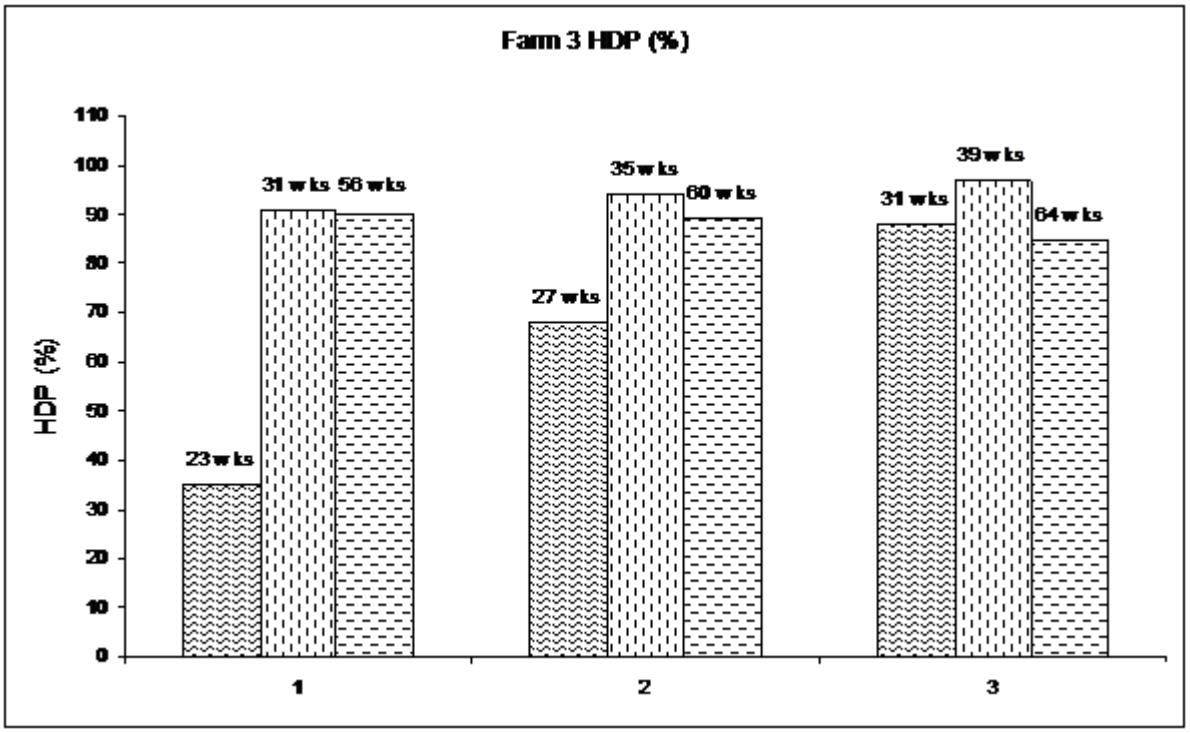


C. Shed 3, flock 3 (56-73 wks)

Figure 4-4 - Data on HDP (%) for Farm 1 (3 flocks: A, B, and C) during 4 months of monitoring



A



B

Figure 4-5 - Data on egg production for Farms 2 (A) and 3 (B) showing the level of production at 3 points of sampling

4.2 Part two of the project: results from experimental model of FLHS

Data on FLHS incidence (as diagnosed in sacrificed birds and birds that died during the experiment), mortality rate, haemorrhagic score and liver fat content of treated and control birds are presented in the Table 4-10.

Birds treated with exogenous E_2 developed advanced clinical signs of FLHS, which were diagnosed in 87.5% of birds in this group. Oestrogen-treated birds in the feed restricted group did develop FLHS in a similarly way with oestrogen treated birds in the ad libitum feed group, however the incidence was lower (87.5% vs. 68.75%, respectively) or ca. 20% less.

Liver weights and liver fat content measurements indicated that E_2 induced significant increases of liver weight and fat content presumably through induction of lipogenesis in the liver. The liver weight to BW (g/100g) ratio was significantly increased in oestrogen-treated hens.

Table 4-10 - Overall data on the frequency, mortality rate, haemorrhagic score liver weights and fat content of treated and un-treated hens[1]

Treatment	N of birds[2]	Birds diagnosed with FLHS (5)	Mortality (%)	Haemorrhagic score	Liver weights (g) fat content (%)	Liver weight to BW ratio (g/100g)
Ad libitum	32	18.75 6.25	0	1-2 3	38.6±5.1 25.2±2.8	2.07
Reduced FI	32	6.25	0	1	39.2±4.8 23.8±2.2	2.19
E_2 (*ad libitum*)	16	87.5 12.5	18.75	4-5 2-3	53.0±6.0 51.4±5.3	2.91
E_2 (reduced FI)	16	68.75 18.75 12.5	6.25	4-5 2-3 1	47.4±5.5 43.6±3.8	2.82

[1]Data were recorded during the whole experimental period; N of birds sacrificed from each treatment at each sampling point was 6. At the end of experimental period all birds were sacrificed and undergone post-mortem examination

[2]There were no significant differences between not-treated and oil-treated groups therefore data are pooled and presented together

4.2.1 Body Weights

Body weight of birds in both feed restricted groups (E_2-treated and non-treated group) decreased starting first week post-treatment (Fig. 4-6), but this decrease was not significant ($P>0.05$). The decrease was more pronounced ($P<0.01$) on the second week of treatment, and continued to remain at this level (without recovering) even 1 week after the treatments was interrupted.

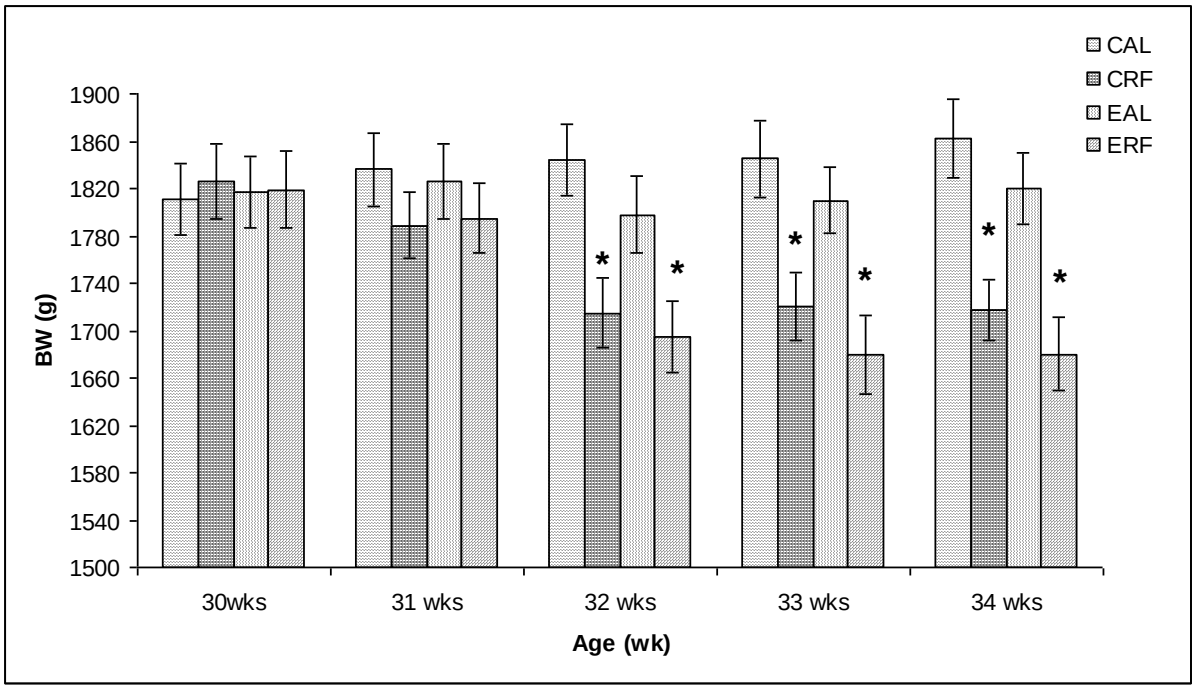


Figure 4-6 - Effects of exogenous E_2 on BW of treated and control birds
CAL = control & ad libitum;
CRF = control & feed restricted
EAL = oestrogen-treated & ad libitum;
ERF = oestrogen-treated & feed restricted.

4.2.2 Egg production

Administration of exogenous E_2 during egg laying (from 30 to 33 wks of age) initially delayed egg production slightly (in both E_2-treated groups), however at week 1 and 2 post-treatments this was not significant (Fig. 4-7). Oestrogen-treated and un-treated hens in both feed restricted groups significantly decrease egg production at week 3. At week 4 (1 week after the treatment was interrupted) all treated hens had reduced HDP as compared with week 1.

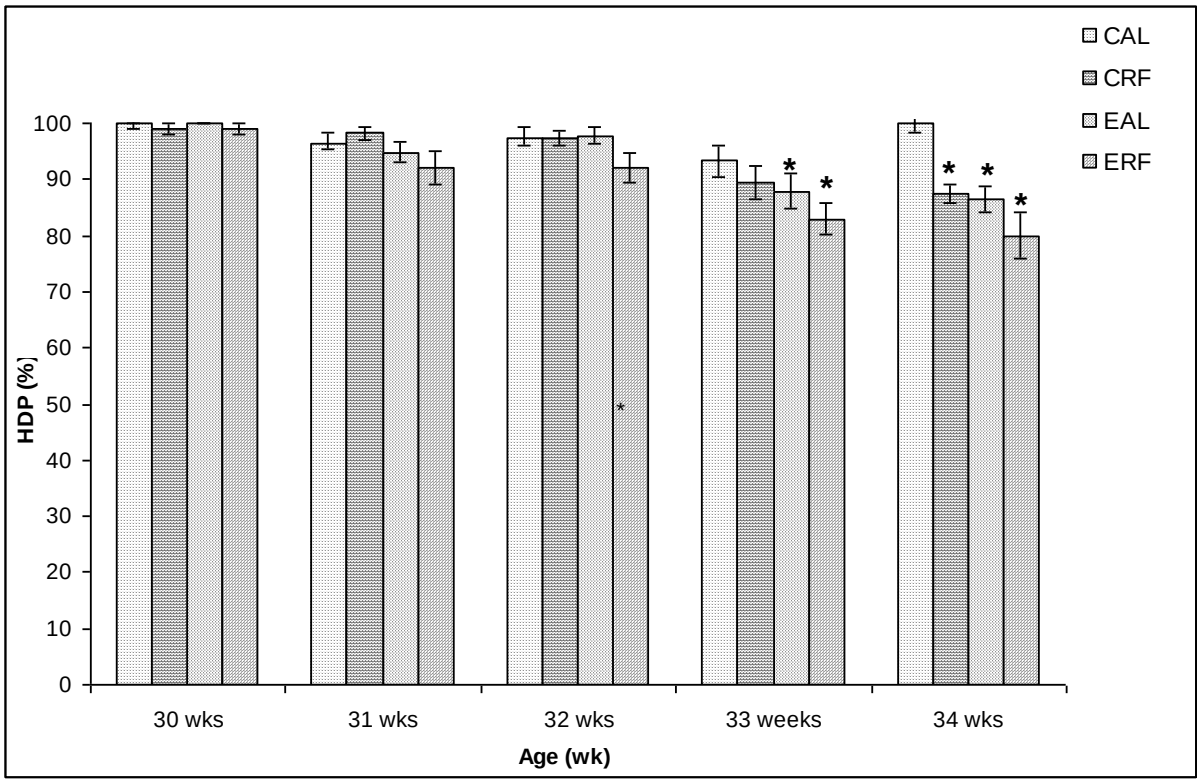


Figure 4-7 - Effects of exogenous E_2 on HDP of treated and untreated & control birds

CAL = control & ad libitum;
CRF = control & feed restricted
EAL = oestrogen-treated & ad libitum;
ERF = oestrogen-treated & feed restricted.

4.2.3 Egg weight

At week 3 post-initial treatment with exogenous oestradiol egg weight of oestrogen-treated & ad libitum fed hens was increased significantly (Fig. 4-8). Egg weights from hens in the restricted feed regimen were decreased at week 4.

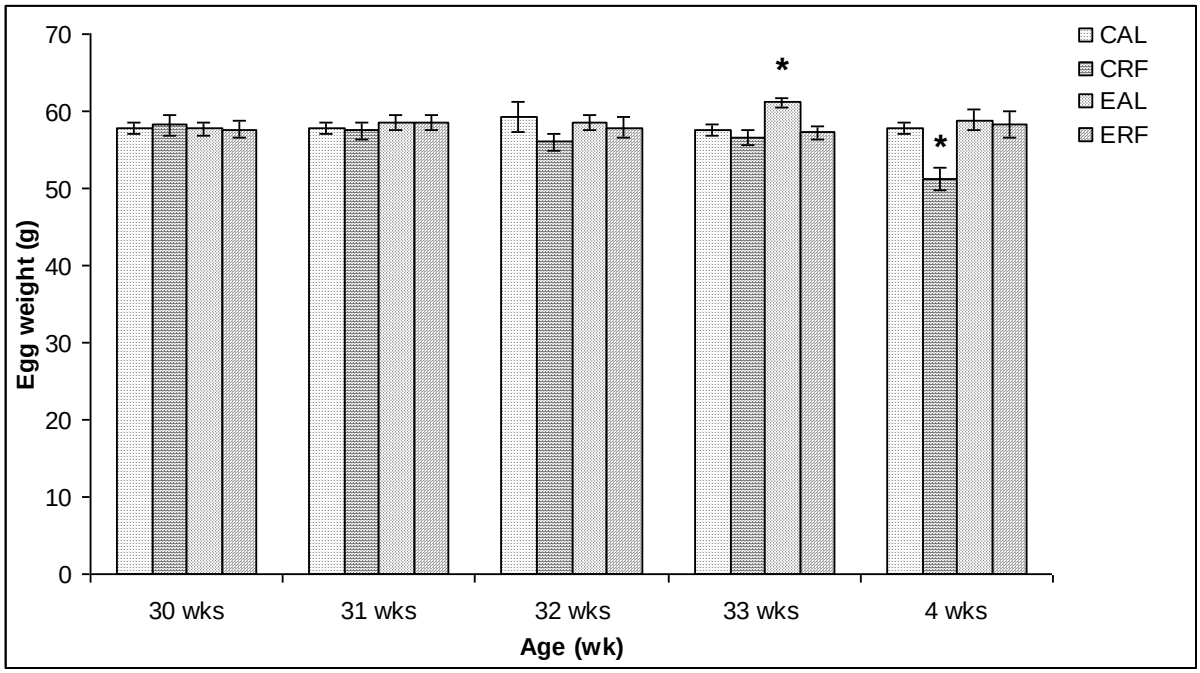


Figure 4-8 - Effects of exogenous E_2 on egg weights of treated and untreated birds
CAL = control & ad libitum;
CRF = control & feed restricted
EAL = oestrogen-treated & ad libitum;
ERF = oestrogen-treated & feed restricted.

4.2.4 Plasma E_2 concentration

The present study demonstrated that plasma oestradiol concentration was elevated by exogenous administration of E_2 (Fig. 4-9). Mean plasma oestradiol concentration in both E_2 treated groups (ad libitum and restricted feed) varied from 4 to70 pg/ml.

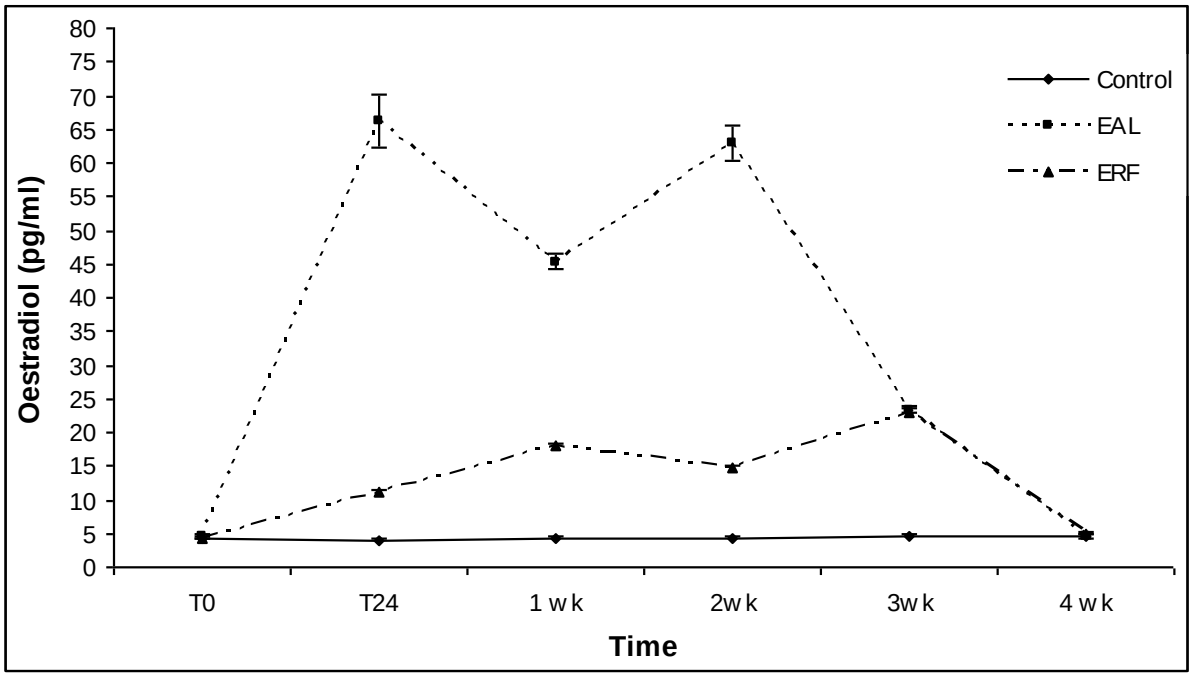


Figure 4-9 - Effects of exogenous E_2 on plasma oestradiol concentration in treated and control birds

Control = data are pooled for all control birds;
EAL = oestrogen-treated & ad libitum birds;
ERF = oestrogen-treated & feed restricted birds.

4.2.5 Peripheral leukocyte and fibrinogen concentration

As shown in Table 4-10, at 24 h, 1 week and 2 weeks post-treatments, oestradiol-treated chickens showed a significant ($P<0.001$) increase in peripheral leukocytes, particularly lymphocytes when compared to basal levels and control hens. Plasma fibrinogen levels were also elevated ($P<0.001$), particularly on the first and second week post-E_2 treatment. Thereafter, both leukocytes and fibrinogen levels decreased.

Table 4-11 - Data on haematological parameters and plasma fibrinogen concentration

Treatment	Time	WBC $x10^3/\mu l$	RBC $x10^6/\mu l$	HCT (%)	Fibrinogen Mg/dL
CAL	0 h	16.5 [c]	2.4	29.6	230[c]
CRF		16.6 [c]	2.6	29.1	250[c]
EAL		16.3 [c]	2.5	29.7	235[c]
ERF		16.1 [c]	2.5	28.6	220[c]
CAL	24 h	17.7 [c]	2.1	26.1	260[c]
CRF		17.5 [c]	2.5	26.5	280[c]
EAL		31.1 [b]	2.2	25.6	550[a]
ERF		25.7 [b]	2.3	26.7	580[a]
CAL	1 wk	17.7 [c]	2.1	26.1	260[c]
CRF		17.5 [c]	2.5	26.5	280[c]
EAL		31.1 [b]	2.2	25.6	550[a]
ERF		25.7 [b]	2.3	26.7	580[a]
CAL	2 wks	15.8 [c]	2.0	25.4	240[c]
CRF		16.6 [c]	2.2	26.2	280[c]
EAL		64.8 [a]	2.3	24.4	520[ab]
ERF		53.3 [a]	2.0	23.8	450[b]
CAL	3 wks	20.0 [b,c]	2.2	26.3	270[c]
CRF		18.2 [c]	2.6	30.0	300[c]
EAL		20.0 [b,c]	3.0	27.6	120[c]
ERF		20.2 [b,c]	2.1	26.1	100[c]

[1]Means with different superscripts (a–c) within a column are significantly different ($P < 0.05$)

[2]EAL = oestrogen-treated & ad libitum; ERF = oestrogen-treated & feed restricted; OAL = oil-treated & ad libitum; ORF = oil-treated & restricted feed; CAL = control & ad libitum; CRF = control & feed restricted

4.2.6 Liver macroscopic evaluation

The liver tissue from euthanised and/or hens that died (during experimental period) were pale, swollen and friable with different grades of haemorrhages and haematomas on both surfaces (dorsal and ventral) and/or in the edges of both lobes. In advances cases (haemorrhage score 4 or 5) liver tissue was ruptured and large blood coagula was found inside the abdominal cavity (Fig. 3-1).

4.2.7 Liver histological examination

Oestradiol treatment resulted in an increased infiltration of hepatocytes and liver tissue with fat and fat vacuoles (Fig. 4-10). Histologically, all livers had significant slight and moderate lipid accumulation in livers, however, E_2-treated birds demonstrated severe fat deposition and large vacuoles containing fat and distending hepatocytes. In addition to fat deposition, histological sections of E_2-treated birds indicated focal inflammatory (heterophilic and/or lymphocytic/ mononuclear) infiltration, haemorrhage and congestion of sinusoids, demonstrating an increased incidence of inflammation and haemorrhage. Massive lipid infiltration, diffuse inflammatory infiltration and congestion was observed especially in the liver parenchyma of birds that macroscopically demonstrated severe lesions of FLHS.

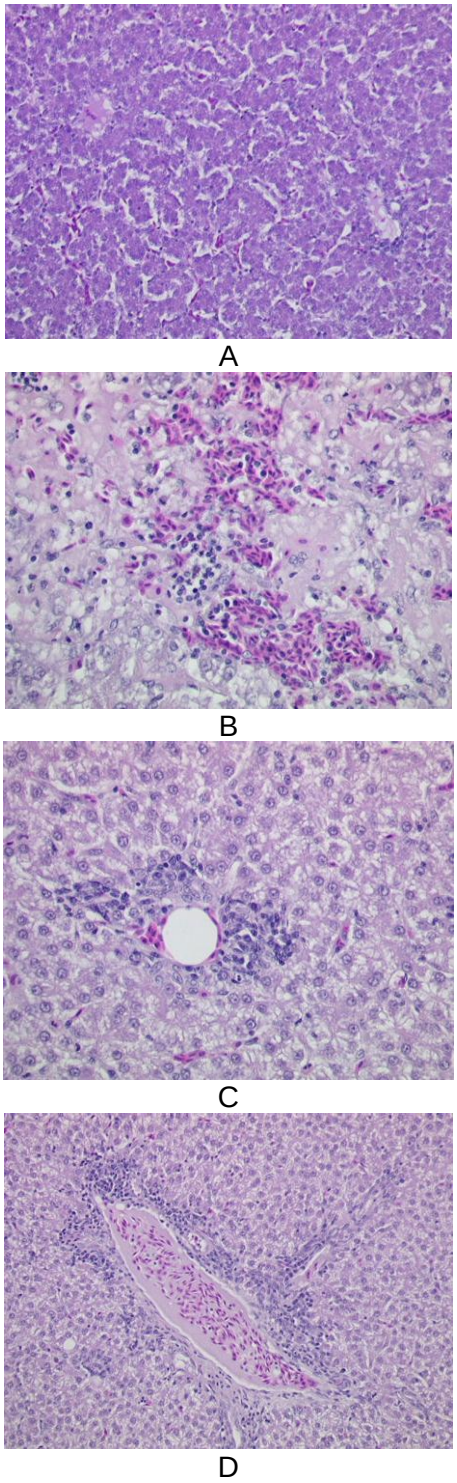


Figure 4-10 - Histological sections showing the normal structure of liver from a control hen (A) and E_2-treated hens (B, C and D). Note focal infiltration with lipid vacuolation and heterophil and lymphocyte (B), mild to moderate periportal congestion and leukocyte infiltration (C and D).

4.3 Part three of the project: Systematic observation of a laying flock

4.3.1 Flock performance

Egg production was monitored for 52 weeks (Fig. 4-16). At 18 weeks of age more than 50% of hens started to lay egg, and at 19 weeks egg production reached over 60%. After the peak (at 25-26 week of age), hens continued to produce over 90% (HDP) until 44 weeks of age. The rate of egg production reduced with increasing age. There was a slight drop in egg production around 47 weeks of age, and another one at around 52 weeks of age probably indicating that some of the hens in a group might have reduced/stopped egg production.

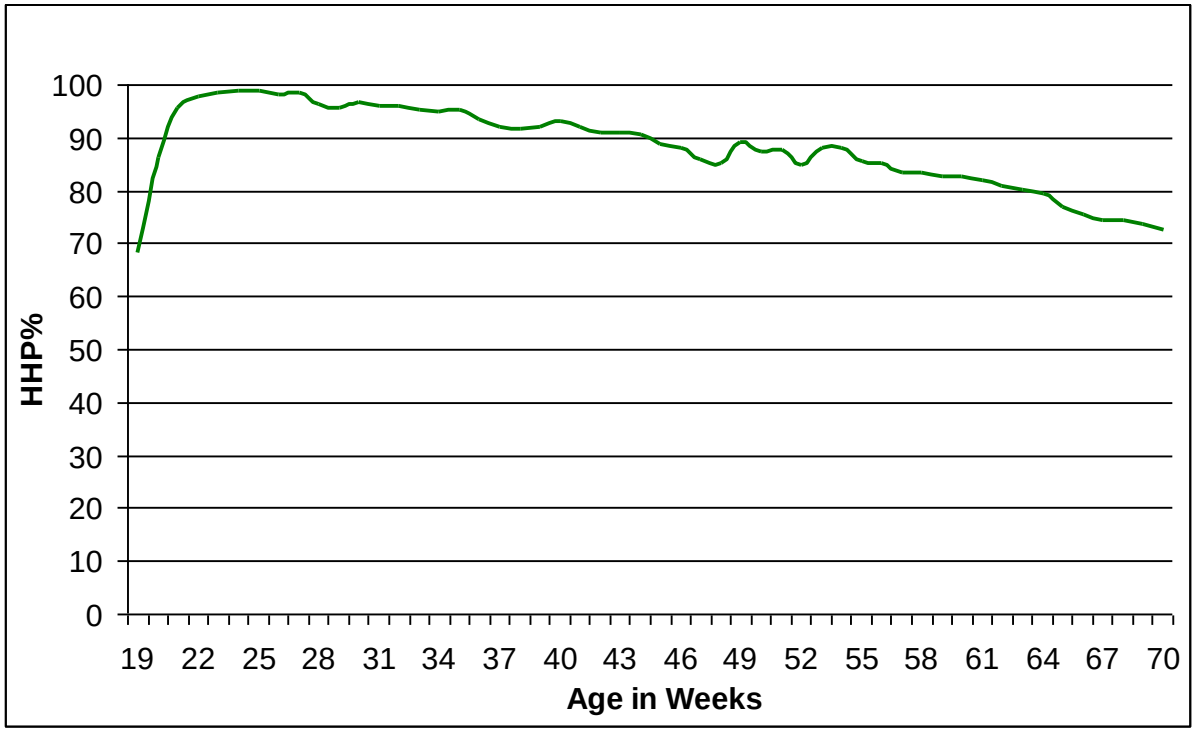


Figure 4-11 - HDP of the flock from 19 to 70 wks of age

The average egg weight of the flock (20% of eggs were weighed) was increased with age (Fig. 4-12), reaching over 60g at 40 weeks of age. The egg weight started to decrease at 60 wks of age, although BW continued to increase until 70 weeks of age.

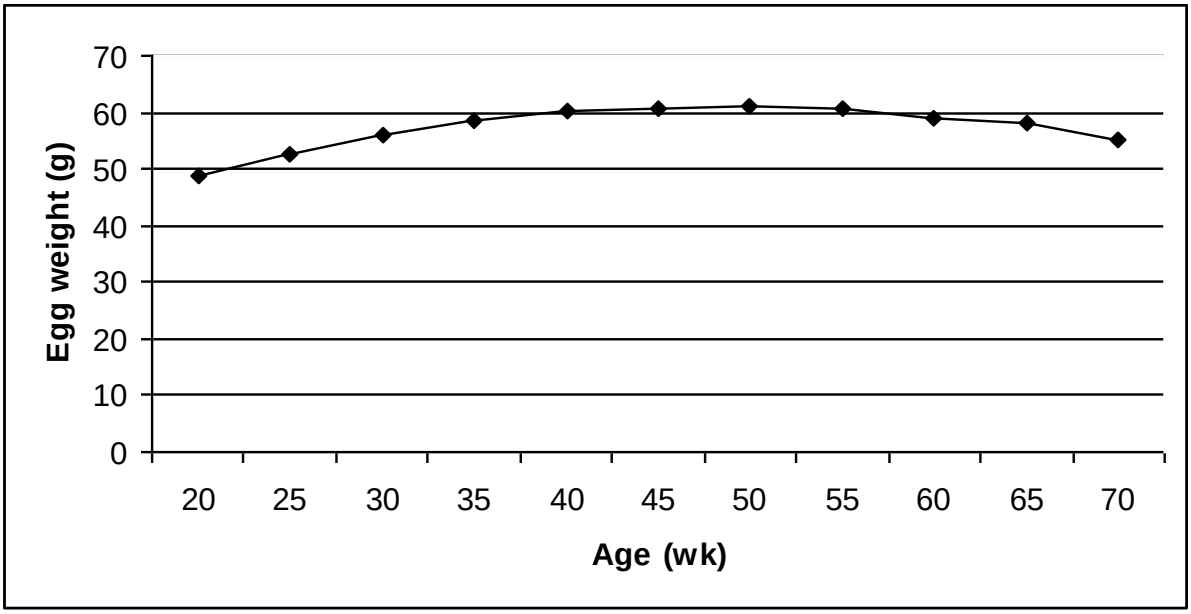


Figure 4-12 - Egg weight of the flock from 20 to 70 weeks of age

At 20 wk of age (Fig. 4-13), the flock had an average BW 1% different to that recommended by the breeder for that age (1657 g vs. 1670 g, respectively). Although BW increased with age, hens weighed 10% less than that recommended by the breeder. Hens were heavier at 70 weeks of age (i.e. 2050g) with a range of 1850 to 2250.

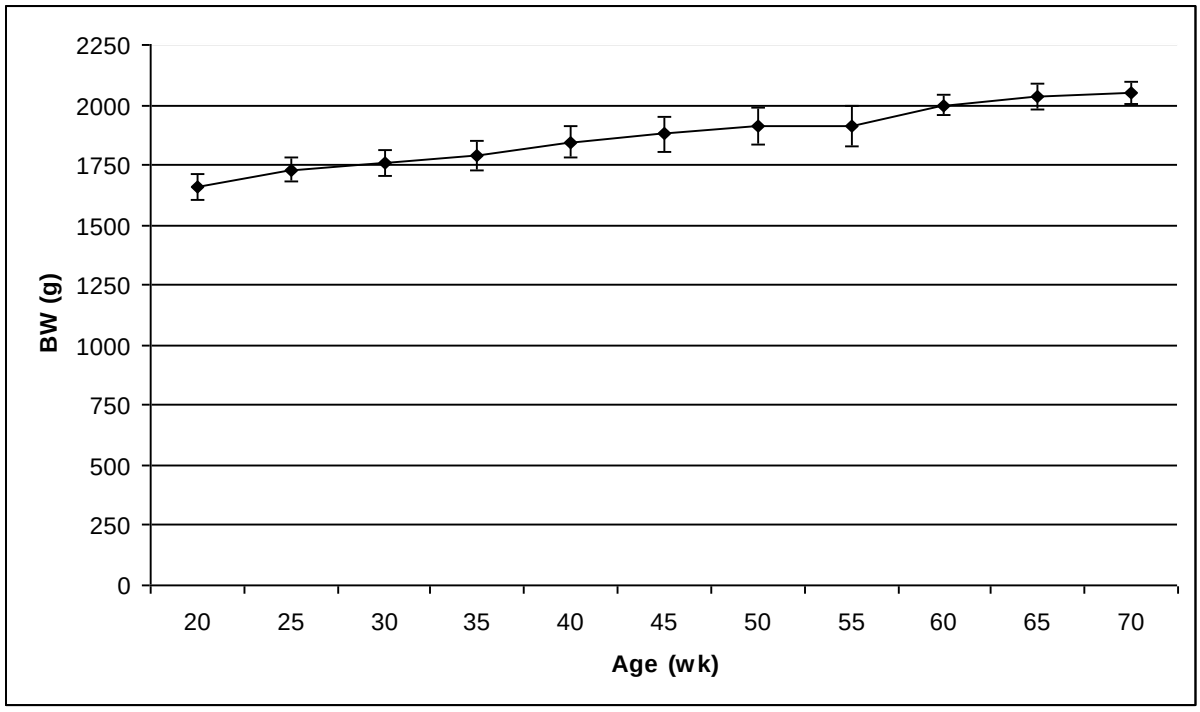


Figure 4-13 - BW of the flock from 20 to 70 weeks of age

4.3.2 Daily feed intake

Average daily feed intake of hens from 19 weeks to 70 weeks of age is presented in Fig. 4-14. At 19 weeks of age average feed intake (DFI) was 98g/hen/day. At 30 weeks of age hens consumed 111±2.2 g per day and this amount increased continuously until 43 weeks

of age when DFI was recorded 117±2.5g. There were two drops in total feed intake (at 40 and 46 weeks of age).

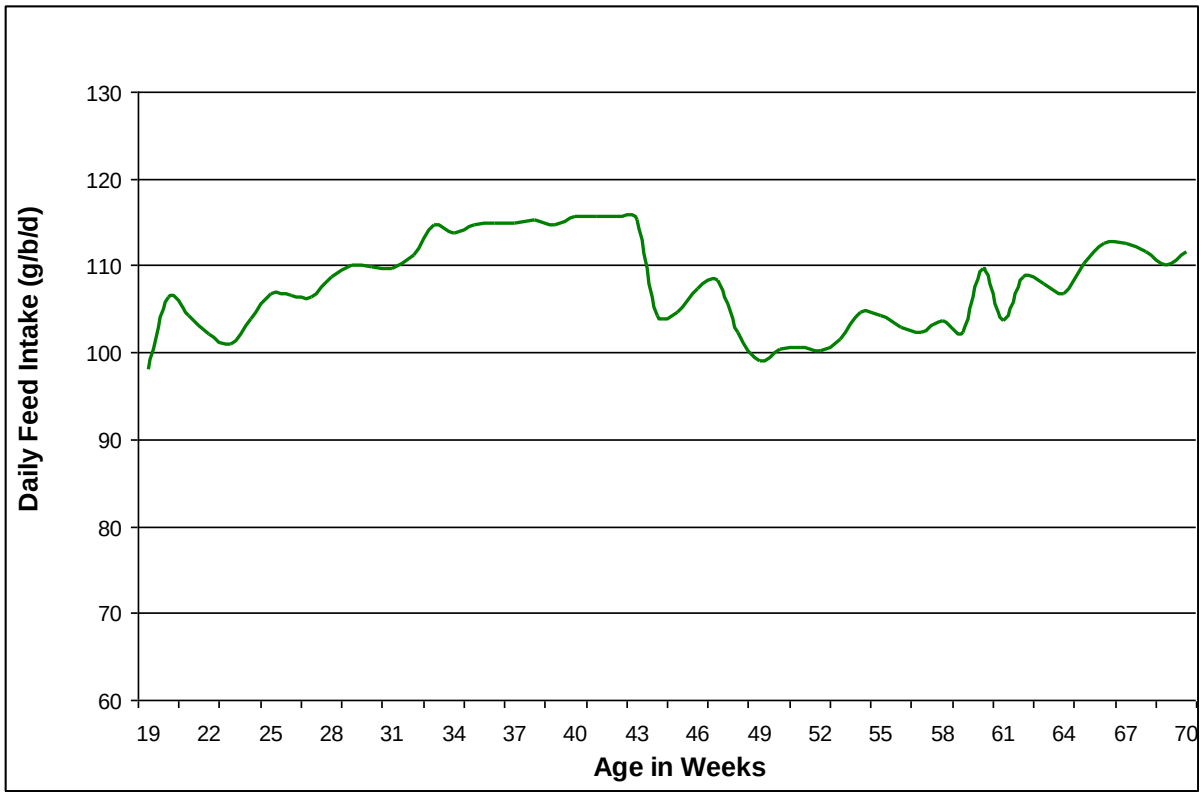


Figure 4-14 - Daily feed intake of hens from 19 to 70 weeks of age.

4.3.3 Blood leukocyte profile and fibrinogen concentration

Both white blood cells (WBC) and fibrinogen levels were significantly changed during the weeks of monitoring. The highest WBC level was measured at 40 weeks of age and the lowest at 70 weeks of age (30.3 x103 cells/µl and 15.5 x103 cells/µl, respectively). The highest fibrinogen levels were found at 35 weeks of age and the lowest at 60 weeks of age.

Table 4-12 - Total leukocyte counts (WBC) and plasma fibrinogen concentration of hens from 25 to 70 weeks of age.

Sampling point (age/weeks) $x10^3$/µl	WBC $X10^3$/ µl	Fibrinogen mg/dL
25	21.5 [b]	150 [c]
30	28.6 [a]	250 [b]
35	27.1 [a,b]	365 [a]
40	30.3 [a]	160 [c]
45	24.7 [b]	150 [b,c]
50	25.5 [b]	180 [b]
55	23.1 [b]	170 [b,c]
60	18.7 [b,c]	120 [c]
65	19.7 [b,c]	150 [c]
70	15.5 [c]	140 [c]

4.3.4 Liver weight to BW ratio and liver examination

From 20 weeks of age to 55 weeks of age liver weights (data not shown here) increased ca. 40% of initial weight (from 28.8 g to 51.3 g, respectively), while BW increased only 15% (from 1657 g to 1914 g, respectively), therefore liver weight to BW ratio increased disproportionally to BW increase (Fig. 4-15). The increase in liver weight can be related to the induction of hepatic production of the yolk precursors which leads to hypertrophy of the liver. In addition to this, hepatic lipogenesis is enhanced in order to meet the demand for vitellogenesis.

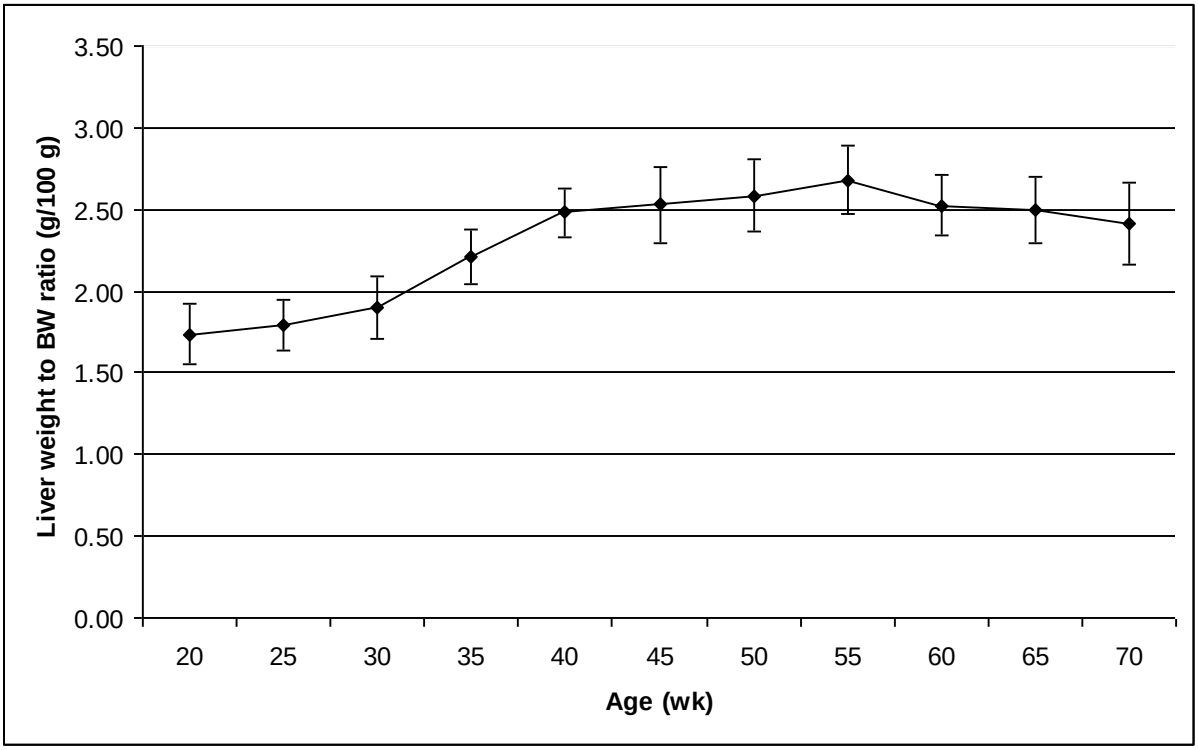
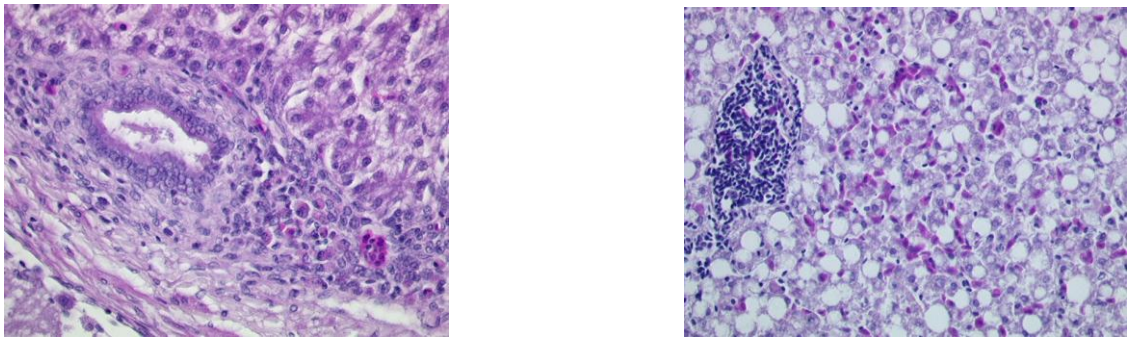


Figure 4-15 - Liver weight of hens from 20 to 70 weeks of age

The examination of livers from hens that were euthanized systematically indicated that the liver colour changed with the increase of production (age) from dark brown to pale brown or yellowish. More than 50% of hens showed focal haemorrhages or haematomas (score 1 or 2); 10% of hens euthanised showed focal necrosis and signs of previous subcapsular haemorrhage (score 3). It appeared that these hens had survived the haemorrhage and continued to produce egg.

Histologically, sections from livers showed severe fatty infiltration and vacuolation (Fig. 4-16 A and B), focal congestion, periportal leukocyte infiltration and fibrosis.



A B

Figure 4-16 - Histological sections of liver from hens sacrificed during flock monitoring

4.3.5 Data on mortality and mortality causes

Figure 4-17 shows mortality rates (cumulative %) of the flock from 19 to 70 weeks of age. At 70 weeks of age the mortality rate was 4.5% (which is lower than breeder's expectation). Interestingly, there were 2 increases in mortality rates (at 38 and 56 weeks of age, respectively).

Of the hens that died (56), 54 necropsied. 70% of these hens had a BW over 1950g. Data from gross examination indicated that 34 hens (or ca. 62.5%) died from liver haematoma and/or liver haemorrhage; 11 hens (or ca. 20%) died from egg peritonitis or eggs blocked in oviduct; 3 hens had mechanical trauma (traumatised in the cage); the cause of death could not be determined for 6 hens.

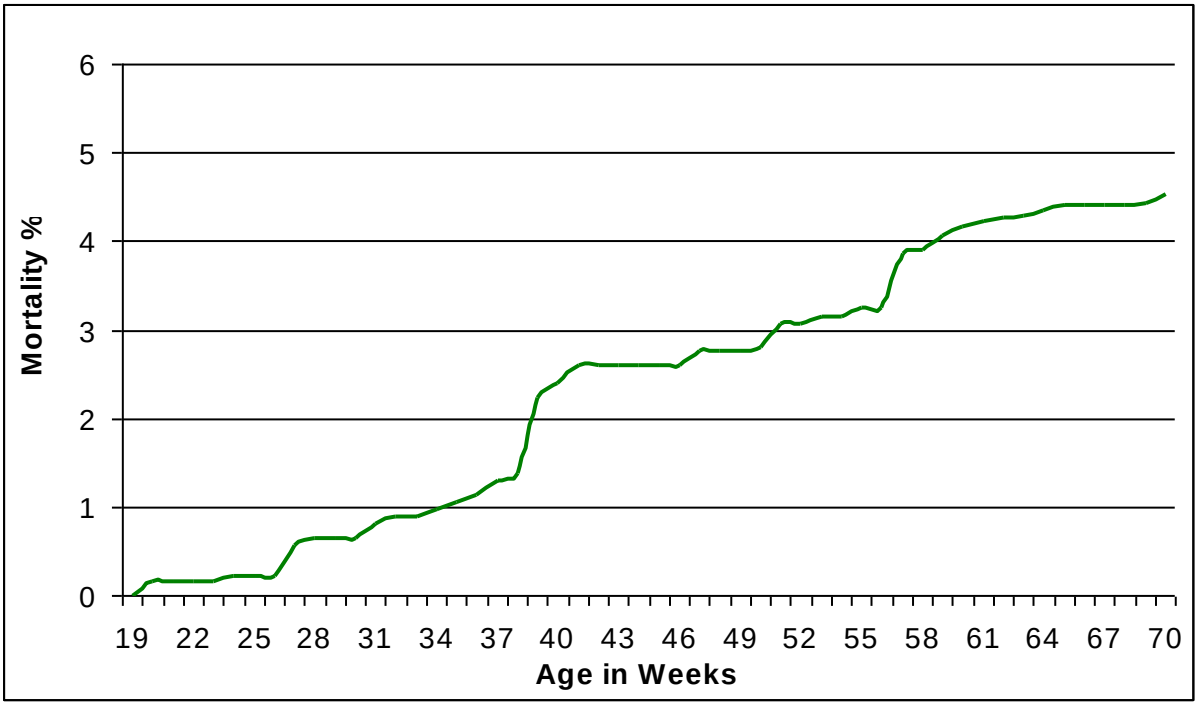


Figure 4-17 - Mortality (cumulative %) of the flock from 19 to 70 weeks of age

5 Discussion

In this project a questionnaire followed by an epidemiological survey were used to determine the occurrence of FLHS in caged laying hens in Queensland. The results demonstrate that FLHS is present in caged birds in Australia. The questionnaire provided important data on hen management practices, and also suggests that most egg producers are not aware of FLHS, but the presence of FLHS was confirmed in the epidemiological study. Post-mortem examination conducted in 3 farms with 7 flocks of different ages indicated that 234 birds (or 36%) of all birds necropsied (597) had FLHS. This indicates that FLHS is the most significant cause of death of laying hens kept in cages. It also confirms our previous observations with a small flock of caged hens at UQ Gatton (Shini et al. 2006) where we found that FLHS was the main cause of death (74% of birds necropsied) in a flock indicating a 6% cumulative mortality rate. The results are also in agreement with previous overseas studies which have shown a high mortality rate (5-20%) due to FLHS in healthy flocks.

Death from FLHS occurs only in extreme cases following massive liver haemorrhage (Squires and Lesson, 1988). Therefore, it is likely that a significant number of hens within a flock are also suffering from “subacute and chronic FLHS” that may cause a drop in egg production but little increase in mortality (Julian, 2005). These hens may exhibit reproductive dysfunction (Chen et al. 2006), due to chronic liver tissue damage and an impairment of the transport of triglycerides, phospholipids, and cholesterol from the liver to the ovary (Walzem, 1996), resulting in decreased yolk formation and egg production. Our data showed that most deaths occurred in heavier hens over 40 wks of age, however data on plasma metabolites demonstrated that abnormal levels of TRG started in hens, ca. 30 wks of age. Moreover, the examination of livers from hens euthanised systematically indicated that more than 50% of hens had focal haemorrhages or haematomas, while 10% of hens euthanised showed focal necrosis and signs of previous subcapsular haemorrhage. Together, the acute and chronic form of the disease suggests that FLHS is a significant source of lost in egg production and confirms our prediction that FLHS is a neglected disease of significant economic importance.

The results of this study also confirm our previous observations that laying hens, in multi-tier cages and in a controlled environment shed, are most at risk of developing FLHS. To our knowledge, we are the first to show the effect of a thermoneutral environmental temperature on the occurrence of FLHS in caged hens. Previous studies that examined the effect of temperature on the occurrence of FLHS were conducted 30 years ago, when controlled environment sheds were not widely used in the industry. In these studies increased mortality due to FLHS was found at temperature extremes. In our study heavier birds in a flock were more likely to have the condition than the lighter birds. The greater BW presumably reflects the lack of activity of caged birds, particularly in a controlled environment shed. Birds are maintained in a thermoneutral zone and have lower energy requirements. Both factors (lack of activity and controlled environmental temperature) contribute to increased BW and increased hepatic lipid deposition.

From the first part of the study, it was concluded that FLHS is present in caged flocks in Australia, and the age of the flock and housing conditions influence the incidence of this metabolic disorder.

Induction of FLHS in the laying hens was investigated to study its pathogenesis and establish the role of oestrogen in the production of FLHS. Ad libitum feed hens demonstrated a higher incidence of FLHS than restricted feed hens, showing that birds with a higher feed and energy intake are more predisposed to the occurrence of FLHS.

Oestrogen-induced hens from feed restricted group also developed FLHS, although with a lower frequency. Body weights and egg production of hens that were restricted to feed was slightly impacted.

In laying hens hepatic lipogenesis is increased dramatically by oestrogen in order to meet the demand for vitellogenesis (Hansen and Walzem 1993). Although the main products of de novo hepatic lipogenesis are triglycerides, the liver is also the major site of cholesterol and phospholipid synthesis. These lipids, along with protein, are the main components of lipoproteins. It is well known that, because de novo fatty acid synthesis in birds takes place mainly in the liver (Annison, 1983), adipose tissue growth and subsequent extrahepatic fattening depend on the availability of plasma triglycerides, which are transported as components of lipoproteins (Hermier, 1997). Many factors, e.g. external (nutritional and environmental factors) and internal (hormones and other mediators) may affect lipid metabolism and disturb metabolic, endocrine and immune interactions resulting in hepatic pathology. Fatty liver occurs in birds when the increase in lipogenesis exceeds the capacity of synthesis and secretion of lipoproteins (Hermier, 1997). Studies in mammals have demonstrated that fat accumulated in the liver and abdominal cavity constitutes an interesting tissue that communicates with other tissues of the body including hepatocytes via adipokines, lipid factors, and lipoprotein particles (Tilg and Moschen, 2008). One of the first organs to be affected when adipose tissue becomes dysfunctional and inflamed is the liver (Attie and Scherer, 2008). In obese humans, fat accumulation in the abdominal region affects both lipid and glucose metabolism, and a fatty liver is insulin resistant. An extremely severe case of a fatty liver will causes an inflammation of the liver cells (steatohepatitis). In chickens, there is a lack of information on the role of a fatty liver in metabolic, endocrine and immune responses.

In this study, elevated leukocyte numbers and fibrinogen levels were highly altered in oestrogen-induced birds and slightly altered in natural cases of FLHS (in birds monitored for 52 wks). As in mammals, in birds the elevation of these parameters demonstrates increased systemic inflammation and tissue repair. Overall, it appears that in addition to the metabolic state of the bird, inflammatory and immune responses might have been involved in the pathogenesis of FLHS. This was also supported from histological data.

Further studies are required to explore the interaction of metabolic, endocrine and inflammatory responses in affected birds, and elucidate their contribution to the pathogenesis of FLHS. It will be important to determine the factors which influence inflammatory processes in hepatocytes and endothelial cells of the liver causing cell damage and rupture. It will be also intriguing to explain why only some laying hens develop FLHS, while all have fatty livers. Data presented here are only preliminary.

A greater understanding of the pathogenesis of FLHS will assist in developing diagnostic tools for early detection of the condition in the field.

6 Conclusion and implications

Studies conducted in this project indicate that FLHS is present in caged layer hens in Queensland and impacts hen health and welfare. Significant economic losses to producers occur because egg production drops and mortality increases. The results demonstrated that FLHS is a major of hen mortality which has the following implications for the industry.

- Egg producers should be made aware of the presence of this syndrome in laying flocks and its significant impact on egg production and hen mortality.

- Egg producers should be advised of the importance of monitoring their flocks for the occurrence of FLHS by conducting post-mortem of dead hens systematically. Monitoring of BW, especially in high producing flocks and heavy breeds, may assist in identifying flocks predisposed to the incidence of the FLHS.

- Development noninvasive techniques to detect FLHS in commercial laying flocks will assist egg producers to detect FLHS and make important management decisions in the relation to this metabolic disease while maximising egg production efficiency.

- Egg producers should be aware that FLHS has a multi-factorial aetiology, including nutritional, hormonal, and environmental factors (i.e. housing conditions). These factors contribute to increased BW and liver fat deposition, resulting in more flock deaths from FLHS.

- Manipulation of the feed intake (decrease of total energy intake) may decrease the incidence of FLHS, but it is not recommended as an industry strategy because it may impact negatively on production.

- Further studies are required to explore the interactions between metabolism, inflammation and endocrinology in the pathogenesis of FLHS, especially, effects of inflammatory factors on liver cells and the occurrence of the condition. This would help explain why only some laying hens develop FLHS, while all have fatty livers.

- A better understanding of the pathogenesis of FLHS will permit development of strategies to reduce the occurrence of this metabolic disorder. Investigations of feed additives that may reduce the production of free radicals, regulate lipid metabolism, and/or protect the liver from the rupture, should be undertaken.

7 Appendices

7.1 Survey-Questionnaire

Fatty Liver Haemorrhagic Syndrome (FLHS) Survey

Farm Name/Address:

Your participation in this survey will be much appreciated and ensure results which will benefit your organisation and the industry as a whole. Please be assured that you will not be identified individually in the survey outcomes. Your confidentiality is respected.
For further information please contact: Dr Shaniko Shini 07 5460 1159 or email s.shini@uq.edu.au. Please return the questionnaire as an email attachment, or fax (07) 5460 1444, or send by mail to: S. Shini, School of Animal Studies, University of Queensland, Gatton QLD 4343.

1. GENERAL

a) Length of time poultry farm has been operational: _____years

b) Average number of caged layers each year / laying cycle: _____

c) Housed in (number of sheds) _____

d) N of flocks currently _____ Age of flock _____

If flocks have different age:
1:
2:
3:

2. HOUSING

a) Cage type _____;

With an environmentally controlled ventilation □ naturally ventilation □ no ventilation □

Size of cages _____

Number of hens per cage _____

b) Shed temperature ____-____°C

3. FEED AND FEED INGREDIENDS/ANALYSES

What kind of feed is used: Commercial □ Self-prepared □

a) Feed Formulation Information.

Please fill in ingredients/feed analyses or attach a list of the diet:

Feed Type	Pre-Lay Diet	Phase 1 Layer Diet	Phase 2 Layer Diet	Phase 3 Layer Diet
Age (week)				
Energy (MJ/KG) or (Kcal/kg)				
Protein (%)				
Fat (%)				
Fibre (%)				
Calcium (g/kg)				
Avail. Phosphorus (g/kg)				
Ca:P				
Methionine				
Methionine + Cystein				
Other				

b) Are there any other chemical components in the feed: yes □ no □

c) If yes, what?
_____

4. HENS AND PERFORMANCE

a) What breed and/or strain do you currently use for your flock:
_____

b) Are hens reared on farm: yes □ no □

If yes, how are hens reared? Floor □ Cage (Wire) □Barn □

c) At what age are pullets placed in cages:
_____

d) At what age do you dispose of hens:
_____

e) Are hens replaced on an all in all out basis:
_____

f) Do you use a lighting regime: yes □ no □

If yes, what is it_____

g) What is your average rate of production_____

h) What is your rate of production at Peak_____

i) What is your rate of production at this time of laying cycle_____

j) What is your average rate of mortality _____

k) What is your average rate of mortality at this time of laying cycle_____

5. MANAGEMENT

a) Are birds beak trimmed yes □ no □
If yes, at what age _____

b) Do you undertake a regular weighing programme yes □ no □
If yes, how often:

c) Do you undertake a regular worming/external parasite eradication/ programme

yes □ no □ If yes, how often: weekly□ monthly□ tri-monthly□ yearly□ as required□

d) What diseases are your flocks vaccinated against: (please tick)

IBV (infectious bronchitis)	
ILT (infectious laryngotracheitis)	
Marek's disease	
Newcastle disease	
Fowl pox	
Coccidiosis	
Infectious coryza	
MG (Mycoplasma gallisepticum)	
MS (Mycoplasma synoviae)	
AE (avian encephalomyelitis)	
EDS (inactivated egg drop syndrome)	
Fowl cholera	
Other	

If known, what were the main causes of mortality (birds found dead & cull) on your farm:

Disease	*Number of mortalities*	*Age (weeks)*
Fowl cholera		
Marek's disease		
Salmonella sp.		
Tracheitis (Mycoplasma sp)		
Coccidiosis		
Spotty liver		
Fatty liver haemorrhagic syndrome		
Egg peritonitis		
Ingluvitis (inflammation of the crop)		
Salpingitis (inflammation of the oviduct)		
Prolapse/protrusion		
Cannibalism		
Physical injury (ie. Broken leg)		
Heat Stress		
Other		
Unknown		

e) Who determines the cause of mortality:

Person	Percentage of time
Owner/Manager	
Veterinarian	
Pathology Lab	

Thank you for your cooperation!

7.2 Diet specifications (feed analysis for Farm 1, 2, and 3)

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Table 7-1 - Farm 1 feed analysis

ANALYSIS			
Volume %	100.0	Na %	0.139
Protein %	17.30	K %	0.531
Fat %	3.91	Cl %	0.174
Fiber %	2.40	Methionine %	0.319
ME_POUL MJ MJ/kg	11.40	METH + CYST%	0.611
Calcium %	3.91	Linoleic	1.207
Phosphorus %	0.66	Choline	878
AV. Phosphorus %	0.38	Soy+FF	10.9
CAL:PHOS	5.95		

Table 7-2 - Farm 2 feed analysis

Nutrient Name	Amount	Units
PROTEIN	19.0	%
Fiber	4.3	%
FAT	5.6	%
CALCIUM	3.82	%
PHOSPHORUS	0.83	%
M.E.POULTRY MJ	11.6	MJ/kg

Table 7-3 - Farm 2 Production formula: Layer 120

Nutrient Name	Amount	Units
PROTEIN	16.69	%
FAT	6.143	%
CALCIUM	3.800	%
AVAIL. PHOSPHORUS	0.450	%
PHYTATE PHOSPHORUS	0.280	%
METHIONINE	0.435	%
METHIONINE + CYSTINE	0.700	%
M.E.POULTRY -MJ	11.50	MJ/kg
CHOLINE	1,300	mg/kg
DIGEST LYSINE	0.669	%
SODIUM	0.180	%
POTASSIUM	0.664	%
CHLORIDE	0.200	%

Table 7-4 - Comparison of laying nutrient levels as recommended by breeders until 44 wks of age (Australian ingredients) and provided in the diets used in farms surveyed

Nutrients	Hy-Line[2]	Farm 1 (farm-mixed)	Farm 2 (farm-mixed)	Isa brown[2]	Farm 3 (commercial)
		Sorghum, wheat and soybean based meal	Corn based meal (100 g)		Sorghum, wheat, soybean, and meat based meal
Metabolisable Energy (MJ/kg) [2]	11.7	11.4	11.6	11.5-11.8	11.5
Protein (g/kg) [2]	16.5	17.3	19.0	17.7	16.7
Fiber		2.50	4.30		
Fat (g/kg)		3.91	5.60		6.14
Calcium (g/kg)	3.50	3.90	3.82	4.1-4.3	3.80
Av. Phosphorus (g/kg)	0.44	0.38	0.48	0.38	0.48
Methionine (%)	0.44	0.32		0.41	0.44
Methionine + Cystein (%)	0.74	0.61		0.71	0.70
Digest. Lysine (%)	0.84	0.80			0.67

[1]Brown-egg-layers at 110 g of feed per hen daily
[2]Isa brown at 110 g of feed per hen daily from 28 wks to the end of lay;
[3]Energy required per hen per day in relation to BW (average of 1750 g) and rate of egg production (80%)
[4]Derived with corn-soybean meal diet

7.3 Performance parameters

Parameter	Hy-Line[1]	Farm1[2]	Farm 2	Isa brown[1]	Farm 3[3]
BW (g) 32 wks 72 wks	1980 2250	1872 2128	2117	1885 1985 (1975 at 64 wks)	1985 2163
HDP (%) 32 wks 72 wks	94 72	94.3 77.4	74%	94.3 75 (79.7 at 64 wks)	91 85
Mortality cumulative (%) 32 wks 72 wks	0.8 4.0	2.0 11.0	7.4	1.2 5.8 (4.9 at 64 wks)	0.8 4.8

[1]At peaking (32 wks) and end of lay (72 wks);
[2]At 29 and 69 wks of age;
[3]At 31 and 64 wks of age

8 References

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9 Plain English Compendium Summary

Project Title:	
• AECL Project No.:	UQ-105
• Researcher:	Shaniko Shini and Wayne Bryden
• Organisation:	University of Queensland, Gatton QLD 4343
• Phone:	07 5460 1159
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• **Objectives**	To determine the incidence of FLHS in caged layer flocks in Queensland, ascertain important factors that predispose hens to this disorder, and understand the impact of this condition on hen physiology, performance and mortality.
• **Background**	FLHS is a metabolic condition occurring worldwide in caged layers and causes significant losses to the egg industry. There is a lack of data on the prevalence, and importance of this condition for Australian Egg Industry. An initial investigation showed a high number of caged birds died due to FLHS. Given that some 80% of Australia's commercial layer flocks are caged, this is a disease of significant economic and welfare importance.
• **Research**	***With the support of the Queensland Egg Farmers Association Inc., a two-step survey was undertaken. Initially, a questionnaire was used to identify farms that had acute sporadic outbreaks of FLHS. For the second part, an intensive epidemiological study was conducted with 7 commercial flocks (from 3 Farms) during 4 months for, egg production, body weight (BW), blood parameters, mortality, and frequency of FLHS. In addition, the development of the syndrome was induced and monitored experimentally.***
• **Outcomes**	The studies confirm that FLHS is present in caged laying flocks in Queensland. Post-mortem examination indicated that 36% of birds' necropsied (ca. 600) had FLHS. The epidemiological and experimental studies showed that most deaths occurred in heavier hens over 40 wks of age. The greater BW presumably reflects the lack of activity of caged birds and most apparent in controlled environmental sheds.
• **Implications**	Death from FLHS occurs only in extreme cases following massive liver haemorrhage. It is likely that a significant number of birds within a flock also suffer from the condition that does not result in mortality but impacts production. Further studies are required to explore the effects of inflammatory factors on liver cells and the occurrence of the FLHS. This would help explain why only some laying hens develop FLHS, while all have fat in livers.
• **Publications**	• 1. Shini, S., Shini, A. and Bryden, W.L. 2008. The occurrence of FLHS in caged layer flocks: results from a survey in Queensland. World's Poultry Science Journal, XXIII World's Poultry Congress, 30 June to 4 July, Brisbane. Book of abstracts. V. 64, Supp. 2:336 2. Shini, S., Shini, A., and Bryden, W.L. 2009. FLHS in laying

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