NUTRIENT DYNAMICS AND PRODUCTION PERFORMANCE OF SHAVER WHITE LAYING HENS HOUSED IN EITHER ENRICHED OR CONVENTIONAL CAGE SYSTEMS OVER AN ENTIRE PRODUCTION CYCLE

By

Mohamed Neijat

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ABSTRACT

The current data provide estimates of the flow of nitrogen (N), calcium (Ca) and phosphorus (P) in Shaver White layers housed under conventional cage (CC) and enriched cage (EC) systems from 19 to 63 wks of age. The experiment was conducted at the University of Manitoba poultry unit. Both feed disappearance ($P < 0.01$) (92.5 vs. 95.0 ± 0.6 g/hen/d, DM basis) and manure output ($P < 0.01$) (79.8 vs. 91.3 ± 1.2 g/hen/d, fresh basis and 27.0 vs. 28.1 ± 0.2 g/hen/d, DM basis) were lower in hens housed in EC compared to CC. Manure DM was 34.1 and 31.0 ± 0.3% for EC and CC, respectively. Feed conversion ratio; body weight; and egg production, weight and mass were not significantly different between the two systems. Although there was no difference in the overall manure N (EC: 1.94 vs. CC: 1.96 ± 0.02 g/hen/d, respectively), N balance was greater ($P < 0.05$) for the CC compared to EC system (85.0 vs. 30.2 ± 13.6 mg/hen/d, respectively). Lower ($P < 0.01$) Ca and P excretions were observed in EC (Ca: 2.11 vs. 2.29 ± 0.04 and P: 0.619 vs. 0.643 ± 0.005 g/hen/d) compared to CC. Overall egg N output was similar between the systems. Although lower Ca deposition ($P < 0.0001$) (2.07 vs. 2.13 ± 0.01 g/hen/d) and output ($P < 0.05$) (38.3 vs. 38.8 ± 0.15 mg/g egg) were noted in eggs from EC compared to CC, shell quality measurements were not different between the two systems. In addition, Ca outputs in eggs expressed as a proportion of Ca intake in both systems were similar (56.5 vs. 56.6 ± 0.51). Although overall mean P retention (-7.22 vs. -7.45 ± 0.71% P intake) was not different between the two groups of hens, Ca retention was higher ($P < 0.05$) in EC than CC hens (-1.37 vs. -4.76 ± 0.89% Ca intake, respectively). In addition to providing environmental enrichment and maintaining

the production performance to the levels of those achieved by CC systems, EC may also help in reducing Ca and P excretions and improving their efficiency of utilization.

DEDICATION

This thesis is dedicated to my late father, Mohamed Said Ibrahim, who was and still is an inspiration to aim higher in my education.

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FOREWORD

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LIST OF ABBREVIATIONS

BW	body weight
Ca	calcium
CC	conventional cage
cm^2	centimetre squared
CV	coefficient of variation
d	day
DM	dry matter
EC	enriched cage
FCR	feed conversion ratio
g	grams
GE	gross energy
h	hour(s)
H^+	hydrogen ions
kg	kilogram
mg	milligram
mL	milliliter
MJ	megajoules
N	nitrogen
P	phosphorus
P	statistical probability
pH	hydrogen ion concentration

R^2	coefficient of determination
SAS	Statistical Analysis Systems
SD	standard deviation
ton	tonnes
vs.	versus
wk	week(s)
^oC	degrees Celsius
$Ca_{10}(PO_4)_6(OH)_2$	Calcium hydroxyapatite
CAN $	Canadian dollar

CHAPTER 1 GENERAL INTRODUCTION

Housing in laying hens has been shown to impact their social behaviour (Albentosa et al., 2007), with subsequent implications for bird welfare (Keeling, 2004) and feed utilization (Lay et al., 2011). Globally, laying hens have been kept predominantly in conventional cage (CC) systems (Tauson, 1998; Awoniyi, 2003; Guesdon and Faure, 2004; Sorensen et al., 2006). Housing birds in CC systems was preferred to keeping birds in flocks on the floor because it provided a lower disease load in the environment (Appleby and Hughes, 1991), an improved working environment for barn employees (Tauson, 1998), reduced aggression and cannibalism in hens (Appleby and Hughes, 1991), while having economic advantages as well (Appleby et al., 2004). However, these benefits are achieved at the expense of very restrictive behavioural expression, leading to a high amount of frustration and stress in the birds (Tauson, 1998; Vits et al., 2005). European countries have increasingly embraced poultry production by following ethical and moral principles such as the phasing out of cage systems (Sumner et al., 2008; Thiele and Pottguter, 2008). Unfortunately, available data on alternative systems such as enriched cages (EC) that were originally developed in Europe may not be suited for extrapolation to North America, due to differences in breed/strain, nutrition, environmental and management factors. Hence, extensive research is being conducted (Mench et al. 2011; Holt et al., 2011; Lay et al., 2011) to provide adequate scientific information on alternative systems for housing laying hens in North America.

The EC systems allow for greater space for the hens compared to the CC type, and are equipped with nesting areas, perches and a scratch pad (European Commission,

1999). Therefore, birds are allowed to express more of their natural behaviours, like nesting, perching, roosting or scratching (Barnett and Hemsworth, 2003). Environmental enrichment also allows for more varied hen behaviour (Hughes and Appleby, 1989; Newberry, 1995; Olsson et al., 2002). Hence, the move towards alternative cage designs for laying hens, such as EC systems, has the potential to lead to differences in hen behaviours, with subsequent impacts on nutrient dynamics.

Minerals constitute about 4% of layers' body weight, of which calcium (Ca) and phosphorus (P) make up 70% (Klasing, 1998). In laying hen nutrition, the role of Ca and P are interrelated in bone development and egg shell formation. In layers, a total Ca output of 28 to 30 times of the hen's total body Ca reserves is required for egg production throughout the entire production cycle (Elaroussi et al., 1994). Similarly, dietary protein from which nitrogen (N) is derived is used in higher proportions in layer diets than for most other farm animals. In addition, N and P are expensive dietary components and potential environmental pollutants (Summers, 1997; Powers and Van Horn, 2001). On average layers excrete 0.55 to 0.75 kg/hen/yr of N (Smith et al., 2000; Leeson and Summers, 2001; Rotz, 2004) and 0.23 kg/hen/yr of P (Spiehs, 2005). For intensive poultry production systems, maintaining efficiency while reducing excretion of these nutrients is a challenge.

With respect to the move towards alternative housing systems, extensive research has evaluated productivity and welfare in birds (Abrahamsson and Tauson, 1995; Vits et al., 2005; Jendral et al., 2008), however studies comparing nutrient dynamics in laying hens housed in different systems are scarce. Therefore, this study was conducted to assess nutrient flow in laying hens housed in cage systems over a full production cycle.

In this study, well managed, healthy and genetically uniform hens (Shaver White laying hens) were used in an intensive egg production system using two distinct cage types: conventional cage and enriched cage systems. Because welfare in laying hens is affected by housing systems, the move towards alternative cage designs for laying hens has the potential to lead to differences in hen behaviours. The project hypothesis and objectives are as follows:

Hypothesis: Alternative cage designs for laying hens have a significant impact on nutrient dynamics.

Aim: To compare nutrient flow (N, Ca and P) in laying hens housed in either enriched or conventional systems through a balance study by taking measurements of nutrient intake, deposition/output in egg components and excretion in manure over an entire production cycle.

Objectives: The general objective of the study was to evaluate various factors in layers housed under traditional and alternative caging system. The specific objectives were to:

1. estimate the efficiency of N, Ca and P utilization in laying hens.
2. quantify nutrient losses in manure and nutrient depositions in egg components over a production cycle.
3. document information that could be valuable reference or database for use in either the evaluation of existing models or development of new models.

4. to identify critical stage of interest, such as high nutrient losses in a full scale production cycle

For the thesis, Chapter 2 summarizes the current literature. In Chapter 3, production performance and N flow of Shaver White layers housed in enriched or conventional caged systems is presented. Chapter 4 discusses production performance and mineral flow in layers housed under conventional and enriched caging systems. Finally, Chapter 5 provides a general discussion and conclusions of the work.

CHAPTER 2 LITERATURE REVIEW

2.1 Cage Systems for Housing Laying Hens

Although laying hens have been kept in wire cages for nearly a century (Rahn, 2001), the use of cage systems developed rapidly during 1960's and 1970's, mainly based on economic and health aspects (Tauson, 1998). However, with more emphasis on humane egg production systems (animal welfare issues), the housing of hens in cages started to be viewed differently by the last decade of the twentieth century (Jalal et al., 2006). Since then, there has been considerable research on modifying conventional cages to minimize welfare risks to laying hens (Appleby et al., 1992; Barnett et al., 2009), yet the application of information in one region of the world warrants its re-evaluation in another area. The choice of a particular housing system for laying hens involves the considerations of production costs, welfare and health of the hen; management capability; food safety and environmental integrity. However, to date, no housing system under consideration is perfect or ideal (Barnett and Newman, 1997; Appleby and Hughes, 1991; Lay et al., 2011). Although the design and space provision within a cage type may differ, there are two major types of cage systems: Conventional cage (CC) and enriched cage (EC) systems.

2.1.1 Conventional Cage Systems

Conventional cage systems (also referred to as standard, traditional, battery or non-enriched cages), are predominantly used to house laying hens (Tauson, 1998;

Awoniyi, 2003; Guesdon and Faure, 2004; Sorensen et al., 2006). In these systems, birds are kept permanently on a sloping wire floor (Matsui et al., 2004). Conventional cages have been used for a number of reasons, including good economic results, less exposure to disease and parasites (due to removal of feces), smaller group numbers than those in non-cage systems (hence eliminating the need for beak trimming as the group size controls aggression and cannibalism), less bird collision (hence fewer broken bones), cleaner eggs, and less management costs (Guesdon and Faure, 2004). However, in CC system, hens have no room to flap, stretch, dust-bathe, or perch hence the hens are unable to fulfill their basic behavioural needs as referred to in the Brambell report (Mench et al. 1986). Conventional cages lack a nesting site, but the hard-wired genetic component enables the bird to express its stereotype behaviour in search for a suitable nesting site (Duncan and Kite, 1989). Nesting behaviour is a hormonal response, which triggers the hens' internal hormonal factors (Wood-Gush and Gilbert, 1975). In addition, sham dust bathing may occur in some strains on the wire floor (Lindberg and Nicol, 1997). The average space used by hens to perform basic behaviours such as standing, ground-scratching, turning, wing-stretching, wing-flapping, feather-ruffling and preening varies between 475 and 1876 cm^2 (Dawkins and Hardie, 1989). Duncan (2001) reported the application of cage densities of 350 and 450 cm^2 of floor area per bird in the U.S. and Canada, respectively, however under the European Union rules, cage space of 550 cm^2 per hen is considered insufficient (D'Silva, 2006).

The welfare concerns in poultry production are the ability to provide freedom of movement, freedom from fear, comfort and shelter, suitable flooring, and freedom to display most normal patterns of behaviour (Guesdon and Faure, 2004; Guesdon et al.,

2006). The welfare of animals with respect to a housing system has been viewed from different aspects based on behavioural, physiological, production and health stand point (Pohle and Cheng, 2009). The CC system may not provide these needs (Appleby and Hughes, 1991). Although the need of an animal has not been clearly defined, basically it implies that the animals' welfare will suffer if they are unable to carry out normal patterns of behaviour (Hughes and Duncan, 1988). Birds may still prefer to perform certain natural behaviours, however, although the limitations of space may vary according to stocking density, this coupled with the bare environment the birds' behavioural repertoire is restricted and bone quality is reduced (Hughes et al., 1993; Fleming et al., 1994; Tauson, 1998; Tauson, 2005).

2.1.2 Enriched Cage Systems

Enriched cages (furnished cages) are equipped with perches, dust baths, and nesting areas allowing the birds to meet the needs for their natural behaviours, such as nesting, roosting, and scratching (Cooper and Albentosa, 2003).They were originally developed in Europe to try to address some of the welfare concerns associated with both conventional cages and non-cage systems. According to specifications by the European Union Council Directive *1999/74/EC*, the EC systems are described to contain a cage area of at least 750 cm^2 of cage area per hen with a height of at least 45 cm as the minimum cage height at the lowest point in the usable area (European Commission, 1999). There are also other cage systems that possess intermediate standards between the conventional and non-cage systems, which Appleby et al. (2002) referred to as furnished cages. These cages provide additional features such as nest area, perches and additional

space, but do not necessarily conform to the specifications laid out in the Directive with respect to actual measurements and design (Appleby et al., 2002).

Enriched cage systems provide environmental enrichment to birds (Hester, 2005; Tauson, 2005; Pavlik et al., 2008; Tactacan et al., 2009) while still retaining the benefits and reduce the disadvantages of floor keeping, including the benefits of a small group size, as in the CC system (Summers and Leeson, 1976; Whitehead and Fleming, 2000; Guesdon and Faure, 2004) and result in better bone mineral density (Newberry, 1995; Kopka et al., 2003; Hester et al. 2004; Tactacan et al., 2009). Therefore, European countries adopted legislative policies that regulate the use of cage systems following the European Union Directive (Commission of the European Communities, 1999) prohibiting all CC in the European Union from 2012 thereafter allowing only EC and non-cage systems (Wang et al., 2009). While in some European countries and the US (e.g. California) cage systems are being phased out (Sumner et al., 2008), the restriction of cage layer operations in Switzerland and Denmark was mentioned as early as 1980's (Lindgren, 1982). In North America, laying hen husbandry practices are not regulated by legislation and the CC still remain the predominant housing system (Jendral et al., 2008). Despite the gap, in Manitoba, following a new housing policy in 2010, legislated by the Manitoba Egg Farmers, the first EC system has recently been installed (Dyck, 2010). Through the Manitoba Egg Farmers initiative, new quota to new entrants into egg production are only provided if installing, at minimum, the EC type of housing and it is expected to transform the present CC systems by 2018 (Dyck, 2010). Systems other than the conventional system, such as the floor system or aviary could also be used.

Enriched (furnished) cage systems provides environmental enrichment (Hester, 2005; Tactacan et al., 2009) which allows hens to express their behavioural characteristics aimed at improving the well-being for birds and maintaining or sustaining production (Pohle and Cheng, 2009). Although it depends greatly on the design, the EC system is considered as an intermediate between the conventional and non-cage systems in terms of their advantages and disadvantages (LayWel, 2006).

2.1.3 Cage Designs and Significance

It is known that the barren environment of CC systems leads to poor welfare of laying hens and that providing them with environmental enrichment improves their welfare (Cooper and Albentosa, 2003). Hence, the importance of allowing the birds to meet their basic natural behaviour in relation to the available features of the cage environment has been extensively researched (Appleby and Hughes, 1991; Cordiner and Savory, 2000; Cooper and Albentosa, 2003). This section provides a brief review of the role of cage design with respect to the features and the space available for laying hens in an enriched environment.

2.1.3.1 Housing Features

Although environmental enrichment of cage systems encompasses a number of features, the main proposed enrichment components for alternative housing systems, including the EC system, include the provision of perches, nesting areas, dust bathing areas/substrate and more space per bird (Ferrante, 2009). Among these components, the lack of a nesting area is the most important deficiency that contributes to a welfare problem for laying hens and this is the case with the CC system (Barnett and Newman,

1997). The presence of nesting area is so important that, before laying an egg, a hen is motivated to gain access to a nest site (Smith et al., 1990) regardless of her previous experience to a nesting area (Cooper and Appleby, 1995). Hens in cages without nests often show severe frustration during pre-laying, as a consequence this effect results into increased pacing, reduced sitting behaviour (Sherwin and Nicol, 1993) or excessive feeding (Meijsser and Hughes, 1989). Nesting behaviour is associated with ovulation (Etches, 1996). The corpus luteum that is involved in the secretion of the estrogen and progesterone in mammals (Niswender et al., 2000) does not exist in birds (Gilbert, 1971). However an analogous feature, the post-ovulatory follicle in the bird (Etches, 1996), which is physiologically active during the first 24 h of its life (Armstrong et al., 1977) is associated with the nesting behaviour and oviposition of the egg (Wood-Gush and Gilbert, 1964). Nesting behaviour was also found to occur in birds in which the functional oviduct had been removed or even in the absence of a nest site, implying that the ruptured follicle is important in expressing the nesting behaviour (Gilbert, 1971). Hence, normal nesting behaviour in laying hens is associated with hormonal secretions (steroid hormones) (Petherick and Rushen, 1997) and not a neural component (Wood-Gush and Gilbert, 1964; Armstrong et al., 1977). In birds, prolactin in conjunction with estrogens and perhaps progesterone is involved during egg laying (Etches et al., 1979).

The introduction of perches in cages is advantageous for the welfare of hens, because they provide an appropriate place to roost especially at night (Appleby et al., 1993) and reduce the discomfort of the wire floor that the birds rest on for the entire production cycle (Hughes and Appleby, 1989; Duncan et al., 1992; Matsui et al., 2004). In addition, it allows an increase in floor space or stocking density compared to systems

without perches (Scott et al., 1999). Furthermore, perches improve bone strength (Abrahamsson and Tauson, 1993; Hughes et al., 1993; Fleming et al., 1994; Jendral et al., 2008), bone mineral density (Newberry, 1995; Hester et al. 2004; Tactacan et al., 2009) and reduce the risk of feather pecking, aggression and cannibalism (Gunnarsson et al., 1999). In addition, depending on the material used for making the perch, it can also improve the footpad condition, especially if wooden types are used (Burger and Arscott, 1984). Although the addition of perches to a cage system provides the advantages mentioned above, on the negative side, the presence of perches may contribute to bone fractures as a consequence of landing failures when jumping between perches (Lay et al., 2011). Similarly, Barnett and Hemsworth (2003) mentioned of the risk of higher egg breakages than those without perches and this would be the case if eggs are laid from a higher level while hens are perching or stepping on the rolling eggs while hens are landing from perches onto the wire floor.

Litter substrate in poultry housing is used for pecking and scratching (exploratory) and dust-bathing behaviours (Appleby et al., 1993). In caged systems, the behavioural need for scratching and foraging are evident in laying hens housed even with wired floors as they perform sham dust bathing behaviours while feeding (Weeks and Nicol, 2006). Pre-laying behaviour has been reported to be normal within cages with litter substrate using artificial turf (Appleby, 1998). This involves stereotypic attempts to dust bathe on the wire floor of their cage in the absences of litter (Van Liere, 1992). Similarly, a dust bathing area (although not a severe disadvantage) should still be applied to cages (Duncan, 2001). For birds, the activity of dust bathing is important for feather conditioning and maintenance because the dust soaks up excess moisture and oil and may

also help to remove tiny parasites that live on feathers (Drisdelle, 2007). Thus, dust bathing maintains the amount and quality of the feather lipids hence benefiting plumage condition (Van Liere, 1992). Another important facility/feature of housing systems for laying hens is a claw trimmer or shortener (abrasive strip) that is used to maintain claw length. This is also considered an enhancement of the cage environment aimed at reducing the risks of long claws being trapped in the various parts of the cage or the direct injury on other birds, which creates the potential for cannibalism (Tauson, 1986).

2.1.3.2 Floor Space

Increased space enables physical exercise such as wing flapping, body shaking, tail-wagging and stretching (Albentosa and Cooper, 2004). The effect of stocking density is a welfare indicator (Ferrante, 2009) because the very limited area that a hen has in a cage suggests that the quality of the design of the cage may have a great effect on both health and production (Tauson, 1986). For example, the lack of movement and exercise contributes to bone weakness (Duncan, 2001). Although results on production and mortality would indicate the need for extra space requirement for hens, the precise area that will provide optimum comfort and better welfare for hens may not be easy to determine (Tactacan et al., 2009). Different stocking densities for layers in cages have been reported from different areas, including cage densities of 350, 450, 700 and 800cm^2 of floor area per bird in the U.S., Canada, Norway and Switzerland, respectively (Tauson, 1998; Duncan, 2001). The need to use different cage densities could be attributed to a number of factors including hen strain (Abrahamsson et al., 1995), environmental condition (Cooper and Albentosa, 2004) and bird activity (Ferrante, 2009).

2.1.4 Effect of Housing on Hen Performance

The effect of housing type on performance of laying hens is well documented (e.g., Moore et al., 1977; Abrahamsson and Tauson, 1995; Tauson, 2005; Banga-Mboko et al., 2010). In this section, selected production variables that have been considered in the current study for this thesis were reviewed.

2.1.4.1 Feed Consumption

The eating behaviour of birds is influenced by the housing environment (Pohle and Cheng, 2009). Results from studies comparing feed intake of hens housed in conventional and enriched/furnished systems are conflicting. Lower feed intake has been observed in hens housed in cages equipped with perches as compared to hens kept without a perch (Tauson and Jansson, 1988; Braastad, 1990; Glatz and Barnett, 1996), suggesting that birds in EC consume less feed compared to those conventionally housed. With respect to group size, social behaviour of birds, indicates that hens prefer to feed synchronously when in a group (Hughes, 1971). This was supported by the findings of Albentosa et al. (2007) who noted that as the number of hens per cage increased, the number of hens feeding simultaneously also increased. Similar findings also showed that hens placed separately in cages spent less time feeding than hens with access to undivided troughs (Huon et al., 1986; Preston and Mulder, 1989). On the other hand, previous studies (e.g., Preisinger, 2000; Pohle and Cheng, 2009) indicate higher levels of feeding behaviour in birds housed in furnished than conventional systems. Similarly, Appleby and Hughes (1991) reported reduced feed consumption as a result of raising layers at high stocking densities. The authors attributed higher feed intake at lower

stocking density in furnished cages to the requirement of more feed to provide energy for heat production to compensate for the lower heat generated by cage mates.

Comparing different housing systems, Johnson et al. (1998) reported that caged hens spend more time feeding than hens in an aviary system. Similarly, studying the interaction between feed intake, dietary protein levels and housing type, Al-Awadi et al. (1995) found that the hens in floor pens consumed significantly more feed than hens in cages, irrespective of the dietary protein levels, indicating the possibility of compensating for extra energy spent by hens in floor systems. Guesdon et al. (2006) relating rate of feed consumption to other factors, such as loss of feather cover, suggested that damage to feather covering (as can result with high stocking density) could lead to increases in feed consumption in order to compensate for the extra heat loss.

2.1.4.2 Feed Conversion Ratio and Body Weight

Feed conversion is poorer in non cage housing systems (e.g., aviary and free range systems) than in cages (Hughes et al., 1985). This is because of lower stocking density in non cage systems, which leads hens to spend more energy in movement and the fact that they may be exposed to lower temperatures which requires the use of energy for heat production (Preisinger, 2000). Singh et al. (2009) found heavier body weights of birds housed in floor pens than those in cages because of better physical condition. Comparing performance of laying hens in cages with and without perches, Tanaka et al. (1993) observed improved feed efficiency but decreased body weight of birds housed in perched cages, however, it did not affect production performance of the hens. This was not due to lack of improvement of physical condition of birds in perched cages but due to

the influence on pacing to eating and drinking that can be affected by the presence of perches.

2.1.4.3 Egg Production

Egg production is strongly influenced by housing systems (Vits et al., 2005). Higher egg production from hens housed in CC than those housed in alternative systems such as aviaries, floor pens, or free range has been reported (Tauson et al., 1999). Although, Jalal et al. (2006) reported a decline of egg production of about 10% as the number of hens per cage was increased from 3 to 6, other studies (e.g. Tactacan et al., 2009) noted that egg production in the EC system (floor space of 642.6 cm^2/hen) is comparable with that of conventional type (floor space of 561.9 cm^2/hen). Differences in egg production under the same housing system (CC) was observed to occur in different strains of birds in which Abrahamsson et al. (1995) showed higher egg production for a white strain than for a brown strain reared in conventional cages.

2.1.4.4 Egg Quality (Egg Weight and Egg Mass)

According to Guesdon and Faure (2004) housing conditions did not affect egg weight. Similarly, using two distinct floor spaces, small (930 cm^2) and large (3700 cm^2) cages, Guru et al. (1974) found that egg weight was unaffected by confinement. However, free range systems produced lighter eggs compared to caged systems (Mostert et al., 1995; Sekeroglu et al., 2010). This could be due to the similar explanation of spent energy as mentioned above. Egg mass follows a similar trend to egg production and declines as cage space decreases (Craig and Milliken, 1989).

2.1.4.5 Egg Shell Quality

Measurements of egg shell quality include egg specific gravity, shell weight, shell thickness, percent shell and shell breaking strength. Egg shell quality measurements (e.g. egg shell breakage, egg shell cleanliness) are highly dependent on cage design, especially for the width of the cage in relation to its depth, the installation and disposition of perches, and group size (Duncan et al., 1992; Abrahamsson and Tauson, 1998). Results on these measures have also been contradictory. For example, a lower incidence of egg shell breakages were recorded in the nest in EC systems compared to CC systems (Tauson, 2003). Studies reporting higher percentage of cracked eggs obtained from CC system than those in furnished cages include that of Guesdon et al. (2006) and Wall and Tauson (2002). Although the addition of perches to a cage system improves bone strength (Hughes et al., 1993; Jendral et al., 2008), they may also lead to egg breakages/cracks depending on whether laid eggs drop on the wire floor while the hen is perching (Lay et al., 2011) or due to collisions between eggs in a small laying area (Wall et al., 2002).

Between the EC and CC systems, no differences in shell breaking strength (Guesdon and Faure, 2004) and specific gravity of the eggs (Tactacan, et al., 2009) have been reported. Although Guesdon and Faure (2004) reported greater shell thickness and strength for free-range eggs (possibly because of smaller sized eggs) compared to those from CC systems, highest shell resistance to breaking was found from CC eggs compared to those from free range, barn and organic production systems (Hidalgo et al., 2008). However, at later stages of production, shell quality was found to be better in floor pens compared to those from cages because of the ability of birds to exercise (greater activity) in floor rearing, which may then benefit the Ca metabolism (Singh et al., 2009). Among

different housing systems, shell strength for CC and EC systems was found to be intermediate between those in aviary (greatest) and free-range (weakest) types (Mertens et al., 2006). Hence, in comparing different housing systems, there is no clear trend for which system leads to the best egg shell quality (Abrahamsson and Tauson, 1998; Holt et al., 2011).

2.2 Protein (Nitrogen) and Macro-mineral Nutrition in Laying Hens

Dietary protein is the predominant form of nitrogen (N) entering the body (on average most proteins contain 16% N) (Ferket et al. 2002). Although there is relatively more information on Ca and P nutrition compared to protein or N in laying hens housed in different systems, these studies are mainly designed to assess productivity and welfare of birds (Abrahamsson and Tauson, 1995; Vits et al., 2005; Jendral et al., 2008). Generally, nutrient dynamics in laying hens housed in different systems has not been well researched. Alternative housing systems also need to be re-evaluated in comparison to the predominant system with respect to the flow of these nutrients in laying hens. Therefore, it is important to understand the requirements and utilization of these nutrients in laying hens.

The following review has two sub-sections dealing with N nutrition, and macro-mineral nutrition in laying hens and finally, the later part of the review provides information of possible influences of housing environment on nutrient utilization and their excretion during the laying cycle.

2.2.1 Protein Utilization and Nitrogen Excretion in Laying Hens

Poultry are more efficient at converting dietary N to products compared to swine and cattle (Ferket et al. 2002). However, only approximately 30 to 40% of the N consumed by birds is retained for maintenance, growth and product output, while the remainder is excreted (Kebreab et al., 2005; Summers, 2008). Manure provides valuable nutrients needed for crop growth. However, the excretion of N, part of which is volatilized as ammonia (due to microbial decomposition of uric acid) and lost to the atmosphere, it becomes an environmental concern causing leaching into the ground water and run off into surface water (Klopfenstein, 2002; Roberts et al., 2007). This implies that substantial amounts of N may be potentially lost from commercial laying hens to the environment. Since N excretion is proportional to the amount consumed (Nahm, 2003), an approach that could potentially reduce N intake without affecting the hen's performance would have a significant impact in reducing N excretion and consequently reducing the potential of environmental impact (Ferguson et al., 1998).

In animal production, the nutritional level of dietary protein is not constant and is influenced by a number of factors including animal, diet characteristics and management/housing type (Summers and Leeson, 1976; Ishibashi and Yonemochi, 2003; Ocak and Sungu, 2009). Hence, dietary manipulation aimed at reducing N excretion while meeting bird's need has been extensively researched (e. g. Summers, 1993; Blair et al., 1999; Roberts et al., 2007). Considering the fact that the vast majority of laying hens (90%; Awoniyi, 2003) are still being reared under cage systems, the influence of management factors with respect to housing on N utilization is not well documented. This information becomes of great importance when evaluating cage systems for laying hens

(such as EC types) as an alternative to traditional (CC) systems. Hence, the following sub-section reviews the general requirement and utilization of protein in laying hens, to understand the biological background of N nutrition in the birds.

2.2.1.1 Protein Requirement in Laying Hen

Given free choice, the hen selects her own protein level (Holcombe et al., 1976; Summers and Leeson 1978; Olver and Malan, 2000). In hens, protein requirements are governed by the energy level in the diet (Fisher, 1998). Similarly, although hens tend to adjust their feed intake according to their energy requirements, if the protein content of the diet is low, birds may increase feed consumption to compensate (Gous et al., 1987). The requirement of protein in laying hens is a function of maintenance and egg formation needs (Moran, 1987) although, the demand for growth particularly in the early stage is also important. Protein requirements based on the physiological needs of laying hens are briefly reviewed in the subsections below.

2.2.1.1.1 Requirement for Egg Production. Protein requirement is reflected most sensitively in egg production (Shapiro, 1968). The egg production rate (laying percentage) is not constant throughout the laying period as it increases to more than 90% of egg lay at peak egg production and declines thereafter. With advancing age, as the rate of egg production declines, egg size increases (Johnston and Gous, 2007). Because the amino acid composition of egg white and egg yolk remains the same, the requirement for this nutrient by hens declines with advancing age through the laying cycle (Leeson and Summers, 2005). This agrees with the fact that older birds are less efficient in utilizing dietary protein and enhanced nutrient inputs to increase egg output at the end of the laying year as compared with young pullets (Jennings et al., 1972; Summers and Leeson,

1983). But from a different perspective, based on the need to sustain any given level of output, the protein requirements for older birds do not decrease as the laying year progresses (Jennings et al., 1972; Yamazaki et al., 1982). This, in part, is possibly aimed at achieving a similar outcome level, because the efficiency of utilization of amino acids for the same process declines as the bird matures (Jennings et al., 1972) hence requiring more protein. In fact, it is noted that birds allowed free choice to diets containing varying protein or energy content voluntarily consume much less protein in early stages of their life and more with increasing age (National Research Council, 1994). In addition, Gous (1986) showed that the hens demand for amino acids are greatest at peak production with no marked difference for the rest of the laying cycle. Therefore, the adjustment of protein level of diets for laying hens, given as in phase-feeding programs (varying levels of protein, according to distinct stage of development), relates to egg production.

In laying birds, the response to protein related to egg production is mainly in terms of egg numbers and size (Fisher, 1998). For example, feeding higher levels of protein at the onset of production may help to increase egg size more rapidly (Leeson and Summers, 2000). It is also important to relate protein requirements to the different levels of egg component outputs. Although egg size increases with age, among the three egg components, it is the proportion of yolk that increases with time while the white and shell decrease. Egg white constitutes more than 50% by weight of the whole egg and contains more than 50% of the total protein in the egg compared to the other egg components (Johnston and Gous, 2006; Zita et al., 2009). In a modeling study of egg production and nutrient responses in broiler breeder hens, protein partition rules are based in the order of priority for maintenance, yolk protein deposition and then for albumen protein (Gous and

Nonis, 2010). However, the overall egg protein composition for an average egg (approximately 60 g of egg weight) remains constant at about 6 g or equivalent to 1.2 g N, on DM basis (Moran, 1987; Ishibashi and Yonemochi, 2003).

Hence, although the nutritional level of dietary protein may be influenced by a number of factors, the crude protein requirement of a laying hen at 100% egg production rate is estimated to be 16.3 g/d, i.e., approximately equivalent to 2.6 g N/d (Ishibashi and Yonemochi, 2003) based on 55% efficiency of protein synthesis for the egg (Scott et al., 1976).

2.2.1.1.2 Requirements for Maintenance and Growth. The hen has a protein requirement above that which is necessary for egg production (Shapiro, 1968) which may then influence the optimum protein requirement for maintenance. The higher protein requirement in older birds compared to young ones is possibly related to greater feather and endogenous protein loss in addition to sustaining egg protein requirement and body weight gain (Jennings et al., 1972). For feather development, in the first production cycle, Scott et al. (1982) suggested a protein requirement (recovering lost feathers) of 0.4 g/d per hen for up to 42 weeks of age, and 0.1 g/d for the later life. For body growth of laying hens, protein requirements in the early stage of egg production is expressed as BW × 0.18 × 0.5 g protein/g of body weight gain (Ishibashi and Yonemochi, 2003). This equates to approximately 13 g/d protein requirement for body growth of the laying hen (considering hen body weight of 1.4 kg and gaining approximately 100 g/d body weight, in the early stages of the production cycle). The laying hen requires about 1 g of protein for maintenance for every 2 g of protein deposited in the egg (Robbins, 1981). The protein requirement for hens in a force molted state aimed at obtaining a recovery from

30% loss of bodyweight and feather losses of 130 g in two weeks after re-feeding is approximately 12.4 to 14.3% which contains total sulfur amino acid of 0.46 to 0.62%, respectively (Ishibashi and Yonemochi, 2003). In general, laying birds exhibit continuous changes requiring varied protein requirement; however, based on N retention studies, Yamazaki et al. (1982) reported that a daily protein intake of 16 to 17 g/hen (equivalent to N content of 2.6 to 2.7 g/hen, respectively) was necessary for maximum N retention.

2.2.1.2 Protein Utilization and Egg Protein Formation

Although hens tend to adjust their feed intake according to their energy requirements (Gous et al., 1987; Fisher, 1998), dietary protein is the main factor influencing feed intake at the onset of production. However, energy becomes the main factor determining feed intake after 23 weeks of age (Valkonen, 2010). Because protein synthesis is an endergonic process (requiring energy), the use of energy for this purpose provides the stimulus for increasing feed intake that occurs during egg production (Morris and Taylor, 1967). As mentioned earlier, the greatest demand for amino acids is at peak egg production (Gous, 1986) and continues until egg production is sustained above a certain threshold value (Holcombe et al., 1976).

When the dietary supply of amino acids is limited, the skeletal muscles provide a source of amino acids in response to the needs for protein synthesis in the oviduct (Smith, 1978). In addition, the liver and plasma proteins have been considered as part of the protein reserves. For example, serum albumin in the fowl may act as a labile (unstable) source of amino acids in a critical nutritional condition (Smith, 1978). Similarly, at a later stage of the production cycle, the level of N retention regulates the amount of egg protein that a hen could produce by decreasing the rate of egg production to prevent tissue

depletion (Chi and Speers, 1976). During this process, egg production decreases gradually because hens could utilize labile endogenous sources of amino acids for egg protein synthesis during a short period which is in negative N balance (Chi and Speers, 1976).

Egg protein in birds, which constitutes about 11.7% of the total egg composition, is composed of 41.9% yolk protein, 53.7% egg white protein, 2.09% membrane protein and 2.38% shell protein (Smith, 1978). The protein content of the yolks and whites, in general varies from 407 to 495 g/kg DM (40.7 to 49.5%). These variations could result from a number of factors including the origin of protein, and the site and processes involved in egg protein deposition over the production cycle. For example, although albumen and yolk have almost equal protein content, the source of protein for the formation of these components differ (Keshavarz, 1998). Whereas the proteins of the albumen and shell are synthesized in the oviduct, the proteins for the deposition of the yolk are continuously synthesized outside the ovary (within the liver) (Morris and Taylor, 1967; Gilbert, 1980). The yolk proteins are largely associated with lipids (very low density lipoproteins) constituting the majority of the DM (Bellairs et al., 1972); hence, its formation is possibly being influenced by lipogenesis. In addition, there are differences in time when the egg components are formed. While yolk formation occurs between night and day, the white (albumen) are deposited at night, when the small intestine is assumed to be empty, because they do not require dietary pigments unlike for yolk protein formation which is an important constituent of the circulating very low density lipoproteins (Moran, 1987). Furthermore, egg components like yolk and ovalbumins are derived directly from the diet while other proteins like ovoglycoproteins and shell

membrane proteins are assumed to be derived by the breakdown of body protein during egg protein synthesis (Fisher, 1998). However, this contradicts with previous studies by Morris and Taylor (1967) who indicated that shell and membrane protein, which is approximately 0.4 g/egg, is provided by the diet. Much of the protein of the shell is contributed by the membrane and not the calcified part (Gilbert, 1980).

2.2.2 Calcium and Phosphorus Nutrition in Laying Hens

The ability of hens to regulate their intake of Ca (Holcombe et al., 1975) and P (Barkley et al., 2004) to match their nutritional requirement demonstrates the significance and preferential selection for these minerals during egg production. Although other minerals and electrolyte balance (e.g., magnesium, chloride, potassium, sodium) may influence or contribute to the egg forming process in hens (Turner and DeBeer, 2009), Ca and P are key elements and their availability is most crucial during the laying period (De Vries et al., 2010).

2.2.2.1 Calcium and Phosphorus Requirement

Calcium for egg shell formation is provided primarily from dietary sources during the day, medullary bone during the night when birds are not actively consuming feed (acute demand) and cortical bone during periods of chronic Ca deficiency or deprivation (Etches, 1987). Dietary sources provide 60-75 % of Ca while the remaining 25-40 % is derived from skeletal stores (Mueller et al., 1964). In hens, Ca requirement are higher during egg shell formation than any other stage of its life cycle (Leeson and Summers, 2000) implying that the level of Ca requirement for egg shell formation depends on the age of laying hens (Lichovnikova and Zeman, 2008). This is linked to the fact that the

medullary bone formation occurs concomitantly with the maturation of the ovarian follicles shortly before the onset of egg production (Dacke et al., 1999). Through the laying period, the medullary bone is readily turned over during times of insufficient dietary Ca levels and replaced when dietary Ca levels are in excess of the hen's requirement (Fleming et al., 1998). In general, daily recommendations for Ca of more than 3.75 g/hen (Roland, 1986), 3.25 g/hen (National Research Council, 1994) and 3.60 g/hen (36.0 g Ca per kg feed based on daily feed intake of approximately 100 g/hen, Chandramoni et al., 1998) have been reported. Calcium levels of 3 to 4% (30 to 40 g/kg diet) are commonly included in commercial laying hen diets (Summers et al., 1976) with specific recommendation of 32.5 g of Ca /kg of diet for caged layers (Chandramoni et al., 1998). On the other hand, P is required in small quantity for eggshell formation (Taylor, 1965), approximately 22 mg per egg shell (Hossain and Bertechini, 1998).

Extreme levels of both Ca and P are detrimental for laying hens. Excessive dietary Ca levels can increase the pH in the gut resulting in decreased absorption of P and other minerals (magnesium, manganese and zinc) from the intestines (Ensminger, 1992; National Research Council, 1994). Excess Ca may also lead to P deficiency by the formation of insoluble Ca phosphates in the digestive tract (Arthur et al., 1983), impaired metabolic functions and a decline in feed intake (Kaplan, 2009). Similarly, low dietary Ca is known to increase feed and water intake by hens in comparison to those consuming adequate dietary Ca (Damron and Flunker, 1995). This can have a consequence on passage rate (Turner and DeBeer, 2009) leading to excessive loss and subsequent deficiency of nutrients.

On the other hand, high dietary level of P is not only expensive (financially and environmentally) but also detrimental to egg shell quality (Chandramoni et al., 1998) as it contributes to the alteration of the acid-base balance (Keshavarz, 1994). Similarly, excess P in the diet forms insoluble Ca phosphate, which renders Ca unusable (Kaplan, 2009). The body then continues to absorb the P resulting in hypocalcaemia and metabolic bone disease (Kaplan, 2009). Imbalanced amount of Ca and P added into a layer diets can impair formation of both medullary (Ca reserve) and cortical (structural) bones hence affecting the integrity and strength of bone (Rath et al., 2000). Limiting available P within the range of 0.3 to 0.4% is ideal for shell quality (Leeson and Summers, 2000). Using caged hens, Guenter (1980) observed optimum egg production at a lower (0.1%) level of added inorganic P to a basal diet (containing 0.38% total P), with no subsequent changes in egg production with further additions (0.2, 0.3 or 0.4%) of inorganic P. However, greater non phytin P may be required at very high temperatures, and optimal concentration at 5 g total P/kg diet were recommended for caged layers in tropical environments (Chandramoni et al., 1998). Harms et al. (1965) reported that high levels of total P (above 0.60%) depressed egg production in hens maintained on litter but not those housed on wire cages. In addition, the author noted that increased P levels did not enhance a Ca deficiency in caged hens that were in high rate of egg lay, hence making it possible for them to tolerate the high levels of P. This implies that the requirement for P of caged laying hens is higher than those raised on litter (Harms et al., 1965).

2.2.2.2 Calcium-Phosphorus Kinetics in Laying Hens

Calcium constitutes approximately 1.5% of body weight and 40% of egg shell (Bolukbasi et al., 2005). The requirement for dietary P in layers is mainly because it

interacts with Ca during bone synthesis (medullary bone) prior to eggshell formation (Tolboom and Kwakkel, 1998; Ahmad and Balander, 2004). In the body 98 to 99% of the Ca is present in the bird's skeleton most of which is in the form of hydroxyapatite, ($Ca_{10}(PO_4)_6(OH)_{2)}$, with small amounts of non-crystalline Ca phosphate and Ca carbonate (Klasing, 1998). The remaining 1 to 2% of Ca in the body is found in plasma and other body fluids.

In the egg shell, Ca is mainly stored as Ca carbonate (Bolukbasi et al., 2005; Bar, 2009). Phosphorus is required in small quantity for eggshell formation, approximately 22 mg per egg shell (Hossain and Bertechini, 1998) and with a Ca:P ratio of approximately 100:1 (Ahmad and Balander, 2004). However, there is a close association of P and Ca dynamics in that the deficiency or over abundance of one during egg shell formation, can affect the proper utilization of the other (Kebreab et al., 2009). For example, during egg shell formation, as the P blood level increases, phosphate excretion by the kidney also increases with the loss of H^+ ions, aiding in the maintenance of bicarbonate levels necessary for egg shell formation (Pelicia et al., 2009). There is some evidence that the inability of the hen to produce an increased amount of egg shell is related to the activity of 25-hydroxycholecalciferol-1-hydroxylase, an enzyme involved in Ca homeostasis (Elaroussi et al., 1994). In addition, the hen's ability to absorb Ca from the intestine and to mobilize Ca from the medullary bones decreases with age (Keshavarz and Nakajima, 1993; Keshavarz, 2003). Because these two conditions deteriorate with age (Elaroussi et al., 1994), it contributes, in part, to poor egg shell quality at the end of the production cycle. However, an increase in egg size with age with a corresponding decrease in percent shell is another explanation.

2.2.2.3 Calcium and Phosphorus Utilization

Simkiss (1961) showed that regardless of the amount of intake, the bird can only acquire little more than 1g of Ca/d, yet the egg shell contains about 2 g of Ca. Similarly, Summers et al. (1976) noted that regardless of the level of dietary Ca fed, the hen would reach a plateau in retention of around 1.5 g of Ca/d. These findings indicate that laying birds consume much more Ca than is being utilized. In addition, Bar et al. (2002) also indicated that the rate of Ca absorption during shell formation is lower than 70% of Ca intake. Hence, the birds depend on the medullary source of a dynamic reserve of Ca for supplementation (Whitehead, 2004). During the egg-laying cycle, periods of intense bone formation and of destruction alternate therefore birds in Ca balance restore the minerals lost during shell calcification when shell formation is not taking place (De Bernard et al., 1980). However, Ca excretion and shell formation are suggested to be competing processes (Mueller et al., 1964). Besides, a number of factors may also influence the optimum level of Ca and P utilization in laying hens. For example, dietary factors alone may include the levels of Ca and P in the diet (Leeson and Summers 2000; Rama Rao and Reddy, 2001), Ca:P ratio in the diet (Singh and Panda, 1996), pH (Ensminger et al., 1990), dietary levels of other minerals (Chan and Swaminathan, 1998; Ledoux and Cheeke, 2005), types and availability of P and Ca (Scott et al., 1999; Ceylan et al., 2003; Leeson and Summers, 2005), particle size of Ca source (Leeson and Summers, 2005), feeding time (Keshavarz, 1998), and levels of other nutrients (e.g., non-digestible carbohydrates, Chen and Chen, 2004; vitamin D_3, Bolukbasi et al., 2005; dietary fat, Maynard et al., 1979).

Although nutritional deficiencies of Ca and P have been shown to result in bone loss (Wilson and Duff, 1991), it is also interesting to note that Ca inadequacy is not a primary cause of osteoporosis in caged hens (Rennie et al., 1997; Whitehead and Fleming, 2000). Increasing Ca retention and incorporation into bone does not prevent the progressive loss of trabecular bone (Rennie et al., 1997); however, it is mainly related to the lack of exercise (Fleming et al., 1994; Tauson, 1998). Studies (e.g., Kopka et al., 2003; Tactacan et al., 2009) indicate improved bone mineral density and bone strength for birds in EC as compared to those in the CC systems, signifying the importance of features such as scratch pad, perches and extra space. Hence, the important role of both Ca and P in maintaining bone strength can be achieved as bone mass and strength increases with use (Lanyon, 1993).

2.2.3 Influence of Housing on Nutrient Utilization and Excretion by Laying Hens

The hen's efficiency to utilize feed in a given environment can be influenced by a number of factors including genetic background, diet, animal condition and management/housing (Summers and Leeson, 1976; Ishibashi and Ohta, 1999; Ocak and Sungu, 2009). There are different commercial egg production facilities that involve a variety of housing and management aspects. Hence different housing systems may provide varied micro-environments to the hens, thereby affecting their comfort, health and the ability to utilize nutrients (Xin et al., 2011).

2.2.3.1 Housing and Nutrient Utilization

Predominantly, birds are housed under cage systems (Tauson, 1998; Awoniyi, 2003; Guesdon and Faure, 2004). In such conditions, housing of hens should provide an

optimal microclimate close to the thermoneutral zone for the birds which is usually between 20 to 25°C (Leeson and Summers, 2005; Leeson, 2010). In alternative housing systems such as EC or modified cage systems, hens have more space for physical activity which increases energy expenditure and heat production. Due to lower stocking densities, temperatures in these systems are sometimes low (Preisinger, 2000). On the other hand, higher temperature in densely caged birds (e.g. CC systems) may also influence production performance as a consequence of decreased feed consumption which may in turn decrease egg weight (Tanor et al., 1984).

With available floor spacing, the hen may maintain a normal temperature through physical mechanisms such as posture (standing position) because it allows the bird to regulate the respiration rate by increasing respiratory evaporation or wing flapping and changing the angles of the feathers so as to retain or disperse more heat (Meltzer, 1987). Other mechanisms such as chemical or metabolic processes may also be involved through the hens' ability to regulate its nutrient consumption (Meltzer, 1987). For example, protein is known to have a higher heat increment than either carbohydrate or fat because consuming the higher protein diets under more severe heat stress leads to a higher heat increment (Sohail et al., 2003). This effect is counteracted by the hen's ability to reduce its energy consumption (Gordon and Roland, 1996). Similarly, this is explained by the hens' preference to avoid excessive protein consumption and the corresponding increase in metabolic heat during the warmer seasons (Holcombe et al., 1976). Lunven et al. (1973) found that environmental conditions can cause quantitative effects in the protein content of eggs and can even alter the ratio of yolk to white of the egg without affecting the amino acid profile. In addition, during heat stress, hens respond by panting and this

leads to a reduction in blood carbon dioxide levels and an increase in blood pH. This subsequently influences Ca homeostasis by lowering blood bicarbonate ion levels, a component of the endogenous buffering system (Turner and DeBeer, 2009; Chukwuka et al., 2011).

Manipulation of the diet has been studied to overcome the negative effect of limited cage space on performance (Owings et al., 1967; Jackson and Waldrup, 1988). With respect to N nutrition, increased dietary protein was found to partially overcome the effect of reduced cage space on egg production in laying hens (Owings et al., 1967). However, Brake and Peebles (1992), using graded levels of dietary lysine (0.68, 0.73 and 0.78%) detected no effects of increased dietary lysine on performance when hens were housed under higher densities (i.e., comparing 3, 2 and 1 hen/cage in a 25.4 cm × 40.0 cm cage space). Similarly, Jalal et al. (2006) using increasing metabolizable energy level in the diet did not reverse the negative effects of crowding due to decreasing cage space on egg production.

The interaction of nutrient and management/housing conditions has resulted in varied hen behaviour or effects. For example, although fragility of the bones in caged hens is severe with progressive periods as the hen remains in reproductive condition (Whitehead, 2004), the requirements for P and Ca do not increase with age. Davidson and Boyne (1970) noted that the requirement for Ca for laying hens maintained in cages, are between 1.7 to 2.8%, suggesting a decrease in Ca requirement with age as level of egg production decreased. Similarly, the same authors indicated that the requirement for total P lies between 0.4 and 0.6% and levels above the minimum requirement (0.5%) did not improve performance. Likewise, increased Ca retention and incorporation into bone did

not prevent the progressive loss of trabecular bone which implies that Ca inadequacy is not the primary cause of osteoporosis in hens (Rennie et al., 1997). Similarly, in caged hens, dietary deficiencies stimulate explorative behaviour in caged hens (Ambrosen and Petersen, 1997) thereby indirectly influencing the performance of hens. For example, Van Krimpen et al. (2005) explained that laying hens tend to spend more time feeding when fed low energy diets.

Although in most cases farmers provide the same diet to hens under different housing systems, the nutrient requirement of birds managed under the different housing types may vary because of differences in the levels of bird activity (Poultryhub, 2010). Similarly, because different housing conditions may elicit stress responses in laying hens (Holt et al., 2011), specific housing-related factors like high stocking density (Roberts, 2010), environmental temperature (Jadhao and Sinha, 1998) and nesting area (Hughes et al., 1986) have been studied and shown to result in differences in production performance (such as egg shell quality).

The type of housing system can significantly influence the regulation of adrenal cortical function **(**Koelkebeck et al., 1986) resulting in fear-related response that induces stress **(**Jones, 1996). In poultry, stress levels can be determined by measuring the plasma or fecal corticosterone levels (Fraisse and Cockrem, 2006). Corticosterone is the main glucocorticoid in birds and it is considered to be an indicator of stress as well as a measure of an animal's perception of the environment (Dantzer and Mormede, 1983). The stimulation of the adrenal cortical tissue, in addition to causing stress, alters energy metabolism (Etches, 1976). Therefore, the type of housing system can influence the level of activity of the birds and consequently their energy requirements (Poultryhub, 2010)

hence influencing nutrient utilization. Similarly, this may relate to differences in the eating behaviour of birds under different housing environment (Pohle and Cheng, 2009). For example, comparing different housing systems, Johnson et al. (1998) reported that caged hens spend more time feeding than hens in an aviary system. Although the exposure of birds to repeated stress conditions may not necessarily lead to an elevation of plasma corticosterone levels (Jones and Faure, 1981), high levels of fearfulness have been negatively associated with egg production, plumage condition, egg shell quality, growth, and feed conversion efficiency **(**Jones, 1996). As indicated previously, laying hens are able to choose an optimum nutrient (such as protein) for egg production during a particular laying stage or physiological status. To investigate these changes during egg production that affect nutritional requirements and subsequent nutritional choice, Sahin and Forbes (1999) studied the effect of corticosterone concentrations in the plasma on diet selection for protein in relation to egg production by laying hens. The authors found that corticosterone reduced egg production and protein efficiency without changing the weight of egg components; however, the birds' preference for high protein ingredients increased. Furthermore, Sahin and Forbes (1999) noted that higher levels of corticosterone resulted in reduced ovarian weight and increased carcass fat deposition, suggesting that the high demand for protein was for fat anabolism rather than egg production.

Results by Pavlik et al. (2008) and Tactacan et al. (2009) found no differences in the corticosterone levels of birds housed in either EC or CC, implying lack of difference between the stress levels in birds under these cage systems. Environmental enrichment has been considered as one of the methods by which fear reaction may be modified

during rearing of birds (Reed et al., 1993). However, Pavlik et al. (2008) indicated that housing systems which are more similar to the animal's natural environment (as being offered by the EC) may not necessarily be associated with decreased levels of plasma corticosterone. In this context, management strategies based on alternative housing to replace conventional systems warrants an extensive understanding and evaluation of nutrient flow in laying hens.

2.2.3.2 Effect of Housing on Nutrient Excretion

Nitrogen and P are two major excretory products in poultry that are of environmental concerns. Of the consumed N, on average, approximately 35% is retained by hens for maintenance, growth and product output (Kebreab et al., 2005; Summers, 2008). Similarly, P excretion at 30 wks of age birds consuming diets containing 0.4 and 0.2% available P (0.57 and 0.37% total P, respectively), resulted in a corresponding P excretions of 79.7 and 73.6% of intake, respectively (Summers, 2008). In general, under commercial production, systems on nutrient outflow related to the amount of nutrient excreted, the level of feed wastage and the quantity of manure output.

In poultry, approximately 15% of N is excreted in feces (as urea) without being digested and absorbed, and about 50% in urine as uric acid (i.e., absorbed N that is not used for body protein deposition) (Ferket et al., 2002). The uric acid excretion is mainly due to the lack of storage mechanisms for amino acids supplied beyond the requirement for protein synthesis (Nahm, 2003) and the lack of a complete urea cycle enzymes in birds (Ledoux and Cheeke, 2005). Manure from caged hens, without litter or bulking agents has low DM content (Dall'Ara et al., 2008). Most of N the (approximately 60-70% of uric acid and urea) is rapidly converted to ammonia, in wet conditions (Klopfenstein,

2002; Roberts et al., 2006). Because poultry are fed diets with higher protein content than most other farm animals; poultry manure can be a potential source of N pollution. This is reflected in the annual excretion levels by layers of about 30% of N (as a percentage of body weight) compared to 7, 12, 22, and 11% of N for gestating sows, beef, dairy and dry cows, respectively (Rotz, 2004). However, with respect to N intake, poultry are better utilizers of N (Ferket et al., 2002) than the other animals stated. For example, from these figures the corresponding N excretion levels are approximately equivalent to 50% of N intake for laying hens (considering daily N intake of 3.0 g/hen and a body weight of 1.86 kg); and 76% of N intake for gestating sows (in second parity, consuming 1.84 kg/d with 12.8% crude protein in diet; National Research Council, 1998). Similarly, higher excretion of N can also result from excess (above requirement) inclusion of protein and amino acids in poultry diets (Ritz et al., 2004). For example, on average, a laying hen with a production capacity of 300 eggs/yr consuming 100 g/d of feed with 18% protein can result in approximately 0.1 kg/yr N excretion in excess than when consuming a diet that is balanced with 15% protein (Rotz, 2004). Hence, indicating the direct link of N excretion to the animal's N intake (Meluzzi et al., 2001).

Under a commercial production, Leeson and Summers (2001) obtained 0.75 kg/hen/yr N excretion in manure of laying birds while Rotz (2004) estimated 0.55 kg/hen/yr N excretion. Differences in the levels of N excretion from the different studies may depend on many factors, including variations in dietary protein contents, age of the bird, as well as management. While Patterson (1994) estimated a total N output of 0.68 g/d per hen for commercial Leghorn hens housed in a barn with a deep-pit manure system, Nicholson et al. (1996) predicted manure N loss of 18.0 kg/ton at 30% DM

(equivalent to 1.80 g/d per hen) for caged birds under intensive production. These results show that large variation exists in N losses in manure from different poultry production systems.

On the other hand, the amount of P excretion in manure in a given laying period can vary due to intake levels, its subsequent release during the mobilization of Ca from medullary bone tissue during the calcification process (Etches, 1987; Bar, 2009; De Vries et al., 2010) and released during other metabolic processes (Pelicia et al., 2009). On average 0.23 kg/hen/yr P is excreted in manure (Spiehs, 2005). Similarly, Ca and P occur in the body in combination with each other most of the time, and an excess or inadequate supply of one of the two in the diet limits the utilization of either mineral (Maynard et al., 1979). Rao et al. (1992) showed that low P rather than excess Ca was more responsible for large increases of Ca excretion.

Furthermore, there is need to consider the quantity of poultry manure volume excreted during the production cycle. Van Horn (1998) reported a total animal manure production of 1.37 billion tons in the U.S. of which chickens account for 14 million tons. Although there is a large demand for excreta as composts, the environmental impact of N and P from intensive poultry production, is in excess of local demand at present (Ishibashi and Yonemochi, 2003). In addition, when poultry manure is applied based on either N or P needs of a crop, it results in an over application of P and under application of N, respectively (Xin et al., 2011) because P in poultry manure is in excess of plant requirements (Preusch et al., 2002). According to Manitoba conservation strategies, manure application rates are based on analysis of the nutrient content of the manure (because manure nutrient concentrations are highly variable) and the soil at the time of

application (Manitoba Agriculture, Food and Rural Initiatives, 2004). Other approaches limiting the application of animal waste as fertilizer has been viewed on the basis of balancing nutrient application with crop utilization (Patterson and Lorenz, 1997) and assessing the carrying capacity of a given agricultural land for animal production (De Boer et al., 2000). Hence, these limitations consequently affect the application rates of the poultry manure and this suggests possible means to reduce manure output.

In summary, although nutrient utilization may vary due to differences in environment, hen genetics, nutrition and management, housing system also imposes its own unique factors (such as bird density, stress levels) which can affect overall nutrition of laying hens. With increasing concern in welfare of hens raised in traditional (conventional) cage systems, this information becomes of great importance when evaluating alternative housing systems for laying hens (such as EC types). In addition, any alternative system should aim at decreasing not only nutrient excretion in manure (especially N and P), but also the total amount of manure output.

CHAPTER 3 PRODUCTION PERFORMANCE AND NITROGEN FLOW OF SHAVER WHITE LAYERS HOUSED IN EITHER ENRICHED OR CONVENTIONAL CAGED SYSTEMS

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3.1 ABSTRACT Despite the large number of studies examining the impact of cage systems on calcium and phosphorus nutrition, data on nitrogen (N) balance of hens when housed under different systems are limited. To this end, an experiment was conducted to assess N balance, manure characteristics, and indices of performance of laying hens housed in two distinct caging systems. A total of 4,836 commercial Shaver White hens were housed in either enriched (EC) or conventional (CC) cages (average floor space per bird of 643 and 468 cm^2, respectively) under semi-controlled environmental conditions. Enriched cages provided hens with a curtained nesting area, scratch pad and perches. Birds in both systems were phase-fed similar layer diets for eleven periods (4 weeks each). Data, expressed on a hen basis, were analyzed as repeated measures using the MIXED model procedure of SAS. Lower feed disappearance ($P < 0.01$) (92.5 vs. 95.0 ± 0.6 g/d DM basis) and manure output ($P < 0.01$) (79.8 vs. 91.3 ± 1.2 g/d as is basis and 27.0 vs. 28.1 ± 0.2 g/d DM basis) were observed in birds housed in EC compared to CC, respectively. Manure DM was 34.1 and 31.0% ± 0.3 for EC and CC, respectively. Egg production, feed conversion ratio, body weight, egg weight and egg mass were not significantly different between the two systems. Overall egg N output decreased with age for both cage systems and was not significantly different between the systems. While

there was no difference in the overall manure N excretion (1.94 and 1.96 ± 0.02 g/d for EC and CC, respectively), hens housed in CC had a significantly ($P < 0.05$) higher N balance compared to those in EC system (85.0 vs. 30.2 ± 13.6 mg/d, respectively), potentially explained by a higher ($P < 0.05$) manure N excretion in the EC at later stages of production. The current data provide estimates of the efficiency of N utilization in laying hens housed under different housing conditions

Key words: caging systems, manure nitrogen, nitrogen balance, egg production

3.2 INTRODUCTION

The move towards alternative cage designs for laying hens has the potential to lead to differences in hen behaviours, with subsequent impacts on nutrient dynamics. Environmental enrichment provides more varied behaviour, which can result in better physical condition (Appleby et al., 2002), space for exercise (Cooper and Appleby, 1996; Gunnarsson et al., 2000; Olsson et al., 2002) and better leg bone strength as a result of using the perch (Hughes and Appleby, 1989). Several studies have evaluated the effect of housing systems on bone quality (Hughes and Appleby, 1989; Fleming et al., 1994; Tauson, 1998) and egg shell quality (Van Den Brand et al., 2004). As such, the focus has primarily been on calcium and phosphorus dynamics. Studies on other nutrients are limited.

Nitrogen (N) is a key element in animal production, being one of the more expensive nutrients in poultry diets. When considering dietary protein, its nutritional

value is influenced by a number of factors including management and housing type (Ishibashi and Yonemochi, 2003). In addition, approximately 30 - 40% or less of the N consumed is retained for maintenance, growth and product output, while the remainder is excreted (Kebreab et al., 2005; Summers, 2008). Furthermore, N has been the focus of several studies related to manure management because (together with phosphorus) it is an environmental concern (Smith et al. 2000; Meluzzi et al. 2001; Nahm, 2007). However, less information is available to compare the effects of environmental enrichment of housing type on N flow and balance of laying hens. Increased dietary nutrient density (Jackson and Waldroup, 1988) and more specifically, increased dietary protein (Owings et al., 1967) was found to partially overcome the effect of reduced cage space on egg production in laying hens. However, Brake and Peebles (1992), using graded levels of dietary lysine (0.68 – 0.78%, increasing by 0.05%) detected no effects of increased dietary lysine on performance when hens were housed under higher densities (i.e. comparing 3, 2 and 1 hen/cage in a 25.4 cm × 40.0 cm cage space). In general, the available comparative data related to nutrient flow as affected by housing systems stems from European experiences (e.g., Groot Koerkamp et al., 1999; De Boer et al., 2000), and may not entirely reflect North American conditions. Strong interest in moving to alternative cage designs in North America necessitates the establishment of data to compare nutrient flow and bird performance under conventional and enriched housing systems, to take into account differences due to such factors as climate, layer strains, and feed ingredient usage. Therefore, the objective of this study was to assess N flow in Shaver White laying hens when housed in either enriched (EC) or conventional (CC) caging systems, over a full production cycle.

3.3 MATERIALS AND METHODS

For this study, 4,836 beak-treated Shaver Whites pullets (obtained from Manitoba Perfect Pulletes Ltd, Rosenort, Manitoba) at 19 week of age were introduced into a caging facility at the University of Manitoba's poultry unit. Birds were maintained under semi-controlled environmental conditions for eleven periods (28 days each) in an intensive egg production system. Handling and care of hens was in accordance with ethical principles of the guide to the care and use of experimental animals (Canadian Council on Animal Care, 1993) and the recommended code of practice for the care and handling of pullets, layers and spent fowl (Canadian Agri-Food Research Council, 2003). All protocols were approved by the University of Manitoba Animal Care Protocol Management and Review Committee.

3.3.1 Cage Design and Description

Cage designs used in this study have been previously described in detail by Tactacan et al. (2009) and briefly described in Appendix I. The enriched cages also referred to as furnished, housed 24 laying hens. The average floor space area per bird was 642 cm^2. Conventional cages also referred to as traditional, housed 6 laying hens. The average floor space area per bird was 468 cm^2.

3.3.2 Experimental Cage Units and Barn Environment

For each cage system, 10 experimental (test) cage units were randomly selected throughout the barn (middle of the house as well as extreme ends of the house) as shown in Appendix II. Each experimental unit consisted of 24 birds (one enriched cage; 4

grouped conventional cages), for a total of 480 Shaver White laying hens on test. All test cages were located on the bottom tier. Barn temperatures and humidity were controlled by air movement regulated through ventilation provided by inlet and exhaust fans mounted in the side walls. Incandescent light was provided by 60 Watt bulbs which produced a lighting intensity of 54 to 67 Lux. As the birds were introduced (19 wks of age), 13.5 h/d lighting was provided and from week 22 to end of lay, the birds were exposed to 15 h photoperiod from 0600 h to 2100 h.

3.3.3 Management and Sample Collection

A phase feeding program, as recommended for the strain, was used for this trial, with hens housed under the two treatments receiving identical nutritional programs. Layer diets, based on a wheat–soybean mix (Appendix III), were formulated according to nutritional recommendation and specification by ISA (2009). The diets included phases I (periods 1 to 6), II (periods 7 to 9) and III (periods 10 and 11) with corresponding N contents of 3.49, 2.93 and 2.86 ± 0.10 %, respectively. As this study was designed to provide baseline nutrient flow data for subsequent nutrient modeling purposes, the diets did not contain added phytase or exogenous enzymes. During the production cycle, a 5-day sample collection period was conducted in the middle of each 4-week period. Apart from the 5-day collection period, all the birds were fed *ad libitum*. During the 5-day collection period, both sides of each test cage units were partitioned using rigid dividers and a known amount of feed was poured manually into the troughs and the final weigh-back was determined on day 5. Feed disappearance, including feed wastage (observed to be minimal) was calculated as the difference between the feed offered and the final weigh-back. To reduce excessive loss/spillage, wire mesh (2.5 cm × 3.8 cm mesh size)

was used to cover the feeding troughs throughout the barn and the feed was rationed in two lots (day-1 and day-4). For the test cage units, feed disappearance as a measure of feed intake was taken on a 5-day basis, and calculated as a mean for each 24-bird replicate per cage unit as the total feed offered minus the weigh-back divided by the number of birds (24) and days of feeding (5). Feed samples were obtained from each batch delivered to the production unit. Sub-samples were ground and sieved through a 1mm screen and stored for analysis.

Water was provided *ad libitum* using nipple drinkers (1 nipple/8 hens and 1 nipple/6 hens for EC and CC systems, respectively) mounted along the centre of each row being shared by both side of the cage unit, which is in line with the recommended code of practice for white layer adults in Canada (Canadian Agri-Food Research Council, 2003). Water (chlorinated) was supplied from a municipal source. Water meters were placed on lines supplying the EC rows and the CC rows, thus permitting the monitoring of total water consumption by cage type over the entire production unit, but not specifically for the experimental cage units. Water consumption readings were taken every morning at 0800 h. The difference in the reading between the two consecutive days divided by the number of hens housed was calculated as the daily consumption per bird.

All hens in the barn were inspected daily and any dead or sacrificed birds were recorded. Losses from test cages were replaced with spare birds of similar weight from non-test cages. Daily measurements of barn temperature, humidity and egg production were recorded. Throughout the production cycle, body weights for birds in five selected test cage units for each system was recorded individually at the beginning of every 5-d collection period.

Manure was removed by a conveyor belt system beneath each cage tier. Manure from test cages was collected separately using plastic trays/sheets placed on the conveyor belts underneath each test cage units during the 5 day collection period. Sheets with dimensions 57 cm width × 258 cm length for EC and 48 cm width × 202 cm length for CC systems were used. Manure was collected twice during the 5-d period. On the 5th day, total manure output per replicate was pooled, homogenized through the use of a mixing implement attached to an electric hand drill, and weighed to obtain total manure weight. Subsamples of 1.5 kg to 2 kg were obtained and frozen at -20^{o}C before being freeze dried and finally ground to pass through a sieve screen of 1mm and stored for subsequent analysis. Consistent with commercial production practices, the collected manure included excreta, spilled water and feed, feathers and broken eggs.

Although egg production data was available for the entire production cycle, for the purposes of this study, egg production was calculated for every 5-d collection period. For egg weight measurements and composition, 4 eggs were sampled daily during the 5-d collection period from each cage unit and immediately stored in an egg cooler (10 to 12^{o}C). On day 6, the eggs were removed from the cooler and weighed (total weight of 20 eggs per cage unit) using a digital scale. Mean egg weight per cage unit was obtained by dividing the total egg weight by 20. Hen-day egg production was calculated by dividing the number of eggs produced by the number of live birds in each cage unit during the 5-d collection period. Egg mass output was calculated by multiplying the actual hen-day rate of egg production by the average egg weight in grams. Feed conversion ratios were calculated by dividing feed (g) by egg mass (g).

3.3.4 Egg Component Assessment

Ten eggs were broken and the yolks carefully separated from the whites (albumen) using an egg separator. The yolk, white and shell samples were pooled and homogenized to yield 2 replicates of 5 eggs each for every cage unit, placed in labeled plastic bags and weighed. The samples were frozen at -20^{o}C and later freeze dried and weighed. Corresponding final freeze dried weights were taken for the different component samples to determine the dry matter (DM) of the samples. Samples were then ground and sieved through a 1mm screen and stored for further analysis.

3.3.5 Chemical Analysis

Feed, manure and egg component weights, on both a fresh and DM basis, were recorded. Nitrogen content was determined using a CNS-2000 carbon, N and sulfur analyzer (LECO®, St. Joseph, MI, USA). To obtain the total egg N output, the individual egg component N outputs were summed. Nitrogen balance or retention indices were calculated taking into account amounts of N ingested, amount retained in the egg components and N excreted in manure.

3.3.6 Statistical Analysis

Data were analyzed as repeated measures using the MIXED model procedure of SAS (SAS 9.2; SAS Inc, Cary, NC). The model consisted of a completely randomized design, modified into a split plot with cages (for each treatment) as the error term for the main effect due to the treatment (type of cage system) i.e. the cage location within the barn was the random effect. In the sub-plot, experimental period and treatment by period interactions were considered as fixed effects and the residuals were used as the error term. The univariate linear model used in the analysis is summarized below:

$$Y_{ijk} = \mu + t_i + t(cage)_j + tp_{ij} + p_k + e_{ijk},$$

where Y_{ijk} = observation of the parameter tested; μ = model constant, t_i = effect of caging system which is the treatment (i = 1, 2); $t(cage)_j$ = effect of the different locations of cage units within a cage system (j = 1-10); p_k = effect of experimental period (k = 1-11); tp_{ij} = interaction between cage system and experimental period (treatment x period) and e_{ijk} = random error variation.

Least square means were estimated for all parameters investigated. There was no difference in the outcomes among the different variance-covariance structures hence for all the analysis the compound symmetry of variance-covariance structure was used. Differences between means were determined using the least squares differences by Tukey's test. Significance level was declared at $P \leq 0.05$ in all comparisons unless otherwise stated. The influence of water intake, temperature and humidity on feed intake and on manure weight was assessed using PROC REG procedure of SAS (SAS 9.2) for determining the covariance. Univariate diagnostics analysis allowing for studentized residuals was conducted to check for outliers.

3.4 RESULTS

3.4.1 Bird Performance Parameters

In all the parameters, period (bird maturity) had a significant ($P < 0.0001$) influence on the performance of laying hens. Overall mean feed disappearance was significantly ($P < 0.01$) greater in birds housed in CC compared to the EC system (overall means of 95.0 and 92.5 g/hen/d, respectively; Table 3.1). However, in the early stages

Table 3.1. Feed disappearance and performance of Shaver White hens under conventional (CC) and enriched cage (EC) systems[1]

	Feed disappearance (g/hen/d)	Body wt (kg/hen)	Egg production (%)	Egg wt (g)	Egg mass (g/hen/d)	FCR (g feed/ g egg)
Cage system[2]						
EC	92.5	1.67	90.6	59.7	54.3	1.76
CC	95.0	1.67	91.7	59.8	55.2	1.78
SE	0.61	0.01	0.43	0.24	0.37	0.01
Period[3]						
1	77.1^{c}	1.45	55.4^{d}	50.3	27.9^{e}	2.83^{a}
2	92.6^{b}	1.56	97.0^{a}	56.3	54.6^{d}	1.70^{b}
3	96.4^{a}	1.60	97.7^{a}	59.2	57.9^{abc}	1.67^{b}
4	95.5^{a}	1.65	97.9^{a}	59.7	58.4^{abc}	1.64^{b}
5	96.0^{a}	1.68	97.4^{a}	60.4	58.8^{ab}	1.63^{b}
6	95.5^{a}	1.71	96.5^{a}	61.3	59.1^{a}	1.62^{b}
7	95.5^{a}	1.74	94.2^{ab}	61.1	57.6^{a}	1.66^{b}
8	95.0^{a}	1.75	94.5^{ab}	61.7	58.3^{ab}	1.63^{b}
9	94.7^{a}	1.74	92.3^{bc}	62.6	57.7^{a}	1.64^{b}
10	96.2^{a}	1.78	90.2^{c}	62.0	55.9^{cd}	1.72^{b}
11	96.8^{a}	1.75	89.4^{c}	62.1	56.3^{bcd}	1.73^{b}
SE	0.61	0.01	0.86	0.27	0.59	0.03
P-value						
Cage	< 0.01	NS	NS	NS	NS	NS
Period	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cage × Period[4]	NS	< 0.01	NS	< 0.001	NS	NS

[a–e] Different superscripts within each column are significantly different at $P < 0.05$

[1]Data are presented as least square means (LSM), their standard errors (SE)

[2]LSM as main effect of cage type

[3]LSM as main effect of period on overall mean of CC and EC systems

[4]Cage × Period indicate interaction of caging system and period for all the parameters tested over an entire production cycle

(i.e., 19 to 27 weeks of bird age), there were no marked differences in feed disappearance between the two systems (Figure 3.1a). The results also showed that after period 2, feed disappearance of hens within a cage system did not fluctuate during the entire laying period. Although the overall egg production (percentage hen-day basis), feed conversion ratio (FCR), egg quality parameters (egg weight and egg mass) and body weight were numerically higher in birds housed in CC system, there was no statistically significant difference between the two systems (Table 3.1). However, there was a significant cage-type by period interaction of body weight ($P < 0.01$) and egg weight ($P < 0.001$). The results indicated that body weight for birds in CC (1.63 ± 0.01 kg/hen) were significantly ($P < 0.01$) greater than those in the EC (1.58 ± 0.01 kg/hen) in period 3 (Figure 3.1b) and no marked differences were observed between the two groups of hens for the rest of the periods. In addition, CC birds produced significantly heavier eggs ($P < 0.05$) than EC birds in period 5 (61.3 vs. 59.7 ± 0.34 g, respectively) and period 6 (61.8 vs. 60.7 ± 0.34 g, respectively; Figure 3.1c) with no difference between the two groups of hens for the rest of the periods.

3.4.2 Manure Assessment

The difference in overall mean manure weight between the two systems (on fresh basis) was significant ($P < 0.0001$), with CC birds excreting 91.3 g/d per hen, 11.5 g/d more than the EC birds. Manure DM from EC hens (34.1%) was significantly ($P < 0.01$) greater than from hens in CC (31.0%) resulting in a significant ($P < 0.01$) difference in the DM based manure weight (27.0 and 28.1 g/hen/d for EC and CC birds, respectively) (Table 3.2). These results were slightly lower than values of manure output predicted by

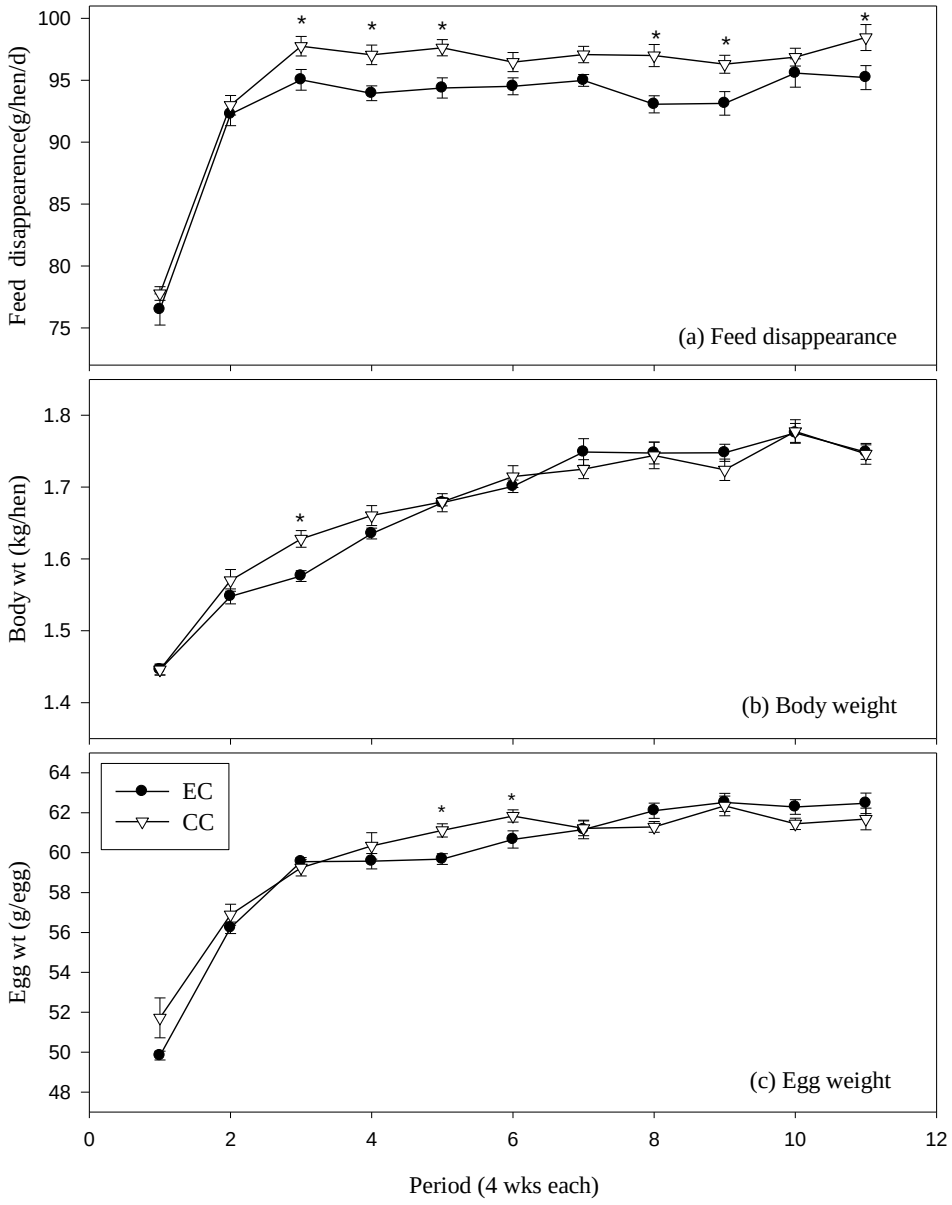

Figure 3.1. Feed disappearance, egg weight and body weight measurements of laying birds reared in conventional (CC) and enriched cages (EC) over an entire production cycle, (*, $P < 0.05$).

Table 3.2. Manure volume, nitrogen and energy assessment during an entire production cycle of Shaver White hens under conventional (CC) and enriched cage (EC) systems[1]

	DM (%)	Manure wt (as is basis) (g/hen/d)	Manure wt (DM basis) (g/hen/d)	Manure N (g/hen/d)	Manure GE content (MJ/hen/d)
Cage system[2]					
EC	34.1	79.8	27.0	1.94	0.35
CC	31.0	91.3	28.1	1.96	0.36
SE	0.32	1.18	0.23	0.02	0.003
Period[3]					
1	35.4^{a}	71.7^{e}	25.1^{d}	1.85	0.31^{d}
2	31.0^{de}	91.8^{a}	28.3^{a}	2.22	0.38^{a}
3	31.5^{cde}	92.8^{a}	29.0^{a}	2.17	0.38^{a}
4	30.5^{e}	93.6^{a}	28.4^{a}	2.15	0.38^{a}
5	31.5^{cde}	90.9^{ab}	28.4^{a}	2.09	0.37^{ab}
6	31.4^{cde}	90.7^{ab}	28.3^{a}	2.04	0.35^{bc}
7	34.1^{ab}	82.8^{cd}	28.2^{ab}	1.84	0.35^{bc}
8	32.8^{bcd}	86.5^{bc}	28.2^{ab}	1.93	0.35^{bc}
9	33.0^{bcd}	81.4^{d}	26.7^{c}	1.71	0.33^{cd}
10	33.1^{bc}	78.9^{d}	26.0^{cd}	1.77	0.35^{bc}
11	33.8^{ab}	80.6^{d}	27.0^{bc}	1.68	0.37^{ab}
SE	0.47	1.31	0.31	0.03	0.005
P-value					
Cage	< 0.0001	< 0.0001	< 0.01	NS	< 0.01
Period	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cage×Period[4]	NS	NS	NS	< 0.0001	NS

$^{a–e}$ Different superscripts within each column are significantly different at $P < 0.05$

[1]Data are presented as least square means (LSM), their standard errors (SE)

[2]LSM as main effect of cage type

[3]LSM as main effect of period on overall mean of CC and EC systems

[4]Cage × Period indicate interaction of caging system and period for all the parameters tested over an entire production cycle

Smith et al. (2000) using caged birds under intensive production (34.4 g/day DM basis or 115 g/day fresh excreta per hen at 30% DM).

Energy loss in manure was lower ($P < 0.01$) for EC caged birds than their counterparts in CC. Overall manure N excretion from the two cage systems was not significantly different. However, there was a housing type by period interaction on manure N content. Detailed assessment of manure output (Table 3.3) showed greater manure N from CC birds than from EC birds between periods 1 to 8, significant ($P < 0.01$) effect was observed in period 3 and then later in period 8 (EC = 2.09 and 1.85; CC = 2.24 and 2.02 ± 0.04, respectively). However, in the later stages of production (periods 9 to 11), birds in EC excreted more ($P < 0.05$) N in manure than CC birds.

3.4.3 Nitrogen Intake, Nitrogen Balance (Retention) and Output/Deposition in Eggs

There was a significantly ($P < 0.05$) higher N intake (Table 3.4), corresponding with higher feed disappearance, by CC hens compared to hens in EC (3.05 and 2.97 ± 0.02 g/hen/d, respectively). Although overall feed disappearance remained constant after the substantial increase noted between period 1 and 2 (Figure 3.1a), overall N intake declined with age of the birds after period 2 (Figure 3.2a). Despite the decrease in N content of the diet from one phase to the next, there was an increase in N intake in period 9, with declines thereafter. Overall, CC hens had a significantly ($P < 0.05$) greater N balance than the EC birds, retaining 2.60 ± 0.46% of N intake compared to 0.65 ± 0.46% for EC birds (Figure 3.2b and Table 3.4).

Results from individual egg analysis showed that there were no significant differences in the N output in egg whites (558 and 564 ± 4.26 mg/hen/d respectively); however, the deposited N in egg white was significantly ($P < 0.01$) influenced by the

Table 3.3. Nitrogen (N) excretion in manure as a function of cage type by period effect (hen basis) and significant differences between conventional (CC) and enriched cage (EC) systems

	Manure N[1] (g/hen/d) ± 0.04 Cage system		Sig. Diff. (*P*-value)
Period	EC	CC	
1	1.83	1.87	NS
2	2.18	2.25	NS
3	2.09	2.24	**
4	2.14	2.17	NS
5	2.06	2.11	NS
6	2.04	2.04	NS
7	1.82	1.85	NS
8	1.85	2.02	**
9	1.80	1.63	**
10	1.83	1.71	*
11	1.74	1.63	*

[1]Least square means (LSM) and standard error (± 0.04)
*$P \leq 0.05$; **$P \leq 0.01$; NS, not significantly different

Table 3.4. Nitrogen flow over the entire production cycle in Shaver White hens placed in either enriched (EC) or conventional (CC) cages[1]

	N intake	N deposition (mg/hen/d)			Whole egg N (output)	N retention	
	(g/hen/d)	Egg shell	Egg white	Egg yolk	(mg/ g egg)	Absolute (mg/hen/d)	Percentage intake
Cage system[2]							
EC	2.97	53.3	558	388	18.4	30.2	0.65
CC	3.05	54.6	564	393	18.4	85	2.6
SE	0.02	0.91	4.26	1.73	0.04	13.6	0.46
Period[3]							
1	2.85^{e}	34.9^{e}	331	166	19.0^{b}	464	16.2
2	3.46^{a}	66.4^{a}	639	367	19.6^{a}	166	4.79
3	3.41^{a}	57.7^{b}	647	390	18.9^{bc}	159	4.65
4	3.23^{b}	53.4^{bc}	629	418	18.9^{bc}	-28	-0.92
5	3.23^{b}	53.1^{bc}	623	436	18.9^{bc}	27.6	0.84
6	3.09^{c}	47.9^{d}	632	426	18.6^{c}	-53.4	-1.73
7	2.82^{e}	52.8^{bc}	551	422	17.8^{de}	-43.7	-1.61
8	2.55^{g}	53.8^{b}	548	426	17.6^{ef}	-414	-16.3
9	2.98^{d}	53.5^{bc}	548	413	17.6^{ef}	259	8.57
10	2.82^{e}	50.7^{cd}	511	409	17.4^{f}	78.8	2.74
11	2.70^{f}	69.7^{a}	511	422	18.0^{d}	18.9	0.60
SE	0.02	1.18	4.97	2.68	0.08	26.5	0.86
P-value							
Cage	< 0.05	NS	NS	< 0.05	NS	< 0.05	< 0.01
Period	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cage x Period[4]	NS	NS	< 0.01	< 0.001	NS	< 0.001	< 0.001

[a–g] Different superscripts within each column are significantly different at $P < 0.05$

[1]Data are presented as least square means (LSM), their standard errors (SE)

[2]LSM as main effect of cage type

[3]LSM as main effect of period on overall mean of CC and EC systems

[4]Cage × Period indicate interaction of caging system and period for all the parameters tested over an entire production cycle

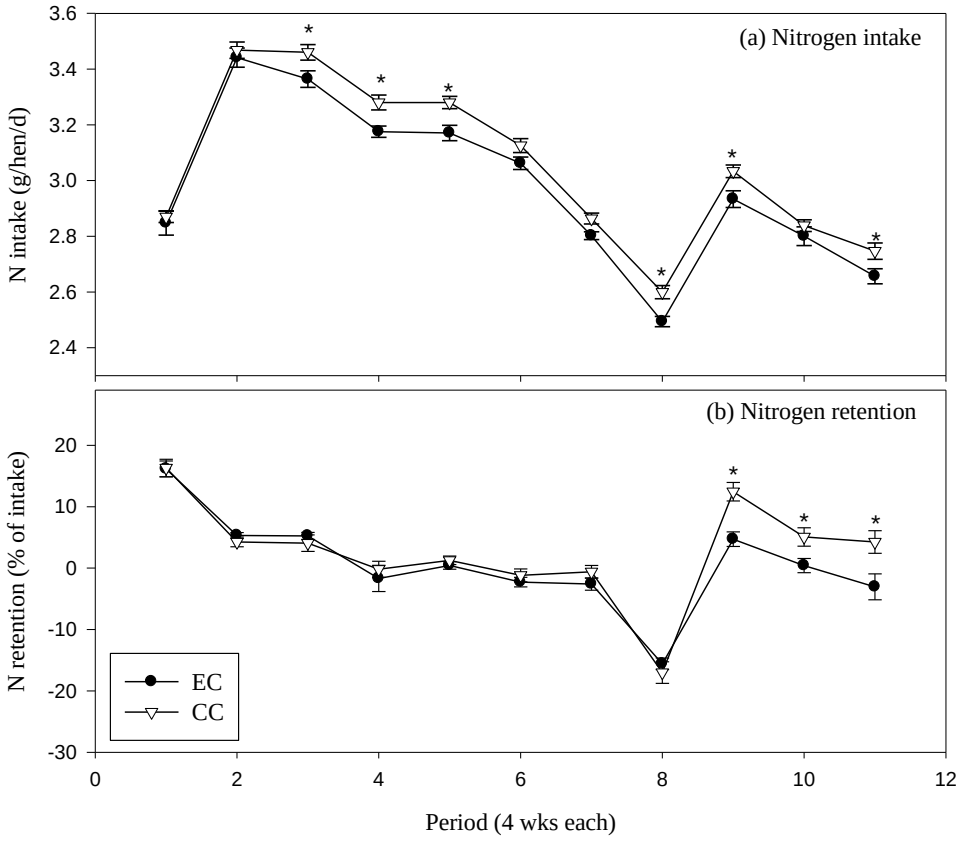

Figure 3.2. Nitrogen (N) intake and percentage retention over an entire production cycle in Shaver White hens placed in either enriched (EC) or conventional (CC) cages, (*, $P < 0.05$).

interaction of cage by period effect. Egg white N was greater for the CC birds (650 vs. 608 ± 7.03 mg/hen/d in CC and EC birds, respectively; Figure 3.3a) in period 4, a period of maximum egg production. Similarly, it was evident that during period 4 - 6, there were significantly ($P < 0.05$) higher egg weights observed in CC compared to EC hens (Figure 3.1c). In both cage systems, N output in egg white declined after peak egg production (Figure 3.3a).

Similarly, there was significantly ($P < 0.05$) greater overall mean N output in egg yolk in CC birds compared to EC birds. Overall, N content in egg yolk was maintained at relatively constant levels over the periods, following the initial increase between periods 1 and 2 (Figure 3.3b). The trends in the N content of egg yolk and egg white may be related to the duration of accumulation or formation of egg components in which egg white deposition takes place over a short period, approximately 6 h (Downing and Bryden, 2002) unlike egg yolk, which accumulates over a longer period of 7 to 12 d (Johnson, 1986), hence the latter being relatively consistent. Overall mean N output in egg shell (53.3 and 54.6 ± 0.91 mg/hen/d respectively) for EC and CC birds was not significantly different (Table 3.4). The N output in individual egg components were summed to obtain the whole egg N output (Table 3.4 and Figure 3.4). There was no significant difference in the egg N output between the two cage systems.

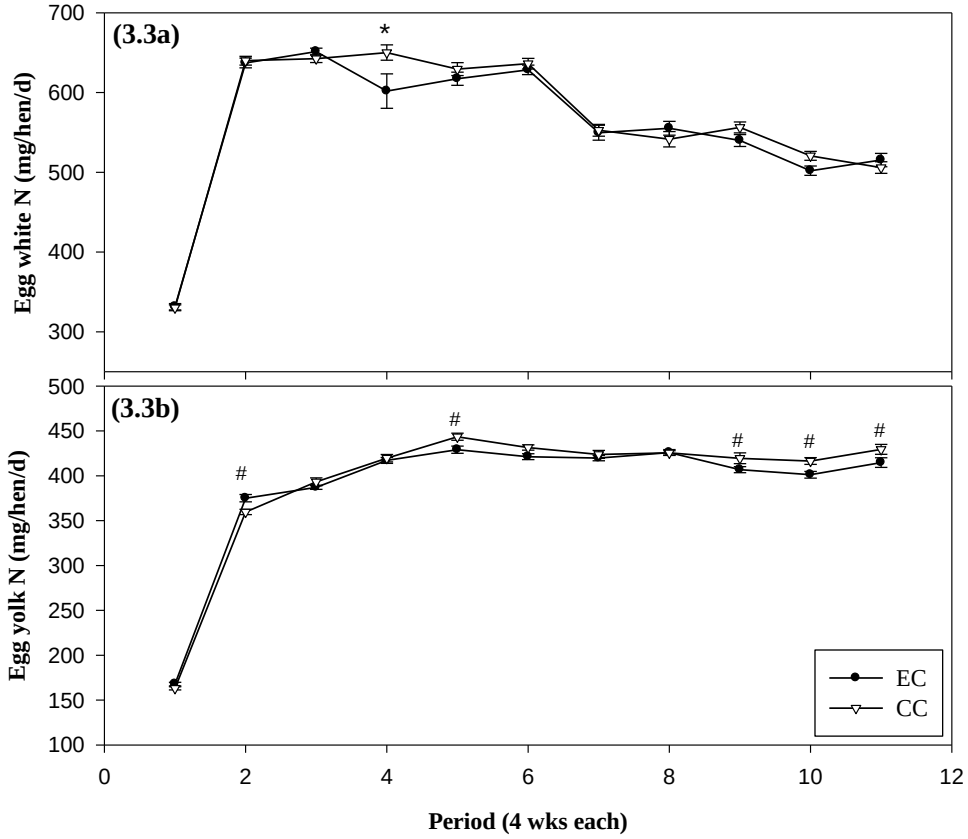

Figure 3.3. Nitrogen (N) deposition (mg/hen/d) in a) egg white and b) egg yolk as a function of cage type by period effect (DM basis). Data are presented as least square means (LSM) with their standard errors (SE). Significant differences marked (* and #) at $P < 0.05$.

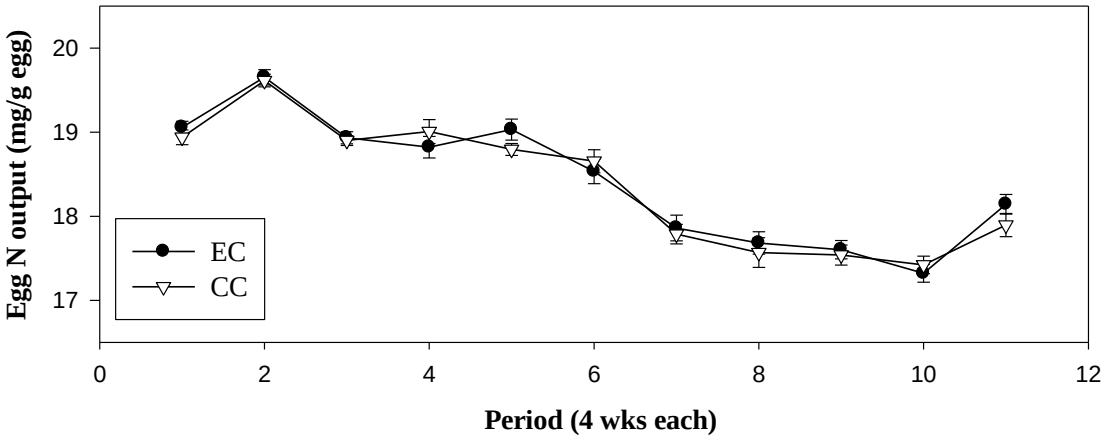

Figure 3.4. Nitrogen (N) output in whole egg (DM basis) in Shaver White laying hens over the entire production cycle under enriched (EC) and conventional (CC) caged systems. Data are presented as least square means (LSM) with their standard errors (SE).

3.5 DISCUSSION

In this study, higher feed disappearance ($P < 0.01$), manure output ($P < 0.01$) and N balance ($P < 0.05$) were found in hens housed in CC compared to the EC systems. Studies comparing feed intake by hens housed in caged systems report contradictory findings. Previous studies (e.g., Preisinger, 2000; Pohle and Cheng, 2009) indicate higher levels of feeding behaviour in birds housed in furnished than conventional systems. Similarly, Appleby and Hughes (1991) reported reduced feed consumption as a result of raising layers at high stocking densities. The authors attributed higher feed intake at lower stocking density in enriched cages to the requirement of more feed to provide energy for heat production to compensate for the lower heat generated by cage mates. However, in the current study, on a daily average basis, there was 2.5 g/hen ($P < 0.01$) more feed disappearance in CC than the EC system.

In line with the current result on feed disappearance, earlier studies (Tauson and Jansson, 1988; Glatz and Barnett, 1996) found lower feed intake in hens housed in cages equipped with perches (as in enriched) than those without a perch (as in conventional). Similarly, bird activity tends to increase with increasing group size when associated with cage area (Carey et al., 1995; Albentosa et al., 2007) explained by the synchronous feeding in hens (Hughes, 1971). In addition, Matsui et al. (2004) and Elson and Croxall (2006) reported lower feed intake in furnished than conventional caged birds. Furnishing cages with perches tends to decrease bird activity (Matsui et al., 2004), increase the amount of resting behaviour occurring in the cage (Tauson, 1998) and provide better insulation of the hens' bodies at night when roosting on the perch (Lill, 1968).

Other explanations besides feed consumption may account for differences in feed disappearance between the two cage systems, including feeder space and design. Approximately 128 and 104 cm^2/hen feeder space was provided by the EC and CC systems, respectively. The smaller feeding space in the CC system may have led to competition and aggressive feeding behaviour which may have contributed to potential differences in feed. Thogerson et al. (2009), relating the effect of feeder space and the behaviour of caged Hy-Line W-36 hens, showed that hens with less feeder space utilized more feed in a short time, suggesting a possible indicator of increased feed wastage. It is also possible that the barren environment in CC systems leads to redundancy and as a consequence the CC birds spend more time eating. These contradictions in feeding behaviour between the two systems may be partly due to differences in the strains of birds used and differences in cage floor space per bird (Adams and Jackson, 1970). Feed disappearance could also be influenced by water intake. In this study, there was a significant ($P < 0.05$, $R^2 = 0.26$) but weak correlation between water and feed disappearance (both provided ad libitum).

All performance parameters were highly influenced by bird maturity (period effects). The two cage systems did not differ in their performance characteristics (egg production, egg weight, egg mass, body weight, FCR). The overall mean egg production was not significantly different ($P = 0.0748$) between the two cage systems. The results agree with Tactacan et al. (2009) who showed no marked differences in egg production between conventional and enriched caged systems used for Shaver White hens. Although the authors used 5 hens in the conventional cages compared to 6 hens used in the current study, their study also showed no significant difference in production performance

between the two housing systems. This agrees with the report that available floor space has more influence on laying performance than number of birds per cage (Marr and Green, 1970). Similarly, results by Benyi et al. (2006) showed significant interactions between strain and floor space of Hyline Brown hens for egg production, egg weight, egg output and mortality, and suggested a floor space of 733 cm^2/ hen for Hyline Brown hens reared in semi-arid areas. Specific results on the effect of stocking density on egg production (Johnson et al., 1974; Gonzalez et al., 1978; Benyi et al., 2006) showed no marked difference. Studies by Abrahamsson et al. (1995), although based on strain differences, showed higher egg production for a white strain than for a brown strain reared in conventional cages. These results supports the claim that the density of hens within the limits (considering hen strain and environmental conditions) tested did not significantly influence production.

Higher feed disappearance with CC birds was likely a factor affecting cage by period interaction that showed significantly ($P < 0.01$) heavier body weights in period 3. After period 3 there was no marked difference in body weight within the CC and between the two systems, possibly because other factors, such as limitations due to dietary energy, could have influenced body weight. Guru et al. (1974), working on two distinct floor spaces provided by small (930 cm^2) or large (3700 cm^2) cages, noted that egg weight was unaffected by confinement. Similarly, Guesdon and Faure (2004) found no effect of cage system on egg weight. Although there was no main effect of cage system on egg weight in this study, there was a cage type by period interaction for egg weight. A significant ($P < 0.05$) increase in egg weight in CC system (Figure 3.1c) was noted following peak egg production (period 5 and 6) probably indicating peak egg mass reserves. In the present

study there was no significant difference in feed conversion between the two caged systems (EC = 1.76 vs. CC = 1.78 ± 0.01 g feed/g egg). Although feed conversion can be influenced by the housing system (Vits et al., 2005), in general, caged birds perform better than those in aviary and free range systems (Hughes et al., 1985).

Manure output from hens housed in EC and CC systems were 27.0 and 28.1 ± 0.23 g/d per hen on DM basis and 79.8 vs. 91.3 ± 1.18 g/d wet basis per hen at 34 and 31 ± 0.32% DM, respectively. As indicated earlier, period (age of hen) had a highly significant ($P < 0.0001$) effect on all parameters, including feed disappearance and manure output. Although this was not true with data estimates collected from previous experiments by Smith et al. (2000) in which the authors found no effect of bird maturity on feed intake or manure output, the authors noted that the latter two parameters were linearly related. This linear relationship between feed intake and manure output, agrees with the results in the current study. Lower feed disappearance (92.5 vs. 95.0 ± 0.61 g/hen/d, for EC and CC birds, respectively) resulted in a corresponding reduction in fresh manure weight ($P < 0.0001$): EC = 79.8 vs. CC = 91.3 ± 1.18; DM basis ($P < 0.01$): EC=27.0 vs. CC=28.1 ± 0.23 g/hen/d). Because there was no significant difference in the bird production performance, the correlation may imply better efficiency of feed utilization by enriched caged birds with the lower feed disappearance.

Similarly, in a study using commercial Leghorn hens housed in a barn with a deep-pit manure system, Patterson (1994) observed manure output of 12.5 ton/1000 birds/yr (equivalent to 34.2 g/d per hen). This value was slightly higher than those obtained in the current study. The author noted significant relationship between feed intake and manure output, with 0.33 kg manure generated per kg of feed intake. In our

study, corresponding values on DM basis were 0.29 and 0.30 kg manure per kg feed disappearance in EC and CC systems, respectively. The results may indicate a better feed efficiency with Shaver White hens placed in either caged systems compared to Leghorns housed in a barn with a deep-pit manure system but it is not clear whether these differences are due to dietary or strain influences. The amount of manure DM was inversely related to ration digestibility (Powers and Van Horn, 2001). Similarly, in our study, manure weight (based on fresh and DM) were ($P < 0.001$) influenced by water intake ($R^2 = 0.54 - 0.55$, respectively). Moisture levels in poultry waste can vary greatly depending on several factors, such as variations in diet, age of the bird, digestive health, and management practices (Patterson and Lorenz, 1997). Hence, manure-feed comparisons may require considerations based on ration specifications and environmental factors. In the current study, the % moisture content of the manure from the CC hens was greater than those housed in EC. The latter observation is likely due to the density of birds and the pattern of excretion on the manure belts, where there is less opportunity for moisture removal from the manure obtained from the CC hens due to the more compact excretion pattern. Consequently, this will have an impact on manure management aspects such as costs for maintenance (weight on the manure conveyor belt), storage (size), transportation and application.

Patterson (1994) estimated a total N output of 243 kg/1000 birds/yr (equivalent to 0.68 g/d per hen) for commercial Leghorn hens housed in deep-pit. Nicholson et al. (1996) predicted (by extrapolation) manure N loss of 18.0 kg/ton at 30% DM. This is approximately equivalent to 1.80 g/d per hen, assuming manure output of 100 g/d per hen with a 30% DM manure output (estimate based on results from the present study). A

number of factors could result in differences in manure N outputs, for example feed N contents which have direct relationship to manure N output (Meluzzi et al., 2001). In this study, there was no significant difference in the overall manure N excretion by birds in both cage systems (1.94 and 1.96 ± 0.02 g/d per hen for EC and CC, respectively). However, a significant ($P < 0.05$) cage by period interaction for manure N was noted in our study. Birds in EC excreted lower N in manure for the first two thirds of the production cycle and higher ($P < 0.05$) manure N excretion in the last stage of the cycle, while the excretion patterns were reversed for CC birds (Table 3.3). The overall mean N content in the eggs was similar (18.4 ± 0.04 mg/g egg) from birds housed in both systems. On average, this was equivalent to 1g N per 59 g egg. This agrees with estimates of 6 g protein/egg indicated by Leveille et al. (1960). Hence, in addition to maintaining egg number and nitrogen content, EC housed birds may possess similar attributes as those based on modern selection criteria (Whitehead and Fleming, 2000) which aims at producing birds that consume less feed and attain low body weight in order to maintain egg production.

Because feed disappearance (after period 2) was not significantly different between periods within each cage system, the N intake relates to the N content in the phase fed diet (analyzed to be 3.49, 2.93 and 2.86 ± 0.10 % for phases I, II and III, respectively). The decline in N intake was inevitable because of a decrease in the rate of egg production after peak production (Shapiro, 1968); yet after this period, the amino acid requirements for maintenance do not increase (Ishibashi and Yonemochi, 2003). Other biological processes, for example the yolk protein precursor lipovitellin, are only detected in the plasma in mature pullets and rapidly fall when birds mature, (Redshaw

and Follett, 1972) reflecting active reproductive stage and peak nutrient requirements. In addition, amino acid N composition of egg white and egg yolk remains the same (Leeson and Summers, 2005); therefore, the requirement for the nutrient decline as the bird progresses through the laying cycle with advancing age.

Birds housed in CC had significantly ($P < 0.01$) higher N balance than the EC birds (2.60 vs. 0.65 ± 0.46 % intake, respectively). In both cage systems, an overall positive N balance was observed; however, in period 8 birds in both systems were at their lowest N balance (-16.30 ± 0.86 % intake). During this period, the hens' in both systems were on the second phase of the diet that contained lower N content than in the first phase diet. Despite the changing of dietary N levels (phase fed diets), there was a gradual decrease in egg production in this experiment towards the end of the laying cycle.

The hen has a protein requirement for more than egg production alone, and the continuous changes in N balance (Figure 3.2b) relates to these demands (e.g., feather growth and protein for body weight gain and also endogenous protein loss). Nitrogen retention provides a valuable measure of the overall protein nutrition of the laying hen. Although there were no significant differences in production performances between the two systems, there was a significant difference in the N balance between them. In this experiment N balance was calculated by input and output relationship (as described by Wu-Haan et al., 2007). Nitrogen intake (inputs) was constituted by the feed and N outputs consisted of N deposited in eggs and excretion in manure. Nitrogen intake was greater in hens housed in CC than in EC given that feed disappearance was significantly ($P < 0.01$) greater in birds housed in CC compared to the EC system (overall means of feed disappearance was 95.0 and 92.5 g/hen/d, respectively). However, the measured N

intake estimated by feed disappearance is likely to be greater than the true intake because the higher feed disappearance with CC hens may not accurately imply that all the feed was consumed by the hens.

On the other hand, although N intake may be overestimated, N loss (excretion) in manure may be underestimated. Housing type has a major effect on manure quality (Smith et al., 2000). Because of cage design (space restriction), individual flock droppings in the CC system heaps on conveyor belts and does not spread out as in the EC system. The thin layer of manure droppings in EC had more exposed surface area which increases the manure drying ability and N loss by volatilization. Manure moisture content for hens in CC (69%) was significantly ($P < 0.01$) greater than those in EC (66%). Lower moisture content in manure corresponds to lower ammonia volatilization and greater retained ammoniacal-N in manure (Yang et al., 2000). In addition, manure N concentrations in the two systems may also vary depending on the proportions of the different components of poultry manure such as excreta, feed, feather and broken eggs (Nahm, 2005).

3.6 CONCLUSION

In conclusion, although we did not assess N retention per hen *per se* but rather N flow within the system, the combination of the analyzed manure N excretion, egg N output and the calculated N retention values may provide valuable estimates of the efficiency of N utilization in laying hens based on a commercial production system. Nitrogen flow in hens housed in EC, indicate that they do not perform poorer than those

in CC system. In addition, from the producers and environmental point of view, the high manure N excretion in EC system may suggest marked potential for linking the role of diet with respect to N flow and managing hens in an enriched cage system especially at a later stage of the laying cycle.

CHAPTER 4 CALCIUM AND PHOSPHORUS DYNAMICS IN COMMERCIAL LAYING HENS HOUSED IN EITHER CONVENTIONAL OR ENRICHED CAGE SYSTEMS

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4.1 ABSTRACT Calcium (Ca) and phosphorus (P) dynamics in Shaver White hens (19 to 63 wks of age) were compared between enriched (EC) and conventional cage (CC) systems. Calcium and P intake and levels in egg components and excreta were considered. Stocked at a commercial density (4,836 hens), ten test cages per system (containing 24 hens), were used as replicate units. Enriched cages provided a nesting area, scratch pad, roost area with perches and more floor space than CC (643 vs. 468 cm^2/hen, respectively). All birds were offered similar phase-fed diets based on wheat-soybean formulation, and housed under semi-controlled environmental conditions for 11 periods (28-days each). Egg weight, production and shell quality indices (egg specific gravity, shell weight, thickness and percent shell) were also measured. Data were analyzed as repeated measures using the MIXED procedure of SAS. Egg production, egg weight and shell quality measurements were not significantly different between the two systems. On a DM basis, EC hens exhibited less ($P < 0.01$) feed disappearance (92.5 vs. 95.0 ± 0.61 g/hen/d), and less ($P < 0.01$) Ca and P excretion in manure (Ca: 2.11 vs. 2.29 ± 0.04 and P: 0.619 vs. 0.643 ± 0.005 g/hen/d) compared to CC hens. Although less Ca deposition ($P < 0.0001$) (2.07 vs. 2.13 ± 0.01 g/hen/d) and output ($P < 0.05$) (38.3 vs. 38.8 ± 0.15 mg/g egg) were observed in eggs from EC compared with CC, both systems

had similar Ca output in eggs when expressed as a proportion of Ca intake (56.5 vs. 56.6 ± 0.51). The overall mean P retention (-7.22 vs. -7.45 ± 0.71% P intake) was not significantly different between the two groups of hens; however, Ca retention was greater ($P < 0.05$) in EC than CC hens (-1.37 vs. -4.76 ± 0.89% Ca intake, respectively). In addition to providing environmental enrichment, EC systems may help to reduce Ca and P excretions, when compared to CC systems, thus improving the utilization of these nutrients.

Key words: cage system, calcium, phosphorus, hen

4.2 INTRODUCTION

Although laying hens have been kept in wire cages since the 1920's (Rahn, 2001), the pressure to shift from conventional cages (CC) to alternative housing systems for laying hens necessitates the gathering of sound performance, health and welfare data based on North American conditions (Holt et al., 2011). Enriched cage (EC) systems provide environmental enrichment to hens (Hester, 2005; Tactacan et al., 2009), however environmental changes may lead to alterations in the efficiency with which hens utilize dietary nutrients (Lay et al., 2011).

In hens, calcium (Ca) and phosphorus (P) are key minerals and their availability is most crucial during the laying period (De Vries et al., 2010). Calcium constitutes approximately 1.5% of body weight of the hen and 40% of egg shell weight (Bolukbasi et al., 2005). It is stored mainly as Ca phosphate in the skeleton (Whitehead and Fleming,

2000) and as Ca carbonate in the egg shell (Bolukbasi et al., 2005; Bar, 2009). Conversely, P, when in excess, is detrimental for egg shell quality (Chandramoni et al., 1998), because it forms insoluble Ca phosphate in the intestine, which renders Ca unusable (De Vries et al., 2010). In addition, excess P in the blood can reduce the formation of the active form of vitamin D (calcitriol) in the kidneys, hence inhibiting bone resorption (the release of bone minerals: Ca and phosphate into the blood) (Etches, 1987). Although P is required in small quantity for egg shell formation (Taylor, 1965), it influences the egg forming process (Ahmad and Balander, 2004) because the metabolism of Ca and P are interrelated (Kebreab et al., 2009). During egg shell formation, P is also used in reducing blood acidosis by flushing out excess H^+ ions during its excretion hence contributing to the maintenance of bicarbonate levels (Pelicia et al., 2009). However, in addition to intake levels, the latter function of P coupled with its subsequent (unintended) release (Whitehead and Fleming, 2000) during the mobilization of Ca from medullary bone tissue (Etches, 1987) in the calcification process (Bar, 2009), can influence the amount of P excretion in manure in a given laying period (De Vries et al., 2010).

Although adequate nutrient intake can help to prevent birds from draining their skeletal Ca reserves for shell formation, structural bone loss is accelerated primarily by the lack of exercise leading to loss of bone volume referred to as osteoporosis (Whitehead and Fleming, 2000). The ability to utilize and deposit Ca and P in hens can differ due to a number of factors, including nutrition (Hurwitz and Bar, 1967; Guinotte et al., 1991), physiological status (Gilbert, 1983; Scott and Balnave, 1991), genetics/breed type (Pandey et al., 1986) and management / housing type (Mench et al., 1986; Norgaard-Nielsen, 1990; Appleby et al., 2002). Although alternative housing systems have been

evaluated for productivity and welfare in birds (Abrahamsson and Tauson, 1995; Vits et al., 2005; Jendral et al., 2008), comparative studies on nutrient dynamics in laying hens housed in different systems are lacking. Therefore, the aim of the present study was to compare Ca and P dynamics in laying hens housed under EC and CC systems in a large scale production unit. The comparison was based on the assessment of Ca and P flow from feed to egg components and excretion in manure.

4.3 MATERIALS AND METHODS

The experiment was conducted at the University of Manitoba poultry farm in accordance with the recommended code of practice for the care and handling of pullets, layers and spent fowl (Canadian Agri-Food Research Council, 2003) and followed the ethical principles of the guide to the care and use of experimental animals (Canadian Council on Animal Care, 1993). All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Protocol Management and Review Committee.

Details on the experimental design for this study have been previously reported (Neijat et al., 2011). In brief, 4836 Shaver White pullets, obtained from Manitoba Perfect Pullets Ltd (Rosenort, MB) were stocked at a commercial level in cages from the age of 19 weeks under semi-controlled environmental conditions that provided 15 h of lighting. Of these, 10 test cages per system were randomly selected in the barn. In this study, an EC unit contained 24 hens providing 643 cm^2/hen floor space, and a CC unit was divided into 4 parts each to contain 6 hens, and providing 468 cm^2 per hen floor space. In

addition, EC provided nesting area, roost area with perches and a scratch pad. A full description of the cages has been reported previously (Tactacan et al., 2009). Bird losses from test cages (if any) were replaced with spare birds of similar live weight from non-test cages in order to maintain bird number per test cage unit constant.

4.3.1 Diet and Sample Collection

All hens were offered similar phase-fed diets based on a wheat-soybean meal mix for 11 periods (28-days each), as recommended for the strain (ISA, 2009). The recommended Ca levels were: 4.2, 4.3 and 4.4% and P (available) levels were: 0.45, 0.43 and 0.41% for Phase I (19-42 wks; periods 1 - 6), Phase II (43-54 wks; periods 7 - 9) and Phase III (55-63 wks; periods 10 - 11), respectively. Table 4.1 shows nutrient composition of the diet provided. Limestone (38% Ca, granular form) was used as the main source of Ca. All the hens were fed *ad libitum* in accordance with the Canadian Agri-Food Research Council (2003); water was also provided *ad libitum* using nipple drinkers (1 nipple/8 hens and 1 nipple/6 hens for EC and CC systems, respectively). Measurements and sampling were taken in the middle of the 28-day period for 5 consecutive days for each of the 11 periods. Hen daily egg production, egg weight and feed disappearance were conducted according to Neijat et al. (2011).

For the analysis of macro-minerals (Ca and P) in feed, manure and egg components, respective subsamples were obtained. In every period, subsamples of 150 to 200 g of feed were obtained from 2 to 3 batches of feed supplied to the unit. These were ground to pass through a sieve screen of 1 mm before analysis. Manure was collected from each test cage unit using plastic trays placed on the conveyor belts underneath each

Table 4.1. Composition of phase fed diets used in the experiment (DM[1] basis). Data represents means for each phase ± standard deviation (SD)

	Phases		
	I	II	III
	(Period 1-6)	(Period 7-9)	(Period 10-11)
Diet composition	(19-42 wk)	(43-54 wk)	(55-63 wk)
DM[1] (%)	91.2 ± 0.26	90.1 ± 0.30	89.9 ± 0.41
Nitrogen (%)	3.49 ± 0.10	2.93 ± 0.10	2.86 ± 0.10
Calcium (%)	4.36 ± 0.42	4.03 ± 0.72	4.67 ± 1.27
Phosphorus (Total, %)	0.72 ± 0.03	0.71 ± 0.08	0.68 ± 0.05
Phosphorus (Available, %)	0.39 ± 0.002	0.38 ± 0.006	0.35 ± 0.003
NDF[2] (%)	12.4 ± 1.04	9.91 ± 0.66	11.7 ± 0.60
ADF[3] (%)	4.28 ± 0.31	3.92 ± 0.16	3.39 ± 0.10
Crude fat (%)	6.17 ± 0.15	3.28 ± 0.23	2.81 ± 0.26
Energy(GE[4], MJ//kg)	20.5 ± 0.25	17.1 ± 0.37	16.7 ± 0.70

[1]Dry matter (DM), [2]neutral detergent fibre (NDF), [3]acid detergent fibre (ADF) and [4]gross energy (GE)

test cage unit during the 5 day collection period. Manure subsamples of 1.5 to 2 kg were obtained and frozen at -20^{o}C before being freeze dried and finally ground to pass through a sieve screen of 1 mm and stored for subsequent analysis. Consistent with commercial production practices, the collected manure included excreta, spilled water and feed, feathers and broken eggs. To obtain a representative sample of the manure, total manure output per cage unit was homogenized through the use of a mixing implement attached to an electric hand drill. In addition, feed spillage was reduced by using wire mesh (2.5 cm × 3.8 cm mesh size) to cover the feed in the troughs and the feed was rationed in two lots (days 1 and 4). The numbers of broken eggs are likely to have been reduced because the cage design in both systems provided a slope for eggs to roll first onto an egg saver that minimized egg breakage, then onto an egg collection belt.

Four eggs were sampled daily during the 5-d collection period from each cage unit and immediately stored in an egg cooler (10 to 12^{o}C). On day 6, the eggs were removed from the cooler and weighed to determine average egg weight. A total of 20 eggs per cage unit were sampled. Of these, 10 eggs were broken and the yolks carefully separated from the whites (albumen) using an egg separator. The yolk, white and shell samples were pooled and homogenized to yield 2 replicates of 5 eggs (one from each day) for every cage unit, placed in labeled plastic bags and weighed. The samples were frozen at -20^{o}C and later freeze dried and weighed. Corresponding final freeze dried weights were taken for the different component samples to determine the dry matter (DM) of the samples. Egg yolk and white samples were crushed using a pestle, and a coffee grinder was used to grind the egg shells before analysis. For each measurement, the averages of two replicates per cage unit were used in the statistical analysis. The

remaining 10 eggs per cage unit were reserved for egg shell quality assessment (i.e., egg specific gravity, shell weight, thickness, percent shell) as described in the following section.

4.3.2 Egg Shell Quality Assessment

Eggs were broken and the shells were carefully washed with shell membranes intact and left to dry for 2 days at room temperature. Shell weight (weight per egg) was determined on dried shells using a digital scale. Shell thickness was measured with membranes intact using a thickness gauge micrometer (B. C. AMES Co.; Waltham, Mass) in which the eggshell thickness of a chip was taken from the equator region of the egg. Ten measurements from the 10 eggs/cage unit were taken and these observations were averaged to determine the egg shell thickness for each cage unit. The obtained thickness values (in thousandths of an inch) were converted into micron units by multiplying by 25.4. Egg specific gravity was determined on day 6 for all eggs collected including those for egg component assessments after being stored in an egg cooler (10 to 12^{o}C) during the sampling period. The Holder and Bradford (1979) method, using sodium chloride solutions ranging in specific gravity from 1.070 to 1.085 in increments of 0.005 units, was used for egg specific gravity determination. The eggs were immersed into solutions with increasing concentrations of salt. The specific gravity is similar to the density of the solution in which the egg floats to break the surface. For statistical analysis, eggs that remained submerged in the specific gravity solution of 1.085 were considered to be 1.090. Percent shell (with membrane intact) was calculated from the dry shell weight and the egg weight.

4.3.3 Calcium and Total Phosphorus Analysis

Samples of feed (1 to 2 g); manure and egg yolk (0.5 to 1 g); egg white (0.5 to 0.6 g); and egg shell (0.1 - 0.2 g) were put in a furnace at 600^{o}C to ash over night according to procedures described by method 942.05 of AOAC (1990). For egg white samples, considering its frothy nature, furnace temperature was gradually increased from 150^{o}C (for 1h) to 260^{o}C (for 1h) then to 427^{o}C (for 1h) before setting it finally to 600^{o}C over night. Following method 985.01 of AOAC (2005), the samples were then digested using 10 mL of 1% HNO_3 and 5N HCl for one hour in a sonication water bath at 60^{o}C. The digests were then vortexed and 10 mL (for feed, manure and yolk samples), 5 mL (for egg shell) and all the digest (for egg white) were used for dilution in a 100 mL volumetric conical flask using de-ionized water and at least 20 mL was filtered through P5 filter paper into a scintillation vial. The Ca and P contents were then measured by an inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA).

4.3.4 Nutrient Balance

Calcium and P balance (retention) were calculated using the amount of nutrient consumed, deposited in the egg and that excreted in the manure. Percentage retention of nutrients were calculated as the amount of nutrient in the manure and in the egg components subtracted from the amount consumed in the feed.

4.3.5 Statistical Analysis

A group of 24 hens were randomly assigned to each of 10 cage units per treatment (cage type of either EC or CC) giving a completely random design for cage units. A total of 480 hens were in the study for both systems. Production performance and other variables were observed as a 5-day average in the middle of every period (28 days each)

for 11 periods. The experiment had a repeated measures design with cage system as the treatment applied to main plot (the cage units) and 11 measurements (periods) taken per cage. Statistical analyses of all dependent variables for the effects of cage systems and time period during the production cycle were carried out using the MIXED procedure of SAS (SAS 9.2; SAS Inc, Cary, NC). The statistical model used in the analysis was as follows:

$$Y_{ijk} = \mu + t_i + c_{ij} + p_k + tp_{ik} + e_{ijk},$$

where Y_{ijk} = observation on a variable; μ = model constant, t_i = effect of cage system which is the treatment (i = 1, 2); c_{ij} = effect of the different cage units within a cage system (j = 1-10); p_k = effect of experimental period (k = 1-11); tp_{ij} = interaction between cage system and experimental period (treatment x period) and e_{ijk} = random error variation. In the repeated measure (split plot design), the c_{ij} is the error term for the factor applied to main plots (type of cage system, t_i). Experimental period and treatment by period interactions were considered as fixed effects applied to sub-plot and were tested using the residual error (e_{ijk}). Least square means were estimated for all variables. Means were compared using Tukey's test. Comparisons giving *P*-values less than 0.05 were considered significant. Studentized residuals were evaluated for each variable and observations with studentized residuals exceeding ±3 were excluded from the analysis. For most of the variables tested, 0 to 6 observations per variable were dropped as outliers, but when missing observations were noted as for Ca intakes for period 7 to 11, the new sample size (n) values were indicated. In all analyses, Shapiro-Wilk test was used to evaluate normality of the residuals.

4.4 RESULTS

4.4.1 Feed Disappearance, Egg Production and Weight

Feed disappearance was greater ($P < 0.01$) for hens in the CC than those in the EC (on DM basis, 95.0 and 92.5 ± 0.61 g/hen/d, respectively; Figure 4.1a). The overall egg production (percentage hen-day basis) and egg weight were not statistically different between the two systems. On average, rate of lay was 90.6 vs. 91.7 ± 0.43% (Figure 4.1b) and egg weight was 59.7 vs. 59.8 ± 0.24 g (Figure 4.1c) for EC and CC systems, respectively. Significant cage by period effects of egg weight, resulting in heavier eggs ($P < 0.001$) in periods 5 and 6 for the CC systems compared to the EC types may have resulted from the consecutive increases in higher feed disappearances from periods 3 to 5 for CC compared to the EC system. During the entire production period, irrespective of housing system, all variables tested were influenced by period (age of hen) ($P < 0.0001$).

4.4.2 Calcium and Phosphorus Intake

During the course of the study, the values for Ca intake in periods 7 to 11 were disregarded because high variation (18 to 27 %) in Ca contents of sample composition was noted (Table 4.2). The limestone (granular form) used as the main source of Ca was found to segregate in the feed for those periods. The lower ($P < 0.01$) feed disappearance by hens in EC than those in CC, resulted in lower daily average intakes of Ca (3.97 vs. 4.06 ± 0.03 g/hen, $P < 0.05$; Table 4.2) and P (0.66 vs. 0.68 ± 0.004 g/hen, $P < 0.01$; Table 4.3) for EC hens than their counterparts in the CC system.

4.4.3 Egg Calcium and Phosphorus Contents

There was significantly less Ca deposition in egg shells from the EC than from the

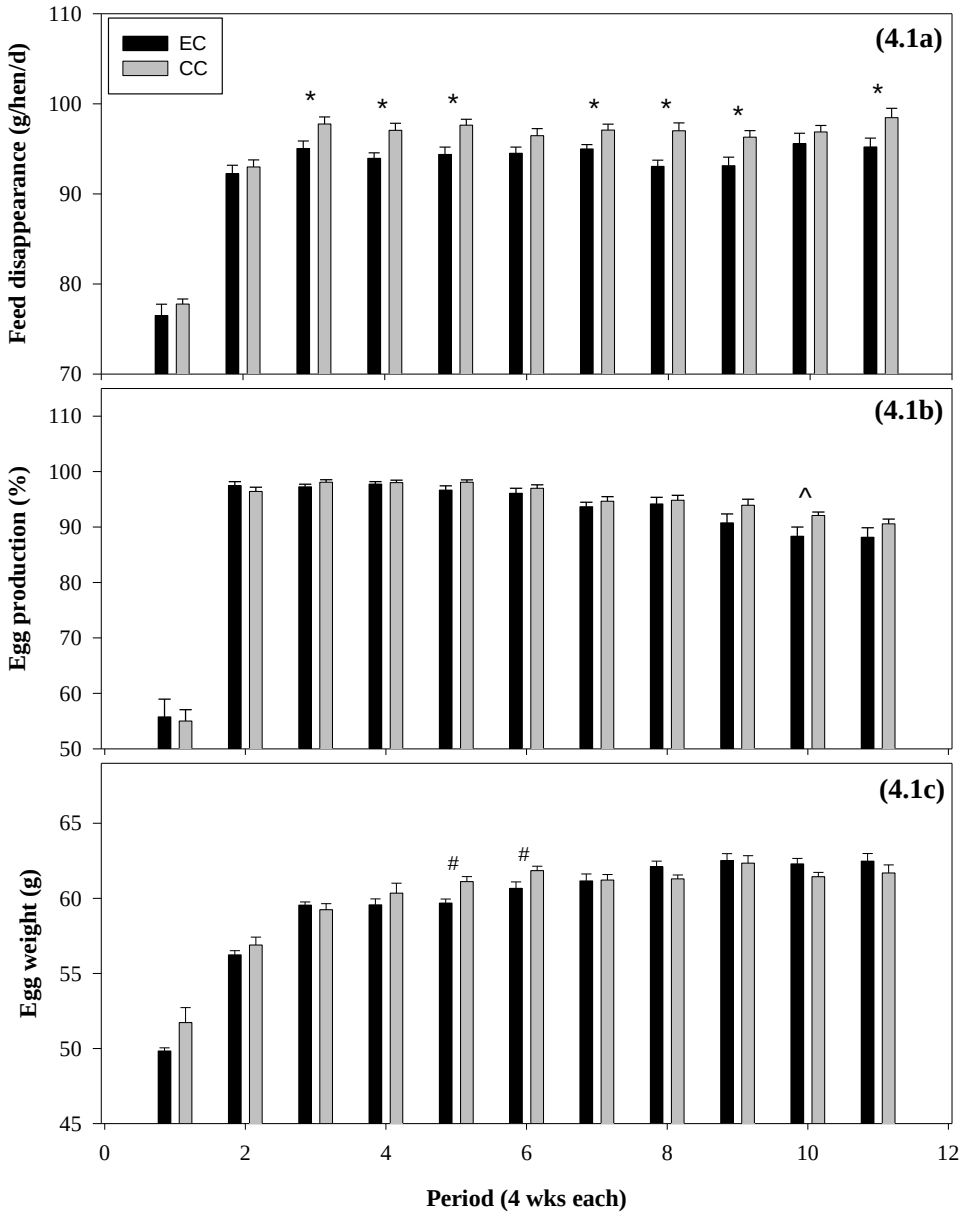

Figure 4.1. a) Feed disappearance (g/hen/d, DM basis), b) egg production (%) and c) egg weight (g) in laying hens housed in enriched cage (EC) and conventional cage (CC) systems. Data are presented as least square means (LSM) with their standard errors (SE). Significant differences at $P < 0.05$, for feed disappearance, egg production and egg weight are marked by *, ^ and #, respectively.

Table 4.2. Calcium (Ca) flow in laying hens housed in enriched (EC) and conventional (CC) cage systems (DM basis) [1]

	Ca intake (g/hen/d)	Manure Ca excretion (g/hen/d)	Ca deposition in			Deposition of Ca in shelled egg (g/hen/d)	Ca balance	
			Egg shell (g/hen/d)	Egg white (mg/hen/d)	Egg yolk (mg/hen/d)		Absolute (mg/hen/d)	% of intake
Cage[2]								
EC	3.97	2.11	2.03	4.14	34.3	2.07	-54.7	-1.37
CC	4.06	2.29	2.10	3.83	33.5	2.13	-185	-4.76
SE	0.03	0.04	0.01	0.04	0.34	0.01	35.3	0.89
Period[3]								
1	3.48^{e}	2.47^{a}	1.12	3.02	19.4	1.15	-143^{b}	-4.14^{bc}
2	3.74^{d}	1.77^{e}	2.14	4.52	43.3	2.19	-210^{bc}	-5.61^{bc}
3	4.00^{c}	1.95^{de}	2.25	4.27	28.3	2.28	-224^{bc}	-5.53^{bc}
4	4.14^{b}	1.93^{de}	2.15	3.44	31.5	2.19	10.7^{ab}	0.43^{ab}
5	4.37^{a}	1.91^{de}	2.19	4.13	35.0	2.23	228^{a}	5.19^{a}
6	4.35^{a}	2.48^{ab}	2.21	6.19	37.4	2.25	-381^{c}	-8.72^{c}
7	-	2.51^{a}	2.20	3.69	37.8	2.24	-	-
8	-	2.31^{abc}	2.20	3.64	37.1	2.24	-	-
9	-	2.55^{a}	2.16	3.82	35.3	2.20	-	-
10	-	2.11^{cd}	2.02	3.63	35.4	2.06	-	-
11	-	2.23^{bc}	2.04	3.50	32.3	2.08	-	-
SE	0.03	0.06	0.01	0.08	0.7	0.02	61.2	1.59
P-value								
Cage	< 0.05	< 0.01	< 0.0001	< 0.0001	NS	< 0.0001	< 0.05	< 0.05
Period	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cage×Period[4]	NS	NS	< 0.0001	< 0.0001	< 0.0001	<0.0001	NS	NS

[a–e] Different superscripts within each variable (column) are significantly different at $P < 0.05$

[1]Data are presented as least square means (LSM) and their standard errors (SE)

[2]LSM as main effect of cage type

[3]LSM as main effect of period on overall mean of CC and EC systems

[4]Cage × Period indicate interaction between caging system and period

For periods 7 to 11 Ca intakes and balances were disregarded (n=120)

Table 4.3. Phosphorus (P) flow in laying hens housed in enriched (EC) and conventional (CC) cage systems (DM basis) [1]

	P intake (mg/hen/d)	Manure P excretion (mg/hen/d)	P deposition in Egg shell[5] (mg/hen/d)	P deposition in Egg white (mg/hen/d)	P deposition in Egg yolk (mg/hen/d)	Deposition of P in shelled egg (mg/hen/d)	P balance Absolute (mg/hen/d)	P balance % of intake
Cage[2]								
EC	657	619	2.57	3.72	76.0	82.3	-46.8	-7.22
CC	675	643	2.71	3.81	74.8	81.1	-49.8	-7.45
SE	4.35	5.23	0.18	0.04	0.46	0.39	4.66	0.71
Period[3]								
1	532^{g}	484^{e}	BD	2.42	29.5	31.9	18.2^{ab}	3.34^{ab}
2	658^{e}	642^{bcd}	BD	4.32	67.7	72.1	-50.5^{cd}	-8.57^{de}
3	675^{d}	634^{bcd}	BD	4.07	75.4	79.4	-37.5^{c}	-5.50^{cd}
4	697^{c}	649^{bc}	BD	4.37	76.4	81.6	-38.7^{cd}	-5.62^{cde}
5	701^{c}	615^{cd}	BD	3.88	81.7	85.6	-1.05^{b}	-0.17^{bc}
6	716^{b}	668^{ab}	4.65^{bc}	4.30	89.1	98.0	-52.5^{cd}	-7.36^{de}
7	659^{e}	642^{bcd}	4.92^{b}	3.72	89.5	98.1	-80.2^{de}	-12.2^{ef}
8	637^{f}	704^{a}	4.76^{bc}	3.56	83.5	89.8	-161^{f}	-25.4^{g}
9	739^{a}	608^{d}	8.44^{a}	4.02	79.7	91.8	35.6^{a}	4.79^{ab}
10	664^{de}	$641b^{cd}$	2.34^{c}	3.33	81.0	86.7	-63.0^{cde}	-9.51^{def}
11	649^{ef}	660^{b}	3.94^{bc}	3.41	76.3	83.6	-94.4^{e}	-14.6^{f}
SE	4.3	8.36	0.36	0.06	0.94	0.98	7.66	1.31
P-value								
Cage	< 0.01	< 0.01	NS	NS	NS	< 0.05	NS	NS
Period	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cage×Period[4]	NS	NS	< 0.0001	< 0.0001	< 0.0001	< 0.0001	NS	NS

[a–g] Different superscripts within each variable (column) are significantly different at $P < 0.05$

[1]Data are presented as least square means (LSM) and their standard errors (SE)

[2]LSM as main effect of cage type

[3]LSM as main effect of period on overall mean of CC and EC systems

[4]Cage × Period indicate interaction between caging system and period

[5]Phosphorus in egg shell were below detection levels (BD) in periods 1 to 5, hence considered as zero in statistical analysis

CC system (2.03 vs. 2.10 ± 0.01 g/hen/d, respectively; $P < 0.0001$) (Table 4.2). A significant ($P < 0.0001$) cage by period effect indicated greater Ca deposition in egg shells for the CC system throughout the production cycle (Figure 4.2). Expressed in mg/g egg, this was equivalent to 37.4 vs. 38.2 ± 0.15, respectively ($P < 0.01$, Table 4.4), with greater outputs observed in periods 3, 8 and 11 for the CC compared to EC system (Figure 4.3). However, Ca output in egg shell, expressed as a percentage of Ca intake was not significantly different between the two systems (55.5 vs. 55.3 ± 0.39 for EC and CC, respectively; Table 4.4).

Calcium deposition in egg yolk peaked in period 2 for both systems and thereafter leveled in the range of 30 to 40 mg/h/d for the rest of the production cycle (Figure 4.4a). A cage by period interaction ($P < 0.0001$), indicated higher Ca depositions in egg yolks obtained from the EC compared to CC system in the earlier (period 2 and 3) and latter (periods 10 and 11) stages of the laying cycle (Figure 4.4a). However, there was no difference in the overall yolk Ca deposition due to the main effect of housing (34.3 vs. 33.5 ± 0.34 mg/hen/d, respectively for EC and CC systems) (Table 4.2). Although Ca deposition in egg white remained relatively constant for both systems (between 3 to 4 mg/hen/d) for most of the duration of the production cycle, higher levels were observed in periods 2 (for EC system) and 6 (for both systems) (Figure 4.4b). Overall, this resulted in hens housed in EC depositing more Ca in the egg white compared to those in the CC system (4.14 vs. 3.83 ± 0.04 mg/hen/d, $P < 0.0001$; Table 4.2).

Overall, (cumulative assessment of Ca content in shelled egg, Figure 4.4c) hens housed in the EC were found to deposit less ($P < 0.0001$) Ca in the egg (shelled) compared to those in the CC system (2.07 vs. 2.13 ± 0.01 g/hen/d, respectively;

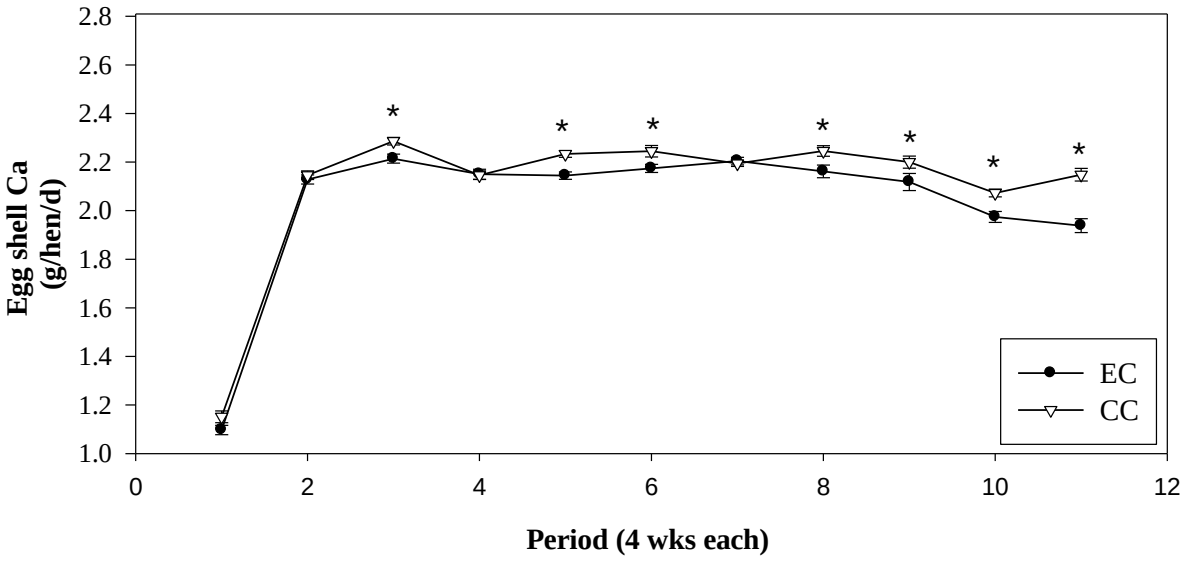

Figure 4.2. Daily calcium (Ca) deposition in egg shell (g/hen/d) by hens housed in enriched cage (EC) and conventional cage (CC) systems. Data are presented as least square means (LSM) with their standard errors (SE). Significant differences marked (*).at $P < 0.05$.

Table 4.4. Calcium (Ca) and phosphorus (P) outputs in egg shell and shelled egg (as mg/g egg and as a percentage of nutrient intakes) in laying hens housed in enriched cage (EC) and conventional (CC) systems [1]

	Nutrient output in egg shell (mg/g egg)		Egg shell Ca output	Nutrient output in shelled egg (mg/g egg)		Nutrient output in shelled egg (% Ca intake)	Nutrient output in shelled egg (% P intake)
	Ca	P	(% Ca intake)[5]	Ca	P[4]	Ca[5]	P
Cage[2]							
EC	37.4	0.042	55.5	38.3	1.5	56.5	14.4
CC	38.2	0.043	55.3	38.8	1.44	56.6	13.8
SE	0.15	0.002	0.39	0.15	0.01	0.51	0.11
Period[3]							
1	40.1	BD	58.5^{b}	40.9	1.15	59.6^{b}	10.9
2	39.1	BD	58.9^{ab}	40.0	1.32	60.3^{ab}	11.3
3	38.9	BD	57.6^{b}	39.8	1.37	59.0^{b}	12.0
4	36.8	BD	53.1^{c}	37.6	1.41	54.2^{c}	12.0
5	37.2	BD	51.5^{c}	37.9	1.45	52.4^{c}	12.5
6	37.4	0.079^{b}	52.6^{c}	38.1	1.66	53.6^{c}	14.2
7	38.2	0.077^{b}	-	38.9	1.69	-	15.8
8	37.8	0.071^{b}	-	38.5	1.55	-	15.2
9	37.4	0.129^{a}	-	38.1	1.57	-	22.3
10	36.2	0.042^{c}	-	36.9	1.55	-	14.5
11	36.8	0.071^{b}	-	37.4	1.49	-	14.5
SE	0.29	0.004	0.47	0.3	0.02	0.62	0.19
P-value							
Cage	< 0.01	NS	NS	< 0.05	< 0.0001	NS	< 0.0001
Period	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cage × Period	< 0.01	< 0.0001	NS	< 0.001	< 0.0001	NS	< 0.0001

$^{a–c}$ Different superscripts within each variable (column) are significantly different at $P < 0.05$

[1]Data are presented as least square means (LSM) and their standard errors (SE)

[2]LSM as main effect of cage type

[3]LSM as main effect of period on overall mean of CC and EC systems

[4]Phosphorus in egg shell were below detection levels (BD) in periods 1 to 5, hence considered as zero in statistical analysis

[5]For periods 7 to 11 values for Ca intakes were disregarded hence affecting the corresponding computations (each variable, n=120)

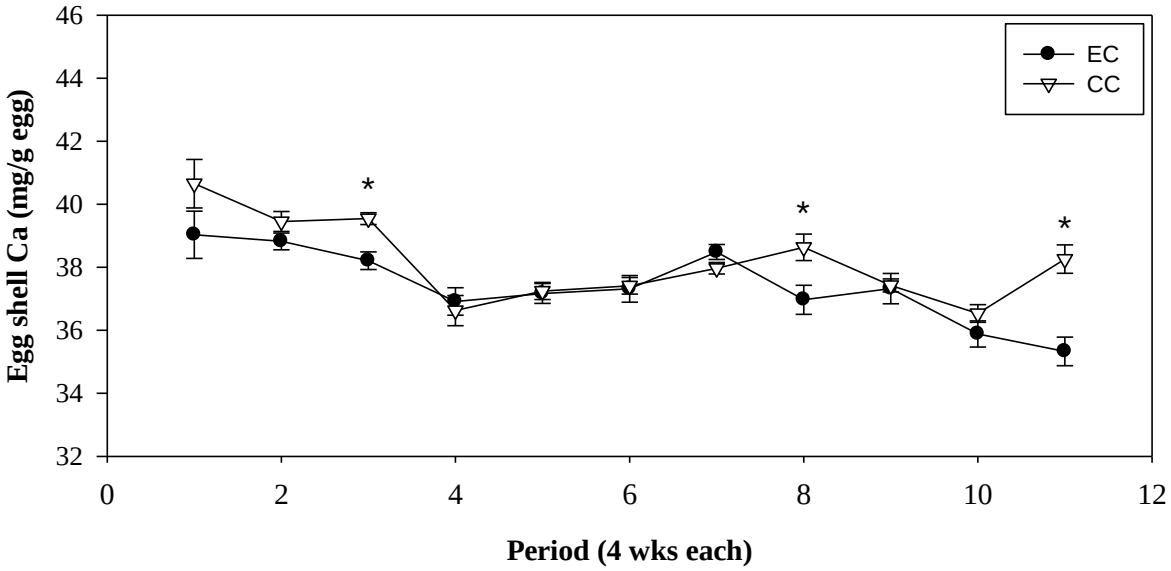

Figure 4.3. Calcium (Ca) output in egg shell (mg/g egg) obtained from hens housed in enriched cage (EC) and conventional cage (CC) systems. Data are presented as least square means (LSM) with their standard errors (SE). Significant differences marked (*) at $P < 0.05$.

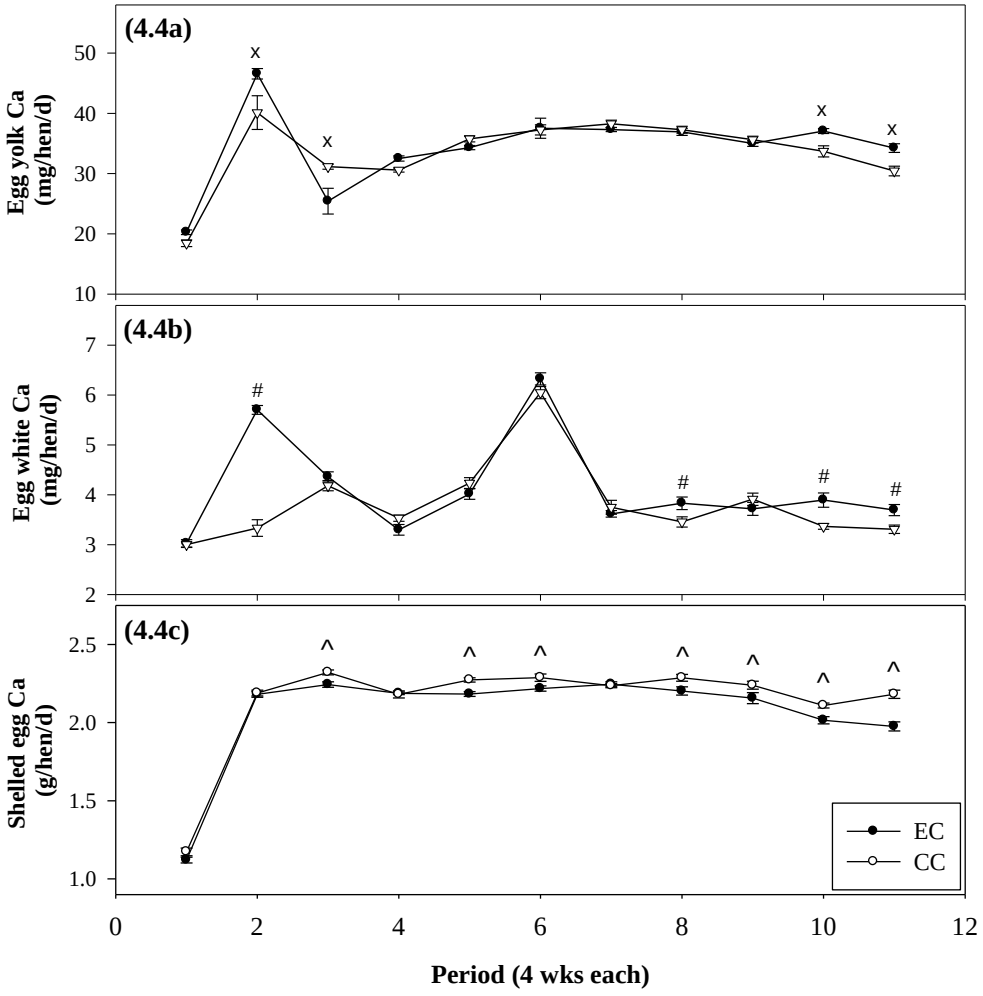

Figure 4.4. Daily calcium (Ca) deposition in a) egg yolk, b) egg white (mg/hen/d) and c) shelled (whole) egg (g/hen/d) by hens housed in enriched cage (EC) and conventional cage (CC) systems. Data are presented as least square means (LSM) with their standard errors (SE). Significant differences at $P < 0.05$, for Ca deposition in egg yolk, egg white and shelled egg are marked by x, # and ^ , respectively.

Table 4.2). The cage by period effect ($P < 0.0001$) indicating greater Ca deposition in shelled eggs for the CC than the EC system for most of the periods (Figure 4.4c) were similar to those noted for the egg shells (Figure 4.2). This was equivalent to an overall Ca deposits of 38.3 vs. 38.8 ± 0.15 mg/g egg, respectively which was also lower ($P < 0.05$) in eggs produced under the EC compared to the CC systems (Table 4.4). A cage by period effect ($P < 0.001$; Figure 4.5) was observed in periods 3, 8 and 11 that was higher for the CC obtained eggs compared to those from the EC system. However, there was no difference on intake basis (56.5 vs. 56.6 ± 0.51 as % Ca intake, respectively) in the content of Ca in shelled eggs between the two systems (Table 4.4).

Phosphorus deposition/output in individual egg components was not statistically different due to the main effect of caging (egg shell: 2.57 vs. 2.71 ± 0.18; egg white: 3.72 vs. 3.81 ± 0.04; egg yolk: 76.0 vs. 74.8 ± 0.46 mg/hen/d; respectively for EC and CC systems) (Table 4.3). However, cage by period interactions ($P < 0.0001$) was observed as shown for egg white (Figure 4.6a) and egg yolk (Figure 4.6b). For egg shell, P levels in the first 5 periods of the experiment were below detection levels (< 0.1%) and were indicated as zeros for statistical analysis (Table 4.3 and 4.4). After period 1, there was a gradual deposition of P in egg white (apart from period 9) and egg yolk ranging between 3.5 to 4.5 and 70 to 90 mg/hen/d for egg white and egg yolk, respectively. Overall, there were higher ($P < 0.05$) levels of P being deposited in eggs from EC compared to CC systems i.e. 82.3 vs. 81.1 ± 0.39 mg/hen/d, respectively (Table 4.3), a result of a cage by period effect (Figure 4.6c) possibly arising from greater P deposits in the egg yolk, it was consistently higher at the latter stages of the production cycle (Figure 4.6b). Similarly, P content in shelled egg, both in mg/g egg (EC: 1.50 vs. 1.44 ± 0.01) and % of P intake

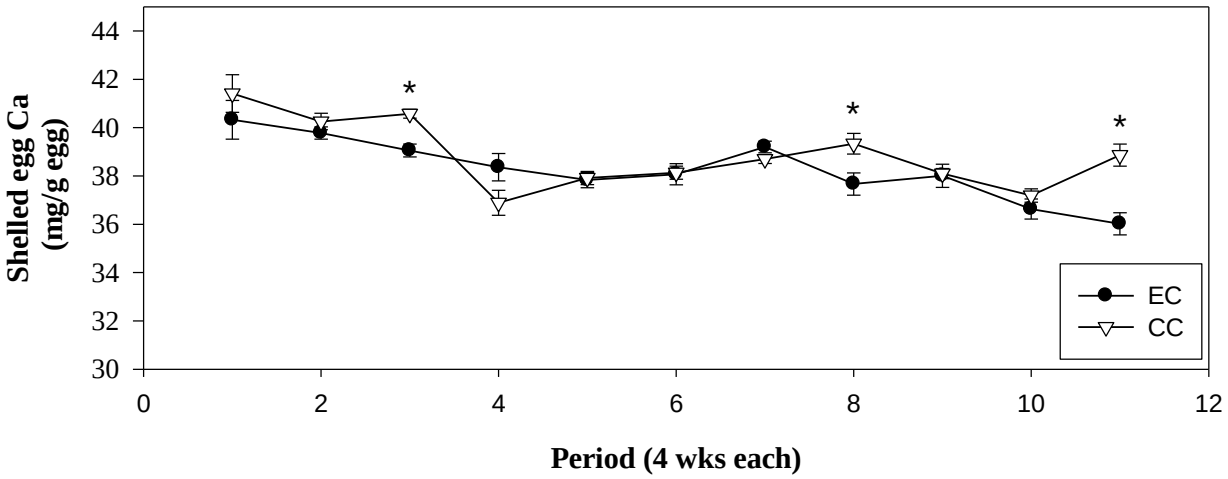

Figure 4.5. Calcium (Ca) output in shelled eggs (mg/g egg) by hens housed in enriched cage (EC) and conventional cage (CC) systems. Data are presented as least square means (LSM) with their standard errors (SE). Significant differences (*) observed at $P < 0.05$.

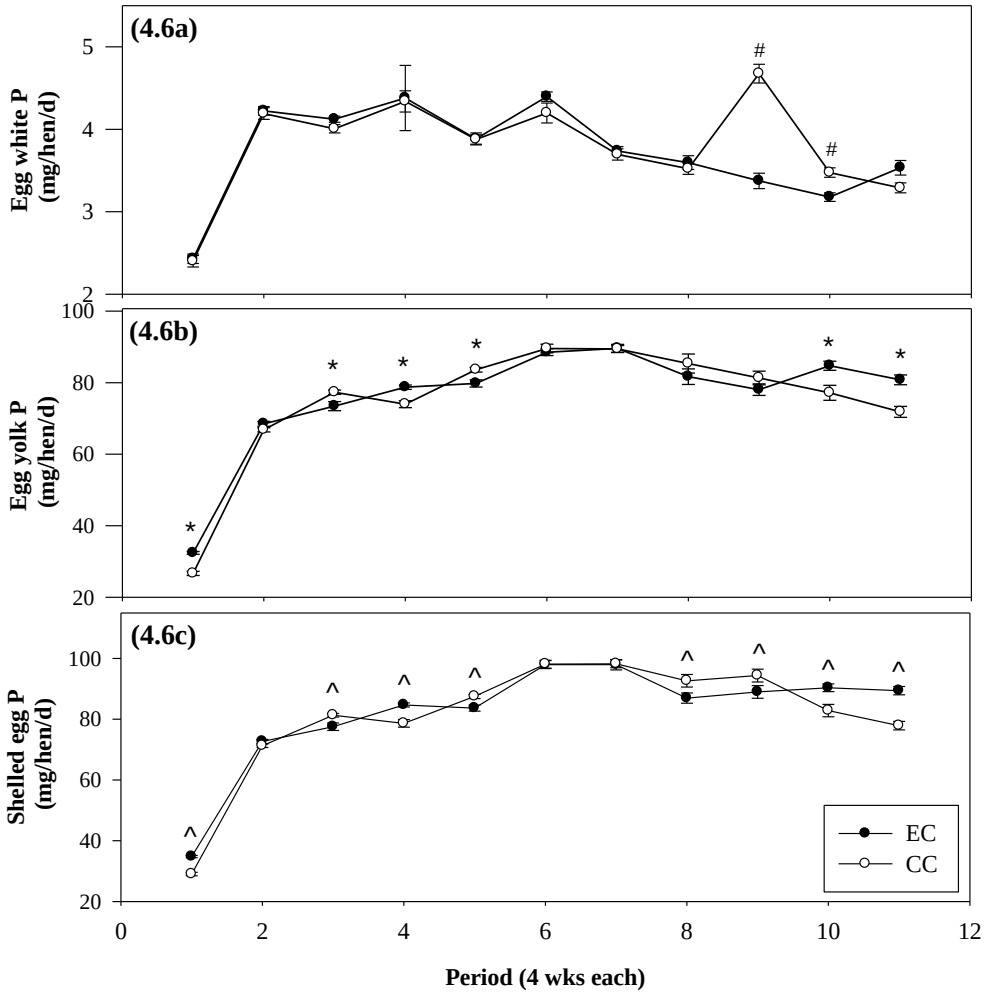

Figure 4.6. Daily phosphorus (P) deposition in a) egg white, b) egg yolk and c) shelled (whole) egg (mg/hen/d) by hens housed in either enriched cage (EC) or conventional cage (CC) systems. Data are presented as least square means (LSM) with their standard errors (SE). Significant differences at $P < 0.05$, for P deposition in egg white, egg yolk and shelled (whole) egg, are marked by #, * and ^, respectively.

(EC: 14.4 vs. CC: 13.8 ± 0.11) were also greater in the EC than the CC system ($P < 0.0001$; Table 4.4). A significant cage by period interactions (Figure 4.7a and b, respectively) indicating higher P in shelled egg in the EC compared to the CC system is likely due to wider differences at the latter stages of the production cycle.

4.4.4 Calcium Balance/Retention and Excretion in Manure

Unlike birds in the EC system, those in CC units were already in a negative Ca balance at the start of the measurements (Table 4.5). However, the levels of feed disappearance at the start of the experiment (periods 1 and 2; Figure 4.1a) were not different in both systems. Egg production peaked in period 4 by which time Ca balance for hens in the EC system became positive after remaining in negative balance in periods 2 and 3 (Table 4.5). Similarly, Ca balance for hens in the CC system started to improve by period 4, although it still remained in negative balance. This can be explained by the reduced Ca excretion in manure (Table 4.2), during periods of higher egg lay (periods 2 to 5; Figure 4.8a), resulting in a high and positive Ca balance in period 5 for both cage systems (Table 4.5). Both groups of hens again experienced another phase of negative balance after period 5 (as indicated in period 6). Although negative, overall Ca balance was higher ($P < 0.05$) with EC caged hens than those in CC (-1.37 vs. -4.76 ± 0.89, as a % of Ca intake; Table 4.2). In addition, overall mean manure Ca excretion was found to be lower ($P < 0.01$) in the EC than CC birds (2.11 vs. 2.29 ± 0.04 g/hen/d, respectively). This was based on analyzed manure DM output of 34.1 vs. 31.0 ± 0.32 % for EC and CC, respectively ($P < 0.0001$) (Neijat et al., 2011).

4.4.5 Phosphorus Balance/Retention and Excretion in Manure

The trends for P balance and manure P excretion are shown in Table 4.5 and Figure 4.8b

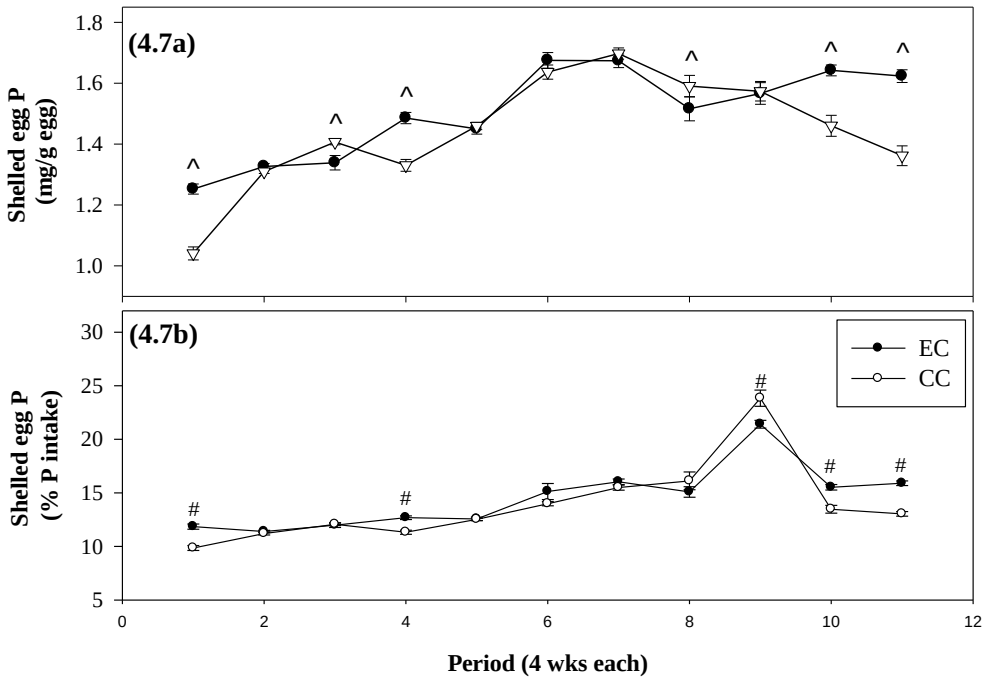

Figure 4.7. Daily phosphorus (P) output in shelled egg in a) mg/hen/d and b) % of P intake by hens housed in enriched cage (EC) and conventional cage (CC) systems

Table 4.5. Average daily intake and balance of calcium (Ca) and phosphorus (P) in laying hens during the entire production cycle under enriched cage (EC) and conventional (CC) systems [1]

		Nutrient intake			Nutrient balance		
Nutrients	Periods	EC	CC	*P*- value[2]	EC	CC	*P* - value[2]
Calcium	1	3.45	3.51	NS	0.035	-0.320	*
(g/hen/d)	2	3.73	3.76	NS	-0.182	-0.238	NS
	3	3.94	4.06	*	-0.161	-0.287	NS
	4	4.07	4.20	*	0.036	-0.015	NS
	5	4.29	4.44	*	0.219	0.236	NS
	6	4.31	4.40	NS	-0.276	-0.487	NS
	7	-	-	-	-	-	-
	8	-	-	-	-	-	-
	9	-	-	-	-	-	-
	10	-	-	-	-	-	-
	11	-	-	-	-	-	-
	SE	0.04			0.068		
Phosphorus	1	528	537	NS	15.8	20.6	NS
(mg/hen/d)	2	655	660	NS	-54.6	-58.3	NS
	3	665	684	*	-20.3	-54.6	*
	4	686	708	*	-58.7	-18.6	NS
	5	689	713	*	-1.9	-0.2	NS
	6	709	724	NS	-51.7	-53.4	NS
	7	648	670	*	-82.6	-77.9	NS
	8	623	650	*	-138.4	-184.4	*
	9	727	751	*	31.0	40.3	NS
	10	660	668	NS	-60.9	-65.0	NS
	11	638	660	*	-92.1	-96.6	NS
	SE	6.09			12.2		

[1]Data are presented as least square means (LSM), their standard errors (SE)
[2]*P*-values compares differences between cage systems for each variable within a period, difference are significant (*) at $P < 0.05$
For periods 7 to 11 Ca intakes and balances were disregarded (n=120)

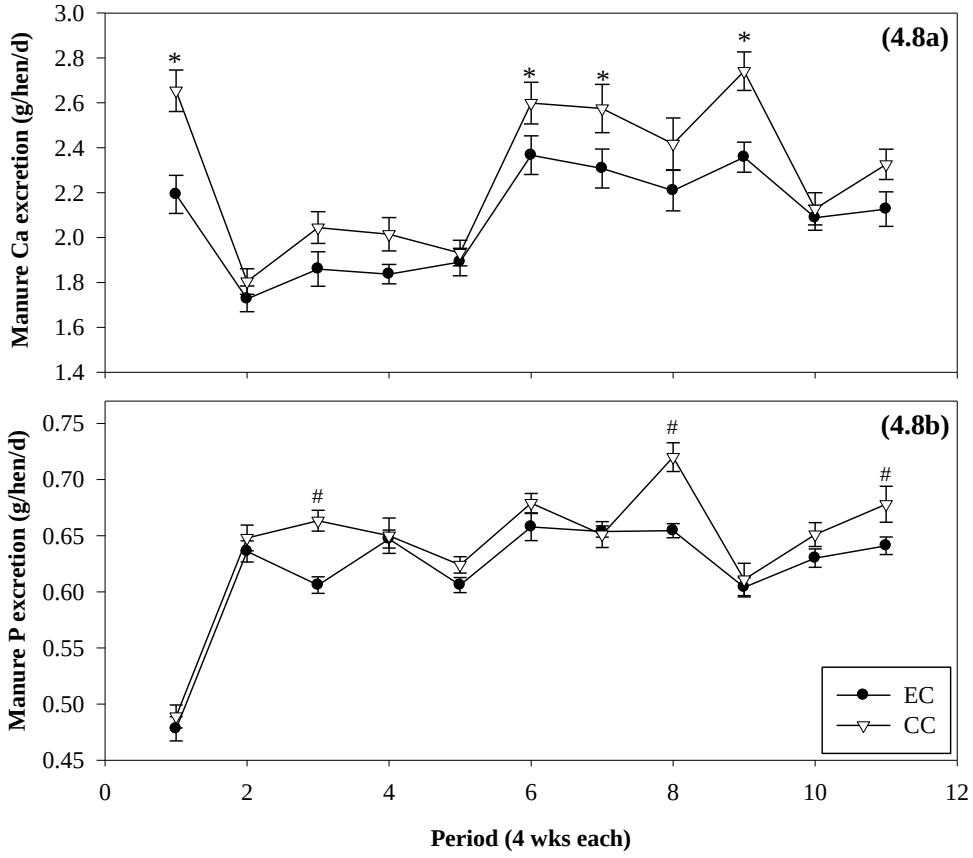

Figure 4.8. Manure a) calcium (Ca) and b) phosphorus (P) losses for Shaver White laying hens housed in enriched (EC) and conventional (CC) cage systems. Data are presented as least square means (LSM). Differences between cage systems within a period are significantly different (* or #) at $P < 0.05$.

Apart from a positive balance in period 1 (both systems), P balance in periods 2 to 6 (in the first half of the production cycle) were similar to the patterns of Ca balance (with a higher balance in period 5; Table 4.5). However, the trend for P balance in the second half of the cycle fluctuated through the remaining periods. Highly positive P balance in period 9 for both groups was likely due to high P intake levels in the same period. The explanation for this is not clear, because there was no difference in the amount of feed disappearance in both systems. Feed disappearance for period 9 was the same as for period 8 in the EC birds (Figure 4.1a) and was slightly less than period 8 for the CC birds, which was reflected in higher retention in both systems for period 9. Moreover, birds in both systems did not cause an increase in manure P excretion in period 9 compared to other periods with lesser P intake levels (Figure 4.8b). Although there was no significant difference in the overall mean P balance between the two systems (-7.22 vs. -7.45 ± 0.71 % of P intake, respectively for EC and CC), manure P excretion was significantly lower ($P < 0.01$) in EC hens than CC ones (619 vs. 643 ± 5.23 mg/hen/d, respectively; Table 4.3).

4.4.6 Egg Shell Quality and Egg Component Weights

There was no significant overall difference in the shell quality assessments between the two systems of housing for laying hens (Table 4.6). These measures were air dried shell weight (5.79 vs. 5.78 ± 0.03 g/egg), shell thickness (385 vs. 385 ± 1.35 microns), egg specific gravity (1.087 vs. 1.087 ± 0.0001) and percentage of shell (9.72 vs. 9.68 ± 0.04 %) for EC and CC systems, respectively. However, there was a cage by period interaction for egg shell weight. Air dried egg shell weight was significantly

Table 4.6. Egg shell quality assessment for Shaver White laying hens housed under enriched (EC) and conventional cage (CC) systems over the entire production cycle (as is basis) [1]

	Egg shell wt (g/egg)	Egg shell thickness (microns)	Egg specific gravity (Absolute)	Percent shell (%)[5]
Cage[2]				
EC	5.79	385	1.0865	9.72
CC	5.78	385	1.0866	9.68
SE	0.03	1.35	0.0001	0.04
Period[3]				
1	5.13	391^{a}	1.0890^{a}	10.20^{a}
2	5.62	392^{a}	1.0878^{bc}	9.98^{ab}
3	5.78	394^{a}	1.0880^{b}	9.75^{bc}
4	5.84	387^{ab}	1.0857^{de}	9.78^{bc}
5	5.90	387^{ab}	1.0865^{d}	9.77^{bc}
6	5.92	386^{ab}	1.0865^{d}	9.66^{cd}
7	5.90	387^{ab}	1.0870^{c}	9.66^{cd}
8	5.87	377^{bc}	1.0858^{de}	9.52^{cde}
9	5.90	372^{c}	1.0861^{de}	9.44^{de}
10	5.95	382^{abc}	1.0854^{e}	9.60^{cde}
11	5.80	375^{bc}	1.0852^{e}	9.33^{e}
SE	0.04	2.81	0.0002	0.06
P-value				
Cage	NS	NS	NS	NS
Period	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cage × Period[4]	< 0.05	NS	NS	NS

[a–e] Different superscripts within each variable are significantly different at $P < 0.05$
[1]Data are presented as least square means (LSM) and their standard errors (SE)
[2]LSM as main effect of cage type
[3]LSM as main effect of period on overall mean of CC and EC systems
[4]Cage × Period indicate interaction between caging system and period
[5]Percent shell was expressed as a percentage of egg weight

greater ($P < 0.05$; Table 4.6) in periods 10 and 11 (6.06 vs. 5.84 and 5.90 vs. 5.71 ± 0.06 g/egg for EC than CC system, respectively). Similarly, a separate assessment of the weights of the different egg components, expressed as mg per g egg (DM basis) was presented in Figure 4.9. There was no overall difference between the two systems in the overall mean egg shell weight (EC: 100.0 vs. CC: 100.9 ± 0.35, mg/g egg; data not shown in Tables). However, there was a cage by period effect ($P < 0.05$) in periods 3, 8 and 11 (Figure 4.9a) showing higher values for CC than the EC produced eggs. Differences in the two measures of egg shell weights could be attributable to the effect of extended cold storage of eggs which is found to decrease egg weight due to moisture loss (Butcher and Miles, 2004; Jones and Musgrove, 2005). This might have accounted for the observed lower overall egg shell weight in our study compared to a previous study by Valkonen et al. (2010). Consequently, this may influence egg specific gravity (Butcher and Miles, 2004); however, these influences apply equally to both cage systems.

While the proportion of egg mass allocated to all three egg components changed with age ($P < 0.0001$; Figure 4.9), there was no difference in the proportional weights of the other two egg components (egg yolk and egg white) between the two systems. On a DM basis, egg yolk ($P = 0.956$; Figure 4.9b) obtained from both systems weighed 135 ± 0.59 mg/g egg and egg white ($P = 0.242$; Figure 4.9c) weighed 71.8 vs. 71.3 ± 0.31 mg/g egg for EC and CC, respectively, although in the latter stages of production (periods 10 and 11), eggs from the EC system had more egg white compared to those from the CC cages.

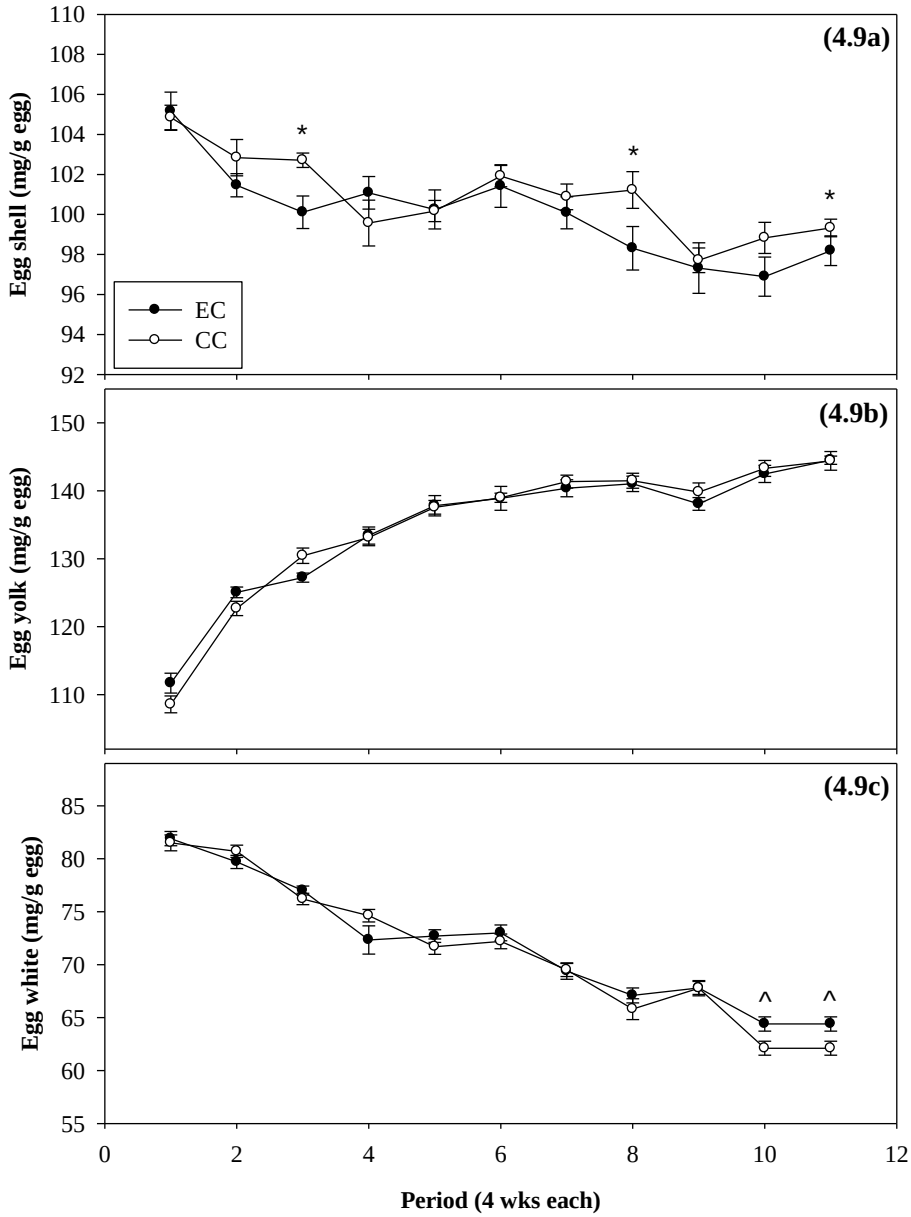

Figure 4.9. Individual egg component weights (DM basis) for Shaver White laying hens housed in either enriched (EC) or conventional (CC) cage systems: a) egg shell, b) egg yolk and c) egg white. Data are presented as least square means (LSM). Differences between cage systems within a period are significantly different (* or ^) at $P < 0.05$.

4.5 DISCUSSION

Overall, hens were provided the recommended intake levels of Ca (3.97 vs. 4.06 ± 0.03 g/hen/d; Tables 4.2) in accordance with Roland (1986), National Research Council (1994) and Chandramoni et al. (1998); and P (657 vs. 675 ± 4.35 mg/hen/d; Table 4.3) in accordance with Miles et al. (1983), Chandramoni et al. (1998) and ISA (2009), respectively for EC and CC hens. Although there was no significant difference in egg production and egg weight between the two systems, lower Ca and P excretion in EC hens coupled with higher Ca balance was observed in EC housed hens. Similarly, there was no significant difference between the two systems in the egg shell quality assessments.

The recommended specifications for Ca in a phase feeding program, according to ISA (2009) should be 4.2, 4.3 and 4.4 % for 19 - 42, 43 - 54 and 55 - 63 wks of age group (i.e., periods 1 - 6, 7 - 9 and 10 - 11), respectively. These levels were obtained during periods 1 to 6. However, representative feed samples for periods 7 to 11 was not obtained due to challenges with diet segregation as a result of the nature of the particulate limestone used. Therefore, data from these periods were not included in the analysis. Feed disappearance, egg production, and egg shell quality measures were not negatively impacted during periods 7 to 11, which implies that the challenges experienced in this study related to obtaining representative feed samples was not due to inaccuracies in feed formulation or delivery of sufficient Ca (or other nutrients) to the hens. Corresponding P intakes for periods 7 to 11 were considered, because total and available P contents in the feed were within the recommended limits for laying hens (Miles et al., 1983;

Chandramoni et al., 1998; ISA, 2009). However, fluctuations in the trend of P balances in both groups of hens in the second half of the laying cycle is likely due to variation in the levels of dietary Ca which has an influence on P retention in laying hens (Scheideler and Sell, 1986).

Although feed disappearance in periods 1 and 2 was not significantly different between the two systems, hens in the CC system were already in a negative Ca balance at the start of the measurements (Table 4.5). In sourcing Ca, the laying hens obtain the majority from the diet (60-75%) while the remaining 25-40% is taken from skeletal stores (Mueller et al., 1964). Hence, during egg formation, this requires the hen to maintain adequate Ca reserves (Roland and Harms, 1973) in the extracellular Ca pool of the small intestine (De Vries et al., 2010). This occurs mainly in the anterior part of the small intestine which is the major site of Ca absorption (Hurwitz and Bar, 1967). However, it is also important to note that the intestinal capability to absorb Ca does not reach its maximum at the onset of production, but gradually increases during the early laying periods (Scott and Balnave, 1991). Therefore, these results imply that, at the beginning of the production cycle, the birds were possibly mobilizing more Ca from bone reserves. This is also supported by the high positive P balance at the beginning of the production cycle (period 1) which implies excess P (from Ca phosphate) has been released when the Ca is removed from the bone for shell formation (Boorman and Gunaratne, 2001; Ahmad and Balander, 2004). This is reflected in the higher negative Ca balance ($P < 0.05$) during the early production periods which was exacerbated by higher Ca deposition in the egg from the CC compared to the EC hens during the same period compared to other periods observed from the trend of Ca outputs in egg or egg shell (mg/g egg; Table 4.4). In

addition, regardless of the amount of intake, the bird can reach a plateau in absorption of around 1 to 1.5 g of Ca/d. Yet, the egg shell of the same bird needs a deposit of about 2 g of Ca daily (Simkiss, 1961, Summers et al., 1976). However, Ca supply from intestinal absorption alone is inadequate (Hurwitz and Bar, 1967). It is also possible that by period 5 (36 to 39 wks of age) when positive Ca balance was achieved, hens in both housing systems could have attained peak Ca retention or their maximum ability to absorb Ca from the intestine. In a previous study, Parkinson and Cransberg (1999) observed peak Ca reserves to occur at 30 weeks of age.

Higher ($P < 0.05$) overall Ca balance and lower ($P < 0.01$) manure Ca excretion observed with EC caged hens compared with those in CC are in line with earlier studies by Guru et al. (1974) who noted the influence of the degree of confinement of laying hens (based on floor space and the duration of caging) on percentage retention of Ca and P, suggesting that the birds kept in severe confinement (smaller cage space) for a prolonged period tend to excrete larger amounts of Ca with a subsequent decrease in P retention. However, in the current study, although manure P excretion was significantly lower ($P < 0.01$) in the EC caged hens compared to those in the CC system, no difference was noted in the overall mean P balance between the two systems. Although the actual loss of nutrients in the manure of a given animal may vary with management, diet and the age of animal; previous studies by Spiehs (2005) measured 30 kg P /ton manure from poultry. This was equivalent to approximately 0.62 g/hen/d manure P (based on a daily average manure excretion of 27 g/hen at 30% DM), similar to the result for our study in which daily manure P excretion in both systems were on average 0.62 and 0.64 ± 0.005 g/hen for EC and CC systems. In this study, although the manure samples contained a

mixture of excreta, spilled water and feed, feathers and broken eggs, it (the collected manure) provides a true representation of manure samples obtained from a standard commercial based poultry production system.

In both systems, the reduction in manure Ca excretions was more pronounced during periods of high egg lay (periods 2 to 5). This agrees with the theory that, in hens, Ca requirement reaches a maximum level during peak egg production when egg yolk material formation (Taylor, 1972) and egg shell formation (Whitehead and Fleming, 2000) are also greatest. During these periods, there was no significant difference between the two systems in manure Ca excretion. Similarly, the occurrence of bone related problems in hens, such as osteoporosis, have been related mainly to the lack of exercise available in cages (Fleming et al., 1994; Tauson, 1998) and not primarily due to deficiency of Ca (Rennie et al., 1997; Whitehead and Fleming, 2000). However, from the current study, a higher overall Ca balance, coupled with the reduction in manure Ca and P excretion as observed in EC hens as compared to those in the CC system, agree with reports from previous studies (Kopka et al., 2003; Tactacan et al., 2009) indicating improved bone mineral density and bone strength for hens housed in the EC system.

As reservoir for Ca, the egg shell (constituting approximately 98% of the total Ca deposition in shelled egg in both systems as in this study) is considered as a natural packaging material (Rodriguez-Navarro et al., 2002). Hence, maintaining a good quality shell throughout the production cycle is of importance not only from a health and economic point of view to the egg consumers and producers (Hughes et al., 1986) but also to the breeder industry where the egg shell porosity is of concern during embryonic development (Reynard and Savory, 1999). The most commonly used indicators of Ca

metabolism in layers relate to shell quality assessment parameters (Gordon and Roland, 1998). In our study, we considered both the chemical (Ca and P levels) and physical (egg shell weight, thickness, specific gravity of egg and percent shell) qualities.

With respect to chemical analysis, although there was lower Ca content in egg shells derived from the EC as compared to the CC system, EC hens were found to be more efficient in depositing Ca in their egg shell than their CC counterparts because Ca output in egg shell, expressed as a percentage of Ca intake was not significantly different between the two systems. This indicates that a lower daily intake of Ca by hens housed in the EC led to the production of eggs with similar egg shell quality as compared to those obtained from the CC system. The influence of housing environment on egg Ca levels were also noted by Mench et al. (1986) who reported higher Ca levels in eggs from caged hens compared with those in floor pen management systems. On the other hand, although P deposition in egg shell between the two groups of hens showed no significant difference due to the main effect of housing, the interaction of cage by period effect was highly significant. This was probably due to the non uniformity of distribution in the amounts of P in egg shell (Hossain and Bertechini, 1998). Similar explanation, could also apply to the inconsistent trends due to the cage by period effects observed for both minerals in egg white because it contained lower levels of the minerals.

Previous reports regarding egg shell quality from caged hens in EC and CC systems have been contradictory. Although Vits et al. (2005) reported stronger egg shells from birds in furnished cages as compared to those from a CC system, Wall et al. (2002) observed a lower percentage of broken eggs in CC housing than those in furnished cages. In the current study, the overall higher Ca content in egg shells from the CC compared to

the EC system agrees with previous studies that reported lower percentage of broken eggs in CC system as compared to those in the EC. However, Guesdon et al. (2006) explained differences in egg breakage may be due to the influence of cage design including the installation of perches (Duncan et al., 1992; Abrahamsson and Tauson, 1998) rather than specific cage effects. This could also be supported by the current results for physical egg shell quality measurements where no significant differences between the two systems were observed. Hence, this supports Guesdon et al. (2006) findings relating an increase in the incidence of egg breakage with EC systems to cage design rather than differences in egg shell quality. Egg shell quality parameters deteriorate with increasing age (Riczu et al., 2004), with the exception of egg shell weight, which increases with age but at a slower rate compared to the weight of egg yolk (Johnston and Gous, 2007). The latter observations agree with the current results for both systems used in this study (Table 4.6). Finally, there was no significant difference in the overall mean weights of the individual egg components between the two systems (Figure 4.9). The influence of storage period on egg weight (Jones and Musgrove, 2005) may also explain the observed variations in egg shell weight (Figure 4.9a) in contrast to the close values for egg yolk and egg white (Figure 4.9b and c, respectively).

4.6 CONCLUSION

In conclusion, the study showed an overall higher Ca balance and reduced manure Ca and P excretion in hens housed in EC compared with CC systems. Although research to explain the biological mechanism by which environmental enrichment may affect

nutrient balance in hens is limited, the current results indicate that EC systems may provide better means of utilizing Ca and P. Hence, such a comparative study of Ca and P measures in Shaver White laying hens housed under EC and CC systems may not only provide more evidence as to the advantages of EC than CC systems for housing hens, but may also serve as a basis for further improvement in the system's design.

CHAPTER 5 GENERAL DISCUSSION

The main objective of the current study was to compare the dynamics of N, Ca, and P flow in Shaver White laying hens housed in two distinct cage systems: EC and CC systems. The comparison was based on the assessment of the flow of nutrients (N, Ca and P) from feed to egg components and excretion in manure. This information is relevant when evaluating alternative housing systems to the traditional (conventional) type. Because the CC systems do not allow hens to fulfill most of their natural behaviours, they pose important welfare concerns to laying hens (Baxter, 1994). To this end, cage systems which enable the birds to carry out behavioural repertoire by including nests, perches and a scratch area/litter, as provided in EC systems, have been considered among the alternative housing systems for laying hens according to the European Union Council Directive *1999/74/EC* (European Commission, 1999). However, the EC systems were originally developed in Europe: The current study contributes to the re-evaluation of its proposed benefits based on North American conditions. Although biological responses in laying hens in relation to cage environment may not be clear, this section focuses on understanding other possible explanations for the differences between the two systems and their implications.

High bird density (decreased cage space) has been used to increase net income in egg production by utilizing available housing facilities, equipment, and labour cost per area of housing to maximum capacity (Adams and Craig, 1985); however, this has been found to result in bird stress and impaired bird welfare (Mtileni et al., 2007; Rios et al., 2009), causing significant effects on their performance (Cunningham et al., 1987; Craig

and Milliken, 1989; Jalal et al., 2006). In addition, since dietary nutrient requirements for hens are based on daily intake, management factors such as stocking density, feeder space and cage features can have a large impact on feed utilization and subsequent nutrient flow.

Results from studies comparing feed intake of hens housed under CC and EC systems are inconclusive. This could result from differences in the eating behaviours of hens under different housing environment (Pohle and Cheng, 2009). High stocking densities of birds have been related to a decrease in feed consumption because of the high heat generated by cage mates (Appleby and Hughes, 1991) which has a direct influence on body temperature (Gonzalez et al., 2008). This may lead to lower levels of intake by birds housed in CC systems compared to those in EC, as reported by Preisinger (2000) and Pohle and Cheng (2009). However, the type of housing system can also influence the level of activity of the birds and therefore their energy requirements (Poultryhub, 2010). It (type of housing) can influence the level of stress in the birds **(**Jones, 1996), altering energy metabolism (Etches, 1976) which may in turn affect feed intake and consequently nutrient utilization by hens.

Environmental enrichment during the rearing of birds has been considered to be one of the methods available to provide the benefits of reduced stress (Reed et al., 1993; Cheng et al., 2003). As discussed in Chapter 3, the lower feed intake by hens housed in cages equipped with perches (as in the furnished types of cages) as compared to those kept without a perch (Tauson and Jansson, 1988; Braastad, 1990; Glatz and Barnett, 1996), is possibly due to reduced stress (Cheng et al., 2003). However, in determining the level of stress between the two systems, Pavlik et al. (2008) and Tactacan et al. (2009)

found no differences in the corticosterone levels in birds housed in EC and CC, suggesting a lack of difference in the stress levels between birds housed in these cage systems. In fact, housing technologies which are more similar to the animal's natural environment (as provided by the different features in EC system) do not necessarily imply decreased levels of plasma corticosterone (Pavlik et al., 2008). Different housing systems have variable abilities to provide the appropriate micro-environment to the hens, thereby affecting her comfort, health and resource utilization efficiency (Xin et al., 2011).

Furthermore, differences in feed disappearance between the two cage systems could be attributable to differences in feeder space and design. In this study, approximately 128 and 104 cm^2/hen feeder space was provided by the EC and CC systems, respectively. In a previous study, Tactacan et al. (2009), using 5 hens/cage for CC system (providing an average floor space of 561.9 cm^2/hen) and 24 hens/cage for EC housing (providing an average floor space of 642.6 cm^2/hen) provided feeder spaces of 128 and 125 cm^2/hen, respectively (Tactacan et al., pers. Comm., 2011). In their study, the authors did not find a statistical difference in the overall feed disappearance due to cage type. However, in the current study, the smaller feeder space in the CC system may have led to competition and aggressive feeding behaviour which may have contributed to potential differences in feed disappearance which is a possible indicator of increased feed wastage (Thogerson et al., 2009).

On a commercial basis, feed wastage can be an important component when comparing different housing systems. For example, it has been shown that in general, there is a 1.5% increase in the excretion of N and P in the manure for every 1% increase in feed wastage (Nahm, 2007). This could have a consequence on nutrient excretion

levels in manure. However, in the current study, the interactions between cage type and period (age of the bird) on manure N excretion showed higher N excretion in manure for the EC compared to the CC housed hens at later stages of production (Table 3.3). This suggests that feed wastage, which was assumed to be higher in the CC compared to the EC system, may not to be a confounding factor, possibly because of the measures undertaken to minimize feed spillage during the course of the study. Although there is no clear scientific evidence for this trend in N flow in the laying hens, this did not reflect in an overall significant difference in either body weight (1.67 vs. 1.67 ± 0.01 kg/hen) or shelled (whole) egg N content (18.4 vs. 18.4 ± 0.04 mg/g egg) or feathering score between the two groups of hens. Feathering score was measured at the end of the experiment, using a 4-point scoring system described by Tauson et al., 1984; EC: 2.33 vs. CC: 2.16 ± 0.33, data not shown in Tables. Overall mean manure N excretion between the two groups of hens was not statistically different (Table 3.2). From this study, overall, Shaver White hens have the potential for excreting approximately, 0.61 kg N /hen/yr when house in either EC or CC systems. This was similar to excretion levels of 18.0 kg/ton manure at 30% DM (equivalent to 0.66 kg N/hen/yr) for caged birds under intensive production predicted by Nicholson et al. (1996). However, lower levels of 0.68 g/d per hen (equivalent to 0.25 kg/hen/yr N) were estimated for commercial Leghorn hens housed in a barn with a deep-pit manure system (Patterson, 1994). Although the differences in the levels of N excretion from the different studies may depend on a number of factors, including variations in dietary protein contents, age of the bird and management; there seems to be a similarity in the levels of N excretion between caged birds.

Although minimal differences were noted in the N flow comparisons between the EC and CC systems (Chapter 3), significant effects of comparative variables on Ca and P flows were noted between the two cage systems (Chapter 4). From this study, Ca and P excretions in manure were 9 and 4% higher, respectively, from hens housed in the CC compare to those in the EC system. The results also indicate that 7.3 kg/1000 hens more P, on an annual basis, is estimated to accumulate under the CC system as compared to the EC system. Poultry manure in general contains higher levels of P than is required for crop use (Manitoba Agriculture, Food and Rural Initiatives, 2004). Many soils can partially absorb much of the excess P, but substantial amounts of soluble and organic P run off into surface waters during heavy rainfall (Keplinger et al., 2005). Therefore, there are limitations regarding the application rates of the poultry manure to agricultural lands (Manitoba Agriculture, Food and Rural Initiatives, 2004), and this study provides evidence that manure obtained from the EC system could result in reduced P loads.

Although the overall mean P retention in hens (Table 4.3) was not significantly different between the two systems, the higher ($P < 0.05$) Ca retention in EC compared to those in CC hens (Table 4.2) supports the findings of improved bone mineral density for hens housed in EC system compared to CC that was observed in studies by Kopka et al. (2003) and Tactacan et al. (2009). It is likely that the combination of higher retention levels, lower excretion in manure and lower depositions of Ca in egg shell shown by hens in the EC system compared to those in the CC may have resulted in its accumulation in the bone. Whereas lack of exercise in cage systems can result in bone loss (Fleming et al., 1994; Tauson, 1998; Whitehead and Fleming, 2000); bone use, that can be facilitated by the provision of perches and more spacing in EC system than in the CC may encourage

the improvement of bone mineral buildup, considering the fact that there is a possibility of compromising bone strength even in non-cage system (Ferrante, 2009).

For egg mineral content, even though Ca deposition in shelled eggs showed a significant ($P < 0.0001$) cage by period effect, which was greater for the CC than the EC system for most of the periods during the laying cycle, egg shell quality measurements (egg specific gravity, shell weight, thickness and percent shell) were not different between the two systems. This is in agreement with the fact that quality features characterizing eggs from the different housing systems do not justify the higher prices for alternative eggs but rather a basis of welfare and ethical motivations (Hidalgo et al., 2008). In addition, the study also proves an economic benefit from the view that while the expression of Ca outputs in shelled eggs as a proportion of Ca intake indicates improved efficiency of utilization of Ca by hens in the EC system compared to their counterparts in the CC systems, the net effect of cage type can result in a reduction of Ca intake by 33 kg/1000 hens/yr for hens housed in the EC compared to those in the CC system. This difference may partly be reflected in offsetting the production costs of eggs for the EC system which increases when the area per bird in the cage is increased.

Finally, a comparison based on the manure weights from the two systems that showed a higher moisture content of manure from CC system compared to those from EC mainly as a result of bird density and the pattern of excretion of manure on the conveyor belts explained in Chapter 3, this will have an impact on manure management aspects such as maintenance cost of manure belt, transportation, size of manure storage and disposal. Furthermore, on a DM basis, a kg of feed disappearance resulted in 0.29 and 0.30 kg of manure output for hens from EC and CC systems, respectively. This indicates

better feed utilization by hens housed in the former compared to the latter system. Hence, the benefits with the move towards the use of EC system, does not only reduce manure nutrient excretion levels but also reduces the bulk of manure volume/weight. On average, DM manure out was reduced by 1.1g/hen/d from the EC systems which was equivalent to a reduction of 1200 tons manure/yr for a population of 3 million layers in Manitoba estimated by Statistics Canada (2009).

CHAPTER 6 SUMMARY AND CONCLUSIONS

The assessment of nutrient flows in laying hens over a production cycle is an important criterion in comparing alternative housing systems for sustainability in egg production while considering hen welfare and managing environment issues. Birds in the enriched cage system showed evidence of improved welfare from the environmental enrichment of increased space in cages and provision of cage features (nesting area, scratch pad and a roost area with perches) without compromising production performance. In addition, the system is also a possible means of reducing environmental pollution.

Based on the results obtained from this study, it can be concluded that with the enriched cage system:

1. Hens perform as well as those housed in the CC systems.
2. Reduction in feed intake and hence feed cost (on annual basis, approximately, 365 CAN $ for every 1000 layers would be saved provided barn environment is maintained around 20.3± 0.46^{o}C temperature and relative humidity of 43.3 ± 2.19%). (On average, feed cost was calculated to be 0.40 CAN $/kg).
3. Improved nutrient utilization because similar (e.g., N and Ca, on % intake basis) or higher (P) concentrations could be achieved in eggs with lower daily intake of these nutrients.

4. Reduced manure volume/weight (approximately, 0.4 ton less manure/1000 layers, annually).
5. Reduced Ca and P excretions in manure (approximately, a reduction of 65.7 kg Ca and 7.3 kg P/1000 layers, annually).
6. The overall higher Ca balance may support the evidence of higher mineral density in bones for EC hens as indicated in previous studies.
7. Lower N balance and higher manure N excretion levels at a later stage of the production cycle may coincide with the need to reduce egg size (not determined in the study) as a possible means for maintaining sustainability in egg production and egg shell quality.
8. For the hatching egg producer, the improvement of interior egg components mineral (Ca and P) contents may be of significance during embryo development.
9. Based on the similarities of egg shell quality, the study may provide basis for further improvement on the system's design especially in relation to managing quality egg production (includes egg breakages as identified in previous studies).

CHAPTER 7 FUTURE RESEARCH DIRECTIONS

This study provided more evidence that there are advantages of EC over the CC systems for housing laying hens. The next logical step is to investigate the biological mechanism(s) of how environmental enrichment may stimulate hens to improve their performance and welfare. In addition, some aspects of future research activity might focus on the following points:

- Shaver White hens are among the highly selected lines for egg production with a smaller body mass. But a similar study with heavier strains of birds such as the Bovan or Brown would be interesting to follow. Even if birds display similar behavioural repertoire in these systems, further study on the Ca-P dynamics relation to maintaing egg shell quality and bone strength in hens with heavier body mass, as compared to Shaver Whites, could be beneficial.

- Higher excretion levels of N in the manure from hens in the EC system and particularly towards the end of the production cycle, requires further understanding.

- Further work on Ca and P dynamics which resulted in higher retention levels and lower excretions in manure as shown by hens in the EC system is warranted to devise systems that would even be more efficient. This may also include the

application of phytase enzyme supplements to improve the utilization of these minerals.

- According to the literature, Ca deficiency does not play a vital role in bone loss in caged hens but in this study improved Ca retention in EC hens might have improved bone strength. Therefore, a study looking at Ca utilization efficiency and bone mineral density is recommended to better understand the relationship between Ca retention and bone strength.

- Furthermore, improvement of the cage design may also enhance exterior egg qualities (for example, reducing egg breakage and achieving cleaner eggs).

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APPENDIX

Appendix I. Descriptions of cage designs

The enriched and conventional cages used for this experiment were of the following design and were previous described in details by Tactacan et al. (2009).

Enriched cages: The enriched cages (EC) were of Hellmann furnished-cage model (Hellmann Poultry GmbH & Co., Telbrake, Vechta, Germany), designed with an area of 241 cm length, 64 cm depth and 48 – 53 cm height. This system contained two perches (along the cage length), strip curtained nest/lay area (58 cm width x 25 cm depth), roost area (122 cm width x 46 cm depth), scratch pad (48 cm width x 20 cm depth) and 2 claw trimmers (2.5 cm thickness x 15 cm length) placed in front of the feeder area. Each cage unit housed 24 laying hens. The average floor space area per bird was 642 cm^2. The scratch-pad area was made of rough artificial turf - AstroTurf (AstroTurf LLC, Dalton, GA), to allow birds to scratch and "scavenge or forage".

Conventional cages: Conventional cages (CC) consisted of 4 compartments each with dimensions of 51 cm length x 56 cm depth, and 38 to 43 cm height. Each compartment housed 6 laying hens. The average floor space area per bird was 468 cm^2.

Both cage systems consisted of feeders that ran along the row with dimensions: width at the base of feeder = 8.9 cm, depth of feeder = 12.7 cm, length of feeder = 241.3 cm (for EC, consisting of 24 hens) and 48.3 cm (for CC, for one compartment consisting of 6 hens). In addition, both designs provided a slope for eggs to roll first onto an egg saver that minimized egg breakage, then onto an egg collection belt.

Appendix II. Barn layout to show the location of experimental/test cage units for each housing system[1]

UNIT 1 (Euro "enriched" cages) (1098 hens = 1056 + 42 spare)

											½ cages
11	10	9	8	7	6	5	4	3	2	1	spares 10
	24 BIRDS / (1 CAGE UNIT) - 642 cm^2/hen										spares 11
	Enriched = nest/perch with roost area/scratch pad/nail trimmer										spares 11
12	13	14	15	16	17	18	19	20	21	22	spares 10

11.5 cage unit/row

UNIT 2 (Conventional/Traditional cages) (1320 birds)

58	57	56	55	54	53	52	51	50	49	48	47	46	45
6 hens /cage unit (24 hens/4 cage units) -468 cm^2													
59	60	61	62	63	64	65	66	67	68	69	70	71	72

14 cage units / row

UNIT 3 (Euro "enriched" cages) (1098 hens = 1056 + 42 spare)

											½ cages
33	32	31	30	29	28	27	26	25	24	23	spares 10
	24 BIRDS / (1 CAGE UNIT) - 642 cm^2/hen										spares 11
	Enriched = nest/perch with roost area/scratch pad/nail trimmer										spares 11
34	35	36	37	38	39	40	41	42	43	44	spares 10

11.5 cage unit/row

UNIT 4 (Conventional/Traditional cages) (1320 birds)

86	85	84	83	82	81	80	79	78	77	76	75	74	73
6 hens /cage unit (24 hens/4 cage units) -468 cm^2													
87	88	89	90	91	92	93	94	95	96	97	98	99	100

14 cage units / row

[1]Dark shades indicate locations of experimental/test cage units in the barn (10 for each cage system)

Appendix III. Ingredients and formulation for phase fed commercial layer diets (Wheat-soybean based diet) for Shaver White hens

	Inclusion levels (%)		
	Phase I (19-42 wk)	**Phase II (43-54 wk)**	**Phase III (55-68 wk)**
Ingredients			
Spring red hard wheat (15.36 % CP)	61.72	70.19	74.02
Soybean (45.14% CP)	20.76	14.4	10.63
Veg. oil (9200 Kcal/kg ME)	4.36	-	-
Soy oil	-	1.86	1.45
Limestone (38% Ca)	9.87	10.2	10.56
Biophos (21/17)[2]	1.6	1.53	1.46
Vitamin premix	1.00	1.00	1.00
Mineral premix	0.50	0.50	0.50
DL-Methionine (99%)	0.17	0.15	0.14
Threonine (98%)	0.02	0.05	0.06
Lysine	-	0.12	0.18
Nutrient specifications			
Protein (%)	19.0	17.5	16.5
Energy (Kcal/kg ME)	2900	2800	2800
Calcium (%)	4.2	4.3	4.4
Phosphorus (Avail., %)	0.45	0.43	0.41
Methionine (Meth + cyst) (%)	0.45(0.83)	0.43(0.80)	0.41(0.76)
Lysine (%)	0.88	0.83	0.79
Threonine (%)	0.70	0.66	0.62
Linoleic acid (%)	2.73	1.58	1.41

[1]Shaver. Nutrition management guide commercials 2009-2010 (ISA, 2009)
[2]Biophos (21/17) refers to monocalcium phosphate (21% P/17% Ca)