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L'évaluation de l'alimentation mélangée et séquentielle à base de matières premières localement disponibles sur les performances des poules pondeuses en France et au Nigéria

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Dedicated to my beloved mother Sa'adatu UMAR FARUK

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Liste des publications et communications issues du travail de thèse.

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RÉSUMÉ

ABSTRACT

Résumé

En production d'œuf, le poste aliment représente plus de la moitié du coût de production. Pour baisser ce coût, l'utilisation des céréales en grain entier produites à la ferme pourrait être une solution efficace. D'un point de vue pratique, la distribution des céréales peut se faire en mélange avec un aliment complémentaire riche en protéine et en calcium ou par séquence dans la journée. L'objectif de cette thèse est d'évaluer chez la poule pondeuse la pertinence de ces deux systèmes d'alimentation (mélange et séquentiel) en maintenant les performances de production. Une série d'essais a été menée en France et au Nigéria comparant ces deux modes d'alimentation à une distribution classique d'un aliment complet. Ceci a permis d'expérimenter ces méthodes dans des contextes climatiques, socioculturels et économiques différents. Le blé et millet sont les céréales utilisées respectivement en France et au Nigéria.

Les résultats obtenus en France avec 50% de blé entier en mélange ou en séquence indiquent une baisse significative de la consommation journalière en alimentation séquentielle comparée au mélange et à l'aliment complet en lien avec une moindre consommation de blé chez les poules alimentées en mode séquentiel. Cependant, la production et la masse d'œufs restent identiques entre les trois modes. Ceci conduit à une amélioration importante de l'indice de consommation pour les poules en alimentation séquentielle par rapport au mélange (-10%) ou au témoin (-5%). Les poules alimentées en séquence ont un gésier plus développé par rapport aux deux autres régimes. Pour dissocier l'effet de la séquencialité et de la digestion améliorée en lien avec l'apport de graines sous forme entière, une étude a été menée en utilisant du blé broyé en alimentation séquentielle. Les résultats confirment que le modèle d'alimentation séquentielle conduit à une baisse significative d'ingestion sans remettre en cause les performances mais que la forme graine entière est plus efficace que la forme broyée.

Dans une étude réalisée au Nigéria avec du millet, disponible dans la région et adéquat nutritionnellement. Il est incorporé à 33% dans le régime, en cohérence avec les niveaux d'inclusion de céréales dans cette région. Comme pour le blé, la consommation en séquentielle a été plus faible que pour le témoin et le mélange, du fait d'une plus faible ingestion de millet. De plus, la production et le poids d'œufs ont été supérieurs en séquentiel comparés aux deux autres, conduisant ainsi à une amélioration de l'indice de consommation avec l'alimentation séquentielle par rapport aux mélange (-20%) et témoin (-10%).

En conclusion, dans deux contextes différents (France et Nigéria), ce système d'alimentation séquentielle permet d'utiliser des graines entières avec une amélioration de l'efficacité alimentaire. Le modèle se présente donc comme une innovation importante pour améliorer la durabilité des élevages de poules pondeuses tant en France qu'au Nigeria, contribuant dans ce dernier pays à une amélioration de la sécurité alimentaire. Cependant, il sera nécessaire de préciser les modalités optimales d'accès aux céréales en alimentation séquentielle : quantité, durée ainsi que l'utilisation d'autres matières premières que celles utilisées ici. Les mécanismes métaboliques sous-jacents sont aussi à préciser. Il est également nécessaire de transposer cette méthode en élevages de pondeuses de taille industrielle.

Mots clés : Alimentation séquentielle, alimentation mélangée, durabilité d'élevage, sécurité alimentaire, matière première locale

Abstract

The cost of feed in egg production is a serious problem. Use of whole cereal grains grown on-farm could be an effective solution. Cereals could be fed to poultry either in a mixture with a protein-mineral concentrate (loose-mix) or by alternating the two diets (sequential). The objective of this thesis is to evaluate the impact of these systems on performance in laying hen. Experiments were conducted in France and in Nigeria under different climatic, socio-cultural and economic conditions.

Results obtained in France using 50% whole wheat indicated a significant decrease in the daily feed consumption with sequential compared to loose-mix and conventional feeding due to a lower consumption of wheat in sequential feeding. However, egg production and egg mass were similar between the three systems. This lead to a significant improvement in the efficiency of feed utilisation with sequential feeding compared to loose-mix (-10%) and conventional feeding (-5%). Hens fed sequentially had heavier gizzard than the two others. To distinguish the impact of sequential feeding and the improved digestibility due to a more developed gizzard, a study was conducted using ground wheat. The results confirmed that sequential feeding led to significant decreases in intake without affecting performance and that it is more efficient to use whole than ground wheat when employing sequential feeding.

In Nigeria, when using 33% whole millet in feed, sequential feeding significantly reduces food consumption, due to low millet intake. In addition, egg production and egg weight were higher with sequential than with loose-mix and conventional feeding. This led to a significant improvement in the efficiency of feed utilization with sequential than with loose-mix (-20%) and conventional feeding (- 10%).

It was concluded that under the two different conditions (France and Nigeria) sequential feeding allowed the use of whole cereals with improved feed efficiency. It is therefore an effective system in improving the sustainability of egg production both in France and in Nigeria, with an improved food security in the latter country. However, it is necessary to further investigate sequential feeding in terms of type of cereal and time duration for feed access. The underlying metabolic mechanisms are also unclear. It is also necessary to investigate the system on a larger or commercial scale.

Keywords: Sequential feeding, loose-mix feeding, sustainable egg production, food security, local feed ingredients

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CHAPTER 1 : INTRODUCTION

Chapter 1: Introduction

Egg is an essential source of animal protein playing a vital role in human nutrition. Its nutritional composition made it ideal in bridging the gap between the demand and supply in good quality animal protein. On a global scale, the intensification of the production systems, along with the development of international egg markets led to remarkable development in egg production in the late 20th century. The global egg production increased from 35.2 million tons to 62.6 million tons from 1990 to 2007 thus, making this branch of animal production the fastest growing (Windhorst, 2009).

The dynamics of global egg production in 2007 showed that the Asian continent is the leading egg producer with a share of 61% followed by European Union and North America having 15 and 12% respectively. Africa contributed only 3.6% (Windhorst, 2009). In the European countries, Russia is the leading producer with a share of 21% followed by Spain and Germany with 8.9 and 8.1% respectively. France with a share of 7.7% is ranked 5th. In Africa, egg production increased from 1.5 million tons to 2.3 million tons between 1990 and 2007. Nigeria is the centre of egg production in this region, with a share of 24.5% followed by South Africa and Egypt with 17.1 and 10.6% respectively. The significant development in egg production is a result of intensive scientific advancement in the areas of poultry genetics, feeds and nutrition, and environment, leading to a better understanding of the biology of the domestic fowl.

However, despite this achievement, egg production is facing an important obstacle in the area of animal feed. Feeding is one of the most important aspects because of its primary role in metabolic processes and its economic impact. In countries like France, feeding cost represents about 60% of the total cost of egg production. This can reach up to 75% in the developing countries like Nigeria. This has stimulated interest in the research for alternative feed ingredients and techniques of reducing it without affecting hen performance. The commonly used feeding system in egg production is the distribution of a single homogenous complete diet formulated to provide the hen with its minimum daily nutrient

requirements. In this type of system, cereals are grinded and mixed with other protein and mineral concentrates. Grinding of the feed ingredients has the advantage of uniformity of the diet (Blair, 1973), and improvement in productive performance, by increasing particle surface area, thus allowing greater access to digestive enzymes and increase digestive efficiency (Goodband et al., 2002). It however, increases cost due to energy need in milling and transportation. Furthermore, the problem of feed segregation may arise, especially when mash diets are used (Tang, 2006). Large particles may separate from the small ones during feed delivery. Since birds prefer larger particles (Schiffmann, 1968), and at all ages (Portella, 1988), this segregation may promote ingredient selection, thus influence the birds ability to meet their daily requirement (Tang, 2006).

In some European countries, the success encountered with the use of whole cereal grains distributed with a protein concentrate in broiler chicken raises interest on its application in the egg type chicken. This type of distribution is effective in reducing the feeding cost because it does not require grinding of the cereals and allowed for a direct use of on-farm grown cereals. In addition, it is a solution to the problem of scarcity of a complete diet encountered in developing countries like Nigeria. It was decided to work with laying hens because unlike poultry meat which is imported in to the country, poultry egg is exclusively produced in the country by the small scale poultry holders. Therefore, if the use of whole cereals is found effective, it will not only help to boost production but also increase the level of income of these families.

The work presented in this document was carried out with the general objective of investigating the possibilities of direct use of cereal grain in the diet of layer hen. The work focused on the evaluation of the impact of feeding systems involving the use of cereal on layer hen performance. It also attempted to understand some of the biological mechanisms for an explanation of the results obtained. Whole cereal grains were fed with a protein-mineral concentrate diet either in sequential or in loose-mix

system. Sequential feeding is a method that involves the alternating of cereal grain and a protein concentrate over a period of time called cycle. In loose-mix, the cereal and the protein concentrate were mixed and offered simultaneously. The experiments were carried out in France and in Nigeria. This allowed the evaluation of the two techniques under different environmental, economic, social and cultural conditions. However, no comparison was done between the results obtained from the two different countries because of the differences in the aspects outlined above. The type of cereal used in all the experiments carried out in France was wheat. Due to cultural, economic and availability reasons, millet was used in Nigeria.

In chapter 2, a brief overview of the biology of egg production and whole grain feeding in laying hen is presented. The context of poultry egg production in Nigeria and the major climatic constraint were also included. A detailed description of an experiment carried out in France was presented in chapter 3. Its objective was to investigate the impact of sequential and loose-mix feeding using whole wheat on the performance of laying hen. The birds were housed in-group after a 3-week period of adaptation. The experimental period was from week 19-46 of age and parameters such as food consumption, egg production, egg weight, egg components weight and digestive organs weight were measured.

Parallel to the above experiment, another one was carried out using birds housed individually and reported in chapter 4. The objective was to investigate the ability of sequential and loose-mix fed birds in regulating their feed intake according to their requirement and the diet composition. Similar measurements to those carried out during the experiment in chapter 3 were taken. Overall results of the two experiments indicated that sequential feeding is a more promising method than loose-mix despite the fact that it reduces the metabolizable energy intake of the birds. Following these observations, it was decided to further evaluate the impact of sequential feeding. Therefore, an experiment was carried out using either whole or ground wheat with the objective of investigating the effect of wheat physical form

as well as the metabolizable energy intake in sequential feeding. Details and results were presented in chapter 5.

To further evaluate sequential and loose-mix feeding under different climatic, social and cultural conditions to France, an experiment was carried out at Sokoto, North-western Nigeria. Apart from the socio-cultural reason which did not allow the use of whole wheat in this region, there is also the high temperature (max 45°C). In this experiment, whole wheat was replaced by whole millet, which is locally available. Therefore, results on the impact of sequential and loose-mix feeding using whole millet on the performance of laying hen were presented in chapter 6. A general discussion on the results obtained in the two countries as well as the perspectives and conclusion were presented in chapter 7.

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CHAPTER 2 : LITERATURE REVIEW

CHAPTER 2: LITERATURE REVIEW

2.0 Introduction

This chapter provides a brief overview of the feeding methods that use whole cereals in poultry feeds. It is by no means an extensive review, but provides the essential information for an understanding of the applications of these methods in laying hen. In the first place, the basic biological principles of egg production were first discussed. In the second place, the birds' feeding behaviours in relation to egg formation and feed distribution were discussed. Finally, the generalities of egg production in Nigeria and the possibilities of improving egg production through the application of the feeding methods using whole cereals were highlighted.

2.1 The egg formation cycle

The anatomy and functions of the female reproductive organ below are summarized from (Sauveur, 1988). The reproductive organ of the female chicken consists of two parts (1) the **ovary**, principal site of gametogenesis, development of the yolk and synthesis of sex steroids and (2) the **oviduct** which captures the egg yolk during ovulation and successively deposits the albumen and the shell as the egg travels down to the cloaca (Figure 1). In an adult hen, only the left ovary and oviduct normally develops. The right ones regress during embryonic development. When the bird approaches sexual maturity, (16 to 20 weeks of age), the ovary increases rapidly in size from 5 to 60g and can reach up to 150g. It has a cluster-like structure with 7 to 10 follicles, each containing a growing yolk. Also, it contained thousands of ovarian follicles in which only some will develop to form a yolk. The development of the oviduct is parallel to that of the ovary because it depends on ovarian steroid secretion. Its growth and cellular differentiation occur primarily at sexual maturity, about 2 to 3 weeks before the first egg production. Its weight increased by less than a gram to over 40 g in two weeks. Its size increases from 12 or 15 cm to over 70 cm extending from the region of the ovary to the cloaca.

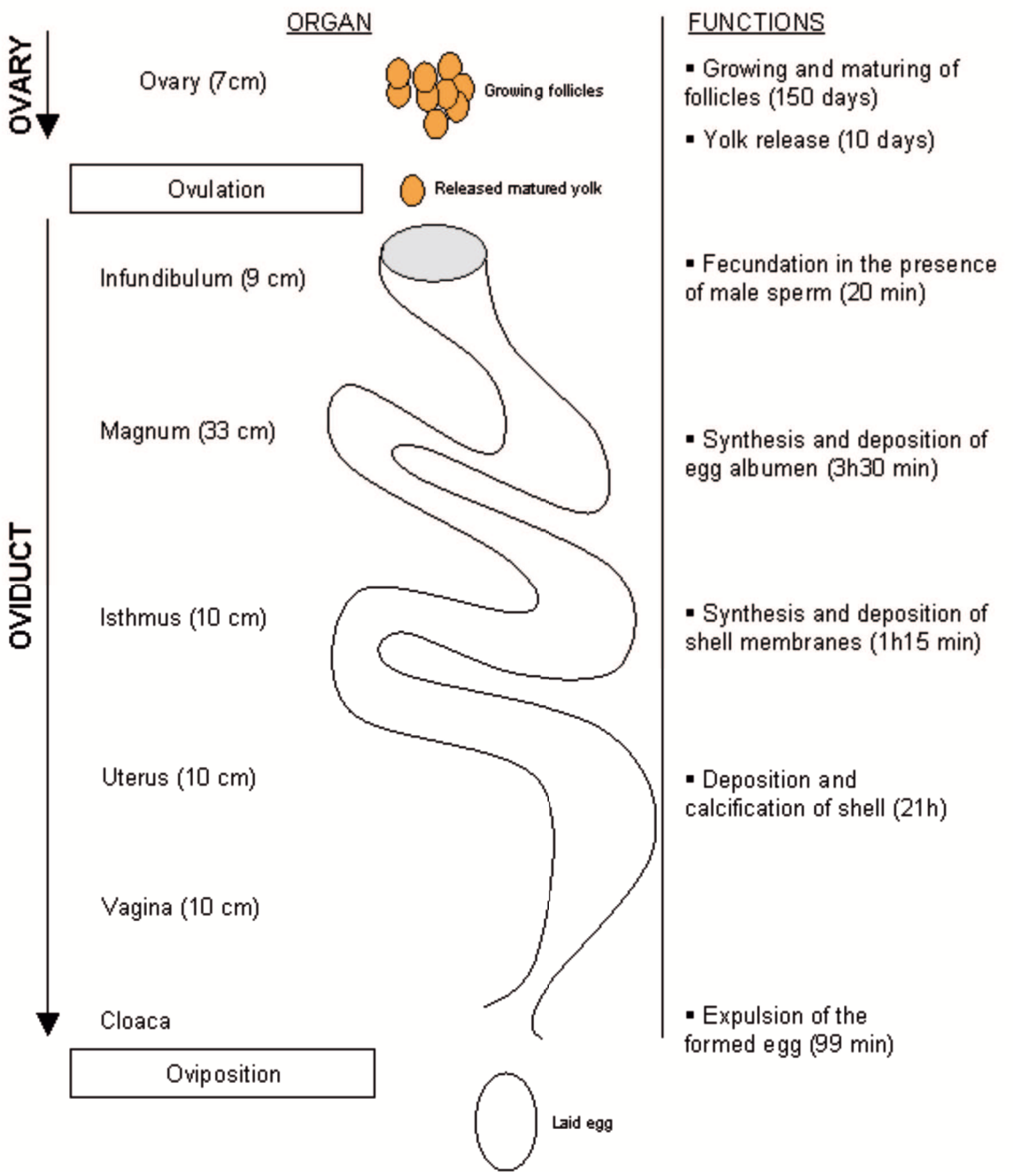


Figure 1. Egg formation in laying hen (Adapted from Sauveur, 1988). The times (h and min) of transit indicated are estimated values. This indicates that it is possible to produce an egg approximately every 24 to 26 hours. If the first egg of a cycle is laid in the morning hours, then the subsequent ovulation occurs about 30 min later. It will take approximately 4h 30 min for the released egg to travel from the infundibulum to the isthmus (shell gland) with most of this time (3h30 min) being spent in the magnum, where albumen is formed and secreted. Thereafter, the egg moves to the shell gland and remained for about 21h where the shell is deposited on the egg before oviposition. The cycle begins for the second egg of the clutch with a new ovulation occurring about 30 min after.

In the proximal portion of the oviduct, near the ovary is a tunnel-like structure measuring about 9 to 10 cm, called the **infundibulum**. Its internal mucosa contains several types of cells having a secretory function, because it participates in the synthesis of the yolk sac and of storing sperm, thus, making it the site of fertilization. Therefore, in the presence of sperm, the yolk is fertilised in the infundibulum. The secretory role of the infundibulum ensures the deposition of a layer on the yolk. This layer is of the same composition as the egg white, and it plays an important role in protecting the transfer of water from the albumen (which will be deposited later) to the yolk. The yolk released during ovulation is captured by the infundibulum and remained in it for about 15 to 20 minutes before moving to the magnum.

The **magnum** is a long and thick tube measuring between 33 and 35 cm long. It has thick internal folds whose appearance changed after the passage of the egg and the secretion of albumen proteins. It is rich in secretory cells and glands. Its inner wall is light gray, nearly transparent in colour, depending on the passage more or less recent of the egg and the associated secretion of proteins. The yolk arriving in the magnum after its stay in the infundibulum is covered with albumen proteins in the magnum. Unlike yolk proteins, whose synthesis is not carried in the ovary but in the liver, albumen proteins are synthesized locally by the wall of the magnum. This synthesis is continuous between the passages of two yolks, although it is accelerated at the time of yolk passage. Therefore, any deficiency in essential amino acids in protein synthesis affects the formation of the albumen and of the subsequent egg size. Albumen protein synthesis is a process much faster than that of the yolk, and this is why in case of a dietary deficiency, the effects are rapidly seen on the albumen. The yolk stays under development in the magnum for about 3 hours and 30 minutes before travelling to the isthmus.

The distinction between the magnum portion of the oviduct and the **isthmus** (measuring 10 cm) is easy due to a substantial narrowing of its diameter and the presence of a narrow strip without glands internally. Leaving the magnum, the yolk is covered with a thick gel protein containing nearly 80% of its final content of sodium, 60 to 70% calcium and 50% chlorine. It stayed for about 1 hour and 15 minutes

in the isthmus, where it begins to be covered with protein fibres, which later constitute the shell membranes. It is also in this part of the oviduct that the calcification of the egg begins at the terminal portion of the isthmus.

The oviduct expands in its end portion to form the **uterus**, where the eggshell deposition takes place. The uterus is a round-like structure and usually called the shell gland. Its walls contain a well-developed thick muscular layer. The uterine lining is dark red in colour and contained several tongue-like folds. The egg stays in the uterus for 20 to 21 hours, where the albumen is hydrated and the shell is deposited, before being expelled (oviposition) through the vagina. The **vagina** is a muscular portion and narrow. Its inner wall contains longitudinal folds, but has no secreting glands. The vagina connects the uterus to the cloaca.

The almost daily production of an egg by the hen is made possible through the simultaneous development of ovarian follicles in a clear hierarchy, leading to the regular presence of a single follicle ready to ovulate. A hen lays an egg every day for 3 to 6 days or more and then stops for a day or more. The sequence of days with an oviposition is called a clutch. Within a clutch, each oviposition is followed about 30 minutes with a new ovulation. This time lapse between ovulation and oviposition made it that eggs are not laid at the same time every day. Thus, the time interval between two successive ovipositions of the same clutch ranged between 24 to 26 hours. In addition, the time lapse made it that under a normal situation it is not likely to find two yolks simultaneously present in the oviduct. However, this does not mean that there is no situation in which the occurrence of double-yolked eggs is observed. The occurrence of double-yolked eggs is highly heritable and a common phenomenon especially at the onset of lay (Christmas and Harms, 1981; Lowry *et al*., 1979) and according to the former author can be influenced by the seasonal effect. Double yolked eggs might also occur in three ways (Conrad and Warren, 1940). First, 65% of them resulted from the simultaneous development and ovulation of two ova. Second, 25% resulted from two ova, which were developing a day apart, being ovulated simultaneously. Third, the 10% resulted either from successive development and simultaneous release

of two ova, one of which should have been released a day earlier, or from an ovum remaining in the body cavity for a day after ovulation and being picked up by the oviduct along with the ovum released on the succeeding day.

Egg formation is mainly regulated by the secretion of steroid hormones (androgens, estrogens and progesterone) from the ovary. It is, however, controlled and synchronized by light, which plays a fundamental role in stimulating the activity of the gonads and synchronizing animals among them. An increase in day length causes an increase in ovarian activity, and its decrease results in the decrease of this activity. When a flock of chickens is illuminated from 6 o'clock in the morning, the eggs are mostly laid between 07 am and 12 noon, with a maximum frequency between 08 hours and 10 hours. From this, it will be seen that the timing of many the egg forming process can be determined when the times of oviposition are known and thus a comparison with bird's behaviour, such as feed intake, could be attempted.

2.2 Pattern of feed intake in relation to egg formation

Food intake of the laying hen in the 24hr period during which egg formation is taking place is higher than in the period when it is not. When the physiological stage of the egg formation is taken into account, food consumption regularly increased from ovulation to the beginning of shell formation. However, Mongin and Sauveur (1974) argued that this observation is valid only if the daylight duration is taken as the reference point as it is always difficult to dissociate the nycthemeral effect from the physiological stage effect. Are they controlled by the same stimulus or are they additive effects? In any case it is well known that birds make use of an endogenous biological nycthemeral rhythm to regulate its feeding behaviour (Bhatti and Morris, 1978). Birds eat their food during the light period (Duncan *et al.,* 1970) because they rarely, if ever, feed in darkness. This is probably one of the reasons why they eat more at the end of the day so as to store food in their crops or oesphagi to last them through the

night (Savory, 1976). If eating doesn't take place in the night, it is therefore necessary for the birds to know when the day will end. Birds living naturally would know when their day was ending by the fading light and may use this as a cue to increase their intake so as to fill their crop. Conversely, birds kept under artificial lighting would have no such warning of imminent darkness. Variations in patterns of feeding may thus be associated with differences in the lighting of the birds' environments. In an experiment, (Savory, 1976) studied the effect of light on the birds' feeding behaviour. A group of chickens was reared with a stimulated "dawn" and "dusk", and another group without this stimulation. The control was reared on a continuous lighting. The non-stimulated group started eating most food in the mornings, but later ate more towards the end of the day, while the stimulated group ate more food at the end of the day during the 20-day experimental period. This showed that birds not only prefer to eat most at the end of the day to ensure adequate food in the night, but they learn when their day would end, and this they did much sooner with the presence of a "dawn" and "dusk" than without it.

Figure 2.1 contained the data plotted from the work of Chah and Moran (1985) who under a 16h lighting period fed a complete mash diet (18% protein, 2800 kcal/kg, 4.12% calcium) to two groups of birds on egg laying and non laying days. Feed intake was about 20% lower on a non-egg laying day (93.3 ± 12.3 g/b/d) compared to an egg laying day (116.1 ± 11.9 g/b/d). The pattern of this daily feed intake was fairly constant for birds on non-egg laying day compared to those on egg laying day. For the latter birds, two peaks of feed intake could be observed (1) between 4 and 6 hours after light-on and (2) between 14 and 16 hours after light-on. However, the above figure contained data obtained since 1985. With the genetic selection and improvements leading to higher productive performances of nowadays birds, it could be questioned if it can be a representative of todays' high productive strain of laying hens. There is a scarcity of recent works comparing feed intake on egg laying and non-laying days. However, the pattern of the daily feed intake was later reported by Keshavarz (1998) and the data were presented on Figure 2.2. It showed that despite the genetic improvement, the daily pattern of feed intake in laying hen fed diets ad libitum is unchanged over the years. Under a 16 hours photoperiod, hens consumed

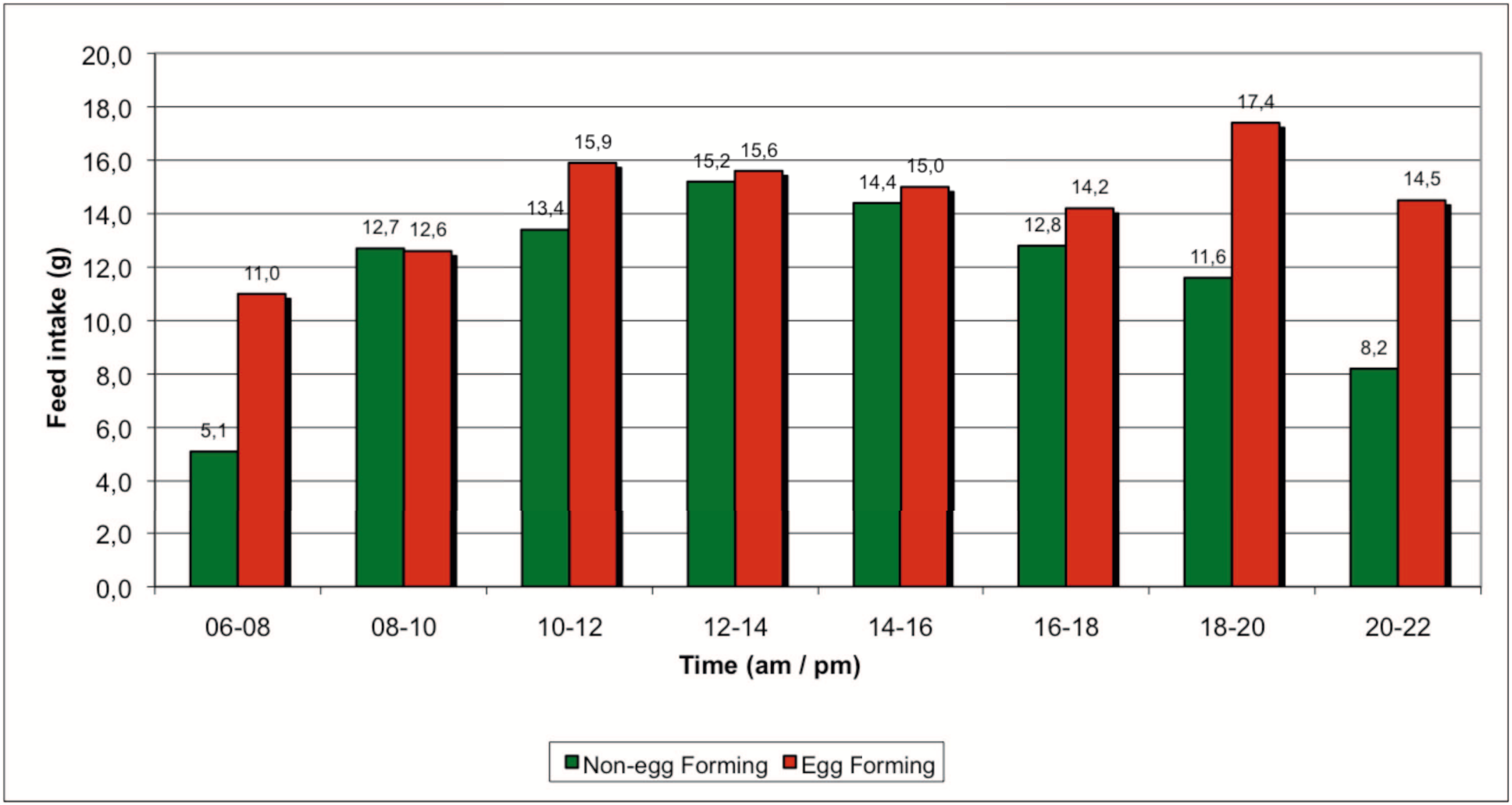


Figure 2.1: The pattern of the daily feed intake (g/bird) on an egg and non egg laying day of hens reared under a 16h photoperiod. Data plotted from Chah and Moran, (1985)

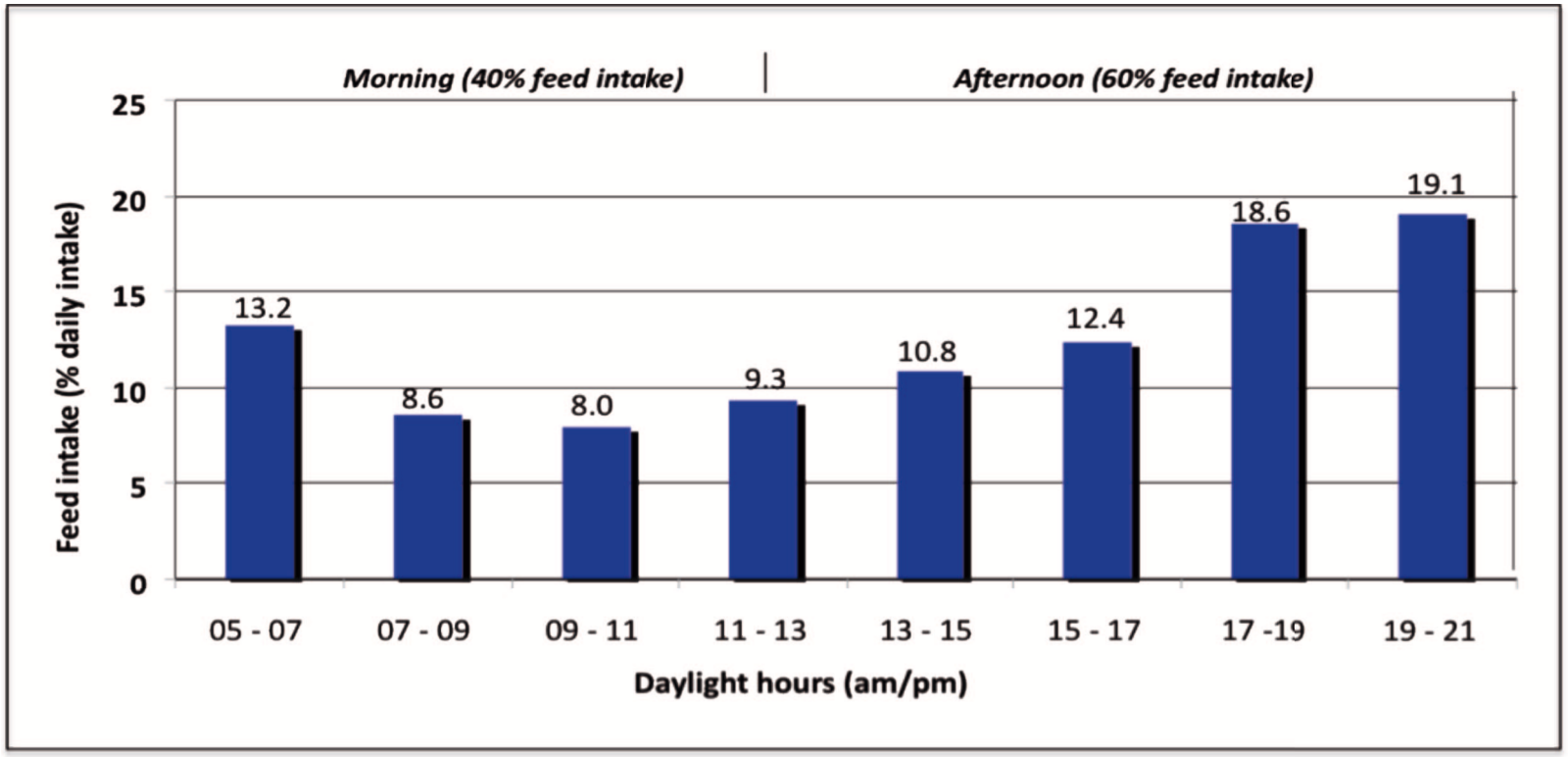


Figure 2.2: The pattern of daily feed intake of birds housed in group and kept under 16h daylight. Data plotted from Keshavarz, (1998).

about 40% of their daily feed during the morning hours (0500-1300 h), and about 60% during the afternoon (1300 to 2100h).

Several authors related the peaks in daily food consumption to egg production by predicting the position of the egg in the oviduct. It is known that the first egg of the cycle is laid during the morning hours. The subsequent ovulation occurs about 30 minutes after oviposition. It takes approximately 4.5 h for yolk to move from the infundibulum to shell gland. According to Morris and Taylor (1967), the morning consumption by birds during albumen secretion (28.1 g/b) is not comparable with that without albumen secretion (19.7 g/b), and related this to an increased amino acid requirement for egg protein synthesis. The study of Shevchenko and Sherapanov (1986) indicated that about 70% of egg white protein is synthesized in the oviduct. These investigators suggested that the hen has a higher protein requirement during the morning, because it is exactly the time of extensive egg white protein synthesis for a majority of hens in their study. Failure of adequate protein synthesis in the magnum during this period may result in reduced egg white and egg size. In fact, the lipoproteins of yolk are continuously synthesized in the liver and accumulated in the follicle until ovulation takes place. On the other hand, albumen proteins are formed and secreted on a daily basis, but during different times of the day (Morris and Taylor, 1967).

Equally, shell formation takes place in the shell gland during the afternoon or evening hours and this can be responsible for higher feed intake in the afternoon (Mongin and Sauveur, 1974), due to an increase in Calcium requirement as a result of the presence of the egg in the shell gland (Figure 2.3).

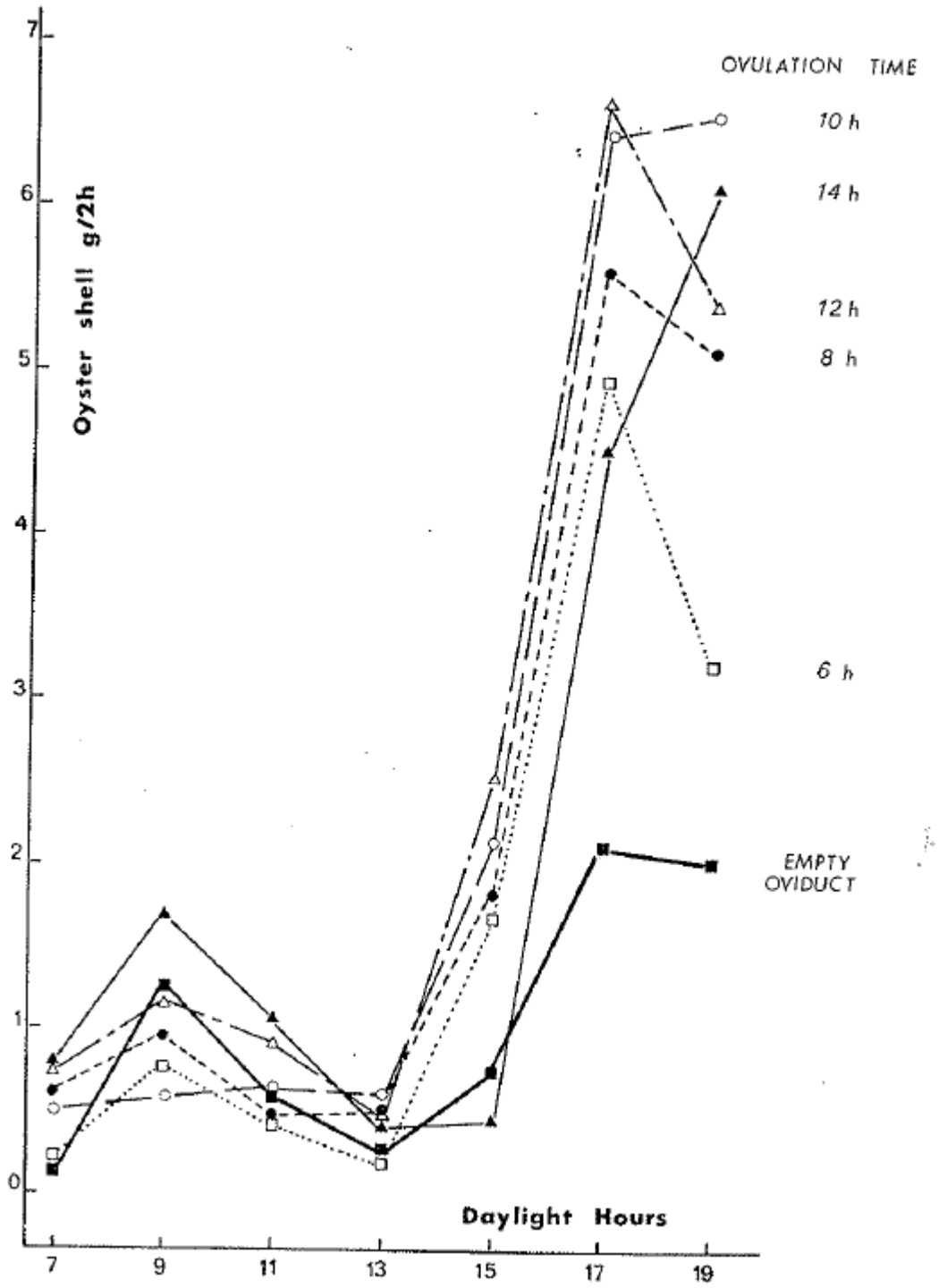


Figure 2.3: Ingestion of Ca (oyster shell) in relation to the stage of egg formation. According to Mongin and Sauveur (1974).

2.3 Is there an alternative feeding system in laying hen?

Two reasons made it possible to have an alternative feeding system in laying hen: birds daily nutrients need and its ability to ingest and utilise unprocessed raw ingredients.

Concerning the first reason, it is clear that nutrients requirement due to egg production plays an important role in the control of the daily food intake and that the nutrients requirement of laying hen is not constant throughout the day. Based on the aforementioned reasoning, it was logical to believe that the requirement for protein, at least for a majority of hens in a flock, would be greater during the morning hours when the ovum is anticipated to be in the magnum and egg white proteins are formed and secreted than during the evening. Here a question can be asked to know what could be the possible advantage or impact of feeding protein-rich diet in the evening other than in the morning? If the synthesis and the secretion of egg protein are not strictly synchronized or done at the same time, then protein will not have a very strict period to be fed as for example calcium. On the other hand, shell is deposited around the albumen during the 18 to 20 h when the egg remains in the shell gland. The period of shell deposition coincides mainly with the evening hours. Therefore, the requirement for Ca should be greater during the evening hours when shell calcification is taking place.

From the foregoing, it is possible to think that feed utilisation could be more efficient if birds are given access to dietary nutrients at the period when they are most required. However, despite this, the feeding of a complete diet predominates today. The reason for this according to some authors is because they are easier to manage in the prevalent cage housing and automated feeding systems (Leeson and Summers, 1979). However, ITAVI (2008) estimated that feeding alone represents about 60% of the total cost of egg production in France (Figure 2.4). This figure is likely to be higher in Nigeria (75%) because of the regular scarcity of a complete diet. In the developing countries such as Nigeria, this single complete diet contained about 35% of cereals that are ground and mixed with other protein and mineral ingredients. However, in the developed countries such as France, cereals represent up to

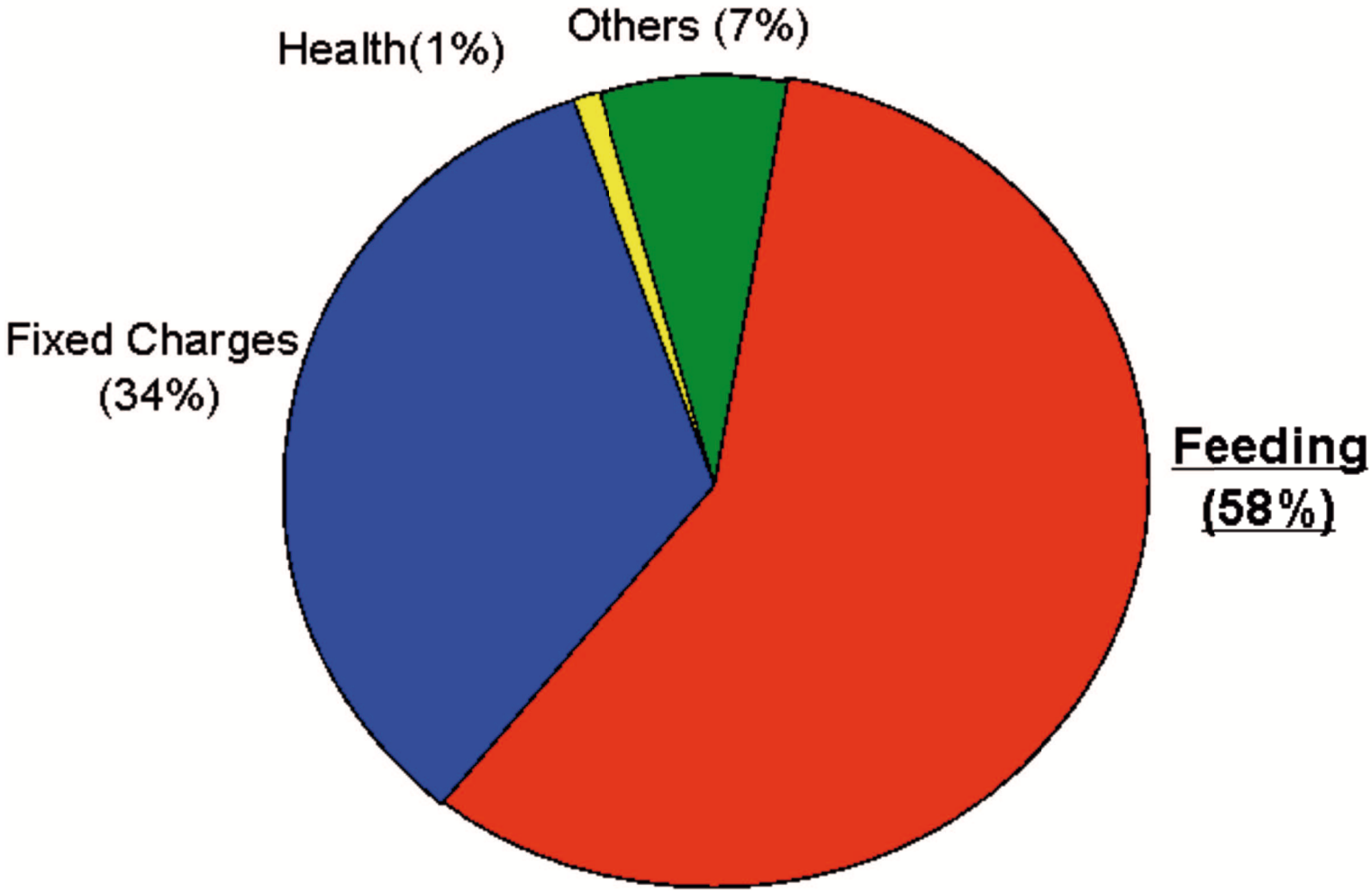


Figure 2.4: Estimated cost of production (%) in caged layers in France (Itavi 2008)

70% in the diet. This implies additional cost in cereal transportation and grinding, keeping in mind that according to Dozier (2002), the cost of grinding cereals represents 25 to 30% of the cost of feed manufacturing.

Concerning the second reason, it was known that poultry digestive system is capable of dealing with whole cereals. Grinding cost will therefore be saved if a system using whole cereals can be developed. Several studies were reported where poultry were fed different diets containing whole cereals and allowed to choose and compose a balanced diet (Blair *et al*., 1973; Karunajeewa, 1978). The proponents of these alternative methods argue that feeding of a single complete diet could be questioned, as individual birds in a given flock will be in a state of either under or over nutritional supply due to individual differences in growth, genetic potential and sex. They further claim that the methods has a value of understanding the specific requirements of the animals, and this could be used to recommend diet specifications (Sinurat and Balnave, 1986). In addition, use of whole cereals using the alternative systems could benefit the environment, by reducing the amount of gas emissions due to transport. Also animal welfare aspects may be taken into consideration such as reduced ascites (accumulation of fluid in the peritoneal cavity) and leg weakness in growing broilers (Bizeray *et al.,* 2002), as well as increasing resistance to coccidiosis due to increased grinding capacity of the gizzard which grinds the oocytes present in it (Cumming, 1994).

The incorporation of cereals in poultry diets is not a new concept as it was a standard practice since the early 20th century when for example, Kempster (1916) and Rugg (1925) observed that laying hens given a choice of diets containing whole cereals produced more eggs than those given a single complete feed. Despite this, very little research had been carried out on this subject. Today, the development of these systems depends on the husbandry skills and feed available to the producer. In addition, the simplicity in application as well as the production goals affects the development of alternative feeding systems. The regional agricultural policies which itself is influenced by historical, social, cultural and economic condition also influence the choice of feeding system. In general, the

notion of "time" and “space” access to food could be used to classify the different feeding systems using cereals into (1) Free choice feeding, (2) Loose-mix feeding and (3) Sequential feeding (Figure 2.5).

2.3.1 Choice feeding system

Among the feeding systems using whole cereals, choice feeding is the most widely studied. As the name implies, this method allows birds to choose from various (usually two) diets presented in different containers. There have been several periods in which interest in choice feeding has been heightened, and whole wheat and a protein concentrate have been given in separate troughs on many commercial units, with results indicating that hens were capable of choosing nutritionally wise mixtures from different diets (Forbes and Covasa, 1995). The basic principle of this method is that individual birds reared in a flock are able to select from various feed ingredients offered. This therefore, assumed that these birds are able to compose their own diet to meet their requirements (Emmans, 1977; Robinson, 1985). This made choice feeding a very appealing technique that seems to be able to meet the wide variety of needs of individual birds within flocks of various types of stock and under different climatic conditions, while having both practical and economic advantages (Cumming, 1994). However this could be questioned especially on the consistency of the results presented below showing that driving a flock using choice feeding is a difficult issue.

Not all choice feeding experiments are successful but most of them show that birds are more or less capable of fairly composing their own diets. Tendencies for impaired plumage conditions and poorer eggshell have been found in some trials (Albustany and Elwinger, 1988). Increased feed intake with choice fed birds was reported (Tauson and Elwinger, 1986), possibly due to stimulation when whole wheat and concentrate are moved in front of the birds as in flat chain feeding in battery cages. However, choice feeding of a protein concentrate and whole wheat to laying hens kept on deep litter resulted to reduction in feed intake (about 13%) with no reduction in rate of lay, egg weight and feed conversion efficiency than those fed the complete mash conventionally over the 48 weeks experimental period (Karunajeewa, 1978). In caged birds, Leeson and Summers (1979) gave a choice of between a diet rich

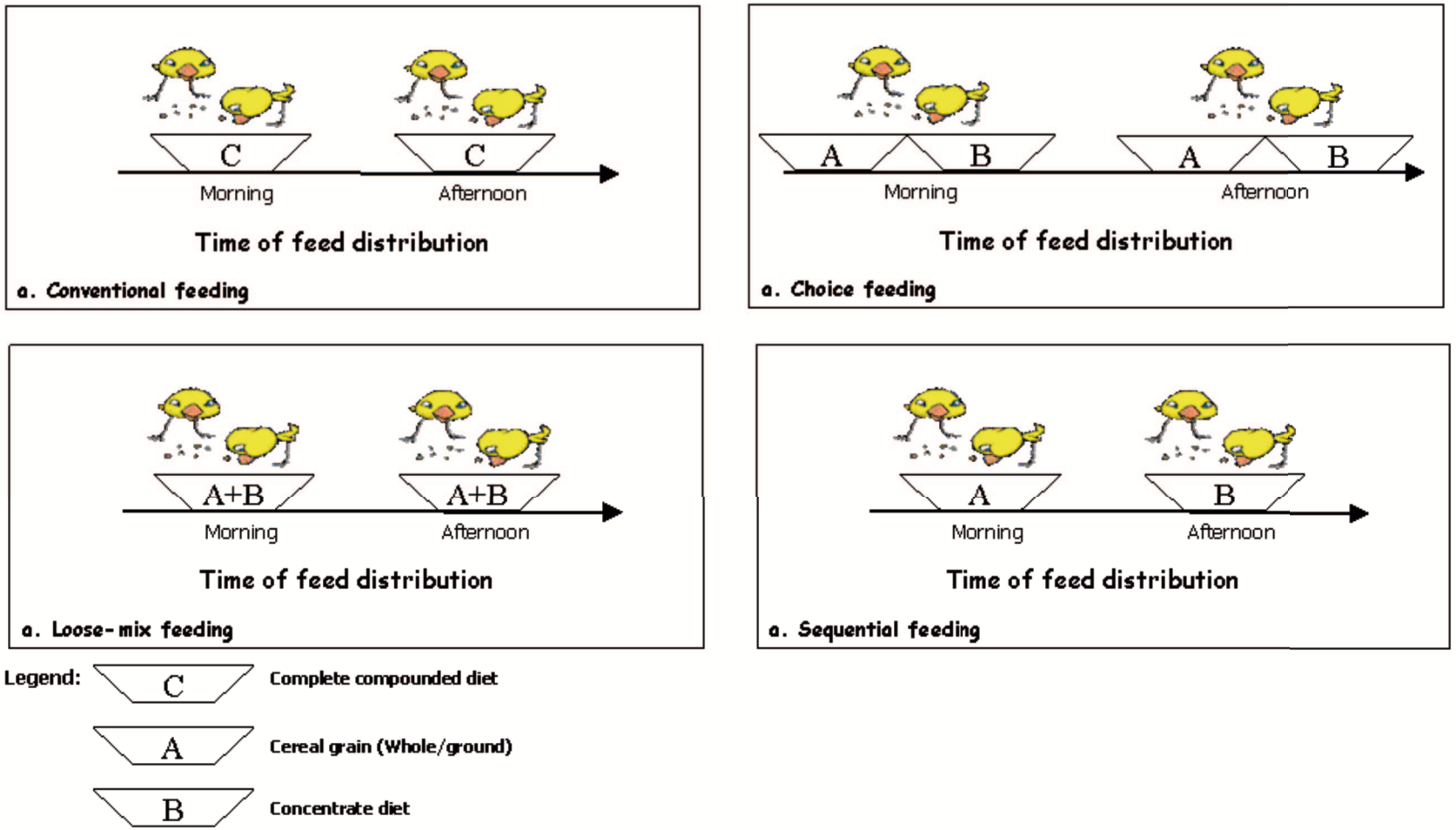


Figure 2.5: Schematic representation of the principle of the present and possible feeding methods in poultry production (conventional feeding, choice feeding, loose-mix feeding, and sequential feeding). **C** is a complete compounded diet containing all the feed ingredients ground and mixed to provide the minimum daily nutrient requirement for a given production target. In a conventional feeding system (the widely used feeding system), this unique diet is fed to birds throughout the day. **A** is an energy rich diet (whole cereal as the case in this work) while **B** is a protein, mineral and vitamin rich diet. The basic principle is that birds given access to the two different diets will consume the right proportion of each in order to have similar nutrient intake as C. Figure adapted from Noirot *et al.,* (1998)

in energy, protein and low calcium and another diet low in energy, protein but high in calcium and observed lower feed intake and similar egg production as the control birds on a complete feed.

In choice feeding, the design of the trough needs to be carefully considered. Forbes and Covasa (1995) reviewed the study comparing the effect of choice feeding and trough design (square bottomed trough with either crosswise or length wise divisions). When a crosswise spilt trough was used and whole grain placed in the centre, egg production was significantly reduced, but when mash was in the centre, egg production was similar to that of control. Tauson *et al.*, (1991) described a chain feeder and mechanized device for feeding restricted amounts of feeds and compared this with separate trough. All the choice fed birds produced significantly heavier eggs than those fed on a conventional mash food. These experiments stressed the need of mechanized feed supply chain to manage correctly choice feeding technique. A further possible beneficial effect of choice feeding is the increased development of gizzard with the resulting increase in digestive efficiency. It also promotes the development of a normal sized proventriculus in chickens fed whole grain, which apparently increases resistance to coccidiosis challenge (Cumming, 1994).

Although the ability of the birds to self-select a nutritionally balanced diet is acknowledged, scientists do not yet understand how birds choose which foods to eat (Forbes and Covasa, 1995). The intake of each of the choice fed diets depends on the location of the feeders in the poultry house (when used in non cage housing system) as well as the nutritional composition of each diet (Noirot *et al.,* 1998). The concept of specific appetite for some nutrients had been widely documented. Selective preference tests have shown that the birds have specific appetites for the major essential nutrients as energy (Hill *et al.,* 1956), protein (Graham, 1934; Holcombe *et al.,* 1976) and calcium (Mongin and Sauveur, 1974; Holcombe *et al.,* 1975). However, possible failures to select an adequate diet provide evidence of an inability of birds to respond adequately under all circumstances as both behavioural and nutritional factors are involved in the process of adaptation to choice feeding. These factors, discussed

below, include the animals' nutritional status and prior experience, training or adaptation to the new feed, social interactions as well as type, form and the nutritive value of the diets.

2.3.1.1 The animals' nutritional status, age and prior experience on choice feeding ability

It is well believed that immediately after hatching a chick have to rapidly learn how to select and ingest feed particles in the absence of maternal guidelines. In addition, chickens associate post ingested effects with physical characteristics or with temporal change of feed. Feed flavor, digestive tract and metabolic signals are combined with visual and tactile cues to progressively build the identity of the feed through experience (Picard *et al.*, 2002). In addition, the genotype (i.e laying or meat type) is known to govern the composition and also the quantity of the feed ingested. For example, Turro-Vincent (1994), demonstrated that at chick stage, layer birds selected and consumed more of a protein-rich than energy-rich diet. Broiler chicks however, contrary to the layer chicks, consumed more of the energy-rich than the protein-rich diet. Adret-Hausberger and Cumming (1985) reported that newly hatched commercial layer and broiler chickens selected sorghum grains while a feral strain pecked at wheat. When the preferred grain was stuck to the floor chicks quickly learn to avoid them, suggesting rapid modification of feeding behavior by experience. As the animal ages, the anatomical and physiological changes occurring at the approach of sexual maturity appear to influence food selection as well as the quantity ingested. During the three weeks preceding the sexual maturity in laying hen, the weight of the ovary increased from about 5g to 50g and the oviduct increases rapidly from 15 to 70 cm long. This change may result in a modification of the nutritional requirements of the chick, and possibly the type and quantity of food consumed.

Sudden change from one feeding system or type of feed to another tends to result to reduction in food consumption in laying hen (Umar Faruk *et al.*, 2008), growth and performance in broiler (Scholtyssek, 1982) due to birds experience with the characteristics of the previous diet. The subject of prior experience had been studied by Covasa and Forbes (1993) who fed wheat grains to broiler chicks from 2 to 4 weeks of age, for either 2 or 6 hours per day, with or without a prior period of deprivation,

with wheat on its own or mixed with the starter crumbs. There was no effect attributable to time of access to wheat or deprivation during the starter phase, but those birds which were given wheat alone for part of each day subsequently ate significantly more wheat during the growing phase, indicating that bird's posses a memory for the type of diet they have been used to.

2.3.1.2 Prior training for choice feeding

Training the birds by accustoming them to whole grain at an early age improves of the birds to select foods that will meet their requirement. Mastika and Cumming (1987) trained one group of birds from 10-21 days after hatching by giving them whole sorghum and protein pellets. No difference in weight gain was noted between the control and untrained choice fed birds. However, the trained birds were significantly more efficient in feed selection, especially as far as protein utilization is concerned. It is therefore, necessary to train the birds to learn the difference between the different diets on offer and hence to learn their nutritional characteristics. Training the birds to distinguish between the properties of the feed can be achieved in different ways. In pigs for example, training by using alternating days of sole access to foods gives good dietary selection when choice feeding is subsequently applied (Gous *et al.*, 1989). Training by giving access to the different diets on a half day basis before the birds were choice fed using two diets differing in protein was also efficient (Shariatmadari and Forbes, 1993). Broiler chicks fed alternately a diet deficient in essential amino acids and a supplemented diet, with change of diet every day needed one week to identify the diets, the distribution pattern or both (Picard, *et al.,* 1999).

However, according to Forbes and Covasa (1995) this technique is not always efficient when whole grains are to be used in choice feeding for several reasons. First, although there are obvious visual differences between the whole grain and the feed, the digestive tract of birds fed whole grain has to adapt and it undergoes physical changes in order to facilitate digestion. For example, a naïve, bird exposed to whole wheat at 3 weeks of age prefers to starve to death rather than to eat wheat, whereas a similar bird which has been accustomed to the grain, is capable of regulating its intake according to

various changes within 24h (Covasa and Forbes, 1994 a). Secondly, alternating methods of training birds are not successful because the bird can avoid eating wheat by learning when the normal feed is on offer (Rose *et al.*, 1994). It is important that the age of the birds is taken into consideration, such that they are introduced to choice feeding at an earlier age (example between 15-18 weeks in laying hen) to avoid failure of the birds in efficient selection of choice fed diets at a later age (Forbes and Covasa, 1995).

2.3.1.3 Social Interactions

Group housed birds are more successful in selecting a diet which meets their requirements than those caged singly. Social interaction between the individual birds provides them with an opportunity to acquire the ability to select appropriate food, through the imitation of congeners (Meunier-Salaün and Faure, 1984), but also by competition between individuals. However, Rose, *et al.,* (1986), found that diet selection of broilers kept in groups of 20, 40 or 60 was not significantly different, while Mastika and Cumming (1987) suggested that birds needs to be in groups of at least eight. Significant differences in terms of wheat intake between single caged birds and pairs of birds given wheat for a 6h period were reported (Covasa and Forbes, 1994 b), despite the fact that the design of the cage allowed the individually caged birds to see each other.

Whether caged singly or in-group, a common feature of choice feeding is the large individual variation between birds (Forbes and Covasa, 1995) and this is observed despite the social motivation. There are always the so called “slow learners” with a higher number in broilers than in egg type chickens (Covasa and Forbes, 1994 b). The process of learning could be accelerated by using experienced birds as “teachers” (Mastika and Cumming, 1987), even though learning is influenced by the presence and behavior of congeners even if they are not experienced. Covasa and Forbes (1994 b) compared inexperienced birds in the presence of experienced birds, with two inexperienced birds put together. Both groups started to eat wheat from day 1, regardless of their experience. When they were split, again into individual cages, they continued to eat about 25% wheat with no difference between

treatments. They therefore concluded that it is not necessary to use experienced birds because simply putting birds together encourages intake.

2.3.1.4 Nutritive value, type and form of feed

Certain grains are more accepted than others. Kempster, (1916) stated that wheat was the favorite cereal for poultry, followed by corn and sorghum. However, the preference of birds towards one type of grain is difficult to access, and this could be related to the form of presentation, palatability and metabolic consequences or, more likely, a combination of all these factors.

The effect of type of grain used in choice feeding was investigated by Karunajeewa (1978), who offered layers either whole triticale, whole wheat, triticale plus wheat or wheat plus oats, each with a protein concentrate food containing 291 g crude protein/kg. The birds consumed 17.6% more triticale per day than whole wheat and maintained the same level of protein concentrate intake, while the consumptions of grain mixture and the protein concentrate were similar for both the grain and the concentrate. These authors associated the higher consumption of triticale to its lower metabolizable energy content. However, Shafey *et al.,* (1992) studied the effect of cereal grain type (wheat, triticale, rye) on performance of laying pullets and observed no significant difference in terms of feed intake and yolk weight between the three types of cereals. Birds fed on wheat or triticale, had higher body weights egg production and egg weight than did those fed rye.

Forbes and Covasa, (1995) reviewed an experiment in which three different grains were compared (whole wheat, cracked corn and whole sorghum) as the source of energy in a choice with either a high or low protein concentrate foods. Feed consumption, body weight, feed conversion, and energy intake were not affected by the type of the grain used. Birds fed on higher protein feed consumed more wheat, corn or sorghum than those fed the lower protein concentrate. This indicates that production performance of birds is not affected by the type of cereal used in choice feeding. However, cereal type affect to some extent the protein concentrate intake which may affect the efficient utilization of protein or other nutrients.

All the above factors outlined above made choice feeding of whole cereals and a protein concentrate diet a very interesting method, but, however, difficult to develop both from the technical to the biological point of views. It therefore becomes necessary to look in to the possibilities of developing other methods that could be more practical in application. These methods could be loose-mix or sequential feeding because they can be simpler in practical terms.

2.3.2 Loose-mix feeding system

This refers to the distribution of a mixture of two or more different diets in a single container. Because whole grain and a protein concentrate (i.e. what is left when the cereal is removed from the formulation of the complete feed) are fed in a single trough, this method was considered as a modification of the choice feeding discussed above and was started in the 1980s as a solution to the practical problem of using different containers such as in choice feeding (Forbes and Covasa, 1995). This method is otherwise called “choice feeding in one trough”. In this method, it is assumed that individual birds will eat the correct quantities for each (whole grain and protein concentrate) to make for itself a balanced diet. However, according to the above authors, this assumption had not been properly tested due to the technical difficulties of measuring the intakes of the two mixed diets.

This method had been mostly and widely practiced in broiler birds. Broiler producers in Northern European countries are including whole wheat grain in their feeds (Noirot *et al.,* 1998). Typically from about 11 days of age, for each 15 tons of grower feed delivered 2 tons are added (Forbes and Covasa, 1995). Adequate mixing takes place during the normal handling of the feed through the augers, bins and feeders. Subsequent batches of feed are ordered with increasing proportions of whole wheat until 4 tons are added to a 15 ton batch from 30 days of age. However, some encouraging results of this method had been reported in laying hen. For example, Cumming (1984), fed three different laying stocks with either a complete commercial food or a mixture of whole wheat and a protein concentrate in a single trough. At 11 months of age whole wheat was replaced with sorghum. He reported that food intake and egg production of the birds fed the mixture was as high as that of birds fed a complete diet. Hopkins and

Dun (1978) fed layers for 52 weeks with either a complete mash diet, a diet with the direct incorporation of 20 or 40% ground wheat, or a choice between mash and whole wheat with either wheat or the mash located nearest to the bird. They reported that the dilution with wheat or by choice feeding reduced egg mass output compared with the complete mash diet.

The major inconveniency of this method is that the birds are likely to systematically operate a feed particle selection. This will lead to increasing the individual variation in terms of intake and performance as the individual birds in a flock are not able to select a balanced diet due to this particle selection (Picard *et al.,* 1997). Therefore, for this method to be more attractive both in economic and biological terms, then the proportion of the different diets needs to be calculated and controlled using computer based system. This made the method more expensive (Filmer, 1991) and possibly the reason why it is less applied in laying hen.

2.3.3 Sequential feeding system

Alternating different diets during the day has been termed "Sequential Feeding" by Gous and Du Preez (1975). This method has been largely studied and applied in broiler chickens with success when for example whole grain wheat was alternated with a protein rich diet (Noirot *et al.,* 1998). More recently, Bouvarel *et al.*, (2008) demonstrated that sequential feeding of broiler chickens using diets of different energy and protein content over a 48-h cycle consumed similar amount of feed compared with the conventional feeding. In addition, walking ability, carcass conformation, breast yield, and abdominal fat did not differ between treatments, thus concluding that growth and slaughter performance similar to standard feeding can be obtained with a 48-h cycle sequential feeding using diets varying in energy and protein contents. This method has also been reported to reduce mortality under acute heat challenge (De Basilio *et al.,*, 2001) and to reduce gait score and increase activity of young broiler chickens.

However, few reports on the impact of sequential feeding in laying hen have been documented and none have been directed at developing this method on a large commercial scale (Forbes and Covasa, 1995). The available reports indicate a broad variation of performance in terms of feed intake,

egg production and the efficiency of feed conversion in sequential feeding when compared with the feeding of a complete single diet. For example, Blair *et al.*, (1973) observed an increased feed intake when laying hens were fed a mixture of whole wheat, barley and crushed maize in the morning hours followed by a protein concentrate in the afternoon hours. However, egg production and egg weight were similar compared with the complete single diet. Robinson (1985) alternated a protein concentrate in the morning and followed by Oats/limestone in the afternoon and observed a reduced feed intake leading to a reduction in egg production and egg weight. The same tendency had been reported by Reichmann and Connor, (1979), when diets containing high energy in the morning followed by a high protein and calcium in the afternoon were used.

According to Forbes and Covasa, (1995) for sequential feeding to work, the birds must learn what and when to eat so that they can select a balanced diet. This means that the length of the time access to cereals is important. When whole wheat and balancer diet were given during alternate 8h periods to birds, there was effective selection, and the higher the protein content of the balancer diet the more the wheat was consumed (Rose *et al.*, 1994). Comparing the performance of birds under 4 or 8h alternating periods, Foote and Rose (1991) observed that aversion to whole wheat was evident with a regimen of 4h alternating period compared to an 8h. However, the later period was found to be more effective because it resulted to performance similar to the control diet, although it increased feed intake.

The few trails in sequential feeding suggest that, provided the birds are trained to consume whole cereal and that an optimum alternating period can be established. Sequential feeding can lead to bird performance equal to that achieved with a complete compounded diet.

2.4 Whole grain feeding and digestive organs

A further possible advantage of whole cereal feeding in poultry is the increased development of the digestive tract (Nir *et al.,* 1990). Whole grain was reported to induce modifications of the upper part (proventriculus, gizzard and pancreas) as well as the lower part (intestine) of the digestive tract. Contrary to whole wheat fed birds, ground wheat fed birds showed a dilation of the proventriculus

(Gabriel *et al.,* 2003). A higher gizzard weight with the use of whole grain was attributed to the increased frequency of contraction of this organ (Hill, 1971) to reduce whole grains to finer particles, and allow them to pass in the small intestine. The gizzard is the "pace maker" of normal gut motility. An increased gizzard size will not only increase the grinding action but also increase the incidence of gastric reflexes that serve to re-expose the digesta to pepsin in the proventriculus, enhance the mixing of digesta with enzymes, improve digestion and also discourage microbial proliferation which may cause disease or compete for nutrients (Ferket, 2000; Gabriel *et al.*, 2003). Improved ileal digestibility (Svihus and Hetland, 2001; Hetland *et al.*, 2002) and apparent metabolizable energy (McIntosh *et al.*, 1962; Preston *et al.*, 2000) in birds given whole wheat compared to those fed diets containing ground wheat had been documented.

The mechanical effect due to gizzard development reduces large feed particles to smaller ones thereby increasing their surface area which enhances their contact with the digestive enzymes and increases digestion of all dietary nutrients. Gabriel *et al.,* (2003) and Engberg *et al.,* (2004) reported that the inclusion of whole wheat in poultry diets leads to a lower pH of the gizzard content and this may lead to increased pepsin (enzyme that breaks down proteins into peptide) activity in gizzard content. The reduced pH indicates the presence of more H^+ ion than OH ions. It was known that the H^+ and pepsin are produced by the same proventricular cells (the oxynticopeptic cells), although they have different regulatory process (Burhol, 1982). The lower pH and potentially higher pepsin activity may increase the denaturation and hydrolysis of proteins.

With a moderate inclusion of whole grain between 200 and 400g/kg, the relative weight of the pancreas increases (Engberg *et al.*, 2004; Wu and Ravindran, 2004). Lower amounts of whole grain (between 100 and 200g/kg) seemed without effect (Ravindran *et al.*, 2006). Whole wheat feeding has also been reported to increase amylase activity in jejunum content, which may contribute to the higher ileal starch digestibility (Svihus and Hetland, 2001). In addition, Svihus *et al.*, (2004) reported a higher bile salt concentration in the jejunal content of whole wheat fed broilers. This increased secretory

(amylase and bile salt) may be due to higher gizzard activity as observed with oat hulls (Hetland *et al*., 2003).

2.5 Feed and feeding under hot climatic condition: Situation in North-western Nigeria

It was intended in the course of this PhD work to evaluate whole grain feeding in the semi-arid northern part of Nigeria using locally available ingredients. Nigeria is bound by Cameroon to the east, Chad to the northeast, Niger to the north, Benin to the west and the Atlantic Ocean to the south. The ecology of the country varies from tropical forest in the south to dry savannah in the far north, yielding a diverse mix of vegetation (Figure 2.6). Although Nigeria lies wholly within the tropical zone, there are wide climatic variations in different regions of the country. Near the coast, the seasons are not sharply defined. Inland, there are two distinct seasons: a wet season from April to October and a dry season from November to March. There are two principal wind currents in Nigeria. The harmattan, coming from the northeast from the month of October to February, is a hot and dry wind and carries a reddish dust from the desert; it causes high temperatures during the day and cool nights. The southwest monsoon wind brings cloudy and rainy weather from the month of February. Temperatures throughout Nigeria are generally high with diurnal variations being more pronounced than the seasonal ones. The highest temperatures and diurnal variation are obtained in the dry season when for example in the north, the daily temperature can reach as high as 45°C during the day and drop as low as 22°C in the night (world climate: http://www.climate-charts.com/).

There is a dearth of reliable information on the current performance of poultry in this region, but it is safe to say that poultry meat and egg production in north-west Nigeria is mainly in the hand of rural dwellers, therefore characterized by traditional low input and output production system. To envisage a migration from this system to a more intensive production system, problems relating to hen performance under harsh climatic condition need to be solved.

Apart from this climatic constraint, there is also the problem of feed scarcity. It is clear that the

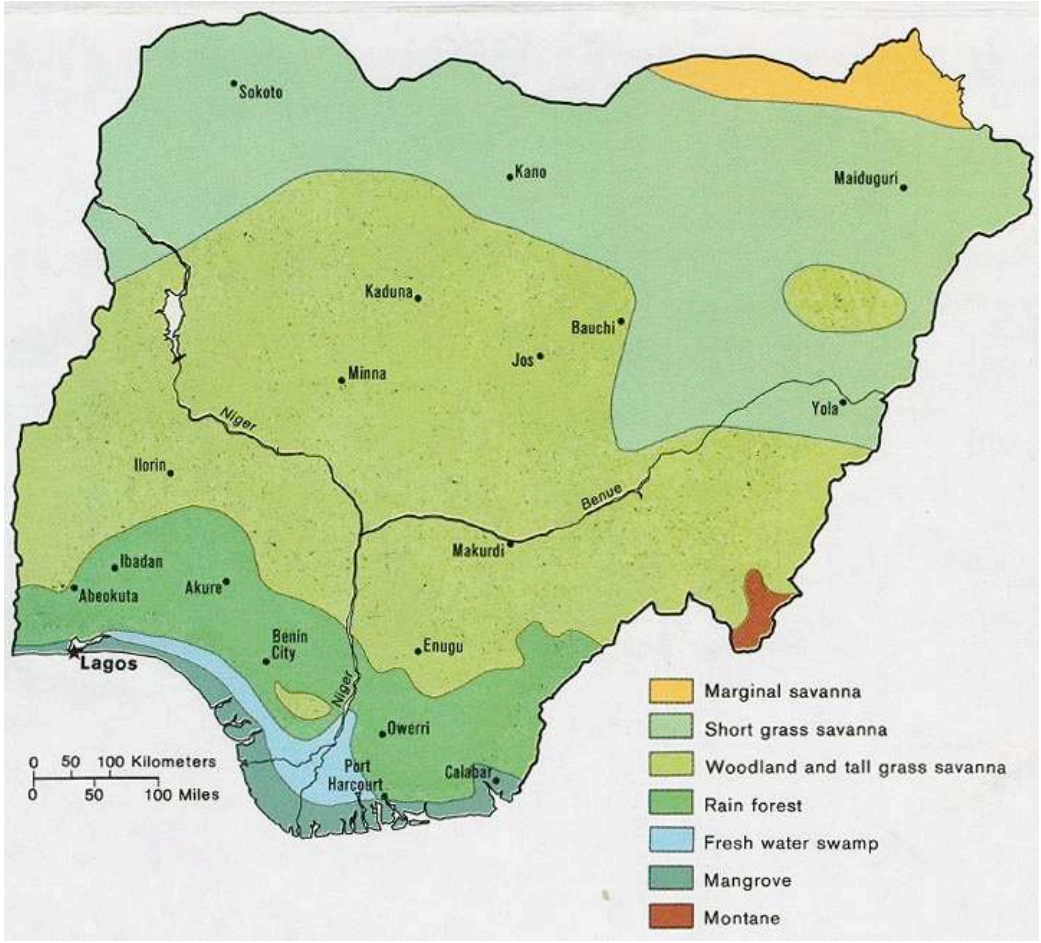


Figure 2.6: Map showing the different ecological zones of Nigeria according to the *Central Intelligence Agency, CIA [http://www.lib.utexas.edu/maps/nigeria.html]* map available at *http://en.wikipedia.org/wiki/File:Nigeria_veg_1979.jpg*. Consulted on the 21st day of November 2009.

climatic conditions of the region made it suitable for the production of drought resistance cereals such as maize, millet and sorghum that can be used as the major ingredients in poultry feed. Equally, protein crops such as groundnut are widely cultivated in this region. In addition, mineral such as limestone, evidenced by the presence of a cement factory in the region, is locally available to be incorporated in poultry egg production. Today, maize is the major cereal used in poultry feed in the region, but the local availability as well as the composition of millet made it logical to think that it can be used to replace maize especially at periods when seasonal variations affect cereal price. Millet has a composition comparable to that of maize. It has a metabolizable energy content of 3367 kcal/kg. Its protein content falls within the range of 10-14%, 2-5% fat, 2-9% fibre, 0.38-0.41% lysine (NRC, 1994). In addition, millet is a crop that appears to be resistant to *aspergillus flavus* infestation (Wilson *et al.*, 1993), thus reducing the problems associated with the contamination of feed with *mycotoxins*.

Despite the local availability of suitable ingredients, poultry farmers in the region continue to rely on commercial compounded feeds because they cannot compound rations for their small number of birds. However, the commercial feed is produced by companies that are only located in the central and southern part of the country. As of today (2010), there is no functioning commercial poultry feed mill in the northern region. The logistic involved in terms of transporting the ingredients to feed mills located in another region and the return of a processed feed back to the northern region made feed supply erratic, thus making them more expensive, thereby putting farmers under great inconveniences during periods of scarcity. Other constraints that were not in the scope of the present study include the lack of management skills from the part of the farmers and the extension services which increases mortality in case of disease outbreak. Based on the fact that feed ingredients are locally available in the region, it was therefore possible to envisage their direct inclusion in poultry feed with minimum level of processing. However, because the direct use of ingredients should not result to low hen performance, it became necessary to understand the nutrient intake and requirements of poultry under high temperature.

The environmental temperature affect level of voluntary feed intake and nutrients utilization, but

it is difficult from the existing knowledge to systematically relate environmental fluctuations to changes in nutrients requirements of animals. Most of the research on changes in feed intake with fluctuations in climatic conditions such as temperature, relative humidity, and the rate of air movement, have been conducted under controlled conditions in laboratory usually with one or two variable under study. These studies have demonstrated modifications in feed intake and production at high temperatures, but transfer of this knowledge to farm practice has been difficult mainly because climatic conditions on farm are considerably more variable than when evaluated in the laboratory.

It should be noted that there is a great deal of disagreement as to what the ideal temperature range for the different classes and age of poultry. This is probably because many factors influence the reaction of poultry to temperature changes. Daghir, (1995) reviewed that the humidity of the atmosphere, light (length of the day and intensity), altitude (air pressure and partial pressures of oxygen and carbon dioxide), wind velocity (air movement), radiant heat and previous acclimatization of the birds are among the most important (NRC, 1981). The environment of the laying hen is composed of these well-known parameters. In general, laying hens perform well within a relatively wide temperature range, extending between 10 and 27°C (Mardsen and Morris, 1987), although recently, (Charles, 2002) reviewed that the optimum temperature for laying hens is likely to be between 19 and 22 °C. In his review, Daghir (1995) argued that the ideal temperature range for growth is not for feed efficiency, and what is ideal for feed efficiency is not ideal for egg weight. This author further stated that feed efficiency is always reduced at temperatures below 21°C. Egg production and growth rate are reduced at temperatures below 10°C. In a nutshell, the overall optimum temperature range is mainly dependent on the relative market value of the product produced, in proportion to feed cost. As the price ratio widens, the best temperature falls, and vice versa.

Studies reporting the effect of high environmental temperature in laying hen are contradicting. On one hand, high temperature is always accompanied by reduction in the amount of food consumed. (NRC, 1981) summarized several papers on laying hens and concluded that the decrease in feed intake is about 1.5% per 1°C, over the range of 5 - 35°C, with a baseline of 20 - 21°C. Heat stress due to high

temperature also depresses body weight (Scott and Balnave, 1988), egg production (Muiruri and Harrison, 1991), egg weight (Balnave and Muheereza, 1997), and egg shell quality (Mahmoud *et al.*, 1996). More recently, Mashaly *et al.*, (2004) worked with three groups of 31 weeks-old laying hens subjected to either 23.9°C, 35°C or a cyclic temperature ranging from 23.9 to 35°C. Feed consumption, egg production, egg weight and body weight were significantly reduced with the 35°C group compared to the two other groups. Equally, shell weight, shell thickness and specific gravity were significantly reduced with the high temperature group. On the other hand, Emery *et al.*, (1984) reported that high temperature did not affect egg production. Furthermore, Muiruri and Harrison (1991) found that heat stress did not significantly affect egg weight or feed conversion. The differences in results can be attributed to differences in treatments or the type of birds used.

Some studies have tried to partition the temperature detrimental effects on performance into those that are due to high temperature and those due to reduced food consumption, by conducting paired feeding experiments. Smith and Oliver, (1972) subjected laying hens to 21 and 38°C and observed that 40-50% reduction in egg production ,and egg weight at 38°C is due to reduced feed intake, while the reductions in shell thickness and shell strength are mainly due to high temperature. Reduced egg production due to high temperature was also associated to the reproductive failure, although the mechanisms involved are not yet understood (Rozenboim *et al.*, 2007).

At this point, a differentiation must be made between the environmental effects on nutrient intake versus its effect on nutrient requirements. Energy intake and requirements decreases with increasing temperature above 21°C and increased with decreasing temperature. Daghir (1973) observed that energy consumption during the summer was significantly (10 – 15%) lower in contrast to winter. Energy requirement for maintenance also decreases with the environmental temperature to reach a low at 27°C, followed by an increase up to 34°C (Hurwitz *et al.*, 1980). However, temperature changes neither increase nor decrease the requirement for protein but reduce amino acids availability. Zuprizal *et al.*, (1993) found that the true digestibility of 12 amino acids were generally depressed in two rape seed and two soya bean meal diets when fed to birds subjected to an increasing ambient

temperature exposure from 21 to 32°C. In an earlier study, Wallis and Balnave (1984), found that the influence of environmental temperature on amino acid digestibility was sex related, with high temperatures decreasing digestibility of amino acids in female birds.

2.6 Conclusion

The major problem encountered in this review is the limited number of literature dealing with sequential and loose-mix feeding in laying hen. Despite this constraint, it is clear that the pattern of feed intake in laying hen in relation to production made it logical to think that if birds are helped to make a selection of the right nutrients, then it is possible to dissociate the time access to different nutrients such that the animals have the right nutrient at the right time, even though the link to egg formation is not so direct as it was believed. In addition, laying hen digestive system is able to deal with whole cereal grain. This made it possible to think that the use of whole grain without transformation is possible in laying hen and that it can bring the benefits as was discussed in the section 2.4 of this chapter. As of today, and despite the aforementioned reasons, no feeding system using whole cereals in laying hen had been developed.

A closer look at the available papers indicated that there are broad variations of performance especially in terms of feed intake, egg production, egg weight and feed conversion efficiency when birds are fed whole cereal grains. The reason for these variations may probably be the large contrasting differences in terms of energy and protein between the different diets the birds were given access to. Selection of nutrients from a dietary choice is a fundamental characteristics of behaviour in animals, although the review of Ashley (1985) on the factors affecting the selection of protein and energy from a dietary choice in animals concluded that the mechanisms controlling the selections could be more precise for energy than for protein, and that the two needs to be kept with a limit to avoid imbalance. In loose-mix, one of the difficulties had been the control of the proportional intake of the different diets while in sequential feeding there had been the problem of making the birds to consume whole cereals. In addition, the birds were nearly always given ad libitum access to these diets as such were supposed

to make a wise selection of their ration. Therefore, for a successful development of feeding methods that incorporates whole cereals in the diet of layer hen, it is necessary to train the birds to consuming whole cereals at an early age. It is also necessary that the contrast in terms of energy and protein in the different diets should not be too large so as to avoid imbalance in the selection of nutrients from the different diets. Equally ad libitum feeding should be avoided so as to guide the birds in selecting the right feed.

The following chapters in this document will deal with the evaluation of loose-mix and sequential feeding using whole cereals and a protein concentrate in France and in Nigeria, taking into consideration the above points.

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CHAPTER 3 :

The impact of Sequential and Loose-mix feeding using whole wheat on the performance of laying hens housed in-group

Influence de l'alimentation mélangée ou séquentielle sur les performances des animaux.

Etude des réactions des poules logées en cages collectives, et nourries en quantité adaptée d'aliment.

Lieu d'essai : INRA UR83 Recherche Avicoles, Nouzilly, France
Durée d'essai : 7 mois précédés de 3 semaines d'habituation à partir de la 16ème semaine d'âge.

Les résultats obtenus lors d'une expérimentation ayant pour objectif d'étudier à long terme (7 mois), l'impact de l'alimentation mélangée et séquentielle sur les performances de production des poules soumises aux deux modes d'alimentation sont présentés dans ce chapitre. Les deux modes ont été comparés avec une alimentation classique.

Les poules ont été habituées à ces modes d'alimentation pendant trois semaines à partir de la 16ème semaine d'âge. Elles sont logées en cages collectives en raison de 5 poules par cage et 16 cages par traitement. La photopériode est de 16h de jour et 8h de nuit. La température est de 21,7 ± 0,7 °C. La quantité d'aliment totale distribuée est de 121 g/poule/jour dont 50 % de blé, ce qui correspond à 105 % des besoins tels que proposés par les sélectionneurs. Toutes les poules reçoivent leurs aliments en deux distributions par jour (4h et 11h après le début de la photopériode). En alimentation séquentielle, les poules reçoivent uniquement le blé lors de la première distribution suivi par un aliment complémentaire riche en protéine lors de la deuxième distribution. Quant à l'alimentation mélangée, le blé et l'aliment complémentaire sont mélangés et distribués tels quels lors des deux distributions. Les poules en alimentation classique ont reçus un aliment complet lors des deux distributions.

Les performances suivantes sont mesurées : la quantité d'aliment ingérée, le nombre et le poids des œufs, ainsi que le poids des animaux. Des mesures des constituants de l'œuf, ainsi que du poids des principaux organes du tube digestif ont été effectuées. Les données récoltées ont été traitées par une analyse de variance (ANOVA) à 5% de niveau de significativité. La comparaison des moyennes est faite par le test de Bonferroni / Dunnet.

Les résultats montrent une réduction significative de la consommation journalière en alimentation séquentielle (109 g/j), contrairement aux alimentations mélangée (116 g/j) et classique (115 g/j). Ceci est dû à une baisse significative de la consommation de blé chez les poules en mode

séquentiel comparées à celles en mélange. Cependant, Le taux de ponte et le poids moyen des œufs restent identiques entre les trois modes. Ceci conduit à une amélioration importante et significative de l'indice de consommation pour les poules en alimentation séquentielle (-10 % et -5 % par rapport à celles en alimentation mélangée et classique respectivement). Cependant, le pourcentage de jaune dans l'œuf a diminué avec l'alimentation séquentielle entre la 25ème et la 38ème semaine d'âge. Cette différence disparaît à la fin d'expérience (semaine 46). Par ailleurs, celles-ci ont un pourcentage de coquille significativement plus important que les deux autres modes. Elles ont également un poids corporel inférieur à celles en mode mélange et en classique. Les poids des principaux organes digestifs tels que le gésier, le pancréas et le foie sont plus importants en alimentation séquentielle.

Les mécanismes ayant conduit à ces améliorations de performances restent à élucider. Néanmoins, il est fort probable que le développement musculaire du gésier plus élevé en alimentation séquentielle a permis une amélioration de la digestion, et a conduit à une meilleure utilisation des nutriments. Une question très intéressante qui ressort de ces résultats est de savoir si cette amélioration est due uniquement à l'hypothèse ci-dessus ou si elle est due à un effet de séquence (c'est-à-dire l'apport des nutriments au bon moment où les poules en ont besoin). Il sera nécessaire de dissocier ces deux effets afin de mieux évaluer les meilleures performances obtenues avec l'alimentation séquentielle.

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Sequential feeding using whole wheat and a separate protein-mineral concentrate improved feed efficiency in laying hens

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ABSTRACT The effect of feeding nutritionally different diets in sequential or loose-mix systems on the performance of laying hen was investigated from 16 to 46 wk of age. Equal proportions of whole wheat grain and protein-mineral concentrate (balancer diet) were fed either alternatively (sequential) or together (loose-mix) to ISA Brown hens. The control was fed a complete layer diet conventionally. Each treatment was allocated 16 cages and each cage contained 5 birds. Light was provided 16 h daily (0400 to 2000 h). Feed offered was controlled (121 g/bird per d) and distributed twice (4 and 11 h after lights-on). In the sequential treatment, only wheat was fed at first distribution, followed by balancer diet at the second distribution. In loose-mix, the 2 rations were mixed and fed together during the 2 distributions. Leftover feed was always removed before the next distribution. Sequential feeding reduced total feed intake when compared with loose-mix and control. It had lower wheat (−9 g/bird per d) but higher balancer (+1.7 g/bird per d) intakes than loose-mix. Egg production, egg mass, and egg weight were similar among treatments. This led to an improvement in efficiency of feed utilization in sequential compared with loose-mix and control (10 and 5%, respectively). Birds fed sequentially had lower calculated ME (kcal/bird per d) intake than those fed in loose-mix and control. Calculated CP (g/bird per d) intake was reduced in sequential compared with loose-mix and control. Sequentially fed hens were lighter in BW. However, they had heavier gizzard, pancreas, and liver. Similar liver lipid was observed among treatments. Liver glycogen was higher in loose-mix than the 2 other treatments. It was concluded that feeding whole wheat and balancer diet, sequentially or loosely mixed, had no negative effect on performance in laying hens. Thus, the 2 systems are alternative to conventional feeding. The increased efficiency of feed utilization in sequential feeding is an added advantage compared with loose-mix and thus could be employed in situations where it is practicable.

Key words: laying hen, sequential feeding, loose-mix feeding, whole wheat, feeding system

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INTRODUCTION

Laying hens are commonly fed a single complete diet. This system has the advantage of uniformity of the diet. One disadvantage is the need for grinding the main dietary components. Energy required for grinding comprises between 25 and 30% of feed manufacturing (Dozier, 2002). It was known that the poultry digestive system is capable of digesting whole grain. Therefore, it is logical to think that the cost incurred in grinding and handling of cereals will be significantly reduced if birds are fed whole grains. Furthermore, the amount of gas emissions due to grinding and transportation could be reduced. In countries in which the cost of transport and diet mixing is expensive, it may be more economical to transport only a protein concentrate. In addition, it allows the use of locally grown cereals in the farm.

Whole grains can be fed with a protein concentrate in different systems (Noirot et al., 1998): simultaneously in different containers (choice feeding), mixed together and fed in single container (loose-mix), or fed at different times of the day (sequential). Choice feeding using unground cereals is accompanied by an improvement in feed utilization because it allows a degree of feed selection by the animal. It presents, however, the inconvenience of having more than one feeding trough to contain the different diets. As such, it is less prac-

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tical in application. Loose-mix and sequential feeding could be practical because only 1 diet and container are required at a time. However, a feeding system using whole grains without reducing bird performance is yet to be developed.

Sequential feeding had been reported to increase total intake when birds were fed a mixture of whole cereals and protein concentrate sequentially (Blair et al., 1973). Egg production and weight were, however, not affected, thereby decreasing efficiency of feed utilization of these birds. Conversely, low feed intakes (Leeson and Summers, 1978; Reichmann and Connor, 1979; Robinson, 1985; Lee and Ohh, 2002) were observed when hens were fed sequentially. Egg production was similar (Leeson and Summers, 1978; Reichmann and Connor, 1979; Lee and Ohh, 2002) or reduced (Robinson, 1985) compared with the conventional feeding system. All of the above authors observed low egg weight in birds fed sequentially. The limited information on loose-mix (Lee and Ohh, 2002) indicated that it reduced feed intake but resulted in similar egg production with a slight decrease in egg weight compared with the conventional system.

Combination of the above studies indicates a broad variation of performance in terms of feed intake, egg production, egg weight, and efficiency of feed conversion in sequentially fed birds compared with conventional ones. With genetic improvement, it could be asked if today's birds are better able to adjust their intake and performance when fed different diets sequentially. The above studies fed diets that contrasted greatly in energy, protein, and calcium. In addition, most experiments gave ad libitum access to these diets and allowed birds to regulate their intake. However, hens might not adapt their intake to fit with their requirements, thereby resulting in some inconsistency in performance. To overcome this, it was postulated that the birds must be guided in their selection by controlling the quantity of the diet offered. Also, the composition of the different diets fed sequentially or loose-mixed should not be too contrasting in energy, protein, and calcium.

The objective of this work was to evaluate the performance of laying hens habituated before point of lay to consume whole wheat and balancer diet in loose-mix or in sequential feeding systems. They were compared with conventional feeding using a single complete diet. Controlled quantities of these dietary components were fed in both the habituation and experimental periods.

MATERIALS AND METHODS

Habituation Period (wk 16 to 18)

Laying birds need a period of learning before becoming proficient in selecting feedstuffs (Forbes and Covasa, 1995). The birds were habituated to the feeding methods using wheat grains and a balancer diet from wk 16 to 18 of age. The objective was to particularly adapt the birds in sequential feeding to whole wheat intake before point of lay (Umar Faruk et al., 2008). The birds were housed individually in bottom-wired cages (25 × 38 cm), equipped with nipple drinkers and individual feeders. This was to allow for an individual follow-up of birds during this period.

The control treatment was fed a single control growing diet (Table 1) containing 2,800 kcal of ME/kg and 16% CP. The loose-mix and the sequential groups were fed whole wheat and a balancer growing diet containing 2,633 kcal of ME/kg and 19% CP. The total quantity of offered diet was progressively increased from 70 to 83 g/bird per day from wk 16 to 18, respectively. The quantity of the balancer growing diet, fed in loose-mix and in sequential groups, was 65% of the total diet given daily. Wheat represents 35% on the assumption that if the birds consumed all of the offered diet, they will therefore consume a similar amount of daily nutrients as the control. In sequential feeding, the wheat was fed in the morning followed by the balancer diet in the afternoon. The wheat was offered 4 h after lights-on for a period of 2 and 3 h during wk 16 and 17, respectively. In loose-mix, the wheat and the balancer diet were mixed and the mixture was fed in the same feeding trough.

Experimental Period (wk 19 to 46)

The 3 feeding systems studied and the hours of feed distribution are illustrated in Figure 1. The experimental period was from wk 19 to 46. During this period, the birds were housed in wire-bottomed cages (550 cm^2/hen) designed to accommodate 5 birds per cage. Each of the 3 treatments was allocated 80 birds divided into 16 cages as replicates. Body weight was used as the criterion for placement such that homogeneous mean BW was placed per cage and per treatment. The birds received 16 h of light/d throughout the experimental period and water was fed ad libitum. Daily temperature was maintained at 21.7 ± 0.7°C.

The control treatment was fed the control layer diet containing 2,750 kcal of ME/kg and 17.5% CP (Table 1). The sequential and loose-mix groups received the balancer layer diet containing 2,380 kcal of ME/kg, 23% CP, and 7.2% calcium (finely ground) sequentially or in loose-mix with whole wheat. This diet was formulated to reach the daily nutritional balance as the control diet, assuming a ratio of 50% wheat and 50% balancer diet. Thus, in sequential and loose-mix treatments, 60.5 g of the diet was fed as whole wheat and the remaining 60.5 g as balancer diet. Each bird received 121 g/d of diet corresponding to 105% of the breeder's guidelines (ISA, 2007).

All of the birds received their daily ration in 2 distributions at 4 and 11 h after lights-on respectively. In sequential feeding, wheat was fed at first distribution, whereas the balancer diet was fed at second distribu-

Table 1. Composition of experimental diets

Item	Habituation period (16 to 18 wk)		Experimental period (19 to 46 wk)		
	Control growing	Balancer diet growing	Control laying	Balancer diet laying	Whole wheat
Ingredient (%)					
Wheat	34.66	—	50.00	—	
Maize	35.00	53.57	16.13	32.08	
Wheat bran	10.00	15.31	2.54	5.01	
Maize gluten	—	—	3.29	6.62	
Soybean meal	16.50	25.25	16.97	34.08	
Soybean oil	—	—	0.80	1.60	
Calcium carbonate	1.84	2.82	8.00	16.04	
Bicalcium phosphate	1.09	1.67	1.16	2.33	
Refined salt	0.20	0.31	0.20	0.40	
Sodium bicarbonate	0.20	0.31	0.20	0.40	
L-Lysine 78	—	—	0.11	0.22	
DL-Methionine	0.01	0.02	0.11	0.22	
Premix[1]	0.50	0.77	0.50	1.00	
Calculated composition (%)					
ME (kcal/kg)	2,800	2,633	2,750	2,380	3,120
CP	16.05	18.33	17.52	23.00	11.90
DM	87.74	87.48	89.06	89.88	86.80
Fat	2.36	2.90	2.51	3.67	1.35
Ash	5.75	8.06	11.71	22.07	1.60
Crude fiber	3.59	4.10	3.01	3.37	2.65
Lysine	0.72	0.93	0.81	1.31	0.31
Methionine	0.32	0.39	0.45	0.71	0.20
Calcium	1.20	1.82	3.61	7.20	0.03
Total phosphorus	0.56	0.71	0.53	0.76	0.32

[1]Vitamin and mineral premix supplied the following amounts per kilogram of premix: vitamin A, 1,600,000 IU; vitamin D_3, 480,000 IU; vitamin E, 2,000 mg; vitamin K_3, 400 mg; vitamin B_1, 109 mg; Zn, 11,000 mg; Mn, 12,000 mg; Cu (sulfate), 1,200 mg; Fe, 4,000 mg; I, 200 mg; Se, 60 mg; DL-methionine, 120 g; and canthaxanthin, 200 mg.

tion after the removal of wheat from the trough using an electric vacuum cleaner (Dyson DC19 vacuum cleaner, Dyson Limited, Malmesbury, UK). In loose-mix and control, the same diets were fed during the 2 distributions. The first distribution was made 4 h after lights-on so as to avoid a negative correlation between feed intake and oviposition (Ballard and Biellier, 1969; Nys et al., 1976). The quantity of wheat offered was based on the conclusions of Bennet (2003) that whole grains should not exceed 50% of the total diet offered to avoid the condition whereby hens will have difficulty in finding the protein concentrate in the ration.

Total feed intake was measured weekly as the difference between feed offered and leftover. In sequential feeding, wheat intake was measured by directly measuring leftover wheat. In loose-mix, wheat intake was measured after separating the wheat from the balancer diet using a manual sieve (2 mm diameter).

Birds were weighed individually at wk 16, 19, 27, 37, and 46. Egg production was measured by recording the

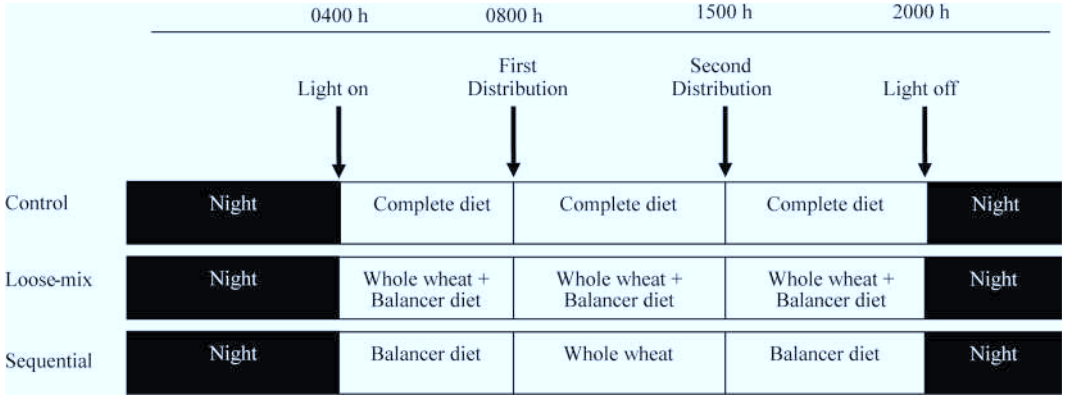


Figure 1. Illustration of the 3 feeding systems (control, loose-mix, and sequential) and the hour of feed distribution. During first distribution, half of the total daily ration was offered, and whole wheat was offered at this time for the sequentially fed birds. The remainder of the total daily ration was fed during the second distribution. The balancer diet was fed at this time for the sequentially fed birds. All feeders were emptied before each distribution.

number of eggs produced daily. Individual egg weight was recorded daily. The weights of the egg yolk, albumen, and shell were measured every 4 wk starting from wk 21 of age. To measure these components, all eggs produced on a given day of the week were collected, weighed individually, and then broken. The albumen and the chalazae were separated from the yolk using forceps before weighing the yolk. The shells were carefully washed and dried for 12 h in a drying oven at 70°C and then weighed. All measurements were taken to the nearest 0.01 g.

The weights of the principal digestive organs were taken at wk 19 and 46. For this measurement, 8 and 16 birds per treatment were used at wk 19 and 46, respectively. At wk 46, eight birds were killed in the morning (0800 h), whereas the remaining 8 birds were killed in the afternoon (1500 h). This was to allow for an evaluation of the effect of daily feeding rhythm on the hepatic lipid, protein, and glycogen contents. The birds were first weighed before being injected with sodium pentobarbital solution (1 mL/kg; CEVA Santé Animale–La Ballastière, Libourne, France). The abdominal cavity was then dissected and the digestive tract was collected and separated into its different segments: proventriculus, gizzard, duodenum, pancreas, jejunum, and ileum. These digestive segments were first emptied and dried using a paper towel before weighing. The proventriculus and the gizzard were placed in an iced container (−4°C) for 3 h to facilitate the removal of the surrounding fat before being emptied. Hepatic lipid (%/liver) was measured according to the Folch procedure (Folch et al., 1957). Hepatic protein (g/g of liver) content was determined by bicinchoninic assay kit (Uptima, Interchim, Montluçon, France) according to procedures of Smith et al. (1985). Analysis of liver glycogen (mmol/g of liver) was conducted according to procedure described by Dalrymple and Hamm (1973) and adapted by Monin and Sellier (1985).

Metabolizable energy requirement (kcal/bird per d) was estimated using the predictive equation of Sakomura (2004):

$$\mathrm{ME} = \mathrm{W}^{0.75} \times (165.74 - 2.37 \times \mathrm{T}) + 6.68 \times \mathrm{WG} + 2.40 \times \mathrm{EM},$$

where ME = ME requirement (kcal/bird per d); T = temperature (°C); WG = weight gain (g/bird per d); EM = egg mass (g/bird per d); and W = BW (kg).

Protein requirement (g/bird per d) was determined using the predictive protein requirements equation (Sakomura et al., 2002):

$$\mathrm{PB} = 1.94 \times \mathrm{W}^{0.75} + 0.480 \times \mathrm{WG} + 0.301 \times \mathrm{EM},$$

where PB = protein requirement (g/bird per d); W = BW (kg); WG = weight gain (g/bird per d); and EM = egg mass (g/bird per d).

Statistical Analysis

Average values from cages were analyzed using StatView (version 5, SAS Institute Inc., Cary, NC). Collected data were analyzed based on 3 periods related to egg production stage (1) before peak, from 19 to 26 wk of age; (2) at peak, from 27 to 37 wk of age; and (3) after peak, from 38 to 46 wk of age. These weeks (19, 26, 37, and 46) also coincide with the weeks in which BW was measured in this work.

A 1-way ANOVA according to the GLM model below was used to test treatment effect on feed intake, egg production and weight, egg components weight, BW, and digestive organs weight:

$$\mathrm{Y_{ij}} = \mathrm{R_i} + \varepsilon_{ij},$$

where $\mathrm{Y_{ij}}$ = measured variables for regimen i and cage j; $\mathrm{R_i}$ = regimen effect (i = sequential, loose-mix, control) and j being the cage number in regimen i; and ε_{ij} = residual.

The hepatic lipid, protein, and glycogen contents were subjected to 2-way ANOVA using the following model:

$$\mathrm{Y_{ijk}} = \mathrm{R_i} + \mathrm{H_j} + \varepsilon_{ijk},$$

where $\mathrm{Y_{ijk}}$ = measured variables for regimen i, hour of slaughter j, and cage k; $\mathrm{R_i}$ = regimen effect (i = sequential, loose-mix, control); $\mathrm{H_j}$ = slaughter time effect (j = morning, afternoon) and k being the cage number in regimen i and hour j; and ε_{ijk} = residual. Results were considered different if $P < 0.05$, and Bonferroni-Dunnet pairwise comparison was used to compare differences in means.

RESULTS

Habituation Period (wk 16 to 18)

The overall average total feed intake from wk 16 to 19 was similar among the 3 treatments: 67.1, 67.4, and 66.3 g/bird per day for control, loose-mix, and sequential, respectively. Wheat intake in sequential feeding increased with increasing age and the quantity offered. It was 12 and 38 g/bird per day for wk 16 and 18, respectively. Body weight gain was similar among the treatments: 8.1, 8.6, and 8.4 g/bird per day for control, loose-mix, and sequential, respectively.

Experimental Period (wk 19 to 46)

As indicated in the statistical analysis section, data collected during the experimental period were analyzed based on 3 periods (before peak: 19 to 26 wk; at peak: 27 to 37 wk; and after peak: 38 to 46 wk). At the onset of the experimental period, 1 replicate belonging to

the control group was eliminated from the study due to technical reasons. This reduced the number of replicates in this treatment to 15, whereas sequential and loose-mix each had 16.

The overall average total feed intake during the experimental period (Table 2) was found to be lower ($P < 0.01$) in sequential feeding (108.7 g/bird per d) when compared with what was obtained with loose-mix (115.9 g/bird per d) and control (115.2 g/bird per d). Wheat intake was found to be lower ($P < 0.01$) in sequential feeding compared with loose-mix, with 51.2 and 60.2 g/bird per d representing about 47 and 50% of the total intake, respectively. Conversely, balancer diet intake was higher ($P < 0.01$) with sequential feeding (60.1 g/bird per d) compared with loose-mix (58.5 g/bird per d).

Similar egg production and egg weight were observed among the 3 treatments (Table 2). Similarly, the average egg mass was similar among treatments. Egg production and weight increased with increasing age; thus, the effect was consistent across the 3 periods. Feed conversion ratio (**FCR**) was lower ($P < 0.01$) for sequential (2.01) than loose-mix (2.21) and control (2.11); FCR of loose-mix and control FCR were not statistically different.

Body weight increased with age and was similar among treatments up to wk 26 (Table 2). However, a difference in BW was observed at wk 37. Sequentially fed birds were lighter in BW (1,724 g/bird) when compared with hens fed loose-mix (1,862 g/bird) and control (1,819 g/bird). Loose-mix was similar in BW to control. No increase in BW from wk 37 to the end of

Table 2. Feed consumption, egg production, egg weight, feed conversion ratio (FCR), and BW of sequential- and loose-mix-fed hens from 19 to 46 wk of age

	Treatment				
Measurement	Control	Loose-mix	Sequential	*P*-value	SEM
Average total feed intake (g/bird per d)					
19 to 26 wk	108.7[a]	109.0[a]	103.4[b]	<0.01	1.3
27 to 37 wk	117.4[a]	118.0[a]	109.6[b]	<0.01	0.8
38 to 46 wk	118.5[a]	119.4[a]	112.8[b]	<0.01	1.0
Overall	115.2[a]	115.9[a]	108.7[b]	<0.01	0.9
Average wheat intake (g/bird per d)					
19 to 26 wk	—	ND[1]	46.0	—	—
27 to 37 wk	—	60.0[a]	49.4[b]	<0.01	0.8
38 to 46 wk	—	60.4[a]	53.6[b]	<0.01	0.8
Overall[2]	—	60.2[a]	51.2[b]	<0.01	0.8
Average balancer diet intake (g/bird per d)					
19 to 26 wk	—	ND	57.4	—	—
27 to 37 wk	—	58.2[a]	60.2[b]	<0.01	0.3
38 to 46 wk	—	58.8[a]	60.0[b]	<0.01	0.4
Overall[2]	—	58.4[a]	60.1[b]	<0.01	0.3
Egg production (%)					
19 to 26 wk	86.9	82.9	87.1	NS[3]	1.2
27 to 37 wk	96.4	94.3	96.2	NS	0.9
38 to 46 wk	94.5	91.3	94.3	NS	1.5
Overall	93.1	90.2	93.1	NS	1.0
Egg weight (g)					
19 to 26 wk	53.6	53.5	53.2	NS	0.4
27 to 37 wk	60.4	61.1	60.3	NS	0.4
38 to 46 wk	62.4	61.9	62.1	NS	0.4
Overall	59.1	59.2	58.8	NS	0.4
Egg mass (g/d)					
19 to 26 wk	46.9	44.8	46.9	NS	0.8
27 to 37 wk	58.3	57.4	58.1	NS	0.7
38 to 46 wk	59.0	57.0	59.0	NS	1.1
Overall	55.2	53.6	55.0	NS	0.8
FCR (g of feed:g of egg)					
19 to 26 wk	2.37[a]	2.53[b]	2.30[a]	<0.01	0.04
27 to 37 wk	2.02[a]	2.07[a]	1.89[b]	<0.01	0.03
38 to 46 wk	2.02[ab]	2.13[b]	1.93[a]	<0.01	0.04
Overall	2.11[a]	2.21[a]	2.01[b]	<0.01	0.03
BW (g)					
19 wk	1,504	1,555	1,522	NS	22.5
26 wk	1,716	1,720	1,655	NS	27.7
37 wk	1,819[a]	1,862[a]	1,724[b]	<0.01	26.1
46 wk	1,823[a]	1,862[a]	1,723[b]	<0.01	26.1

[a,b]Values within the same line with no common superscripts differ significantly ($P < 0.05$).

[1]The respective intakes of wheat and balancer diet between wk 19 to 26 were not available for loose-mix treatment.

[2]Overall intakes of wheat and balancer diet were for values from wk 27 to 46.

[3]Not significant ($P > 0.05$).

the experimental period was observed and hen weight in the sequential feeding group remained lower than the 2 other groups ($P < 0.01$).

In sequential feeding, overall yolk weight (both in g and %) was similar to that of control but inferior to loose-mix (Table 3). Control was similar to loose-mix in yolk weight. Similar egg yolk weight was obtained among treatments before and after peak in egg production (wk 19 to 26 and 38 to 46, respectively). Yolk weight during the peak period (wk 27 to 37) was inferior in sequential compared with loose-mix, but the 2 treatments were similar to the control.

Overall, sequential feeding resulted in heavier eggshell weight (g and %) compared with that obtained in hens fed loose-mix and control (Table 3). Eggshell weight was similar among treatments before peak. At peak, eggshell weight (g) was similar between sequential and loose-mix, and both were higher than the control. However, during this period, eggshell weight (%) was higher in sequential feeding followed by loose-mix and control in descending order. Eggshell weight (%) was similar between control and sequential treatment after peak. There was no treatment effect on the albumen weight (both in g and %) throughout the experimental period (Table 3).

At the end of the habituation period (wk 19), significant differences in the relative weight of digestive organs were observed only for the duodenum and ileum (Table 4). Sequential feeding resulted in heavier duodenum and ileum when compared with loose-mix and control. At the end of the experimental period (wk 46), sequential feeding resulted in heavier gizzard, liver, and pancreas. However, proventriculus, duodenum, ileum, and jejunum weights were similar among treatments at this period. However, no effect of slaughter hour was observed on the weight of these organs. Likewise, no interaction between slaughter hour and treatment on the weight of digestive organs was observed.

Hepatic glycogen content (mmol/g of liver) was similar between sequential and control (Figure 2). However, it was higher in loose-mix compared with the 2 other treatments. In all of the treatments, birds killed in the morning had lower glycogen content compared with those killed in the afternoon. Similar hepatic protein (g/g of liver) content was observed in all of the treatments (Figure 3). Hepatic protein content was affected by slaughter hour; hence, it was higher for birds killed in the morning. Liver lipid content was not affected by treatment or slaughter hour (Figure 4).

DISCUSSION

During the habituation period, increased wheat intake with increasing age and quantity offered indicated a successful adaptation of the birds to consuming whole cereals. This confirms our hypothesis obtained from a previous study (Umar Faruk et al., 2008), in which we observed low wheat intake at wk 25, due to sudden in-

Table 3. Weight (g) and proportion (%) of egg yolk and shell of laying hens fed whole wheat sequentially or in loose-mix from 19 to 46 wk of age

		Treatment				
Measurement	Unit	Control	Loose-mix	Sequential	*P*-value	SEM
Egg yolk						
19 to 26 wk	g	11.4	11.5	11.2	NS[1]	0.13
	%	21.4	21.7	21.2	NS	0.20
27 to 37 wk	g	14.8^{ab}	15.1^{a}	14.3^{b}	<0.01	0.15
	%	24.9^{ab}	25.1^{a}	24.1^{b}	<0.01	0.24
38 to 46 wk	g	16.2	16.2	16.0	NS	0.14
	%	25.9	26.3	25.6	NS	0.22
Overall	g	14.4^{ab}	14.5^{a}	14.1^{b}	<0.01	0.10
	%	24.3^{ab}	24.6^{a}	23.9^{b}	<0.01	0.18
Eggshell						
19 to 26 wk	g	5.5	5.5	5.6	NS	0.06
	%	10.4	10.4	10.6	NS	0.09
27 to 37 wk	g	5.5^{a}	5.9^{b}	6.1^{b}	<0.01	0.08
	%	9.3^{a}	9.8^{b}	10.3^{c}	<0.01	0.11
38 to 46 wk	g	6.2^{ab}	6.1^{a}	6.4^{b}	<0.01	0.06
	%	10.0^{ab}	9.9^{a}	10.3^{b}	<0.01	0.09
Overall	g	5.8^{a}	5.9^{a}	6.1^{b}	<0.01	0.05
	%	9.9^{a}	10.0^{a}	10.4^{b}	<0.01	0.07
Egg albumen						
19 to 26 wk	g	36.2	35.7	35.6	NS	0.37
	%	68.3	67.9	67.6	NS	0.30
27 to 37 wk	g	39.2	39.4	38.7	NS	0.41
	%	65.8	65.1	65.1	NS	0.31
38 to 46 wk	g	39.8	39.1	39.8	NS	0.38
	%	64.0	63.7	64.1	NS	0.25
Overall	g	38.6	38.2	38.3	NS	0.36
	%	65.7	65.3	65.4	NS	0.26

[a–c]Values within the same line with no common superscripts differ significantly ($P < 0.05$).

[1]Not significant ($P > 0.05$).

Table 4. Effect of sequential and loose-mix feeding on the weight of digestive organs (% BW) at wk 19 and 46 of hens fed whole wheat sequentially or in loose-mix with a balancer diet from 16 to 46 wk of age

Organ (% BW)	Week 19[1]					Week 46[2]				
	Control	Loose-mix	Sequential	*P*-value	SEM	Control	Loose-mix	Sequential	*P*-value	SEM
Proventriculus	0.31	0.27	0.31	NS[3]	0.01	0.35	0.31	0.35	NS	0.01
Gizzard	1.84	2.16	2.19	<0.05	0.10	1.21^{b}	1.38^{ab}	1.46^{a}	<0.05	0.05
Duodenum	0.54^{ab}	0.51^{b}	0.64^{a}	<0.05	0.03	0.57	0.56	0.58	NS	0.02
Jejunum	0.85	0.85	1.00	<0.05	0.05	0.94	0.90	0.95	NS	0.03
Ileum	0.63^{b}	0.62^{b}	0.75^{a}	<0.05	0.03	0.76	0.72	0.70	NS	0.03
Liver	2.47	2.43	2.51	NS	0.13	2.78^{b}	2.96^{ab}	3.10^{a}	<0.05	0.08
Pancreas	0.21	0.22	0.22	NS	0.01	0.16^{b}	0.18^{ab}	0.19^{a}	<0.05	0.01

^{a,b}Values within the same line with no common superscripts differ significantly ($P < 0.05$).

[1]Eight birds/treatment were killed at wk 19. All of the birds were killed at the same hour (0800 h).

[2]Sixteen birds/treatment were killed at wk 46. Eight were killed in the morning (0800 h) and the remaining 8 birds in the afternoon (1500 h). No slaughter hour effect ($P > 0.05$) was observed on the measured measurements.

[3]Not significant ($P > 0.05$).

troduction of wheat grains in the diet of birds fed mash up to this age. Therefore, a learning period remains necessary when birds are to be fed with wheat grains (Forbes and Covasa, 1995).

Total feed intake was reduced when diets were fed sequentially. Blair et al. (1973) observed an increased feed intake in the sequential treatment compared with the control. However, they fed pellet balancer diet ad libitum as compared with mash balancer diet fed in controlled quantity in the present work. Reduced feed intake of sequentially fed birds in this work agreed with reports of Leeson and Summers (1978), even though the morning diet was both high in protein and energy whereas the afternoon diet was low in these nutrients. Our results also agreed with Reichmann and Connor (1979), and Lee and Ohh (2002), who had an experimental design similar to our study.

In the present study, low feed intake in sequential feeding was a result of reduced wheat intake. Wheat intake was significantly lowered (−9 g/bird per d) in the sequential than in the loose-mix treatment. Higher wheat intake in loose-mix may be attributed to the feed particle selection (Picard et al., 1997; Umar Faruk et al., 2008). Increasing particle size increases feed intake (Safaa et al., 2009). Particle selection is more likely to be seen in loose-mix because heterogeneity in particle

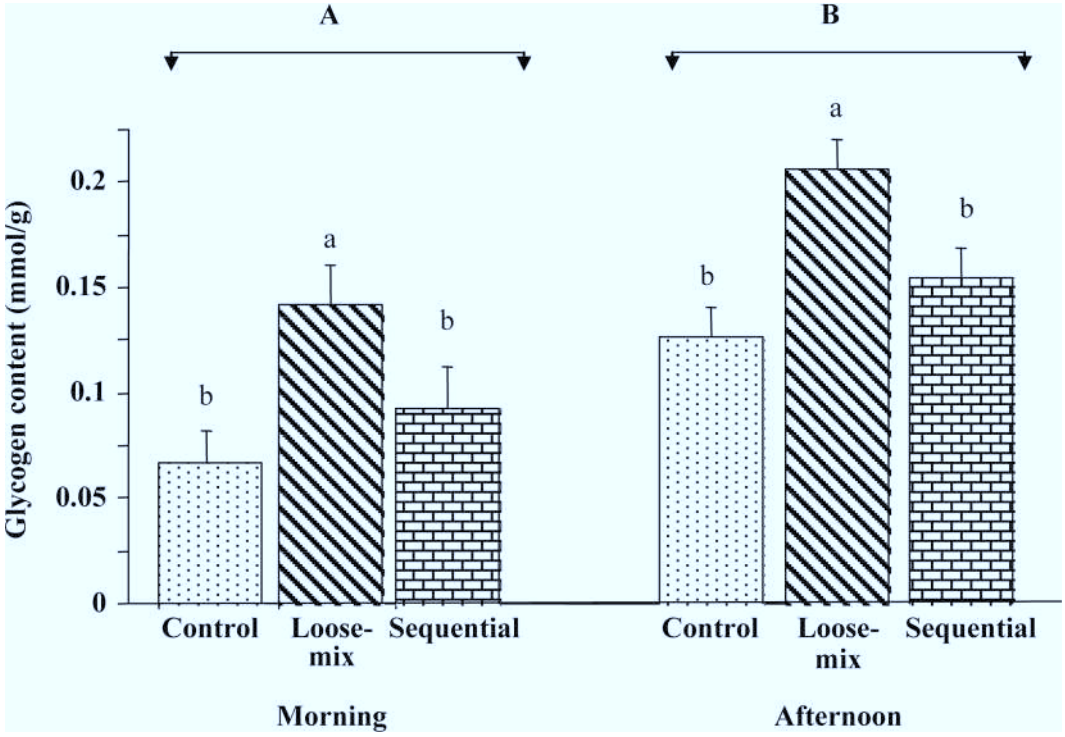


Figure 2. Glycogen content (mmol/g of liver) at wk 46 of birds fed a complete diet (control), whole wheat and balancer diet together (loose-mix), or whole wheat and balancer diet alternately (sequential). Eight birds each were killed in the morning and afternoon. Lowercase letters indicate differences between treatments, whereas uppercase letters indicate differences between the time of day (morning vs. evening).

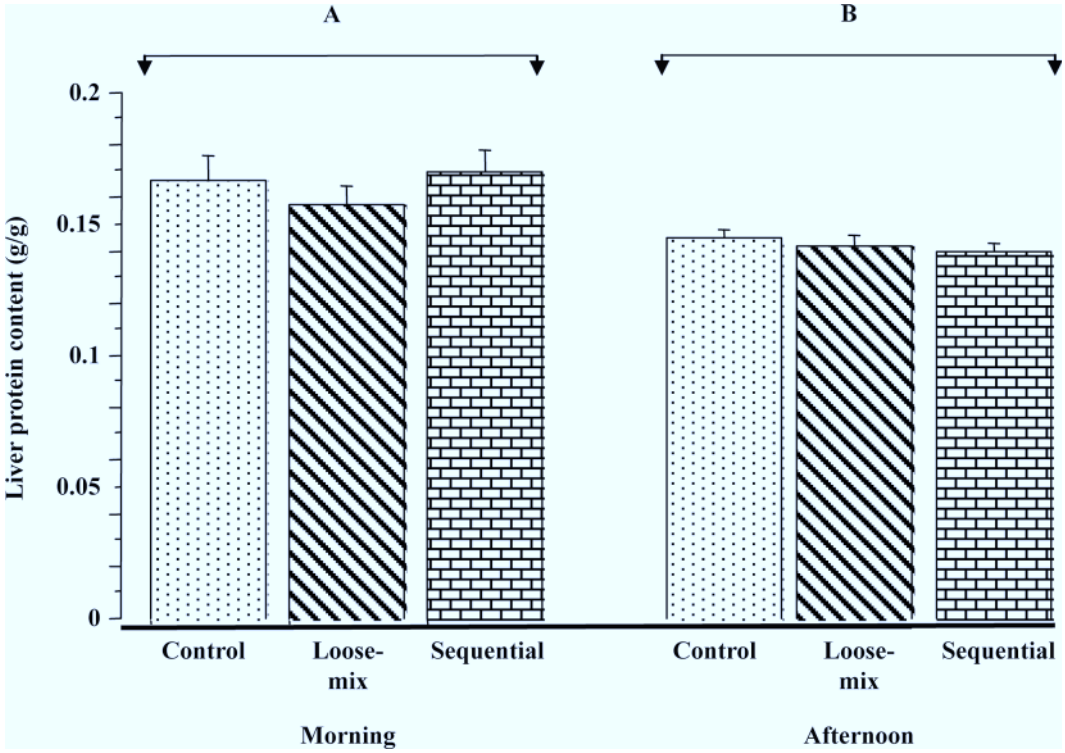


Figure 3. Liver protein content (g/g of liver) at wk 46 of laying hens fed a complete diet (control), whole wheat and balancer diet together (loose-mix), or whole wheat and balancer diet alternately (sequential). Eight birds were killed both in the morning and afternoon. Uppercase letters indicate the difference between morning and afternoon. The absence of lowercase letters indicates no difference between treatments.

size is increased by the addition of wheat grains. This, therefore, confirmed the suggestion of Bennet (2003) that only half of an offered diet should be in the form of grains, to avoid overconsumption of grains.

Although balancer diet intake was significantly higher (+1.7 g/bird per d) in sequential feeding, this was not enough to make up the difference in total feed intake. This was due to the limitation in the daily quantity of the diet offered (105% of the daily spontaneous feed consumption of the genotype currently used). Higher balancer diet intake in sequential feeding could be attributed to its protein and mineral (especially calcium) contents. These were amplified by the time of the day at which this diet was offered. The pattern of daily feed

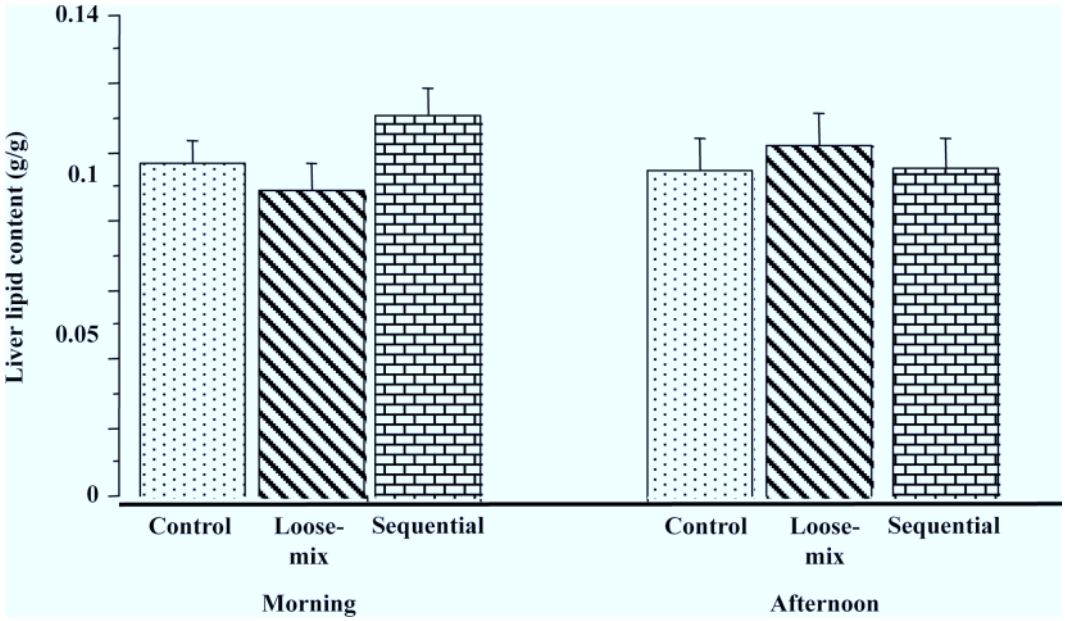


Figure 4. Liver lipid content (g/g of liver) at wk 46 of laying hens fed a complete diet (control), whole wheat and balancer diet together (loose-mix), or whole wheat and balancer diet alternately (sequential). Eight birds were killed both in the morning and afternoon.

Table 5. Calculated ME (kcal/bird per d) and protein (g/bird per d) intakes and requirements of laying hens fed whole wheat sequentially or in loose-mix from 19 to 46 wk of age

		Treatment				
Measurement	Daily intake level	Control	Loose-mix	Sequential	*P*-value	SEM
ME (kcal/bird per d)						
19 to 26 wk	Intake[1]	299.0[a]	299.8[a,2]	280.1[b]	<0.01	3.7
	Requirement[3]	304.4	295.1	291.9	NS[4]	3.6
	Difference[5]	−5.4[a]	4.7[b]	−11.8[a]	<0.01	2.0
27 to 37 wk	Intake	322.9[a]	325.7[a]	297.6[b]	<0.01	2.4
	Requirement	322.8[ab]	325.8[a]	313.6[b]	<0.01	2.7
	Difference	0.1[a]	−0.1[a]	−16.0[b]	<0.01	1.8
38 to 46 wk	Intake	325.9[a]	328.1[a]	309.8[b]	<0.01	2.5
	Requirement	322.6	319.6	313.9	NS	3.3
	Difference	3.3[ab]	8.5[a]	−4.1[b]	<0.01	2.5
Overall (19 to 46 wk)	Intake	317.0[a]	319.1[a]	296.5[b]	<0.01	2.6
	Requirement	317.5	315.0	307.5	NS	2.9
	Difference	−0.5[a]	4.0[a]	−11.0[b]	<0.01	1.8
Protein (g/bird per d)	Intake	19.0	19.0	18.7	NS	0.1
19 to 26 wk	Requirement	18.9[a]	17.9[b]	18.1[ab]	<0.01	0.3
	Difference	0.1[a]	1.1[b]	0.6[ab]	<0.05	0.2
27 to 37 wk	Intake	20.6[a]	20.5[a]	19.7[b]	<0.01	0.1
	Requirement	21.2	21.2	21	NS	0.2
	Difference	−0.6	−0.6	−1.0	NS	0.2
38 to 46 wk	Intake	20.8[a]	20.7[a]	20.2[b]	<0.05	0.1
	Requirement	20.8	20.1	20.5	NS	0.3
	Difference	−0.1	0.6	−0.4	NS	0.3
Overall (19 to 46 wk)	Intake	20.2[a]	20.2[a]	19.6[b]	<0.05	0.1
	Requirement	20.4	19.9	20.0	NS	0.2
	Difference	−0.2	0.3	−0.4	NS	0.2

[a,b]Values within the same line with no common superscripts differ significantly ($P < 0.05$).

[1]Metabolizable energy and protein intakes were calculated by multiplying the quantity of diet consumed and the calculated ME and protein contents of the diet, respectively (Table 1).

[2]Due to the nonavailability of actual intake of wheat and balancer diet during the period from wk 19 to 26, energy and protein intakes were estimated based on equal intake of wheat and balancer diet during this period. This concerns only birds fed in loose-mix.

[3]Requirements in ME and protein were calculated according to Sakomura (2004) and Sakomura et al. (2002), respectively. The temperature values were used according to actual temperatures for each period (i.e., 21.82, 22.00, and 21.28°C for periods 19 to 26, 27 to 37, and 38 to 46 wk, respectively).

[4]Not significant ($P > 0.05$).

[5]Difference between requirements and intakes was calculated as the intake minus the requirement.

intake in laying hens is influenced by the egg-forming cycle as well as by photoperiod (Ballard and Biellier, 1969; Nys et al., 1976; Choi et al., 2004). Thus, hens consumed more diet in the afternoon (Keshavarz, 1998), mainly to account for calcium required in eggshell formation, especially on egg-forming days (Mongin and Sauveur, 1974).

In our work, egg production and weight increased with hen age and were consistent with the breeders' guidelines (ISA, 2007). We observed similar egg production and egg weight among all 3 treatments. This indicates that the reduction in feed intake of sequentially fed birds had no effect on their egg production, egg weight, and mass during the period of study. Blair et al. (1973), Reichmann and Connor (1979), and Lee and Ohh (2002) all reported similar egg production when hens were fed diets sequentially. Robinson (1985) reported a decrease in egg production related to the difficulty in timing protein meal at a particular time of the day. Conversely, Leeson and Summers (1978) reported an increase in egg production due to increase in protein and energy intake of birds fed sequentially. Egg weight was similar (Blair et al., 1973) or reduced (Leeson and Summers, 1978; Robinson, 1985; Lee and Ohh, 2002) in sequential compared with conventional feeding. The latter authors reported decreased egg weight of birds fed in loose-mix. Egg weight and rate of lay are reduced when protein intake is reduced (Morris and Gous, 1988). In the present work, overall protein intake was estimated to be statistically higher for hens fed control (20.2 g/bird per d) and loose-mix (20.2 g/bird per d) compared to the sequential (19.6 g/bird per d) treatment (Table 5). This difference in protein intake had no effect on egg production and weight because the estimated daily intake of protein was similar to the estimated daily requirements in all 3 treatments.

The reduced intake, while maintaining similar egg production and weight in sequential feeding, resulted in a consistent improvement of FCR compared with the 2 other treatments: 5 and 10% relative to control and loose-mix, respectively.

There was no treatment effect on growth performance during the prelaying stage. Cowan et al. (1978) and Karunajeewa and Tham (1984) reported no difference in BW between hens fed a choice of whole grains and a protein concentrate and those fed a control com-

plete diet during the prelaying period. As a result of a lower BW gain from wk 19 to 37, sequentially fed birds had low BW at the end of the experimental period (wk 46). Body weight gain during this period was significantly lower in sequential feeding (1.6 g/d) than loose-mix (2.4 g/d) and control (2.4 g/d). The difference in BW tended to appear about a week after peak production (wk 26), suggesting that body deposition was lowered in sequential feeding to balance the feed intake and the demand in energy required for egg production (Scanes et al., 1987). Estimation of energy intake (Table 5) indicated that sequentially fed birds ingested less energy compared with loose-mix and control. Similar energy intake was observed between loose-mix and control. Energy intake compared with their respective requirements showed a consistent balance for loose-mix and control. However, for sequential feeding, ME intake was lower than requirement. This suggested a slight increase in digestion efficiency in these birds because egg production performance was not affected. In sequential feeding, the reduction in ME intake agreed with results of Lee and Ohh (2002).

The proportion of egg components observed in this work was in line with the changes in the egg components reported by Harms and Hussein (1993). They observed that with increasing hen age, egg weight increases but the eggs contain proportionally more yolk and less albumen and shell. This is because the albumen weight with hen age increases but at a decreasing rate, whereas the yolk increases at a faster rate (Johnston and Gous, 2007). Percentage egg yolk was low in sequential feeding from 27 to 37 wk of age compared with the 2 other treatments. Increase in BW was also low in sequential feeding during this period. The difference in yolk weight was no longer significant at the end of the trial period as was also observed with BW gain. Dietary protein affected egg weight (Fisher, 1969) due to reduction in all components, but yolk and shell weight changed proportionately less than the total and albumen weight.

The percentage eggshell was found to be higher in sequential feeding. This was not surprising because calcium intake fed as flour was largely reinforced during the later part of the photoperiod, as a consequence of a higher level of calcium in the balancer diet compared with the control diet. Calcium absorption in laying hens is affected by stage of shell formation and is higher during the second part of the photoperiod (Etches, 1986; Nys, 1993; Kebreab et al., 2009). Because calcium supplied in this work was ground and mixed in the balancer diet, the results also confirmed the findings of Balnave and Abdoellah (1990) that granular sources of calcium are not an essential prerequisite in nutrient-fractioned feeding systems such as sequential feeding.

At the end of the experimental period, sequentially fed birds had heavier gizzards, pancreas, and liver. Dietary particle size is known to influence the avian digestive tract such that the gizzard weight increases with increasing particle size (Nir et al., 1990). Increase in gizzard weight of sequential and, to some extent, loose-mix-fed birds suggests an increase in grinding capacity compared with control. It could be suggested that this increased grinding capacity in sequential feeding allowed the efficient utilization of feed and this could explain to some extent the improved performance. Change in liver weight agreed with observations of De Basilio et al. (2001), who, under warm conditions, observed heavier livers in broiler birds fed sequentially compared with control. Karunajeewa (1978) observed similar liver weight between birds fed a choice of whole wheat and those fed control.

Similar liver DM and lipid contents between diets were in agreement with Karunajeewa (1978). According to Maurice and Jensen (1979), liver lipid content is affected by type and quantity of dietary cereal. It was not expected to currently differ among treatments because we only introduced 1 type of cereal. Wolford and Polin (1974) observed that increase in feed intake of birds resulted in increased liver lipid content. We observed no difference in hens fed either loose-mix or sequential feeding, although the former had higher feed intake than the latter. Glycogen content is generally believed to vary in function of the feeding state of the animal (Greenberg et al., 2006). Birds killed in the afternoon had higher hepatic glycogen and this was related to their feeding status and also the presence of the digestive enzyme amylase (Rideau et al., 1983). This author showed that amylase concentration is high between 4 and 10 h after oviposition. In this work, the birds were killed in the period corresponding to the peak presence of amylase. Modification in liver glycogen content between groups suggested that changes in liver glucose utilization occurred. This may result from a better digestive utilization (grinding capacity, starch digestion, as well as involvement of gastrointestinal hormones) as suggested by the higher weight of gizzard and pancreas in both sequential and loose-mix groups. The significant effect with the loose-mix group may result from the higher intake of wheat as compared with the sequential group.

However, feeding management (Van Krimpen et al., 2005), especially sequential feeding (Jordan et al., 2009), may affect feather pecking in laying hens, possibly due to reduction in time spent on feed intake. There is, therefore, the need to investigate the optimal duration of the sequence (wheat-balancer diet) in sequential feeding. Equally, the quantity, form (ground or unground), and type of cereal [millet, sorghum, and maize (*Zea mays*)] to be offered need to be explored. It is also necessary to better understand the changes in digestive and liver functions based on feeding system. Sequential feeding imposes constraints in terms of feed allowance, but in particular, conditions can largely improve feed conversion. This feeding system is therefore a promising one in terms of performance but also in facilitating use of local feedstuffs introduced as whole grains.

This trial covers the first half of the egg production cycle; thus, it could be concluded for this period that when birds were fed a controlled quantity of whole wheat and balancer diet sequentially or loosely mixed, similar egg production performance was observed. Loose-mix resulted in similar performance to the classic feeding in terms of feed intake and efficiency of feed utilization, whereas sequential feeding largely increased feed efficiency. This can be used to advantage in reducing feeding cost.

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CHAPTER 4 :

The impact of Sequential and Loose-mix feeding using whole wheat on the performance of laying hens housed individually.

Influence de l'alimentation mélangée ou séquentielle sur les performances des animaux.

Etude des réactions individuelles des poules logées en cages individuelles et nourries à volonté en alimentation séquentielle ou mélangée

Lieu d'essai : INRA UR83 Recherche Avicoles, Nouzilly, France

Durée d'essai : 6 mois précédés de 3 semaines d'habituation à compter de la 16ème semaine d'âge.

L'expérimentation présentée dans ce chapitre est réalisée en parallèle avec celle du chapitre 3 avec des poules logées en cages collectives. La présente avait pour objectif d'étudier les performances individuelles des poules en alimentation mélangée ou séquentielle. Egalement, la capacité individuelle des poules à réguler leur ingestion en fonction de la composition de l'aliment dans ces modes d'alimentation a été étudiée. Tous les animaux ont été nourris à volonté. Trois types d'aliment ont été apportés dans cinq régimes expérimentaux durant 24 semaines (de la 19ème à la 42ème semaine d'âge). Un aliment complémentaire (50) optimisé pour un apport de 50% de blé a été apporté avec 50% de blé en alimentation séquentielle (S50) ou mélangée (M50). Un autre aliment complémentaire (25) optimisé pour un apport de 25% de blé a été proposé avec 50% de celui-ci en alimentation séquentielle (S25) ou mélangée (M25). Ces quatre régimes ont été comparés avec un régime témoin (T) contenant un aliment complet classique. Chaque régime est donné à 25 poules de souche ISABROWN logées en cages individuelles. Les paramètres étudiés sont l'ingestion, la production et le poids de l'œuf, le poids des constituants de l'œuf et des organes digestifs.

Une baisse de consommation d'aliment a été observée avec les poules dans le régime M25 comparées à celles en T, S50 et S25, mais elles sont similaires à celles nourries avec M50. Egalement, nous observons une baisse d'ingestion de protéine pour M25 comparée aux 4 autres régimes.

Cependant, l'ingestion d'énergie metabolizable est comparable entre les quatre régimes. La production ainsi que le poids de l'œuf sont réduits avec M25 comparés à S50 et S25. Une baisse du poids corporel est observée pour M25 par rapport à T, S50 et S25. Cependant, une très forte variation individuelle est observée dans tous les régimes.

Quant aux systèmes d'alimentation, les poules alimentées par séquence ont sous consommé largement le blé et ont dérivé progressivement vers une surconsommation de complémentaire, 50 ou 25. Avec une distribution en mélange, les poules ont consommé en priorité les particules de blé tout au long de la journée et ne se sont pas focalisées sur le complémentaire, qui est sous consommé. Dans cette expérience, globalement, quel que soit le mode de distribution des aliments, mélange ou séquentiel, les poules n'ont pas régulé leur ingestion pour atteindre une efficacité optimale de leur production. Ceci indique qu'avec ces modes de distribution, il est indispensable de piloter les apports respectifs des deux fractions.

Ce chapitre a fait l'objet d'un article en cours de publication dans la revue scientifique *British Poultry Science.* L'article a été accepté pour parution le 08/06/2010.

Running Title: FEEDING SYSTEM AND PERFORMANCE IN HENS

Adaptation of wheat and protein-mineral concentrate intakes by individual hens fed ad libitum in sequential or in loose-mix systems.

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Full-length article

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Abstract 1. Feed intake and performance of birds under sequential or loose-mix feeding was investigated from 19 to 42 weeks of age. A complete diet was fed as control (C). A balancer diet (50) was fed either sequentially (S50) or in loose-mix (L50) with wheat. This diet was to provide similar nutritive value as C assuming a 50:50 diet and wheat intake. Another balancer diet (25) was fed sequentially (S25) or in loose-mix (L25) with wheat. The diet was to provide similar nutritive value as C assuming 75:25 diet and wheat intakes. In sequential feeding, only wheat was fed in the morning (4hrs after lighting) and the balancer diet in the late afternoon (4hrs before light-off). In loose-mix, a mixture of the two diets was fed throughout the 16 h daily light. Each treatment was allocated ad libitum to 25 birds in individual cages.

2. Birds fed L25 had lower total feed intake compared to C, S50 and S25. Protein intake (g/b/d) was reduced with L25 compared to C, S50, S25 and L50. ME (kJ/b/d) intake was however, similar among treatments. Egg production and weight were reduced with L25 compared to S50 and S25. BW was lowered with L25. However, there was high individual variation on all parameters.

3. Feeding system (sequential vs loose-mix) had no effect on ME intake. However loose-mix reduced feed and protein intake due to lower balancer diet intake. It also resulted to low egg production, egg and body weights compared to sequential feeding. The weights of pancreas and gizzard were heavier with sequential and loose-mix compared to the control.

4. Loose-mix reduced performance. Sequential feeding resulted to similar performance as conventional feeding thus could be used to advantage in situations where it is applicable.

Key words: Laying hen, sequential feeding, loose-mix feeding, whole-wheat, feed intake adaptation.

INTRODUCTION

Use of whole cereal grain in poultry ration is a popular practice in Northern Europe. The reason has been the economic benefit as well as the local availability of these feedstuffs. In countries where the cost of feed mixing hinders production, direct incorporation of cereal grains could be an alternative. Although the benefits depend on the relative price of cereals, it is important to have a clear knowledge of the impact of incorporating cereal grains in poultry ration on bird performance. Different methods such as choice feeding, loose-mix feeding and sequential feeding can be used to offer cereal grain to poultry (Noirot et al., 1998). Choice feeding is the simultaneous feeding of grains and a protein-mineral concentrate placed in different containers. Loose-mix feeding is the distribution of these dietary components in a single container. Sequential feeding involves the distribution of the two dietary components separately at different times of the day. Choice feeding using cereal grains is accompanied by an improvement in feed utilisation because it allowed a degree of feed selection by the animal (Forbes and Covasa, 1995; Noirot et al., 1998). It presents however, the inconvenience of having more than one feed trough to offer the different diets. Loose-mix and sequential feeding however, could be practical since only one trough is required.

Studies evaluating sequential and loose-mix feeding in laying hen are limited. Loose-mix feeding of laying hen was reported to reduce both feed and protein intakes when a mixture of whole wheat, whole barley, kibbled maize and pellet protein concentrate were offered (Blair et al., 1973), or when fed a (50:50) mixture of high energy/protein Ca diets (Lee and Ohh, 2002). Although the former observed similar egg production and egg weight to conventional feeding, the latter reported a decrease in egg weight, related to a decrease in energy intake. Loose-mix feeding of 60% barley and a protein concentrate was reported to increase feed, energy and protein intakes, but it reduced egg production while increasing egg weight (Bennet and Classen, 2003). However, when the quantity of whole wheat in a loose-mix diet is limited to 20%, similar intake and production were observed (Kermanshashi and Classen, 2001; MacIsaac and Anderson, 2007).

Sequential feeding of a mixture of whole cereals followed by a pellet protein concentrate resulted in increased feed and protein intakes while maintaining similar energy intake, egg production and egg weight to control (Blair et al., 1973). However, sequential feeding was reported to reduced feed, energy and protein intakes when birds were fed a protein concentrate in the morning followed by whole oats in the afternoon (Robinson, 1985), or when fed high energy diet in the morning and protein concentrate in the afternoon (Leeson and Summers, 1978; Reichmann and Connor, 1979; Lee and Ohh, 2002). Except the work of Blair et al. (1973) all the other authors reported that sequential feeding resulted in lower egg production and egg weight.

Recent investigations on the sequential and loose-mix feeding of whole wheat and a protein-mineral concentrate in laying hen revealed that sequential feeding is more efficient compared to loose-mix and the conventional feeding of a complete diet (Umar Faruk et al. 2010). However, it was possible that this increased efficiency was linked to the experimental protocol used (1) controlled feed intake by feeding a limited quantity (121 g/b/d) of a diet containing 60.5 g each of whole wheat and the concentrate (2) Birds were kept in-group, which facilitate social interactions among individuals, and modify birds feeding pattern through imitation among them (Meunier-Salaün and Faure, 1984). However, this mode of housing may also prevent the study of individual response to sequential and loose-mix feeding, since only the average cage values could be analysed.

The present work was carried out to extend our above recent report. The objective was to investigate the impact of sequential and loose-mix feeding of birds kept in individual cages and fed diets ad libitum. Furthermore, the study investigates the ability of birds under these feeding systems to adapt their feed intake according to their requirements.

MATERIALS AND METHODS

Pre-experimental period (week 16 – 18)

A total of 149 Isa Brown layer hens were trained (Forbes and Covasa, 1995) from wk 16 to 18, to get them habituated to the feeding systems studied. The specific objective was to adapt the

sequentially fed birds to whole-wheat intake before point of lay (Umar Faruk et al., 2008). The animals were housed in a three-tier battery having individual cages (25 x 38cm). Each cage was equipped with a feed trough (20 cm per hen) and a nipple drinker. A complete diet "control habituation" (Table 1) containing 11.7 MJ/kg and 16% CP was fed to a group of 33 birds as control (CH). Another diet called "balancer diet habituation" containing 11.0 MJ/kg and 18% CP was fed sequentially (SH) with wheat (13.0 MJ/kg) to another group containing 58 birds. Hens in this group were given access to whole wheat (*triticum aestivum)* in the morning and the balancer diet in the afternoon. The same diet "balancer diet habituation" was mixed with whole wheat and fed in loose-mix (LH) to another group containing 58 birds. The "balancer diet habituation" was formulated on the assumption that if the birds consumed 65% of it and 35% whole wheat, they will ingest equal amount of nutrients as those receiving diet CH.

Birds were fed ad libitum in line with breeders' guidelines (Nutrition Management Guide, ISA Hendrix Genetics, 2007). The total quantity of diet offered was progressively increased to account for increase in feed intake of a growing bird. Thus, it rose from 70 to 83 g/b/d from week 16 to 18 of age respectively. In sequential feeding, the duration at which birds were given access to wheat was equally increased from 3 h (week 16) to 7 h (week 18). Photoperiod was 10L: 14D at wk 16 and reached 16L: 8D at wk 18. Water was fed ad libitum throughout the study.

Experimental period (week 19 - 42)

Five treatments were formed at week 19 of age. Each of the sequential and loose-mix groups was divided into two groups to form four treatments of 25 birds per treatment. Other 25 birds from the CH group were selected to form the control treatment. During the experimental period, the birds were housed in the same poultry house and individual cages used during the habituation period. Body weight was used as criterion for the placement of the birds so as to obtain a homogenous intra treatment BW. However, due to limited number of birds habituated and the random choice of birds dissected for the measurement of the digestive organs, initial BW for the treatment L50 was inferior compared to C, S50

and S25. Photoperiod was 16L:8D and temperature was maintained at 20.6±1.8°C throughout the experimental period.

A complete diet (Table1) containing 11.5 MJ/kg and 17.5% CP was fed conventionally as control (C). To investigate the ability of laying hens to adapt their intake according to their energy and protein requirements, the balancer diet B50 (10.0 MJ/kg, 23.2% CP) was formulated to provide similar nutritive value as C on the condition that the birds ingest equal quantities (50:50) of the diet and whole wheat. This diet was fed to two experimental groups either sequentially (S50) or in loose-mix (L50). Another balancer diet B25 (11.0 MJ/kg, 19.5% CP) was formulated to provide similar nutritive values as C on the condition that the birds ingested it on a 75:25 basis with wheat. It was fed to other two groups either sequentially (S25) or in loose-mix (L25). Diets were fed ad libitum (180g/b/d) containing equal amount of each of the fractions (wheat/protein mineral concentrate). Birds fed the B50 diet were expected to have a 50% wheat intake, while only 25% wheat intake was expected for those fed the B25 diet. Calcium (Ca) was grounded and incorporated in the balancer diet since provision of granular Ca is not a prerequisite when birds are fed nutrient fractioned diets (Balnave and Muritasari, 1990).

Measurements

Feed intake was measured weekly. In the two S fed treatments, wheat and balancer diet intakes were determined separately. During the experimental period, wheat intake in L was measured after separation using manual sieve (2 mm).

Eggs produced were collected and weighed individually on daily basis. The weight of egg components (yolk, albumen and shell) was determined at an interval of four weeks starting from wk 21. The albumen and the chalazae were separated using forceps prior to weighing the yolk. The shells were washed and dried for 12 hours in a drying oven at 70°C, and then weighed. All measurements were taken to the nearest 0.01g.

Birds were weighed individually at 16, 19, 26 and 37 weeks of age. The weight of the digestive organs was recorded at the end of the pre-experimental (week 19) and experimental (week 42) periods

to test the effect of feeding system on these organs. The birds were weighed before being injected with Na Pentobarbital solution[1] (1ml/kg body weight). The abdominal cavity was then dissected and the digestive tract collected and separated into proventriculus, gizzard, duodenum, pancreas, jejunum and ileum. The segments were first emptied and dried using a paper towel before weighing. The proventriculus and the gizzard were placed in an ice container (-4°C) for 3 hours to facilitate the removal of the surrounding fat. The organs were emptied prior to weighing.

Metabolizable energy (kJ/b/d) and protein (g/b/d) intakes were estimated as a product of feed intake and ME and protein contents of the experimental diets respectively. ME requirement was estimated according to Sakomura (2004)

$$ME\ (kJ/b/d) = W^{0.75}*(165.74 - 2.37*T) + 6.68*WG + 2.40*EM$$

Where;

ME = energy requirement (kJ/b/d), W = Body weight (Kg), T = Temperature (°C), WG = weight gain (g/day), EM = Egg mass (g/b/d).

Protein requirement was estimated according to Sakomura et al., (2002)

$$PB = 1.94*W^{.075} + 0.480*G + 0.301*EM$$

Where;

PB = protein requirement (g/b/d), W = body weight (Kg), G = daily weight gain (g/d) and EM = egg mass (g/b/d).

Statistical Analysis

Individual values from cages were analyzed using StatView (version 5, SAS Institute Inc., Cary, NC). A one-way ANOVA was performed using the GLM model to test treatment effect on feed intake, egg production, egg weight, body weight, digestive organs weight, ME intake and requirements. A 2X2 factorial ANOVA was carried out to test the effect of feeding system (loose-mix vs sequential) and

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balancer diet effect (50 vs 25). Results were considered significantly different if $p<0.05$, and Bonferroni-Dunnet pairwise comparison was used to compare differences between means. Repeated measures Anova was performed on BW, feed and wheat intakes, and egg mass to test the effect of treatment over time. For the ME intake and requirements, a t-test was performed to compare ME intake and ME requirement for each treatment.

RESULTS

During the habituation period, similar total feed intake was observed between treatments: 62.4, 62.5 and 62.4 g/b/d for CH, LH and SH respectively. Wheat intake of SH increased from 13.0 to 40.0 g/b/d from week 16 to 18 respectively. Their balancer diet intake however, decreased with increasing age and wheat intake: 51.6 to 40.0 g/b/d from week 16 to 18 respectively. This increase in wheat intake with the resulting decrease in balancer diet intake during this period suggested a successful adaptation to wheat intake for SH fed birds. Similar BWG from week 16 to 18 was observed: 8.1, 8.3, and 8.6 g/b/d for CH, LH and SH respectively.

During the experimental period, the average total feed intake was lower for birds receiving L25 compared to C, S50 and S25, although it was similar to L50 (Table 2). Figure 1 (b) showed a detailed evolution in feed intake according to treatment from week 19 to 42. There was a significant increase in feed intake in all treatments with time, although from week 30 of age, birds fed L25 and L50 had lower feed intake with higher group variation than what was observed with the other three treatments. Wheat intake was higher for birds receiving L50 followed by L25, S50 and S25 in descending order (Table 2). Detailed wheat intake (Figure 1c) showed a progressive but significant decrease in wheat intake especially with treatments S25 and S50.

Egg production and egg weight were lowered with L25 compared to S50 and S25 (Table 2). Egg production and weight were however, not statistically different between L25, L50 and C. Birds in treatment L25 had lower egg mass and egg yolk weight compared to the other four treatments. Egg mass in this treatment was progressively reduced from week 23 and was generally not stable through out the experimental period (Figure 1 d). Eggshell weight was reduced with L25 when compared to S25, S50 and L50, but it was similar to C. No treatment effect on albumen weight was observed. FCR was similar among all treatments.

BW at week 19 was lower for birds receiving L50 than C, S50 and S25, although they were similar to L25 (Table 2). This was associated to this treatment low BW upon arrival at week 16 (Figure 1

a). Although L50 was initially low in BW, the final BW (week 42) was inversely lower for L25 compared to C, S25 and S50. The evolution showed in figure 1 (a) indicated that birds receiving L50 had a growth rate similar to all treatments, thus ending at similar weight. Inversely, L25, which was similar in BW to all treatments at the beginning of the experiment, had reduced growth thus having lower BW than C, S50 and S25 at the end of the experiment.

Feeding system affected birds' intake and performance with birds fed sequentially having higher total feed intake than those fed in loose-mix (Table 2). The latter had higher wheat intake compared to the former. Hens fed sequentially had higher egg production, egg weight and albumen weight than those fed loose-mix. BW was higher with birds fed sequentially than with those fed loose-mix and this was associated to higher initial BW. However, FCR and BWG were similar among the two systems.

Birds receiving balancer diet optimized for 25% wheat intake had lower feed intake compared to those receiving diet optimized for 50% (Table 2). The former had lower wheat intake than the latter. Egg production, egg weight, egg albumen weight, FCR and final BW were similar between these two diets. However, BWG was higher with birds fed balancer diet optimized for 50% wheat intake compared to that optimized for 25%.

Similar ME (kJ/b/d) intake was observed between treatments (Table 3). Estimated ME requirement for treatment L25 was lower to that of C, S50 and S25, but similar to L50. The difference between ME intake and ME requirement was higher for birds fed L25, than S50, S25 and C. It was however, similar to L50 indicating a surplus in ME intake of birds in these treatments. Protein intake (g/b/d) was reduced with L25 fed birds compared to the other four treatments. Protein intake of birds fed L50 was lower than C and S50 but similar to S25. The estimated protein requirement was lowered in birds fed L25 compared to C, S50 and S25 but similar to L50. The difference between protein intake and requirement was low for birds fed L25 and S25 compared to those fed S50. Birds receiving L50 and C were similar to all treatments in the difference between protein intake and requirement.

ME intake was similar between the sequential and loose-mix systems (Table 3). Estimated ME requirement of sequentially fed birds was higher to that of loose-mix. The difference between ME intake and estimated requirement was lower for birds fed sequentially. Both intake and estimated requirement of protein were higher in birds fed sequentially than those fed loose-mix.

ME intake and estimated requirement and the difference between ME intake and estimated requirement were similar between the two balancer diets (Table 3). Protein intake was low for birds receiving balancer diet formulated for 25% wheat intake compared those receiving diet formulated for 50%. Protein requirement was similar between the two diets.

At the end of habituation period (week 19), similar relative weights of proventriculus, liver and pancreas were observed between CH, SH and LH (Table 4). The relative weight of duodenum was lower for birds in LH group compared to SH. The relative weight of ileum was heavier for birds in SH group than with those in LH and CH. Gizzard and jejunum weight were different among groups (Anova $p<0.05$). However, this difference disappeared using Bonferroni-Dunnet pairwise comparison test ($p>0.05$), probably due to limited number of birds killed (8/treatment).

At the end of the experimental period (week 42), proventriculus was heavier for birds in S compared to C and L treatments. Gizzard was heavier for S and L treatments compared to C. Duodenum was heavier for S compared to C. Liver was heavier with L treatment than with C. Pancreas was heavier for S and L treatments compared to C. There was no significant difference in the weights of jejunum and Ileum between the three treatments.

DISCUSSION

None of the four treatments receiving whole wheat had a daily wheat consumption corresponding to the expected 25 or 50% of the total daily feed intake. On one hand, birds receiving diet formulated for 50% wheat intake consumed slightly less wheat (47%) than the anticipated quantity. On the other hand, those fed diet formulated for 25% wheat intake consumed more wheat (41%) than the anticipated 25%. However, no difference in ME intake was observed between treatments, suggesting an adjustment by the sequential and loose-mix fed birds to consume similar ME to those fed the complete diet C. This lends support to work of Blair et al., (1973), Kermanshashi and Classen (2001) and MacIsaac and Anderson, (2007), reporting similar ME intake of birds fed in loose-mix or complete diet. However, in sequential feeding, Leeson and Summers (1978), Reichmann and Connor (1979), Robinson (1985) and Lee and Ohh (2002) reported a decrease in ME intake simultaneously with a reduced feed intake, which was not the case in the present work.

Birds fed sequentially had higher feed intake compared to loose-mix. This was not in accordance to our earlier work with birds housed in-group (Umar Faruk et al., 2010), in which higher feed intake was obtained with loose-mix compared to sequential. In the above work, birds were housed in group and fed limited quantities of the two dietary fractions which was not the case in the present work. Lower feed intake observed with loose-mix in the present work was mainly attributed to treatment L25 because of its similar balancer diet intake compared to L50. Although L25 fed birds consumed more wheat than expected, their balancer diet intake remained similar to L50, thereby reducing their total intake.

Higher wheat intake when birds are fed in loose-mix than in sequential is in line with our earlier observation under different condition (Umar Faruk et al., 2010). Increased wheat intake was associated to “larger feed particle preference” in laying hen (Picard et al., 1997; Umar Faruk et al., 2008). Portella et al., (1988) observed a marked disappearance of larger particles when birds were fed regular crumbles, and the smaller particles disappeared only as the concentration of large ones decreased

indicating a preference of larger particles by the birds. In the present work, the inclusion of whole wheat in the concentrate diet is likely to increase the heterogeneity of the diet, thus increased selection of the larger particles (whole wheat) first.

Birds fed sequentially consumed more balancer diet compared to those fed in loose-mix. Blair et al. (1973) observed an increased feed intake when birds were fed sequentially, associated to high intake of protein concentrate. However, sequential feeding using an energy-rich diet in the morning and a protein concentrate in the afternoon (Leeson and Summers, 1978; Reichman and Connor, 1979) lowered feed intake. Equally, feed intake was reduced when oats/sorghum were sequentially fed with a protein concentrate (Robinson, 1985), Lee and Ohh (2002) also reported a reduction in feed intake when birds were fed a high energy/protein and low Ca diet in the morning followed by a low energy/protein and high Ca in the afternoon. In the present work, high balancer diet intake in sequential feeding agreed with the feeding pattern in laying hen (Ballard and Biellier, 1969; Nys et al., 1976; Keshavarz, 1998a, b; Choi et al., 2004). Birds consume larger amount of food in the afternoon partly in association with the hen Ca appetite which coincides with the initiation of eggshell formation during this period. For example, Keshavarz (1998a) found that hens subjected to a 16-h light consumed 40% of daily feed intake in the first 8 h after light-on and 60% during the second 8 h period before light-off with a drastic increase recorded 4 h before light-off (Keshavarz, 1998b).

Laying hen adapts its feed intake relatively well to the energy value of its feed, although this regulation is not perfect (Joly and Bougon, 1997), as the hen is influenced by the form and method in which the feed is presented. Our data were subjected to a t-test with ME intake and ME requirement as variables for each treatment. Results showed that no difference between the ME intake and ME requirements for birds fed C and L50, suggesting that they adapted their ME intake to their ME estimated requirement. However, birds fed S50, S25 and L25 had significant difference between ME intake and requirement: birds receiving S50 and S25 had lower ME intake than required but those receiving L25 had an excessive ME intake, although their efficiency of utilising this energy was poor

because they had the lowest performance. Despite their inferior ME intake than what they require, birds fed S50 and S25 maintained similar performance as those fed conventionally. This was also observed in our previous work (Umar Faruk et al, 2010). This is in line with earlier works (Leeson and Summers, 1978; Reichman and Connor, 1979) who also observed a reduction in feed intake in sequential feeding without reduction in production relative to the control and associated it to lower maintenance requirements due to lighter body weight in sequential feeding. In the present work, it is probably linked to an improvement in the rate of utilisation of nutrients evidenced by heavier gizzard, proventriculus and duodenum observed with sequential than with loose-mix and control.

Loose-mix fed birds had fewer eggs with lighter weight than those fed sequentially. Reduction in protein intake is accompanied by reduction in both egg production and egg weight (Morris and Gous, 1988). Our results showed that protein intake was lower with loose-mix treatment. Ad libitum access to the diets mixture enhanced higher wheat consumption in loose-mix as was earlier suggested (Umar Faruk et al., 2008). This therefore led to low protein-mineral concentrate (balancer diet) intake and eventually protein intake.

At the end of the experimental period, sequential and loose-mix fed birds had heavier gizzard than those fed the control diet. This was expected because feeding whole wheat grain increased the dietary particle size of the diets fed sequentially or in loose-mix. Dietary particle size was known to influence the avian digestive tract. Gizzard weight increases with increasing particle size (Nir et al., 1990). This increase in gizzard weight was hypothesized to contribute to better performance of sequentially fed hens housed in-group (Umar Faruk et al., 2010). In the present work, no improvement in performance was observed and this can be associated to broad intra treatment variations, as well as ad libitum feeding used. Changes in liver weight observed were in line with De Basilio et al., (2001), who observed heavier livers in broilers fed cereal grains. It was also in line with Umar Faruk et al., (2010) who observed heavier liver weight in birds fed whole wheat.

In conclusion, the present study confirmed our earlier report (Umar Faruk et al., 2010) that sequential feeding has no adverse effect on egg production, thus outlining its potential interest in feed management in egg production. In the present experimental conditions involving ad libitum access to concentrate diets formulated for different proportion of wheat intake, it is clear that birds will not consume the expected proportion of the respective fractions. In sequential feeding, similar feed intake and performance as the conventional feeding was obtained irrespective of the concentrate diet used. This system could therefore be used to advantage in situations where the cost of grinding and feed mixing hinders production. In loose-mix feeding, distributing concentrated diets optimized for 25% wheat intake reduces nutrient intake and performance because animals will not ingest the correct proportion of the dietary fractions, thus should be avoided.

The advantages of sequential feeding needs to be further explored in terms of the physical form (whole, ground) and type of cereals (millet, sorghum, maize etc) to be used. Ad libitum feeding in sequential feeding resulted to excessive intake of the concentrate diet and this may increase cost, therefore, requires additional investigation. Feeding management (Van Krimpen et al., 2005), especially sequential feeding (Jordan et al., 2009) induce feather pecking in laying hens, due to reduction in time spent on feeding activity. Therefore, the optimal duration of the sequence (wheat –balancer diet) in sequential feeding needs to be established.

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Table 1. *Composition of experimental diets*

Ingredient (g/kg)	Habituation Period (16 18 week)		Experimental Period (19 42 week)			Both periods
	Control habituation (CH)	Balancer diet habituation (LH)	Control (C)	Balancer diet B50	Balancer diet B25	Whole Wheat *(Triticum aestivum)*
Wheat	346.6		500.0			100
Maize	350.0	535.7	161.3	320.8	498.4	
Wheat bran	100.0	153.1	25.4	50.1	45.0	
Maize Gluten			32.9	66.2	24.0	
Soya bean meal (T48)	165.0	252.5	169.7	340.8	285.0	
Soya bean oil			8.0	16.0	10.0	
Calcium Carbonate	18.4	28.2	80.0	160.4	105.1	
Bi calcium phosphate	10.9	16.7	11.6	23.3	17.4	
Refined salt	2.0	3.1	2.0	4.0	2.7	
Sodium Bicarbonate	2.0	3.1	2.0	4.0	2.7	
L Lysine 78			1.1	2.2	1.3	
DL Methionine	0.1	0.2	1.1	2.2	1.8	
Premix (Sup 64 J 02)	5.0	7.7	5.0	10.0	6.7	
Calculated Composition (g/kg as fed basis)						
EM (MJ/kg)	11.7	11.0	11.5	10.0	11.0	13.0
CP	160.5	183.3	175.2	233.2	194.7	119.0
DM	877.0	875.0	891.0	899.0	889.0	868.0
Fiber	35.9	41.0	30.1	33.7	32.7	26.5
Lysine	7.2	9.3	8.1	13.1	10.7	3.1
Methionine	3.2	3.9	4.5	7.1	5.7	2.0
Calcium	12.0	18.2	36.1	72.0	48.0	0.3
Total P	5.6	7.1	5.3	7.6	6.4	3.0
Analyzed Composition (g/kg)						
DM	890.0	887.0	888.0	895.0	890.0	867.0
CP	157.0	186.0	173.0	230.0	195.0	119.0

Vitamin and mineral premix supplied the following amounts per kilogramme of diet: 1200 mg of Cu (sulphate), 4000 mg of Fe, 200 mg of I, 60 mg of Se, 120 g of DL Methionine, 200 mg of Canthaxanthine; 11000 mg of Zn, 12000 mg of Mn; Retinol 480 mg, Cholecalciferol 12 mg, DL ∝ tocopherol acetate 2000 mg, Menadione 400 mg, Thiamine mononitrate109 mg.

Table 2. *Effect of treatment, feeding system, and balancer diet composition on the feed intake and performance of hens fed whole wheat sequentially (S) or in loose-mix (L) with a balancer diet formulated for either 50 or 25% wheat intake from 19 to 42 weeks of age*

	Feed Intake (g/b/d)	Wheat intake/Feed intake	Eggs produced per hen per d	Egg weight (g)	Egg mass (g/d)	FCE (g egg/ g feed)	BW wk 19 (g)	BW wk 42 (g)[2]	BWG (g/d)	Egg Yolk (g)	Egg Albumen (g)	Eggshell (g)
Treatments [(1)]												
C	114.4 ab		0.946 ab	57.3 ab	54.2 a	0.473	1560 a	1832 a	1.7 a	14.2 a	36.9	6.0 ab
S50	115.7 a	0.384 c	0.950 a	58.2 a	55.5 a	0.481	1588 a	1810 a	1.4 ab	14.0 a	38.0	6.1 a
S25	111.5 ab	0.315 d	0.947 a	58.4 a	55.3 a	0.498	1603 a	1809 a	1.1 ab	14.1 a	38.0	6.3 a
L50	107.0 bc	0.563 a	0.924 ab	57.2 ab	53.4 a	0.486	1485 b	1722 ab	1.4 ab	14.0 a	37.2	6.1 a
L25	102.8 c	0.502 b	0.886 b	55.0 b	49.0 b	0.476	1545 ab	1622 b	0.6 b	13.2 b	35.4	5.7 b
P	*<0.01*	*<0.01*	*<0.05*	*<0.05*	*<0.01*	*NS*	*<0.01*	*<0.05*	*<0.05*	*<0.05*	*NS*	*<0.05*
SEM	0.97	1.20	0.69	0.35	0.50	0.004	8.10	17.73	0.09	0.09	0.30	0.04
Feeding system [(2)]												
L	104.8 b	0.532 a	0.904 b	56.0 b		0.480	1515 b	1688 b	1.1		36.4 b	
S	113.7 a	0.350 b	0.948 a	58.3 a		0.490	1596 a	1810 a	1.3		38.0 a	
P	*<0.01*	*<0.01*	*<0.05*	*<0.05*		*NS*	*>0.01*	*<0.05*	*NS*		*<0.05*	
SEM	1.11	1.20	0.83	0.40		0.005	9.52	20.23	0.09		0.34	
Balancer Diet effect												
50	111.3 a	0.474 a	0.937	57.8		0.483	1535 b	1766	1.4 a		37.7	
25	107.0 b	0.412 b	0.916	56.6		0.487	1573 a	1716	0.9 b		36.9	
P	*<0.05*	*<0.05*	*NS*	*NS*		*NS*	*<0.05*	*NS*	*<0.05*		*NS*	
SEM	1.11	1.20	0.83	0.40		0.005	9.52	20.23	0.09		0.34	
Interaction												
FS x DC	*NS*	*NS*	*NS*	*NS*	*<0.05*	*NS*	*NS*	*NS*	*NS*	*<0.05*	*NS*	*<0.05*

[(1)] C = Control, S50 = Sequential feeding of diet formulated for 50% wheat, S25 = Sequential feeding of diet formulated for 25% wheat, L50 = Loose mix feeding of diet formulated for 50% wheat, L25 = Loose mix feeding of diet formulated for 25% wheat.

[(2)] Result were not presented where interaction between feeding system and balancer diet effect were observed

[a,b,c] Values within the same column with different superscript differ significantly ($p<0.05$), *NS:* Not significant ($p>0.05$)

Table 3. *Effect of treatment, feeding system, and balancer diet composition on the feed, ME and Protein intakes and ME and Protein requirements of hens fed whole wheat sequentially (S) or in loose-mix (L) with a balancer diet formulated for either 50 or 25% intake of wheat from 19 to 37 weeks of age* [1]

	Feed intake (g/b/d)	Wheat intake/Feed intake	ME intake (kJ/b/d) [2]	ME Requirement (kJ/b/d) [3]	Difference ME (kJ/b/d) [4]	Protein intake (g/b/d)	Protein Requirement (g/b/d)	Difference Protein (g/b/d)
Treatments [5]								
C	113.1 a		1300.0	1321.4 a	21.1 b	19.8 b	19.9 a	0.1 ab
S50	113.6 a	0.387 c	1264.2	1311.0 a	47.0 b	21.4 a	20.1 a	1.3 a
S25	109.9 a	0.320 d	1280.0	1308.1 a	28.2 b	18.7 bc	20.0 a	1.3 b
L50	106.6 ab	0.569 a	1274.0	1241.0 ab	33.0 ab	18.4 c	18.5 ab	0.1 ab
L25	101.5 b	0.503 b	1226.0	1178.1 b	47.7 a	15.9 d	17.4 b	1.5 b
P	*<0.01*	*<0.01*	*NS*	*<0.01*	*<0.05*	*<0.01*	*<0.01*	*<0.01*
SEM	1.00	1.17	9.20	10.30	9.00	0.23	0.23	0.22
Feeding system [6]								
L	103.9 b	0.533 a	1248.0	1207.0 b	41.0 a	17.1 b	18.0 b	
S	111.8 a	0.354 b	1272.0	1310.0 a	37.7 b	20.1 a	20.0 a	
P	*<0.05*	*<0.01*	*NS*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	
SEM	1.12	1.17	10.50	11.61	10.60	0.27	0.27	
Balancer Diet effect								
50	110.4 a	0.472 a	1269.0	1279.2	11.0	20.0 a	19.4	
25	105.5 b	0.415 b	1252.3	1242.0	11.0	17.3 b	18.7	
P	*<0.05*	*<0.05*	*NS*	*NS*	*NS*	*<0.01*	*NS*	
SEM	1.12	1.17	10.50	11.61	10.60	0.27	0.27	
Interaction								
FS x DC	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*<0.05*

[1] Values shown are averages from week 19 to 37 because body weight at week 42 was only measured for the birds used in the measurement of digestive organs.
[2] Estimation of ME (Kcal/b/d) and Protein (g/b/d) intakes were calculated as the product of feed intake and diet composition.
[3] Requirements in ME (kcal/b/d) and protein (g/b/d) were estimated according to Sakomura, 2004 and Sakomura *et al.,* 2002 respectively.
[4] Difference between requirement and intake were estimated as intake minus requirement.
[5] C = Control, S50 = Sequential feeding of diet formulated for 50% wheat, S25 = Sequential feeding of diet formulated for 25% wheat, L50 = Loose mix feeding of diet formulated for 50% wheat, L25 = Loose mix feeding of diet formulated for 25% wheat.
[6] Result were not presented where interaction between feeding system and balancer diet effect were observed
[a,b,c] Values within the same column with different superscript differ significantly ($p<0.05$), *NS* Not significant ($p>0.05$)

Table 4. *Effect of feeding system on weight of digestive organs (g/kg body weight) at weeks 19 and 42 of birds fed whole wheat sequentially or in loose-mix with a balancer diet*

Organ (g/kg)	Week 19					Week 42[(1)]				
	Feeding system [(2)]			*P*	SEM	Feeding system			*p*	SEM
	CH (n=8)	SH (n=8)	LH (n=8)			C (n=16)	S (n=24)	L (n=24)		
Proventriculus	3.1	3.1	2.8	*NS*	0.08	3.3 b	3.7 a	3.3 b	*<0.05*	0.06
Gizzard	18.5	21.9	21.7	*<0.05*	0.62	11.8 b	13.8 a	14.7 a	*<0.05*	0.27
Duodenum	5.4 ab	6.4 a	5.1 b	*<0.05*	0.21	5.2 b	5.9 a	5.5 ab	*<0.05*	0.11
Jejunum	8.5	10.1	8.5	*<0.05*	0.30	9.4	9.5	9.7	*NS*	0.14
Ileum	6.3 b	7.5 a	6.2 b	*<0.05*	0.22	7.6	7.2	7.4	*NS*	0.12
Liver	24.7	25.1	24.4	*NS*	0.74	25.5 b	26.8 ab	28.9 a	*<0.05*	0.54
Pancreas	2.1	2.2	2.2	*NS*	0.06	1.6 b	1.8 a	1.8 a	*<0.05*	0.04

[(1)] No difference in organs weight between treatments of the same feeding system and no interaction (feeding system x balancer diet level) was observed. Therefore, animals receiving diet 25 were put together with their corresponding 50 treatments to increase the population size from 16 to 24.

[(2)] CH= Control Habituation, SH= sequential habituation, LH= loose mix habituation, C = Control, L = Loose mix, S = Sequential

a,b,c: Values within the same line with different superscript differ significantly with Bonferroni Dunnet ($p<0.05$), NS: Not significant ($p>0.05$)

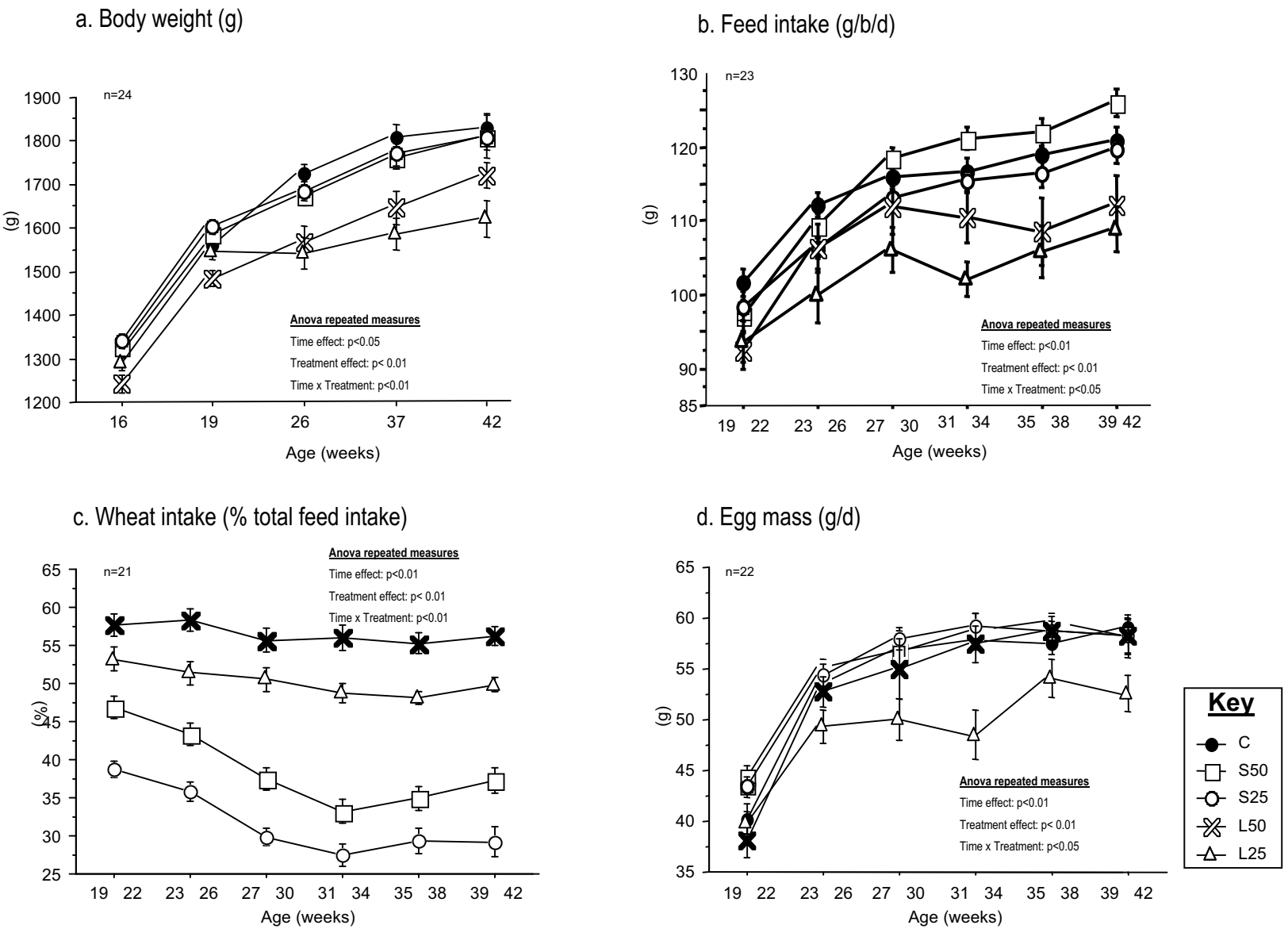


Figure 1. graphs showing individual variation within treatment in (a) BW, (b) Feed intake, (c) wheat intake and (d) egg mass of birds fed whole wheat sequentially (S) or in loose mix(L) with a balancer diet formulated for either 50 or 25% intake of wheat from 19 to 37 weeks of age.

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CHAPTER 5:

Further studies on Sequential feeding: Impact of wheat physical form and energy content of the complete diet on the performance of laying hens housed in-group.

Influence de l'alimentation séquentielle sur les performances des animaux.

Influence de la forme du blé et de la concentration énergétique de l'aliment sur les performances des animaux soumis à l'alimentation séquentielle

Lieu d'essai : INRA UR83 Recherche Avicoles, Nouzilly, France

Durée d'essai : 7 mois précédés de 3 semaines d'habituation à compter de la 16ème semaine d'âge.

Suite aux résultats obtenus dans les chapitres 3 et 4, il est apparu important de se focaliser sur le modèle d'alimentation séquentielle. Une expérimentation a été mise en place avec les objectifs suivants :

1. Valider l'amélioration des performances pour les poules soumises à l'alimentation séquentielle avec du blé entier, comparées à l'alimentation complète classique. Pour parvenir à cet objectif, un régime contenant du blé entier distribué en séquence avec un aliment complémentaire à raison de 50% de la ration journalière a été apporté. Les performances obtenues ont été comparées avec celles des animaux recevant un aliment complet classique.

2. Etudier l'effet de la forme du blé sur les performances des poules soumises à une alimentation séquentielle. Pour cela un régime contenant du blé broyé en alimentation séquentielle a été introduit.

3. Comparer les performances des poules soumises à une alimentation séquentielle ou une alimentation classique, dans une situation où l'ingéré énergétique en alimentation classique est diminué. En effet, lors de l'étude du chapitre 3, une baisse de la consommation d'énergie a été observée chez les poules alimentées de façon séquentielle. Il sera donc intéressant de savoir si une réduction d'ingéré énergétique chez les poules en alimentation classique conduira à des résultats comparables avec ceux obtenues en mode d'alimentation séquentielle. Pour cela, en plus de l'aliment

témoin formulé pour répondre à l'objectif 1 (contenant 2753 kcal/kg), un autre aliment complet contenant moins d'énergie, 2576 kcal/kg, a été apporté.

Toutes les mesures réalisées lors des expériences précédentes ont été répétées. De plus, une mesure du gras abdominal a été réalisée pour avoir quelques éléments de réponse sur le poids faible enregistré chez les poules alimentées de façon séquentielle. Egalement, des mesures d'histologie et de l'activité enzymatique du jéjunum ont été effectuées.

En ce qui concerne le premier objectif, les résultats confirment une baisse de la consommation totale lorsque les animaux sont soumis à en alimentation séquentielle avec du blé entier (103 g/poule/jour) comparée à l'alimentation complète (110 g/poule/jour). La production et le poids moyen d'œuf étant identiques entre les deux régimes confirment l'amélioration de l'indice de consommation en faveur de l'alimentation séquentielle avec du blé entier. Les poules soumises à l'alimentation séquentielle avec du blé entier étaient plus légères que celles soumises à une alimentation classique. Cela peut être relié partiellement à un dépôt moins important de gras abdominal (105 vs 49 g/poule). Les différences de poids observées lors de cette expérience sont identiques à celles observées dans l'expérience du chapitre 3. De plus, le poids du gésier est plus important chez les poules alimentées en séquence avec du blé entier par rapport à l'alimentation classique. Le pourcentage de jaune dans l'œuf a été légèrement inférieur chez les poules soumises à l'alimentation séquentielle avec du blé entier, tandis que leur œufs présentent des pourcentages d'albumen et de coquille supérieurs à ceux d'animaux soumis à une en alimentation classique avec l'aliment complet.

Quant à la forme du blé, le blé broyé a conduit à une baisse non significative mais systématique de la consommation totale (101 vs 103 g/poule/jour) en lien avec une moindre ingestion de blé broyé. La consommation d'aliment complémentaire entre les deux formes du blé était identique. Le poids de l'œuf est inférieur avec lorsque les animaux ont reçu du blé broyé (57 vs 59 g) comparé au blé entier, mais le taux de ponte reste identique entre les deux formes de présentation. Le poids vif des poules recevant le blé broyé est inferieur à celui des poules recevant le blé entier (1601 vs 1650 g/poule). Cette

différence est apparue dès la 26ème semaine d'âge, et est conservée jusqu'à la fin de l'expérience (semaine 46). Le poids du gras abdominal chez les poules alimentée de façon séquentielle avec du blé broyé est plus faible que celui des poules recevant du blé entier. A l'inverse, elles ont un gésier moins développé. Les pourcentages de jaune et d'albumen dans l'œuf sont plus importants chez les poules alimentées en séquence avec du blé entier.

La consommation totale est significativement supérieure pour les poules recevant l'aliment complet contenant moins d'énergie que pour les trois autres régimes. Le taux de ponte ainsi que la masse d'œuf n'ont pas été affectés par le niveau énergétique du régime. Le poids des animaux recevant l'aliment moins énergétique est inférieur par rapport aux animaux témoins mais supérieur à celui des poules recevant du blé broyé distribué en séquence. Aucune différence n'a été observée entre les régimes quant à l'histologie et l'activité enzymatique du jéjunum.

Les résultats de cette expérience montrent l'intérêt de l'alimentation séquentielle avec du blé entier entraînant une réduction de l'ingestion sans remettre en cause les performances de production.

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animal

Is sequential feeding of whole wheat more efficient than ground wheat in laying hens?

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The impact of sequential feeding of whole or ground wheat on the performance of layer hen was investigated using ISABROWN hens from 19 to 42 weeks of age. In addition, the effect of reduced dietary energy content of a complete diet was also investigated. Four treatments were tested. Whole wheat was alternated with a protein–mineral concentrate (balancer diet) in a treatment (sequential whole wheat: SWW), while another treatment alternated ground wheat (sequential ground wheat: SGW) with the same balancer diet. The control (C) was fed a complete layer diet conventionally. Another treatment (low energy: LE) was fed a complete diet conventionally. The diet contained lower energy (10.7 v. 11.6 MJ/kg) compared to the C. Each treatment was allocated 16 cages and each cage contained five birds. Light was provided 16 h daily (0400 to 2000 h). Feed offered was controlled (121 g/bird per day) and distributed twice (2 × 60.5 g) at 4 and 11 h after lights on. In the sequential treatment, only wheat (whole or ground) was fed during the first distribution and the balancer diet during the second distribution. Left over feed was always removed before the next distribution. The total feed intake was not different between SWW and SGW, but the two were lower than C ($P < 0.05$). Wheat intake was however, lowered with SGW compared to SWW ($P < 0.05$). Egg production and egg mass (EM) were not different between treatments. Egg weight was lower with SGW than with SWW ($P < 0.05$), but the two were similar to C. Body weight (BW) was lowered ($P < 0.01$) with SGW relative to SWW and C, SWW BW being also lower than the C one. The efficiency of egg production was increased ($P < 0.01$) with the SWW and SGW relative to the control. Birds fed LE had higher feed intake ($P < 0.05$) but they had similar egg production and EM compared to the two sequential treatments. The efficiency of feed utilization was also reduced ($P < 0.01$) with LE compared to SWW and SGW. It was concluded that sequential feeding is more efficient than conventional feeding. In addition, whole wheat appeared more efficient than ground wheat in terms of egg and BW.

Keywords: sequential feeding, whole wheat, ground wheat, feed intake, egg production

Implications

The cost of feeding represents approximately 60% of the total cost of egg production. Sequential feeding is a techni que that alternates on farm locally produced whole cereals with a separate protein mineral concentrate diet. It is proved to reduce feed intake without reducing egg produc tion, thereby, leading to lower feed cost and increased feed efficiency. The technique also decreases the amount of energy required for grinding and transportation. This work compared sequential feeding of whole *v.* ground wheat. Results showed similar efficiency of feed utilization between the two forms although egg weight was reduced with ground wheat. Efficiency with sequential feeding was therefore improved compared to conventional feeding.

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Introduction

Sequential feeding is a feeding management technique that alternates two nutritionally contrasted diets (usually whole cereals and a protein mineral concentrate (balancer diet)) over a given period or cycle. In laying hen, this technique improved the efficiency of feed utilization by 5% when compared to the conventional feeding of a complete com pounded diet (Umar Faruk *et al.*, 2010). This is because it reduced the total feed intake, due to a decrease in wheat intake, without lowering egg production and weight. In addition, sequential feeding allowed for a direct utilization of on farm grown cereals, thus reducing the cost of energy required for grinding and transportation of whole cereals.

Other techniques can be employed to offer whole grains and a balancer diet to poultry (Noirot *et al.*, 1998). They can be fed simultaneously in different containers (choice feeding)

or mixed together and fed in single container (loose mix). Choice of feeding whole cereals is accompanied by an improvement in feed utilization because it allows a degree of feed selection by the animal, even though this selection could lead to increased heterogeneity between individuals. It presents, however, the inconvenience of having more than one feeding trough to contain different diets. As such, it is less practical. Very recently, sequential feeding was reported to improve the efficiency of feed utilization by 10% com pared to loose mix (Umar Faruk *et al*., 2010) when laying hen were fed whole wheat and a balancer diet either sequentially or in loose mix.

Earlier work on sequential feeding revealed conflicting results in terms of feed intake, egg production and egg weight. Leeson and Summers (1978), Robinson (1985) and Lee and Ohh (2002) reported reduced feed intake when hens were given access to different diets sequentially. The first two stu dies reported reduced egg production and egg weight while the latter reported similar egg production with reduced egg weight compared to conventional feeding. Thus, this approach does not improve the efficiency of feed utilization. In the opposite, Blair *et al*. (1973) reported increased feed intake without any effect on egg production and weight, thereby, deteriorating the efficiency of feed utilization.

Among the factors leading to the improved feed efficiency in the work of Umar Faruk *et al*. (2010) above, are the control of the daily feed supplied (restricted or *ad libitum*) to the birds and the diet composition, especially the level of con trast in terms of energy and protein between the two alter nating diets. Improved efficiency can also be a result of an appropriate timing of the daily nutrients supply in connection with egg formation cycle. Therefore, the origin of the effect of the sequential feeding is not clear: is it linked either to the time of nutrient supply (alternating *v.* continual) or to the wheat physical form? Feed particle size modifies hen feeding behaviour (Portella *et al*., 1988) and to a large extent, giz zard function (Nir *et al*., 1990). A more developed gizzard leads to greater digestion of nutrients (Amerah *et al*., 2007). Deaton *et al*. (1989) compared with the performance of laying hens fed corn ground using a hammer mill or a roller mill, resulting in different feed particle size. They reported no effect of feed particle size on feed intake and performance over three consecutive trials. However, Scott and McCann (2005) used diets obtained from wheat ground using differ ent screen sizes (2, 5 and 8 mm), observed higher feed intake for the hens receiving diets containing large particle (8 mm) compared to those fed the smaller ones (2 mm). Egg weight was higher for birds receiving the 2 mm particles compared to 8 mm, and egg production tended to be reduced with diets containing the 8 mm size particles. Therefore, the form of cereals in sequential feeding needs to be investigated.

Umar Faruk *et al*. (2010) have reported that sequential feeding using wheat as the main cereal results in lower energy intake than the conventional feeding. According to Blair *et al*. (1973), sequential feeding of a mixture of whole wheat and a pelleted balancer diet gave similar energy intake compared to conventional feeding. However, decreased energy intake was observed (Robinson, 1985) when whole oats and a protein concentrate are fed sequentially. The sequential feeding of high energy and a protein concentrate diet (Leeson and Summers, 1978; Reichmann and Connor, 1979; Lee and Ohh, 2002) reduces energy intake and performance relative to conventional feeding. It is therefore of interest to evaluate the effect of energy reduction when comparing conventional to sequential feeding.

The objective of the present work was to investigate the effect of wheat physical form (whole *v.* ground) in sequential feeding. To achieve this objective, two treatments were fed with either whole or ground wheat sequentially distributed with a balancer diet. Another objective was to compare the effect of energy reduction in conventional feeding with sequential feeding. For this objective, a second control diet containing lower energy than the normal control was fed conventionally as treatment.

Material and methods

Birds and housing

All the birds used in the experiment (328 ISA Hendrix Brown growing hens) were acclimatized to sequential feeding from 16 to 18 weeks of age. They were given access to whole wheat in the morning followed by a protein mineral con centrate 'balancer diet growing' in the afternoon (Table 1). The birds were housed in wired bottomed cages designed to accommodate five hens per cage (550 cm^2/bird). Tem perature was maintained at 22.0°C $\pm$ 0.5°C. Photoperiod was 12L : 12D at week 16 and reached 16L : 8D at week 18 (Light on at week 16 was from 05 to 1700 h and from 04 to 2000 h at week 18). Birds were given *ad libitum* access to feed and water throughout the acclimatization period.

The birds were treated according to the European Union's Council Directive of 24 November 1986 (86/609/EEC) through out. All procedures described here fully comply with French legislation on research involving animals. The experimental period was from week 19 to 46 of age. 320 birds were randomly selected and divided into four groups. Each group contained 80 birds divided into 16 cages as replicates. Each replicate con tained five birds. Birds were allotted to replicates on the basis of body weight (BW) such that as homogenous BW as possible was obtained within each cage and treatment. Birds were housed in the same poultry house and kept in the same cages that were used during the acclimatization period.

Experimental treatments

The experiment consisted of four treatments. The composi tion of the diets fed in adaptation and experimental period is described in Table 1. To test the effect of wheat physical form (whole *v.* ground) in sequential feeding, two treatments received diets alternating either whole (SWW) or ground (SGW) wheat in the morning (0830 h) and the protein mineral concentrate 'balancer diet laying' in the afternoon (1530 h). The balancer diet contained 23% protein. It was optimized for a 50% wheat intake for the birds to have similar nutrient intake as those given the control complete diet (C). The control

Table 1 *Dietary composition, nutrient content (as-fed) and particle size distribution of the experimental diets*

	Growing period	Laying period				
	Balancer diet growing	Control (C)	Low energy (LE)	Balancer diet laying	Wheat	
Ingredient (g/kg diet)						
Wheat	–	500.00	462.80	–	100.00	
Maize	535.70	161.30	95.70	342.70		
Wheat bran	100.00	25.40	81.10	–		
Maize gluten	–	32.90	55.00	24.40		
Soya bean meal	252.50	17.00	159.90	410.60		
Soya bean oil	–	8.00	17.90	16.00		
Sunflower meal	–	–	75.00			
Limestone	28.20	79.60	74.90	152.30		
Dicalcium phosphate	16.70	11.60	11.60	26.80		
Salt	3.10	2.00	2.00	4.30		
Sodium bicarbonate	3.10	2.00	1.90	4.00		
L-Lysine 78	–	1.10	1.00	7.00		
DL-Methionine	2.00	1.10	1.60	2.70		
Threonine	–	–	1.00	–		
Premix[1]	5.00	5.00	5.00	10.00		
Pigment[2]			4.10	5.50		
Calculated composition (%)						
Metabolizable energy (MJ/kg)	11.00	11.60	10.70	10.00	13.10	
CP	18.00	18.00	18.00	23.00	12.90	
Dry matter	87.48	88.29	88.50	89.80	86.80	
Fat	2.90	2.49	3.50	3.62	1.35	
Ash	8.06	11.72	12.00	22.03	1.40	
Crude fibre	4.10	2.98	5.00	3.24	2.65	
Total lysine	0.93	0.82	0.83	1.31	0.34	
Total methionine	0.39	0.45	0.49	0.73	0.20	
Calcium	1.82	3.64	3.58	7.20	0.03	
Total P	0.56	0.53	0.61	0.81	0.32	
Analysed composition (%)						
CP		17.73	17.91	22.93	11.90	
DM		89.65	90.37	90.83	86.7	
Proportion of feed particle size (%)						
Diameter (mm)[3]					Whole	Ground
>2.00	6.78	12.02	12.62	4.97	98.1	26.43
<2.00	93.21	87.98	87.39	95.03	18.9	73.57

[1]Vitamin and mineral premix supplied the following amounts per kilogram of premix: vitamin A 1 600 000 IU; vitamin D3 480 000 IU; vitamin E 2000 mg; vitamin K3 400 mg; vitamin B1 109 mg; Zn 11 000 mg; Mn 12 000 mg; Cu (sulphate) 1200 mg; Fe 4000 mg; I 200 mg; Se 60 mg; DL Methionine 120 g; Canthaxanthine 200 mg.
[2]Pigment contains per kilogram: canthaxantine (E161g) 300 mg; luteine (E161b) 1633 mg; zeaxantine (E161h) 91 mg; cryptoxanthine (E161c) 36 mg.
[3]Particle size was determined using the sieving method in dry conditions adapted from Melcion (2000).

(C) corresponded to a complete ground diet containing 11.6 MJ/kg and 18% crude protein. This diet contained 50% wheat ground and mixed with other protein and mineral ingredients. To test if reducing metabolizable energy (ME) intake in conventional feeding will result to similar perfor mance as sequential feeding, another control diet (LE) con taining lower energy (10.7 MJ/kg) but similar protein (18%) to the normal control treatment (C) was fed conventionally.

Each bird received 121 g of feed distributed twice daily. This quantity represented 105% of the theoretical intake of 115 g/bird per day (ISA Hendrix Genetics, 2007). In sequen tial feeding, 50% of the daily fed diet was either whole or ground wheat. In conventional feeding, half of the diet was fed in the morning and the remaining half in the afternoon. Feed left over from previous distribution was always removed before the next distribution. Water was fed *ad libitum* during all periods.

Parameters measured

Feed intake and production. Feed intake was recorded weekly. In sequential feeding, the intakes of wheat and balancer diet were measured separately. The profile of feed particle size for the experimental diets was determined using the dry sieving method adapted from Melcion (2000). A sample of 100 g of feed was sieved during 3 min in a an electric shaker (Restch AS 200 didgit) having sieves with

diameters of 3.15, 2, 1.18, 0.6 mm and bottom tray (<0.6 mm). The profile of feed particle size contained in each of the experimental diets is given in Table 1.

The birds' ME requirement was estimated over two periods corresponding to (i) peak of egg production (week 25 to 31) and (ii) end of the experimental period (week 40 to 46). The estimation was done using the predictive equation of Sakomura (2004);

$$ME = W^{0.75} \times (165.74 - 2.37 \times T) + 6.68 \times WG + 2.40 \times EM$$

where ME = metabolizable energy requirement (Kcal/b per day), T = Temperature (°C), WG = weight gain (g/bird per day), EM = egg mass (g/bird per day) and W = body weight (kg).

Energy exported in the egg (EE) was calculated according to Larbier and Leclercq (1992):

$$EE = (Yw \times 0.33 \times 9.2) + (0.174 \times 5.66 \times Yw) + (Aw \times 0.105 \times 5.66)$$

where Yw = Yolk weight (g), Aw = Albumen weight (g),

The ratio between the EE and the energy intake was then calculated as EE/ME.

BW was recorded at week 19, 26, 37 and 46. Egg production was recorded daily and egg weight was recorded twice weekly by weighing all the eggs produced on the measuring day. The weights of egg yolk, albumen and shell were determined on all eggs at an interval of 4 weeks starting from week 21 of age. For these measurements, the albumen and the chalazae were separated from the yolk using forceps. Egg shells were washed and dried for 12 h in an oven at 70°C, and then weighed. All measurements were taken to the nearest 0.01 g.

The weights of the proventriculus, gizzard, pancreas, spleen and liver were recorded at the end of the experimental period using eight birds per treatment. The birds were randomly selected, weighed and euthanized by the injection of Na Pentobarbital solution[1] (1 ml/kg). The intestine was removed and divided into different segments: duodenum, jejunum and ileum. The intestinal segments were emptied and dried using a paper towel before weighing. The proventriculus and the gizzard were placed in an iced container (−4°C) for 3 h to facilitate the removal of the surrounding fat before being emptied and weighed. Jejunal segments were collected and histological and enzymatic measurements were performed.

Histological measurements. A 1.5 cm segment taken at the middle part of the jejunum was opened longitudinally, rinsed with cold saline (NaCl 9 g/l) and fixed in a buffered formalin solution overnight. It was then rinsed in distilled water and stored in ethanol/water (70/30, v/v) at 4°C until further analysis. The jejunal samples were prepared as described by Goodlad *et al.* (1991). The measurements were made using an optical microscope (Leitz, Laborux), a camera (CFW 1308C, Scion Corporation, Frederick, MD, USA) and image analysis software (Visilog 6.3, Noesis). The length and width of 10 villi and crypts were measured from each bird. The surface area was calculated for each villus and crypt. An average value was calculated for each bird. Villus to crypt length and surface ratios was then calculated.

Enzymatic activity. The jejunal scraping extract were analysed for enzymatic activity of alkaline phosphatase (AP; EC 3.1.3.1) and leucine aminopeptidase (LAP; EC 3.4.11.2). The middle part of the jejunum (one third) was split longitudinally, rinsed with cold saline, wiped on a paper towel and the mucosa scraped off before freezing in liquid nitrogen and stored at −70°C. Samples taken from the frozen intestinal tissues were homogenized at a ratio of 50 mg/ml in phosphate buffer saline (pH 7.4) using an Ultra turrax® (IKA) for 3 × 10 s and centrifuged (10 000×**g**, 15 min, 4°C). The supernatants were stored at −70°C until further analysis. Before the enzyme analysis, the samples were diluted 1/3. A continuous method with 96 well micro plates was used for each enzyme tested.

For the measurement of AP activity, a 0.1 ml of the diluted homogenate was mixed with 0.2 ml of substrate (8.8 μm of *p* nitrophenyl phosphate (Sigma N 4645; Sigma Aldrich Corp., St. Louis, MO, USA) per millilitre of glycine buffer 93 mM containing 50 mM $MgCl_2$, pH 8.8). Readings were carried out at 2 min intervals for 30 min with a multi scan spectrophotometer (TECAN Infinite M200)[2] at 405 nm (37°C) using a standard curve with *p* nitrophenol (Sigma N 7660).

To measure the LAP activity, a 0.03 ml of the diluted homogenate was mixed with 0.25 ml of substrate (1 μm of L leucine *p* nitroanalide (Sigma L 2158) per millilitre of phosphate buffer 0.1 M, pH 7.2). The plate was read at 405 nm (37°C) at 2 min intervals for 10 min *p* Nitroaniline (Sigma N 2128) was used for the standard curve. The results were noted as Units (U)/mg of the weight of intestinal mucosa, one unit corresponding to one nanoMole of the product of the enzymatic reaction (*p* nitrophenol for PA and *p* Nitroaniline for LAP) per time unit (mn).

Statistical analysis

Average values from cages were analysed using StatView (version 5, SAS Institute Inc., Cary, NC, USA). A one way ANOVA was performed using the below GLM model to test treatment effect on all the measured parameters. Results were considered different if $P < 0.05$, and Bonferroni Dunnet multiple comparison test was used to compare differences between treatment means.

$$Y_{ij} = T_i + \varepsilon_{ij}$$

where Y_{ij} = measured variables for treatment i and cage j, T_i = treatment effect (i = C, LE, SWW and SGW) and j being the cage j in treatment i, and ε_{ij} = residual.

[1] CEVA Santé Animale – La Ballastière – 33500 Libourne, France.

[2] TECAN France SAS. 26, av Tony Garnier F-69007, Lyon, France.

Results

Significant reduction in the total feed intake ($P < 0.05$) was observed with SWW and SGW compared to C and LE (Table 2). Lower energy level elicited higher feed consumption in LE diet compared to C diet. In sequential feeding, wheat form did not affect the overall total feed intake, which was similar between SWW and SGW. However, wheat intake was higher ($P < 0.05$) with SWW compared with SGW. No difference in egg production and EM were observed among all the treat ments. Egg weight was higher ($P < 0.05$) for SWW compared to SGW but similar to C and LE. Feed conversion (FCR) ratio was similar between SWW and SGW, but lower than C and LE ($P < 0.01$). The final BW was heavier ($P < 0.01$) for birds fed SWW than SGW, but the two were lower ($P < 0.01$) than C and LE. The heaviest abdominal fat weight was obtained with C, while the lowest was obtained with SGW ($P < 0.01$). The latter was similar in abdominal fat weight to SWW. LE had lower abdominal fat than C, but it was similar to SWW and higher to SGW. Egg yolk weight was higher ($P < 0.01$) for SWW than SGW, but the two were lower than C (Table 3). LE was similar in yolk weight to SWW and C, but higher to SGW ($P < 0.01$). Egg albumen weight was similar between SGW and C, but lower than SWW ($P < 0.05$). LE was similar to all treatments in albumen weight. Eggshell weight was similar between SWW, SGW and LE. However, it was higher for SWW compared to C ($P < 0.05$).

A significant reduction in the overall ME intake was observed with SGW and SWW compared to C and LE (Table 4; $P < 0.01$). ME intake was lowered with LE compared to C ($P < 0.01$), which had the highest ME intake. The overall ME requirement was higher for C compared to SWW and SGW ($P < 0.01$). It was lower for SGW than for SWW ($P < 0.01$). The relative difference between ME intake and requirement was lower for C and higher for SWW and LE ($P < 0.05$), while SGW was similar to all treatments. The amount of EE for egg

Table 2 *Treatment effect on feed intake and performance of birds fed either normal (C) or reduced (LE) energy diet conventionally, or whole (SWW) or ground (SGW) wheat sequentially with a protein–mineral balancer diet from 19 to 46 weeks of age*

		Treatment					
Parameter	Age (weeks)	C	LE	SWW	SGW	*P*-value[1]	s.e.m.[1]
Wheat Intake (% total feed intake)	19 to 26	–	–	44.8^{a}	42.7^{b}	*	0.35
	27 to 37	–	–	43.4	42.6	ns	0.41
	38 to 46	–	–	44.5^{a}	43.1^{b}	*	0.42
	Overall	–	–	44.2^{a}	42.8^{b}	*	0.33
Total feed Intake (g/bird per day)	19 to 26	102.7^{b}	107.6^{a}	99.0^{c}	96.0^{c}	**	0.94
	27 to 37	112.5^{a}	114.9^{a}	104.6^{b}	102.8^{b}	**	0.72
	38 to 46	112.5^{a}	115.5^{a}	106.2^{b}	104.0^{b}	**	0.81
	Overall	109.6^{b}	113.0^{a}	103.5^{c}	101.1^{c}	*	0.69
Egg production (%)[2]	19 to 26	82.6	82.8	82.3	82.5	ns	1.15
	27 to 37	97.7	95.6	96.5	95.5	ns	0.87
	38 to 46	93.9	93.7	89.7	89.7	*	1.39
	Overall	92.0	91.2	90.0	89.7	ns	0.84
Egg weight (g)	19 to 26	53.1	53.9	54.2	52.7	ns	0.42
	27 to 37	59.9^{ab}	59.9^{ab}	60.4^{a}	58.6^{b}	*	0.38
	38 to 46	60.7^{ab}	61.3^{ab}	61.9^{a}	60.2^{b}	*	0.40
	Overall	58.2^{ab}	58.6^{ab}	59.1^{a}	57.4^{b}	*	0.38
Egg mass (g/day)	19 to 26	43.8	44.6	44.5	43.5	ns	0.70
	27 to 37	58.5^{a}	57.2^{ab}	58.3^{ab}	56.0^{b}	*	0.65
	38 to 46	57.1^{ab}	57.5^{a}	55.5^{ab}	54.0^{b}	*	0.90
	Overall	53.8	53.7	53.5	51.8	ns	0.61
FCR (g feed/g egg)	19 to 26	2.31^{a}	2.42^{a}	2.23^{b}	2.21^{b}	**	0.03
	27 to 37	1.93^{b}	2.01^{a}	1.79^{c}	1.84^{c}	**	0.02
	38 to 46	1.98	2.01	1.92	1.93	ns	0.03
	Overall	2.04^{b}	2.11^{b}	1.94^{a}	1.96^{a}	**	0.02
BW (g/b)	19	1518	1537	1532	1532	ns	12.89
	26	1646^{ab}	1679^{a}	1657^{a}	1602^{b}	*	13.94
	37	1776^{a}	1753^{a}	1678^{b}	1625^{b}	**	16.03
	46	1888^{a}	1838^{a}	1734^{b}	1647^{c}	**	18.32
Abdominal fat (%)	46	5.5^{a}	3.1^{b}	2.8^{bc}	1.6^{c}	**	0.25

FCR feed conversion ratio (feed intake/egg mass).
[1]s.e.m. standard error of the mean; $**P < 0.01$; $*P < 0.05$; ns (non-significant) $P > 0.05$; values within a row with no common letters (a, b, c) differ significantly using Bonferroni–Dunnet test at 5% significance level.
[2]Egg production for week 38 to 46 was significantly different between treatments (ANOVA $P < 0.05$), but it was similar on pair-wise comparison Bonferroni–Dunnet ($P > 0.05$).

Table 3 *Treatment effect on egg yolk, albumen and shell weights of birds fed either normal (C) or reduced (LE) energy conventionally, or whole (SWW) or ground (SGW) wheat sequentially with a protein–mineral balancer diet from 19 to 46 weeks of age*

		Treatment					
Parameter	Age (weeks)	C	LE	SWW	SGW	*P*-value[1]	s.e.m.[1]
Egg yolk (g)	19 to 26	11.76	11.79	11.77	11.55	ns	0.09
	27 to 37	15.44^{a}	14.99^{b}	14.69^{b}	14.10^{c}	**	0.11
	38 to 46	16.11^{a}	15.78^{ab}	15.57^{b}	15.11^{c}	**	0.10
	Overall	14.67^{a}	14.45^{ab}	14.25^{b}	13.82^{c}	**	0.08
Egg albumen (g)	19 to 26	35.60^{b}	36.40^{ab}	36.80^{a}	36.00^{ab}	*	0.29
	27 to 37	38.32	38.40	39.30	38.10	ns	0.33
	38 to 46	38.50^{b}	39.52^{ab}	40.30^{a}	39.20^{ab}	*	0.30
	Overall	37.70^{b}	38.20^{ab}	39.10^{a}	37.90^{b}	*	0.26
Egg shell (g)	19 to 26	5.50^{b}	5.60^{ab}	5.73^{a}	5.70^{ab}	*	0.05
	27 to 37	6.08^{ab}	6.02^{b}	6.22^{a}	6.11^{ab}	*	0.05
	38 to 46	6.24	6.33	6.34	6.20	ns	0.04
	Overall	5.95^{b}	6.02^{ab}	6.13^{a}	6.02^{ab}	*	0.04

[1]s.e.m. Standard error of the mean; $^{**}P < 0.01$; $^{*}P < 0.05$; ns (non significant) $P > 0.05$; Values within a row with no common letters (a, b, c) differ significantly using Bonferroni–Dunnet test at 5% significance level.

Table 4 *Estimated metabolizable energy (MJ/bird per day) intake, requirement (MJ/bird per day) and the ratio of EE and ME intake of birds fed either normal (C) or reduced energy (LE) diet, or whole (SWW) or ground (SGW) wheat sequentially with a protein–mineral balancer diet from 19 to 46 weeks of age[1]*

		Treatments					
Age (weeks)	ME (MJ/bird per day)	C	LE	SWW	SGW	*P*-value[2]	s.e.m.[2]
25 to 31	Intake[3]	1.29^{b}	1.24^{a}	1.18^{c}	1.15^{c}	**	0.008
	Requirement[4]	1.32^{a}	1.30^{ab}	1.28^{b}	1.24^{c}	**	0.008
	Difference[5]	0.03^{c}	0.06^{bc}	0.10^{a}	0.09^{ab}	**	0.008
	EE[6]	0.32^{a}	0.31^{ab}	0.32^{a}	0.30^{b}	*	0.004
	EE/ME intake[6]	0.25^{b}	0.25^{b}	0.27^{a}	0.26^{ab}	*	0.003
40 to 46	Intake	1.30^{a}	1.24^{b}	1.22^{bc}	1.19^{c}	**	0.011
	Requirement	1.37^{a}	1.35^{a}	1.29^{b}	1.24^{c}	**	0.011
	Difference	0.07^{b}	0.11^{a}	0.07^{b}	0.05^{b}	**	0.008
	EE	0.34^{a}	0.34^{ab}	0.32^{bc}	0.31^{c}	*	0.006
	EE/ME intake	0.26	0.27	0.26	0.27	ns	0.001
Overall	Intake	1.29^{a}	1.24^{b}	1.20^{c}	1.17^{c}	**	0.008
	Requirement	1.34^{a}	1.32^{a}	1.28^{b}	1.23^{c}	**	0.008
	Difference	0.05^{b}	0.08^{a}	0.08^{a}	0.07^{ab}	*	0.008
	EE	0.33^{a}	0.32^{a}	0.32^{ab}	0.31^{b}	*	0.004
	EE/ME intake	0.26	0.26	0.27	0.26	ns	0.001

EE energy exported in the egg; ME metabolizable energy.
[1]Estimation was done over two periods corresponding to (1) peak of egg production (week 25 to 31) and (2) end of the experimental period (week 40 to 46).
[2]s.e.m. standard error of the mean; $^{**}P < 0.01$; $^{*}P < 0.05$; ns (non-significant) $P > 0.05$; values within a row with no common letters (a, b, c) differ significantly using Bonferroni–Dunnet test at 5% significance level.
[3]ME intake was calculated by multiplying the quantity of diet consumed and the calculated ME content of the diet (Table 1).
[4]ME requirement was calculated according to Sakomura (2004). The temperature values used were the actual values recorded for each period (week 25 to 31 21.90°C; week 40 to 46 22.57°C).
[5]Difference between ME intake and ME requirement was calculated as (ME intake – ME requirement).
[6]EE was calculated according to Larbier and Leclercq (1992) and the ratio of EE and ME intake was calculated as EE/ME intake.

production was similar between SWW and C or LE, but lowered with SGW ($P < 0.05$), although the latter was similar to SWW. However, there was no difference in the ratio of the exported energy for egg production and ME intake between treatments.

At the end of the experimental period (week 46), pro ventriculus weight was similar between LE, SWW and SGW (Table 5). However, C was similar to LE, SWW, but lower than SGW in proventriculus weight ($P < 0.05$). Gizzard weight was heavier for SWW relative to SGW ($P < 0.01$). The latter

Table 5 *Treatment effect on weight of digestive organs (% BW), histology and enzyme activity of Jejunum at week 46 of birds fed complete (C) or reduced energy (LE) diet classically or a protein–mineral balancer diet sequentially with whole (SWW) or ground wheat (SGW) from 19 to 46 weeks of age*

	Treatments (week 46)					
Parameter	C	LE	SWW	SGW	*P*-Value[1]	s.e.m.[1]
Organ weight (% BW)						
Proventriculus	0.319[b]	0.364[ab]	0.353[ab]	0.387[a]	*	0.01
Gizzard	1.186[c]	1.449[b]	1.799[a]	1.535[b]	**	0.05
Duodenum	0.538[b]	0.616[ab]	0.628[ab]	0.631[a]	*	0.02
Jejunum	1.077	1.106	1.124	1.063	ns	0.03
Ileum	0.677	0.685	0.682	0.723	ns	0.04
Liver	2.544	2.440	2.337	2.330	ns	0.07
Pancreas	0.175[b]	0.217[a]	0.197[ab]	0.219[a]	*	0.01
Spleen	0.083	0.089	0.092	0.073	ns	0.01
Histology						
Villus height (mm)	1.056	1.098	1.073	1.124	ns	0.059
Villus width (mm)	0.979	0.989	0.915	0.888	ns	0.051
Villus surface (mm^2)	1.025	1.080	0.974	0.997	ns	0.064
Crypt height (mm)	0.162	0.165	0.179	0.173	ns	0.008
Crypt width (mm)	0.054	0.052	0.056	0.053	ns	0.001
Crypt surface (mm^2)	0.009	0.009	0.010	0.009	ns	0.001
Villus/crypt height	6.535	6.654	6.077	6.555	ns	0.286
Villus/crypt surface	118.0	126.8	100.2	110.9	ns	7.720
Enzyme activity (U/mg)						
Alkaline phosphatase	7.68	7.11	8.38	5.02	ns	1.079
Leucine aminopeptidase	0.10	0.11	0.14	0.12	ns	0.011

[1]s.e.m. standard error of the mean; $**P < 0.01$; $*P < 0.05$; ns (non-significant) $P > 0.05$; values within a row with no common letters (a, b, c) differ significantly using Bonferroni–Dunnet test at 5% significance level.

was similar to LE. The lowest gizzard weight was observed with C ($P < 0.01$). Duodenum and pancreas were heavier for SGW and LE than for C ($P < 0.05$), while SWW was similar to all treatments. No difference in the weights of jejunum, ileum, liver and spleen was observed between all treat ments. Histological measurements of the jejunum showed no difference in the height, width and surface of villus and crypt. Equally, the enzymatic activities of AP and LAP were similar among the four treatments.

Discussion

The current work confirms that sequential feeding improved feed efficiency and reduced BW as recently shown by the authors (Umar Faruk *et al*., 2010). In addition, it demon strated that the form of wheat presentation in sequential feeding have limited influence on the positive effect of sequential feeding. Feeding whole or ground wheat in sequential feeding had no significant effect on the overall total feed intake, egg production, EM and FCR. However, hens fed ground wheat had lower wheat intake (about 5%) compared to those fed whole wheat, and this may be asso ciated to larger particles size of whole compared to ground wheat. Laying hens prefer larger particles to smaller ones (Portella *et al*., 1988; Umar Faruk *et al*., 2008). They pre ferentially consume feed particles sufficiently large to be picked up efficiently by their beaks (Picard *et al*., 1997). Thus, whole wheat, due to its larger particle size, is easier to pick than ground wheat.

Ground wheat reduced egg weight, egg yolk and egg albumen weight compared to whole wheat. Equally the hens fed ground wheat had lower final BW compared to those fed whole wheat. This can be associated to slightly lower wheat intake leading to a numerical lower energy intake. This trend was observed during the whole experimental period, but was never statistically significant. Egg weight is known to be dependent on energy and protein intakes of the birds (Fisher, 1969; Morris and Gous, 1988). Birds fed diet containing 3% reduced dietary ME and protein had lower egg weight than those fed the control (Novak *et al*., 2008). This is confirmed regarding the energy intake in the present work, as the slight reduction in ME intake of birds fed ground wheat led to low egg weight. Although the daily ME supply was close between the two diets, there was a strong difference in final BW after 27 weeks of experiment, and this was accompanied by a numerical difference in the abdominal fat content.

The difference in performance observed (egg and BW) between the two wheat forms in sequential feeding could not be explained only from the digestive functions point of view. Except that gizzard weight was higher for birds fed whole wheat, no modification of the villus and crypt mor phology was observed. The morphology of intestinal villi and crypts in poultry has been associated to intestinal function and bird growth (Sun, 2004). Adverse changes in the content

of the digesta due to alimentation could lead to changes in the surface of intestinal mucosa, because of their close proximity. A lower villus height/crypt depth ratio has been associated with the presence of toxins, poor nutrient absorption and increased secretion in the gastrointestinal tract, diarrhea, reduced disease resistance and lower overall performance. A large crypt indicates a fast tissue turnover and a high demand for new tissue (Xu *et al*., 2003). In this study, there was no modification of the villus and crypt morphology due to treatment and this suggests that sequential feeding had no effect on these parameters. These results agreed with Wu *et al*. (2004) who found that the inclusion of whole wheat either before or after pelleting in the diet of broilers did not affect the villus height or the crypt depth.

In the present experiment, LAP, a brush border enzyme and AP used as an indicator of enterocyte maturity (Weiser, 1973) were tested to see if the improvements of FCR might be partly explained by an increased enzymatic activity. It was, however, found that the activities of these enzymes were not different between treatments, thus, cannot be used as an explanation. Gabriel *et al*. (2003) reported the reduced level of LAP in the duodenum when feeding whole wheat, and they also found reduced levels of AP in the duodenum and maltase in the ileum. However, they also found that the inclusion of whole wheat resulted in larger crypt related to an increase of the cellular renewal, which is not the case in this study.

Another aim of this study was to determine if reducing the energy intake in conventional feeding, through the reduced dietary energy content and limited amount of the offered diets, will give similar performance as sequential feeding. It is clear that birds fed LE had reduced energy intake compared to C. They also consumed more ME than those fed sequentially and this is associated to the increased feed intake (+9.5 g/bird per day) of birds receiving the low energy complete diet compared to the sequential treatments. This was not surprising as hens adjust their feed intake to the energy content of the diet as shown by Sohail *et al*. (2003), who observed an increase in feed intake of 4.8 g/bird per day for a decrease of 1 MJ/kg. Egg production, egg weight and EM were similar between LE and the sequential treatments. However, an improved FCR was observed with birds fed sequentially compared with those fed the low energy diet. Therefore, reducing the energy density of the complete diet did not lead to similar FCR ratio as sequential feeding.

Birds fed sequentially had lower hen BW compared to conventional feeding and this agreed with Umar Faruk *et al*. (2010). These authors hypothesized that the reduced feed intake in sequential feeding combined with eventual similar performance to conventional feeding suggests that body fat deposition would be lowered to balance the energy requirement for egg production (Scanes *et al*., 1987). When birds consume more energy than is required for maintenance, growth and egg production, the excess energy is deposited as fat, which in turn increases BW (Smith, 1973). The results of the relative weight of the abdominal fat observed in the present work supported this hypothesis with sequentially fed birds having lower abdominal fat than those fed conventionally although this is more relevant for the SWW than with SGW. Another hypothesis leading to the improved feed utilization in sequential feeding was a more efficient digestive system. The observation that gizzard weight was heavier for birds fed whole wheat sequentially supports the hypothesis. This was expected, since feed particle size was known to influence poultry digestive organs (Nir *et al*., 1990) and digestive efficiency (Amerah *et al*., 2007). The gizzard is the main retention organ of the solid component of the diet. Its purpose is to make them suitable for intestinal digestion by muscular activity. Vergara *et al*. (1989) observed that the larger the size of the feed particles, the longer the period of their retention in the gizzard. The retention time is particularly important in regulating the rate at which these comes in contact with the digestive enzymes and absorptive surface (Hill and Strachan, 1975). Owing to the presence of wheat grains in the sequential treatment, a longer retention time could be assumed. This eventually led to a more efficient digestion and absorption of nutrients thereby improving the performance of these birds.

The low feed intake in sequential feeding was a result of low whole wheat intake, although similar quantity (60.5 g/bird per day) of each of the two fractions was offered. No statistical difference in balancer diet intake was observed (result not shown). These birds were expected to increase their balancer diet intake according to the generally agreed daily feed intake pattern in laying hens, as well as the nutritional composition of this diet. The pattern of daily feed intake in laying hens is influenced by the egg forming cycle and by photoperiod (Nys *et al*., 1976; Choi *et al*., 2004). Thus, hens consumed more diet in the afternoon (Keshavarz, 1998; Dezat *et al*., 2009), to account for calcium required in eggshell formation (Mongin and Sauveur, 1974). However, the protocol used in the present experiment might limit the birds from overconsuming the balancer diet since this diet was fed in a limited quantity.

It was concluded that the use of ground wheat has no better benefit compared to whole wheat in sequential feeding since it was shown to have a negative effect on egg and bird weights. The experimental period was limited to 47 weeks of age. Further investigations are therefore needed on the observed negative effects of such feeding system on BW as it might negatively affect the sustainability of this technique on a longer production period. This required an investigation on the energy budget in the laying hen under sequential feeding, so as to understand the ranking between the metabolic functions, that is, growth, maintenance and egg production. Nonetheless, sequential feeding improved feed efficiency and allows the use of whole cereals with minimum processing. This is of particular interest in situations where the availability of a complete diet impedes production, or where whole cereals are locally available. The use of other types of cereals in sequential feeding is another area requiring attention, as this will allow for an extensive application of this promising technique in a large range of climatic or economical conditions.

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CHAPTER 6 :

The impact of Sequential and Loose-mix feeding using whole millet on the performance of laying hens housed in-group under hot climatic condition

Influence de l'alimentation mélangée ou séquentielle sur les performances des animaux.

Impact de l'alimentation séquentielle et mélangée avec du millet entier sur les performances des poules élevées en cages collectives en zone chaude du nord du Nigéria

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Durée d'essai : 4 mois précédés de 6 semaines d'habituation à compter de la 16ème semaine d'âge.

Les modes d'alimentation ont été expérimentés à Sokoto, une ville située à l'extrême nord du Nigéria, chez des poules pondeuses de souche ISABROWN de la 23ème à la 42ème semaine d'âge. Le millet, de par sa composition et sa disponibilité dans la région, est utilisé à la place du maïs. Il est incorporé à un taux de 33% dans le régime, pour respecter le taux d'inclusion des céréales classiquement utilisé dans l'aliment complet destiné aux volailles dans cette région. La mise en place du protocole est faite dans des conditions proches du terrain. Les poules ont été placées dans des cages collectives à raison de 6 poules dans 2 cages adjacentes (unité de mesure) dans un bâtiment ouvert. Chaque régime comporte 17 répétitions. La photopériode est de 16h de lumière et de 8h de nuit. Les premières 12h de lumière sont assurées par la lumière naturelle, et les 4h restantes par des lampes halogènes. La ventilation étant naturelle, la température du bâtiment est dépendante des conditions météorologiques du jour. Les conditions dans la zone d'étude lors de l'expérience sont caractérisées par une forte amplitude de la température journalière et ont atteint plus de 36°C dans la journée et moins de 22°C dans la nuit.

Quatre régimes expérimentaux ont été testés. Deux d'entre eux sont des témoins : un aliment complet à base de maïs et un autre aliment complet à base de millet. Ils ont été apportés de manière classique. L'objectif est d'étudier la possibilité de remplacer le maïs par le millet, car le maïs malgré son

prix élevé par rapport au millet, est la céréale utilisée dans l'aliment destiné aux volailles dans cette région. Deux régimes contenant le millet en grain entier ont été apportés soit en séquence soit en mélange avec un aliment complémentaire. Les poules ont été habituées avec le millet en grain entier à partir de la 16ème semaine d'âge, où elles ont reçu 100 g/poule/jour d'aliment dont 30% de millet. La quantité d'aliment distribuée lors de la période expérimentale est de 130 g/poule/jour dont 33% de millet. Les mesures réalisées sont l'ingestion, le nombre et le poids de l'œuf, et le poids des constituants de l'œuf. Le poids des poules est enregistré aux 19ème et 38ème semaines d'âge. Les données analysées, à l'exception du poids vif, correspondent aux semaines 23 à 42, car l'entrée en ponte s'est faite à partir de la semaine 23.

Les résultats obtenus avec les deux aliments témoins sont comparables en ce qui concerne l'ingestion totale et le gain du poids corporel. Cependant, la production et le poids de l'œuf sont supérieurs avec l'aliment témoin contenant le millet comparé à celui contenant le maïs. De plus, la masse d'œuf ainsi que le poids corporel des poules sont supérieurs lorsque les poules ont été alimenté avec du millet comparé avec au maïs. Ces résultats ont conduit à une amélioration de l'indice de consommation avec l'aliment complet contenant le millet indiquant que celui-ci peut remplacer le maïs dans l'aliment complet dans des conditions de la zone d'étude.

Comme pour le blé en France, la consommation des animaux en mode d'alimentation séquentielle a été plus faible que pour dans le groupe témoin recevant du millet et le mélange complémentaire, du fait d'une faible ingestion de millet. La production d'œufs est comparable entre les animaux recevant l'alimentation sous forme séquentielle ou séquentielle et mélangée, mais l'alimentation en mode séquentiel conduit à une baisse significative de la production d'œufs par rapport au système témoin millet. Cependant le poids moyen de l'œuf est supérieur lorsque les animaux sont alimentés en mode séquentiel par rapport à l'alimentation mélangée, les deux régimes étant comparables au témoin. Aucune différence quant à la masse d'œuf n'a été constatée entre les trois régimes. Il en résulte qu'une amélioration de l'indice de consommation lorsque l'alimentation est

réalisée en mode séquentiel par rapport aux deux autres modes (témoin millet et mélange). Le poids du jaune dans l'œuf était supérieur pour les poules en alimentation séquentielle, et inférieur pour le témoin millet, le mélange étant intermédiaire. Quant au poids de l'albumen, aucune différence n'a été observée entre les régimes. Le poids de la coquille à l'âge de 28 semaines est inférieur pour les poules recevant l'aliment témoin à base de millet par rapport à celles en alimentation mélangée. Le poids de la coquille à l'âge de 34 semaines est supérieur pour les poules alimentées en séquence puis le témoin, les mélanges ayant des coquilles de poids inférieurs.

L'ensemble des résultats montre que le millet peut remplacer le maïs dans l'aliment destiné aux poules pondeuses dans des régions chaudes telles que le nord du Nigéria. De plus, le millet peut être utilisé sous forme de grain entier alimentation séquentielle ou mélangée. On observe amélioration plus importante des performances en alimentation séquentielle par rapport à l'alimentation mélangée.

Ce chapitre a fait l'objet d'un article qui sera prochainement proposé pour la revue *Archiv für geflügelkunde.*

Running Title: WHOLE GRAIN FEEDING IN LAYER HENS

Sequential and loose-mix feeding of whole millet grains and a protein concentrate is an efficient feed management system under hot climatic conditions

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Abstract 1. The objectives of the present work were (1) To evaluate the suitability of substituting maize by millet under hot semi-arid climatic condition. (2) To evaluate the impact of loose-mix and sequential feeding of whole millet and a protein-mineral concentrate on performance of laying hen under this climatic condition. The experiment consisted of a total of four treatments. To achieve the first objective, two complete diets (a) Maize-based diet containing maize as the principal cereal and (b) Millet-based containing millet (replacing maize on equal weight basis) as the principal cereal were fed conventionally. To achieve the second objective, two other treatments were fed. (c) A loose-mix in which a mixture of whole millet and a protein-mineral concentrate were offered in a single trough (d) A sequential treatment in which only whole millet was fed in the morning followed by the concentrate diet in the afternoon. IsaBrown laying hens were used and data was collected from week 23-42 of age. Each treatment was allocated 17 cages as replicates and each replicate contained 6 hens. Water was offered ad libitum and the daily photoperiod was 16L:8D. Diets were fed ad libitum in two distributions (08h30 and 14h30) corresponding to 2h30 minutes after light on.

2. Feed intake and body weight gain were not significantly different between the maize-based and the millet-based complete diets. However, egg production, egg weight, egg mass, and final body weight were higher with the millet-based than with maize-based diet. The efficiency of feed utilization was significantly improved with the millet-based than with maize-based diet.

3. Sequential feeding resulted to a significant reduction in feed intake compared to loose-mix and the millet-based complete diet. Millet intake was lowered with sequential compared to loose-mix. Egg production was similar between loose-mix and sequential treatments, but the latter had lower egg production compared to the millet-based complete diet. Egg weight was higher with sequential than with loose-mix, but the two were similar to the millet-based complete diet. Egg mass was not significantly different between the three treatments. BW was lowered with the loose-mix compared to sequential and millet-based complete diet. The efficiency of feed utilization was significantly improved with sequential than with loose-mix and the millet-based complete diet.

4. It was concluded that under hot climatic conditions, maize could be substituted (on an equal weight basis) with millet in complete diet without reducing performance. In addition, the use of whole millet and a protein-mineral concentrate in sequential feeding is more efficient than in loose-mix, thus sequential feeding can be employed for an effective feed management under the hot climatic condition.

Key words: Laying hen, sequential feeding, loose-mix feeding, whole millet, hot climate, locally available feed ingredients.

INTRODUCTION

Under hot climatic conditions, poultry production has been hindered by a number of constraints. For example, in the northern part of Nigeria, apart from the semi-arid climate, there is the erratic supply of a complete compounded diet. This is because under practical situation, ingredients required for feed manufacturing are cultivated in the north and transported to the distant southern part of the country for feed manufacturing. It becomes therefore imperative to find alternative solutions that could allow the use of feed ingredients with a minimum level of processing and transport, without compromising the production potentials of the birds.

Any solution to feed problems in this region must take into account the hot climatic condition. Temperature is the major factor limiting the development of egg production (Picard *et al.,* 1993). Higher temperature reduces feed intake and slow down production rate. Some workers tried to partition the detrimental effects on performance due to high temperature *per se* and due to reduced feed intake. For example, using laying hens subjected to 21°C and 38°C, (Smith and Oliver, 1972), showed that reduction in egg production and egg weight at 38°C is due to reduced feed intake, while reductions in shell thickness and shell strength are mainly due to the high temperature. Methods to alleviate heat stress and improve hen performance in hot climates have been proposed. Nutritional manipulation such as the use of vitamins and minerals gave encouraging results (Daghir, 1995), although this cannot provide a sustainable solution, due to the additional cost (Gous, 1995).

Feeding techniques such as loose-mix and sequential feeding could help to provide a sustainable solution to the problem of scarcity of a complete compounded diet (Umar Faruk *et al.,* 2010). Loose-mix is the technique of feed distribution using a mixture of grains and a protein concentrate. Sequential feeding on the other hand, is the alternating of these dietary fractions over a given period or cycle. These techniques allow the direct use of locally available ingredients, thereby reducing the cost of grinding and transportation of ingredients. Recently, Umar Faruk et al., (2010), reported that loose-mix feeding of whole wheat and a separate concentrate, resulted to similar intake

and performance compared to the conventional feeding a complete compounded diet. However, sequential feeding of a limited amount of these dietary fractions reduced intake without affecting performance. This increases the efficiency of feed utilization compared to a complete compounded diet.

In most African dry regions, maize (*Zea mays*) is the major cereal grain used in poultry feed. However, the use of millet (*Pennisetum glaucum*) could be envisaged, because of its availability, due to its adaptability to the local climatic condition. Millet is one of the world's drought resistant plants that grow in a short, dry summer season, even in infertile sandy soils. Millets' nutritional characteristics in terms of protein (Burton, 1972) and energy (more or less similar to maize (Adeola and Rogler, 1994: Davis *et al.,* 2003) make it an attractive ingredient in poultry feeding (Luis & Sullivan, 1982).

The main objective of this work was to evaluate the impact of loose-mix and sequential feeding of whole millet and a protein-mineral concentrate on the performance of laying hen under hot climatic condition. In the first instance, the suitability of replacing maize with millet (on an equal weight basis) in a conventional laying hen diet under this climatic condition was investigated. Subsequently, the impact of feeding whole millet grain under sequential or loose-mix feeding on performance was investigated.

MATERIALS AND METHODS

The climatic conditions of the study area (northern part of Nigeria) exhibit constant temperature between days. There are, however, wide diurnal ranges in temperature (between nights and days). The mean monthly temperature during the day exceeds 36°C while it falls at night, at most times, below 22°C. Humidity is relatively low throughout the year.

Birds and Housing condition

A total of four hundred and eight, 19 weeks-old ISA Brown laying hens were randomly allocated to one of four treatments. Each treatment contained 102 birds divided into 17 replicates with 6 birds per replicate (550 cm^2/ bird). From 16 to 19 weeks of age, all the four hundred and eight birds were habituated to whole millet intake in-line with recommendations of (Umar Faruk *et al.,* 2008). Water was

fed ad libitum. The experiment was carried out in an open sided laying house, thus difficult to control temperature, humidity and light. The experiment runs from October to February with an average temperature of 27°C ± 8°C. Light was 16L8D with 5h of light being supplemented using electric powered fluorescent lamps.

Experimental Treatments and feeding method

The experiment consisted of four treatments. A control treatment (control maize) was fed a complete maize based diet (Table 1). This diet corresponds to a typical complete feed used in the region. The formula, was based on maize, groundnut meal and wheat offal, and was obtained from the department of Animal Science of Usmanu Danfodiyo University, Sokoto, Nigeria. Another control treatment (Control millet) was fed a complete millet based diet. This diet was obtained by replacing all the maize in the maize based control diet above with millet on an equal weight basis. Another treatment (Sequential) was fed by alternating whole millet with a protein-mineral concentrate (balancer diet). The fourth treatment (Loose-mix) was fed a mixture of whole millet and the balancer diet.

All treatments were fed ad libitum (133 g/b/d or 116% of the theoretical daily intake of 115 g/b/d). The daily ration was offered in two distributions. The first distribution was done at 08h30 corresponding to 02h30 after light on and the second at 14h30. Birds were fed 35% of the daily ration during the first distribution and the remaining 65% during the second distribution. In the case of the sequential treatment, only millet was fed during the first distribution and the balancer diet during the second distribution. Feed left over from previous distribution was always removed before the next distribution in all treatments. Since laying hens were involved, calcium was added to the protein concentrate to account for calcium required for eggshell formation (Mongin and Sauver, 1974). For the other three treatments, it was the same diet that was fed during the two distributions.

Measurements

Body weight (BW) was recorded at weeks 19 and 38 of age. Feed intake was measured weekly as the difference between total feed offered and the cumulative left over. In sequential treatment millet

and balancer diet intakes were measured separately. In loose-mix treatment, the two fractions (millet and balancer diet) were determined by separation using a manual sieve (2 mm) in order to determine their respective intake.

Egg production was recorded daily. Egg weight was recorded twice a week by weighing all eggs produced in the measuring day. The weight of the egg components (shell, yolk and albumen) was measured at week 28 and 34. For this measurement, all eggs produced in a given day of the measuring week were first weighed individually and then broken. The albumen and the chalazae were separated from the yolk using forceps prior to weighing the yolk. The shells were carefully washed and dried for 12 hours in a drying oven at 70°C, and then weighed. All measurements were taken to the nearest 0.01g.

Statistical Analysis

Average values from cages were analyzed using StatView (version 5, SAS Institute Inc., Cary, NC). A one-way analysis of variance (ANOVA) GLM model was used to test treatment effect on the measured parameters. Bonferroni/Dunnet pair-wise comparison was used to compare differences between treatment means. The GLM model was:

$$Y_{ij} = R_i + _{ij}$$

where Y_{ij} = measured variables for treatment i and cage j, R_i = treatment effect (i = maize based, millet based, loose-mix and sequential) and j being the cage number within treatment i, and $_{ij}$ = residual.

RESULTS

In all the experimental treatments, birds came into lay at the middle of week 22 of age. Data collection began at the beginning of week 23 of age. Therefore, results were presented according to two periods linked with the stages of egg production: before peak (week 23-26) and after peak (week 27-42).

Effect of replacing maize with millet on equal weight basis in the complete diets:

Maize-based control versus Millet-based control: The overall after-peak results showed that the average daily feed intake was not significantly different between the treatment fed maize-based and that fed millet-based complete diet (table 2). Average feed intake was similar throughout the experimental period. Similarly, no difference in initial BW and BWG was observed between these two treatments. Overall after-peak egg production was reduced with the treatment receiving maize-based diet compared to that receiving millet-based complete diet. During the period before the peak of egg production (weeks 23-26 of age), similar egg production was observed between the two treatments. However, after the peak, egg production was reduced with the maize-based diet between the 27-30 and 35-38 weeks of age and this reduced the overall egg production for this treatment. Overall after-peak egg weight and egg mass were lowered with the treatment receiving the maize-based complete diet and this was the case from week 27 up to week 38 of age. Egg weight and mass were not different between the two treatments during the 23-26 weeks and 39-42 weeks of age. The final BW was slightly lowered with the treatment fed the maize-based diet compared to millet-based diet. The overall after-peak efficiency of feed utilisation was improved with the treatment receiving the millet-based diet compared to the maize-based diet, even though feed efficiency was only statistically different between these two treatments during the 27-30 and 35-38 weeks of age. Egg yolk, egg albumen and eggshell were not different between the two treatments for the measuring periods (weeks 28 and 34 of age).

Effect of the feeding system

Millet-based control versus Loose-mix versus Sequential: The overall average feed intake was significantly lowered with sequential than with loose-mix and the control millet. This reduction was observed throughout the experiment. Overall millet consumption was significantly lowered with sequential than with loose-mix feeding and this was true for all the measured periods.

The overall after-peak egg production was significantly lower with sequential feeding than with the millet-based control but it was similar to loose-mix. The latter had similar overall egg production to the millet-based complete treatment. Except, for the period corresponding to the 35-38 weeks of age, egg production was not different between these three treatments. The overall after-peak egg weight was significantly lowered with loose-mix relative to sequential and the millet-based control, although egg weight was similar between the three treatments up to week 34 of age. Moreover, eggs from sequential for week 39 to 42 were heavier than millet-based control and loose-mix. There is no significant difference in the overall egg mass between the three treatments although the millet-based control treatment had higher egg mass than loose-mix and sequential treatments from week 35 to 38 of age. The overall after-peak efficiency of feed utilisation was significantly improved with sequential treatment than with loose-mix and the millet-based control. This was true for the whole measuring period except for between weeks 39-42, where, the millet-based control was similar in efficiency to sequential treatment. Yolk weight at week 28 of age was similar between sequential and loose-mix and the two were higher than the millet-based control treatment. However, egg yolk weight at week 34 of age, was heavier for sequential followed by loose-mix and millet-based control in decreasing order. Albumen weight was not significantly different between the three treatments both at week 28 and 34 of age. Eggshell was heavier with loose-mix than with the millet-based control at week 28 of age, while sequential treatment was similar to the two others. Eggshell weight at week 34 of age was heavier for sequential treatment followed by millet-based control and loose-mix in descending order. Initial BW was not different between the three treatments. Final BW was lowered with loose-mix than with the other two

treatments. BWG was lowered with loose-mix relative to millet-based control but it was similar to sequential treatment.

DISCUSSION

The average total food consumption in the present experiment was not expected to reach the recorded amount of 115 g/b/d, due to the high environmental temperature in this region. However, this could be explained by the low dietary ME content (2500 kcal/kg) of the experimental diets associated to the seasonal effect. Daghir (1995), reviewed that intake during the summer drops significantly (10-15%) in contrast to winter or spring. The period in which this experiment was carried out (October to February) corresponds to the coolest period of the year in the region with a temperature of about 27±8°C, although it can go below 22°C.

Our assumption that replacing maize with millet on an equal weight basis will not meaningfully affect the diet nutrient composition is not correct concerning the protein content in favour of millet-based diet. In the present work, when maize was replaced with millet on an equal weight basis, there is no difference in the total feed consumed. Equally, BW and BWG were not significantly affected. However, birds millet based diet had higher egg production, egg weight and egg mass than those fed the maize-based diet. This is partially in agreement with the reports of Collins *et al.,* (1997) and Abd-Elrazig & Elzubair (1998), who observed no difference in all the measured parameters including the egg weight and egg mass, when hens were fed diets containing millet as a substitute to maize. However, our results were in agreement with Kumar *et al.,* (1991) who also observed increased egg weight when birds were fed a diet containing millet. In the present work, the actual ME content of the millet was not measured. It was probably higher than the calculated content. Inversely, lower egg weight was observed when maize was completely (Amini & Ruiz-Feria, 2007) or partially (Mehran *et al.,* 2010) replaced with millet, and it was associated it to the lower levels of linoleic acid in millet compared to maize. Linoleic acid is known to improve egg weight and maize is a good source of linoleic acid containing 22 g/kg, while millet contained only 8.4 g/kg (NRC, 1994). However in our study, it might be assumed that higher egg weight might be related to the additional supply of protein in the millet-based diet leading to increases in albumen synthesis even though albumen content were not statistically different. The increased egg production and egg weight obtained with the millet-based relative to the maize-based treatment led to higher egg mass and consequently improved the efficiency of feed utilisation. The results therefore, suggested that millet is not only suitable in replacing maize part for part in laying hen diet but better

performance could be obtained in hot climate. However feedstuffs analysis should be systematically performed to predict relevant nutritional supply.

There is no report on the impact of loose-mix and sequential feeding of whole millet in hot climate. The present work found that sequential feeding lowered the total feed intake by about 15% compared to loose-mix and the millet-based complete diet. Loose-mix, had similar feed intake to the millet-based diet. This was consistent to Garcia & Dale (2006) who offered diets containing whole millet in loose-mix at different inclusion levels and observed no difference in feed intake and egg production compared to the control. In our study, the low feed intake in sequential feeding was a result of lower whole millet intake (-25%) compared to loose-mix. This agreed with previous works done on wheat (Umar Faruk *et al.,* 2010) in which a significant reduction in the total feed intake of sequentially fed birds was observed due to low wheat intake compared to birds fed on loose-mix. This can be associated to the birds' large feed particles selection behaviour (Picard *et al.,* 1997). Laying hens choose more of the larger feed particles than the smaller ones (Portella *et al.,* 1988). The addition of whole millet to the fine mash balancer diet as was the case with loose-mix treatment increases the feed particles size, thus increasing millet intake through this selection phenomenon. The low feed intake of sequential feeding in the present work could be result of a combined effect of the environmental temperature and the duration of access to the millet. During the experiment, a large presence of the birds at the trough immediately after feed distribution (08h30) before the daily temperature rise was observed. However, 2 hours later, when the daily temperature begins to rise, only few birds were present. As the millet was removed at 14h30 before the temperature come down (at around 16h30), sequentially fed birds may not have sufficient time to consume the millet. This phenomenon by which sequentially fed birds ate more food immediately after distribution and less feed 2h later, probably due to satiety provoked by whole cereals, had been investigated by (Jordan *et al.,* 2010). As was shown in table 2, there was a reduction in millet intake between weeks 31-34. Sequentially fed birds reduced both millet concentrate diet intake while loose-mix fed birds reduced only their millet intake. In all the two treatments, the reduction was mainly on week 31 and 32 and it was a result of a sanitary factor, which was controlled immediately. This was evidenced by the increase in intake after this period.

Reduction in the total feed intake in sequential feeding did not affect proportionally egg production and this resulted to an improved efficiency of feed utilisation compared to loose-mix (18%) and the millet-based control (12%). This was because the sequential feeding of whole millet led low feed intake and increased egg weight. Birds fed in loose-mix had reduced BW compared to those fed sequentially. This was not consistent to the reports of Umar Faruk *et al.,* (2010), who observed the opposite. This could be explained by the intake of energy and protein by the birds since they do not consume similar fractions however additional feedstuffs analysis should have been performed to validate this assumption. Another interesting result is the slight but significant increase in yolk weight in sequential feeding compared the other treatments and this might be associated to the feeding system since loose-mix was similar to the millet based complete diet. Eggshell weight was also increased with sequential feeding than loose-mix and maize-based control. The data on feed intake suggest that sequential treatment consumed more of the balancer diet than millet while the reverse was obtained with the loose-mix. This then assumed that the former consumed more Ca than the latter, thus depositing more calcium for egg formation (Mongin & Sauveur, 1974). This effect on eggshell is particularly important since the quality of eggshell under hot climatic conditions is a problem due to reduce Ca intake and affects the solidity of eggshell during transportation over long distances.

CONCLUSION AND PERSPECTIVES

It was concluded that millet is a suitable substitute for maize in the hot semi-arid conditions. Equally, the use of whole millet in laying hen diet is a possible solution to the problem of feed scarcity in which some developing countries, such as Nigeria are facing. Millet grain can be fed with a protein-mineral concentrate and similar (loose-mix) or improved (sequential) efficiency as the complete compounded diet containing millet could be obtained. The present work fed diets that are low in energy. It is therefore, necessary to evaluate these systems using different energy levels so as to establish the optimum dietary energy level that will optimise production under these temperatures. Similarly, there is the need to investigate these feeding systems using the different millet varieties locally available, as well as other cereals (e.g. sorghum). It would

be of interest to carry out further experiments on a longer period (to cover the hot season or at seasonal transition points) to test the resilience of sequential feeding to these harsh climatic conditions.

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Table 1. Composition of experimental diets

Ingredient (g/kg)	Maize based control diet	Millet based control diet	Balancer diet [1]	Millet
Maize	328.0	-	-	
Millet		328.0		1000
Groundnut meal	193.0	193.0	287.2	
Wheat offal	365.0	365.0	543.2	
Limestone	89.6	89.6	133.3	
Bone meal	17.0	17.0	25.3	
Premix [2]	2.5	2.5	3.7	
Salt	2.5	2.5	3.7	
Methionine	1.6	1.6	2.4	
Lysine	0.8	0.8	1.2	
Calculated composition (%)[3]				
ME (Kcal/kg)	10.5	10.5	8.2	15.1
CP	18	18	21	11.9
Lysine	0.75	0.75	0.99	0.25
Methionine	0.35	0.35	0.43	0.18
Calcium	3.6	3.6	5.35	0.02
Available Phosphorus	0.35	0.35	0.47	0.10
Analyzed composition (% DM)				
CP	16.3	17.9	21.6	11.2
Calcium	3.1	3.0	5.8	-
Dry matter	91.7	93.5	93.3	93.1

[1] This diet does not contain millet. It was fed to two treatments either in a mixture (loose-mix) or on alternating (sequential) with whole millet grain.

[2] Vitamin and mineral premix supplied the following amounts per kilogramme of premix: Vitamin A 1600000 IU; Vitamin D3 480000 IU; Vitamin E 2000 mg; Vitamin K3 400mg; Vitamin B1 109 mg; Zn 11000 mg; Mn 12000 mg; Cu (sulphate) 1200 mg; Fe 4000 mg; I 200 mg; Se 60 mg; DL Methionine 120 g; Canthaxanthine 200 mg

[2] Based on the assumption that replacing maize with millet on an equal weight basis will provide similar nutritive value

Table 2. Feed intake and performance of layer hens fed maize or millet based complete diets from 23 to 42 weeks old [(1)]

Parameter		Stage of Egg Production	Age (weeks)	Treatments		*P*
				Control Maize	Control Millet	
Total feed intake (g/b/d)	(17)	Before peak	23 26	99.00 ± 0.68	98.60 ± 0.49	*ns*
	(16)	After peak	27 30	120.80 ± 0.81	119.40 ± 0.73	*ns*
			31 34	121.70 ± 1.27	123.10 ± 1.33	*ns*
			35 38	124.30 ± 1.50	127.10 ± 1.07	*ns*
			39 42	121.30 ± 0.80	120.80 ± 1.42	*ns*
		Overall	27 42	122.00 ± 0.88	122.60 ± 0.86	*ns*
Hen day egg production (%)	(17)	Before peak	23 26	36.60 ± 3.20	40.20 ± 1.80	*ns*
	(16)	After peak	27 30	78.80 ± 2.04 b	84.50 ± 1.44 a	*<0.05*
			31 34	70.80 ± 1.70	74.50 ± 1.24	*ns*
			35 38	66.00 ± 2.40 b	75.30 ± 2.60 a	*<0.05*
			39 42	66.70 ± 2.10	72.20 ± 3.01	*ns*
		Overall	27 42	70.60 ± 1.50 b	76.50 ± 1.35 a	*<0.01*
Egg weight (g)	(17)	Before peak	23 26	49.00 ± 0.53	50.30 ± 0.75	*ns*
	(16)	After peak	27 30	54.00 ± 0.34 b	55.20 ± 0.32 a	*<0.05*
			31 34	52.30 ± 0.50 b	53.60 ± 0.30 a	*<0.05*
			35 38	55.00 ± 0.52 b	56.80 ± 0.55 a	*<0.05*
			39 42	54.30 ± 0.34	55.40 ± 0.39	*ns*
		Overall	27 42	54.00 ± 0.34 b	55.30 ± 0.34 a	*<0.05*
Egg mass (g/d)	(17)	Before peak	23 26	18.20 ± 1.55	20.70 ± 0.99	*ns*
	(16)	After peak	27 30	42.60 ± 1.12 b	46.70 ± 0.91 a	*<0.05*
			31 34	37.20 ± 1.04 b	40.00 ± 0.73 a	*<0.05*
			35 38	36.30 ± 1.32 b	42.80 ± 1.62 a	*<0.05*
			39 42	36.20 ± 1.10	39.90 ± 1.59	*ns*
		Overall	27 42	38.10 ± 0.80 b	42.30 ± 0.81 a	*<0.05*
FCE (egg mass/feed intake)	(17)	Before peak	23 26	0.183 ± 0.015	0.210 ± 0.010	*ns*
	(16)	After peak	27 30	0.353 ± 0.009 b	0.391 ± 0.008 a	*<0.05*
			31 34	0.307 ± 0.010	0.326 ± 0.008	*ns*
			35 38	0.292 ± 0.011 b	0.337 ± 0.013 a	*<0.05*
			39 42	0.299 ± 0.010	0.330 ± 0.013	*ns*
		Overall	27 42	0.312 ± 0.007 b	0.345 ± 0.008 a	*<0.05*
Egg yolk (g)	(16)	After peak	28	13.30± 0.128	13.60 ± 0.155	*ns*
			34	13.80 ± 0.136	13.70 ± 0.153	*ns*
Egg albumen (g)	(16)		28	35.50 ± 0.053	36.60 ± 0.042	*ns*
			34	34.30 ± 0.039	34.80 ± 0.041	*ns*
Egg shell (g)	(16)		28	5.50 ± 0.064	5.50 ± 0.055	*ns*
			34	4.97 ± 0.063	5.05 ± 0.061	*ns*
BW (g/b)	(17)		19	1352 ± 17.2	1352 ± 16.72	*ns*
	(16)		38	1550 ± 8.5 b	1590 ± 15.89 a	*<0.05*
BWG (g/b/d)	(16)		19 38	1.40 ± 0.1	1.70 ± 0.11	*ns*

[1] Values are Means ± SEM, number of replicates given in parentheses.
[a,b,c] Values within the same line with no common letters differ significantly (P<0.05); *ns:* Not significant (p>0.05).

Table 3. Feed intake and performance of layer hens fed whole millet in loose-mix or in sequential feeding with a protein concentrate diet from 23 to 42 weeks old [(1)].

Parameter		Stage of Egg Production	Age (weeks)	Treatments			*p*
				Control Millet	Loose mix	Sequential	
Millet intake (% of total)	(17)	Before peak	23 26		NA [2]	36.70 ± 0.42	
	(16)	After peak	27 30		42.70 ± 0.34 a	35.70 ± 1.00 b	*<0.01*
			31 34		40.00 ± 0.25 a	24.80 ± 0.74 b	*<0.01*
			35 38		39.50 ± 0.47 a	31.40 ± 1.06 b	*<0.01*
			39 42		40.20 ± 0.34 a	31.40 ± 0.80 b	*<0.01*
		Overall	27 42		40.60 ± 0.17 a	30.0 ± 0.66 b	*<0.01*
Total feed intake (g/b/d)	(17)	Before peak	23 26	98.60 ± 0.49 a	97.50 ± 0.60 a	93.30 ± 0.78 b	*<0.01*
	(16)	After peak	27 30	119.40 ± 0.73 a	117.20 ± 0.94 a	98.20 ± 1.13 b	*<0.01*
			31 34	123.10 ± 1.33 a	124.10 ± 0.87 a	95.30 ± 1.54 b	*<0.01*
			35 38	127.10 ± 1.07 a	126.10 ± 1.49 a	102.20 ± 1.84 b	*<0.01*
			39 42	120.80 ± 1.42 a	120.50 ± 1.58 a	102.30 ± 1.51 b	*<0.01*
		Overall	27 42	122.60 ± 0.86 a	121.90 ± 0.73 a	99.50 ± 1.10 b	*<0.01*
Hen day egg production (%)	(17)	Before peak	23 26	40.20 ± 1.78	30.50 ± 2.99	35.60 ± 4.03	*ns*
	(16)	After peak	27 30	84.50 ± 1.44	80.00 ± 1.81	78.90 ±1.78	*ns*
			31 34	74.50 ± 1.24	73.30 ± 1.86	71.30 ± 1.64	*ns*
			35 38	75.30 ± 2.59 a	67.10 ± 2.11 ab	66.10 ± 2.29 b	*<0.05*
			39 42	72.20 ± 3.01	66.20 ± 1.93	65.00 ± 2.32	*ns*
		Overall	27 42	76.50 ± 1.35 a	71.70 ± 1.50 ab	70.30 ± 1.46 b	*<0.05*
Egg weight (g)	(17)	Before peak	23 26	50.30 ± 0.75	51,40 ± 0.51	50,50 ± 0.52	*ns*
	(16)	After peak	27 30	55.20 ± 0.32	55.40 ± 0.29	56.10 ± 0.41	*ns*
			31 34	53.60 ± 0.30	53.80 ± 0.41	54.50 ± 0.29	*ns*
			35 38	56.80 ± 0.55 a	54.60 ± 0.37 b	56.00 ± 0.45 ab	*<0.05*
			39 42	55.40 ± 0.39 b	55.10 ± 0.38 b	57.10 ± 0.43 a	*<0.01*
		Overall	27 42	55.30 ± 0.34 ab	54.80 ± 0.29 b	55.90 ± 0.33 a	*<0.05*
Egg mass (g/d)	(17)	Before peak	23 26	20.70 ± 0.99	15.90 ± 1.48	18.30 ± 2.14	*ns*
	(16)	After peak	27 30	46.70 ± 0.91	44.40 ± 1.05	44.30 ± 1.11	*ns*
			31 34	40.00 ± 0.73	39.60 ± 1.09	39.00 ± 0.99	*ns*
			35 38	42.70 ± 1.62 a	36.70 ± 1.17 b	37.10 ± 1.37 b	*<0.05*
			39 42	39.90 ± 1.59	36.50 ± 1.16	37.10 ± 1.29	*ns*
		Overall	27 42	42.30 ± 0.81	39.30 ± 0.88	39.40 ± 0.88	*ns*
FCE (egg mass/feed intake)	(17)	Before peak	23 26	0.210 ± 0.010	0.163 ± 0.015	0.195 ± 0.022	*ns*
	(16)	After peak	27 30	0.391 ± 0.008 b	0.379 ± 0.010 b	0.452 ± 0.010 a	*<0.01*
			31 34	0.326 ± 0.008 b	0.318 ± 0.08 b	0.409 ± 0.009 a	*<0.01*
			35 38	0.337 ± 0.013 a	0.291 ± 0.008 b	0.364 ± 0.013 a	*<0.05*
			39 42	0.330 ± 0.013 ab	0.303 ± 0.009 b	0.363 ± 0.013 a	*<0.05*
		Overall	27 42	0.345 ± 0.008 b	0.323 ± 0.007 b	0.396 ± 0.007 a	*<0.01*
Egg yolk (g)	(16)	After peak	28	13.60 ± 0.016 b	14.20 ± 0.015 a	14.50 ± 0.013 a	*<0.05*
			34	13.70 ± 0.0153 c	14.40 ± 0.013 b	15.10 ± 0.019 a	*<0.01*
Egg albumen (g)	(16)		28	36.52 ± 0.042	36.03 ± 0.036	35.80 ± 0.045	*ns*
			34	34.80 ± 0.041	34.51 ± 0.043	33.70 ± 0.041	*ns*
Egg shell (g)	(16)		28	5.50 ± 0.055 b	5.80 ± 0.044 a	5.6 ± 0.056 ab	*<0.05*
			34	5.05 ± 0.061 b	4.70 ± 0.071 c	5.30 ± 0.065 a	*<0.01*
BW (g/b)	(17)		19	1352 ± 16.72	1361 ± 14.92	1384 ± 24.02	*ns*
	(16)		38	1590 ± 15.89 a	1513 ± 20.22 b	1578 ± 18.05 a	*<0.05*
BWG (g/b/d)	(16)		19 38	1.7 ± 0.11 a	1.1 ± 0.16 b	1.4 ± 0.20 ab	*<0.05*

[1] Values are Means ± SEM, number of replicates given in parentheses.

[2] Not available.

[a,b,c] Values within the same line with no common letters differ significantly (P<0.05); *ns:* Not significant (P>0.05).

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CHAPTER 7:

Discussion Conclusion and Perspectives

7.1 Introduction

This chapter outlined and discussed the results obtained with loose-mix and sequential feeding in an attempt to evaluate the impact of these systems on the performance of layer hen under the different study conditions in France and in Nigeria. The discussion led to some conclusions as well as perspectives for the future of these systems. The genesis of this work was the need to provide information, which was very scarce, on the possible alternative feeding systems other than the conventional feeding system in egg production. It is necessary for the alternative to provide a solution to the problems associated with feeding without reducing bird performance. These problems can be classified as either economic or logistic depending on their geographical origins. In France, the problem is an economic one. The cost of feeding represents about 60% of the cost of producing an egg as illustrated in figure 1. A look at the situation in Nigeria revealed that in addition to the economic problem, there is also the logistic problem, which is translated by the regular scarcity of a complete compounded diet in the zones distant from the feed manufacturing areas.

In countries like France, a complete diet contained about 60% of cereals that provides the bulk of the metabolizable energy in the diet. In countries like Nigeria, however, a complete diet contained less than 40% cereal. In any of the two cases above, the cereal requires to be transported from the farms to the feed mill. Once at the feed mill they need to be grounded before being mixed with the other ingredients that will provide protein, minerals and vitamins necessary for a given production target. The cost of grinding was estimated to be between 25 to 30% of the cost of feed manufacturing (Dozier, 2002). Logically, this implies that if a feeding system that can allow the direct use of cereal grain with minimum processing can be developed in laying hen, then the cost of transporting the cereal from farm to feed mill as well as grinding can be saved.

In addition to the economic problem above, there is also the problem of scarcity of a complete diet in Nigeria. Figure 2.6 is a map showing the different vegetation and climatic zones in Nigeria. Feed ingredients (i.e energy source such as maize, millet and sorghum or protein sources such as groundnut)

are produced in the northern part of the country because of the favourable climatic condition for these plants. Majority of the feed mills however, are situated in the middle and southern zone due to political and strategic reasons that are out of the scope of this work. In a nutshell, it implies that the ingredients produced in the northern part of the country needs to be transported some hundreds of kilometres for feed manufacturing. This however, imposes a prevalent problem of feed scarcity in the north due to logistics linked to other social factors. Therefore, a system that can allow the use of locally available feed ingredients in egg production will help in saving these difficulties.

From the foregoing, this work was carried out with the objective of evaluating the possibilities of direct incorporating of whole grain cereal in layer hen feed. Specifically, this work looked at the impact of feeding whole wheat (France) and whole Millet (Nigeria) sequentially or in loose-mix with a protein mineral concentrate on layer hen performance. Also an attempt was made to understand some underlying biological mechanisms that can be used to explain the results obtained.

7.2 Are Sequential and Loose-mix feeding systems two nominators from a common denominator?

Loose-mix and sequential feeding are two different systems sharing a common base. Although the two are based on the principle of fractioning the diet, their impacts on the performance in laying hen are different. Feed intake of birds under loose-mix and sequential feeding was evaluated.

The first difficulty that was encountered was to make the birds fed sequentially to consume whole cereal after point of lay (week 19) especially if they have never been given access to it. During preliminary experiments (Annex 1a and 1b), 24 week old Isa Brown laying hens were subjected to loose-mix and sequential feeding using mash and pellets diets ad libitum and the kinetics of feed intake was measured at ½ h, 3h, 6h and 24h after feed distribution. Although the total feed intake was not modified between treatments of the same feed type, it was observed that globally, birds fed sequentially consumed less wheat than loose-mix and that wheat intake represent less than 1% of the total feed intake of about 30% of the birds in sequential feeding. In loose-mix the birds ingested more wheat than

the protein concentrate. This outlined the need for early adaptation of the birds to grain consumption if an optimum intake is to be achieved during the later stage. In the subsequent experiments presented in this document, the efficiency of the habituation period had been confirmed as was seen in chapter 3, 4, 5 and 6, with sequentially fed birds consuming reasonably enough whole cereal. These preliminary studies confirmed by the experiment reported chapter 4 (i.e. with *ad libitum* supply) also highlighted the importance of limiting the feed offered so as to avoid over consumption of one diet than the other.

With a successful adaptation and limitation of the total feed offered to the birds, it was globally observed that the total feed intake was reduced with sequential compared to loose-mix and conventional feeding. The only experiment in which sequential feeding resulted to higher feed intake compared to loose-mix was presented chapter 4. However, it should be noted that in this experiment, the total feed offered was not controlled as it was the case with the other experiments. Lower feed intake in sequential feeding was always a result of low cereal intake. This cannot be seen as a failure to successfully adapt the birds to cereal intake since it was observed that they consumed more than 40% of their diet as wheat. Loose-mix was always having higher cereal intake compared to sequential feeding. This had been linked to the fact that birds prefer larger particles as reported by several authors (Portella *et al.*, 1988 ; Picard *et al.*, 1997). A hypothesis that can be used to explain the lower wheat intake in sequential feeding is that there is probably a combined effect coming from the duration of access to wheat or millet and the rate of feed transit in the digestive tract. First, the birds in sequential feeding were given access to whole cereals for a given limited time during the photoperiod, while in loose-mix they were having a 24h access to wheat. A visual observation during the experiments suggested that the birds quickly consume wheat immediately after it is distributed (Annex 2). However, about 2 hours later, very few birds are seen at the feed trough. This means that they quickly consume wheat which is stored in the crop waiting for it to be reduced to a smaller size by the mechanical action of the gizzard, before passing through the digestive tract. During this period, these birds seize eating

because they may have a feeling of satiety. It is likely that before the end of the grinding process in sequential feeding, those fed in loose-mix continued to consume wheat.

It was observed that sequentially fed birds increased more their balancer diet intake than the loose-mix suggesting that they attempted to compensate for the lower wheat intake. Although this increase was never enough, it was associated to the limitation of the quantity of the diet given to the birds while when they were fed ad libitum, they increased continuously their balancer intake. It was argued that since birds consume more diet in the second part of the photoperiod, then this should be expected. In addition, this diet contained Ca, thus increase in its intake agrees with the specific Ca requirement during this period. The limitation in the quantity of the diet offered is necessary here because as it was seen on chapter 4, birds fed ad libitum tends to over consume this diet and as such may lead to nutrient imbalance if allowed to continue. This showed that although birds can attempt to select and balance their diet when given access to different diets, it is necessary to guide them in the process to avoid nutrient imbalance according to an optimal performance.

The lower cereal intake in sequential feeding led to lower ME intake compared to loose-mix. It also led to a slight but significant decrease in protein intake. Despite this reduction, egg production and egg weight were not affected. This led to the remarkable increase in performance as seen in this work. Lowering the amount of feed required to produce an egg will have a high economic impact especially when a large production unit is considered or when the availability of the diet is a problem. It can be assumed that genetic research had programmed these birds to attain this level of production with the minimum input. If this assumption is correct, then two possible explanations can be attempted. In the first instance, if the birds consumed lower feed than those fed a complete diet, then they needed to find an alternative way in order to respond to the demands in nutrients to satisfy production. As such they will have to mobilize their body reserves to meet up this demand. The results are clearly seen on the body weight of these birds. It could be recalled that in this work we observed a reduced body weight with sequential feeding. It can go further to say that there was also reduced body fat deposition since

the abdominal fat content was lowered with sequential feeding compared to the control as was seen in chapter 5. Secondly, the improvement in digestion evidenced by heavier gizzards obtained with sequential feeding could explain to some extent the improvement in the efficiency of nutrients digestion with sequentially fed birds as was discussed in chapters 3,4,5 and below further in this chapter. The question that comes after this is that are they able to maintain this level of intake and production over the whole production period. Looking at the results of this work, the only answer that can be attempted is that as from week 37 of age (refer to chapter 3), the body weight was stable in all the treatments up to the end of the experiment. This suggests given that the feeding and production conditions are kept constant, then it is likely that they will maintain this BW for a longer period. In this case this will become an added advantage especially in the developing countries like Nigeria. It was known that in these countries, after the production period, laying birds are sold as meat birds and consumed. These birds will be more appreciated when they contain less body fat. In other words, sequential feeding is a dual system that allows having both egg and meat producing birds. However, it is necessary to further investigate the body weight when millet is to be used because observe a slight increase in BW of sequentially fed birds when compared to loose-mix in Nigeria. This raised an important issue, which is the need to have a sound knowledge of the feed ingredients available in this region.

Recent predictive equations were used to investigate protein (Sakomura *et al.*, 2002) and energy (Sakomura, 2004) requirements in laying hens. Protein requirement was more difficult to determine because of the requirement in specific amino acids, although these equations were found to fit relatively well as was seen in chapter 3. Predicting energy requirement was not perfect as was seen in chapter 4. Unbalanced figures were obtained for all treatments. This underlined the fact that it is necessary to investigate the energy requirement as well as partition in laying hen. In a nutshell, this observation highlights the need to go further in determining the actual amounts of nutrients need and supply, as well as understanding the factors that determine birds' priority in making its nutrient budget,

especially when sequential feeding is to be applied in connection with nycthemeral changes of nutrient utilization.

To evaluate these feeding systems, weight of egg components was measured. Egg yolk weight is an important egg internal quality parameter used in food industries. Egg yolk was found to be lowered with sequential feeding using whole wheat in one of the two experiments carried out in France with birds housed in-group. However, when birds were housed individually, loose-mix reduced yolk weight in connection with the dietary balance as seen in chapter 4. Sequential feeding of whole millet in Nigeria was even found to increase yolk weight, while loose-mix lowered it. This made it difficult to draw a general conclusion on the impact of these feeding systems on egg yolk weight. However, it should be noted that in all the studies, the proportion of yolk in the egg was in the acceptable range of 25% of the total egg weight (Nys *et al.*, 2008).

The impact of sequential and loose-mix feeding on egg albumen is however clear. When whole wheat was used, egg albumen was not affected in two of the three experiments. Using whole millet had no impact on the albumen weight as was seen in chapter 6. Concerning eggshell weight, it is clear that sequential feeding increased shell weight in all the experiments, including those carried out using whole millet. This had already been linked to increase Calcium intake as a result of higher balancer diet intake in the afternoon by these birds. The increased eggshell weight is an added opportunity in sequential feeding because the quality of the shell is an important economic parameter that could not be neglected. As an example, during the hotter periods of the year in Nigeria, egg production unit records the highest number of broken eggs. It is therefore necessary to further experiment sequential feeding since it is a means of improving the eggshell solidity, thus may provide a solution to egg breakages. It should be noted that the changes in the weight of egg components observed in the course of this work were in line with earlier reports (Harms and Hussein, 1993) indicating that with the increasing hen age, egg weight increases but the egg contain proportionally more yolk and less albumen.

The remarkable increase in the efficiency of the sequentially fed birds had been related to an increased gizzard weight, which assumes an improved digestibility and probably utilisation of nutrients. This is not new, as several authors had reported increased gizzard weight with increasing feed particle size (Nir et al., 1990 ; Amerah *et al.*, 2007). However, this only indicates the digestive capacity, thereby increasing the surface area of feed particles, but this does not mean that nutrient absorption is improved. The principal site for nutrient absorption is the small intestine. Unfortunately, this work was not able to put into evidence any modifications of the jejunum morphology due to feeding system as was measured by villus and crypt in chapter 5. Equally, no modification in the enzymatic activity of LAP and AP enzymes was observed.

If these parameters were not affected, then it could be hypothesized that a lower rate of transit increased feed particles retention time in the digestive tract thereby improving their digestion and absorption. Increased feed particles retention time is particularly important in regulating the rate at which these comes in contact with the digestive enzymes and absorptive surfaces (Hill and Strachan, 1975).

7.3 Is there an alternative to conventional feeding in egg production?

Yes there is an alternative to conventional feeding. The different studies presented in this document demonstrated that loose-mix and sequential feeding using whole cereals have no negative impact on performance in laying hen. The two systems provides an opportunity to utilise locally available feed ingredients with minimum processing, thereby helping to find solutions to the two problems (feed cost and scarcity) in which this study was set to solve. In addition, sequential feeding is more promising than loose-mix because it reduces the amount of feed required producing an egg.

7.4 Perspectives

For a broader application of this system, some important points need to be taken into account. The present work evaluated the impact of these feeding systems during the first half of the egg

production cycle. It is therefore necessary to extend the experimental duration in order to have information on these systems impact on during the later part of the cycle.

It is necessary to further investigate the digestive efficiency and its relation to nutrients absorption in hens under these feeding systems in an attempt to provide information on the underlying mechanisms that led to increased efficiency in sequential feeding without affecting meaningfully the morphology of the intestine. There is also the need to carryout an extensive study on the energy need and partition in hens under this system. It appears that the birds had a hierarchical repartition of energy according to the different process (growth, maintenance, production) this needs to be investigated because results of this work especially those with sequential feeding suggest that production was the priority to the expense of growth.

This work was carried out under experimental condition (especially studies carried out in France), where housing, feed and animal factors were controlled. It is therefore necessary to investigate these systems under large poultry production unit conditions to ascertain their impact in these situations. This does not mean that different results are to be expected but may rather consolidate the present findings. It should be recalled that the experiments in Nigeria are carried out in situations very close to those obtained in egg production units.

Due to a significant reduction in the amount of time spent on feeding activity, birds fed sequentially had poor feather condition as was shown in annex 2. This may affect hen welfare, especially if the duration of time of access to wheat was long. Therefore it is necessary to investigate this aspect with a view to increasing birds feeding activity (such as reducing the time of access to wheat) or other strategies that may distract birds' attention to avoid feather pecking.

To widen the systems application, the impact of the use of other locally available cereals such as sorghum, maize etc as well as local breed of laying hen present in the developing countries is required. Another aspect that will be useful especially under hot climatic conditions is the interaction between performance and heat stress when birds are subjected to these feeding systems. This needs

to be investigated with a view to establishing the necessary steps to be employed for an improved egg production in these regions.

Because of the difficulties encountered with regards to the composition of the feed ingredients available in Nigeria, there is an urgent need to establish a data base containing the composition of these feed ingredients. In the course of this work, 30 local ingredients from Nigeria had already been analyzed for dry matter, protein, ash, fat, phosphorus, fibre and energy. This needs to be continued because knowledge on these ingredients is the first step in finding ways to incorporate them in poultry feed for improved egg production.

In an attempt to provide more information on these systems, it is necessary to carryout economic as well as environmental studies that will serve as a decision-making tool for farmers under different conditions both in France and in Nigeria. The tool should take into account the environmental aspect such as gas emission from cereal transport and grinding as well as discharge of non utilised / absorbed feed nutrients in the faeces. Today, method of accounting for emissions of greenhouse gases had been developed and could be integrated to this tool. With respect to discharge of pollutants, it could be hypothesized that birds fed a nutrient (e.g. Phosphorus) at the moment they needed it most will discharge less of it in the faeces as such reduce its impact on the environment.

For this system to be more applicable it is necessary for it to be simple in application. Therefore, it is important to look at the technical aspect that will allow the farmer to use this system without difficulty. For example, feed chain, silos and trough design needs to be investigated.

Finally, this work is a first step in the evaluation of the impact of loose-mix and sequential feeding using whole cereals and a protein-mineral concentrate in laying hen. The work showed that the methods are an innovation having both practical and economic advantages that could be used to improve and sustain egg production and improve food security.

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Annexes

Annex 1a

REACTION A COURT TERME DE POULES PONDEUSES FACE A UN MELANGE DE BLE ET D'ALIMENTS DE GRANULOMETRIE DIFFERENTE

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RÉSUMÉ

Ce travail avait pour objectif d'évaluer le comportement de poules pondeuses Isa Brown recevant des aliments sous différentes présentations et nutritionnellement équivalents. Sept aliments ont été comparés : quatre sous forme de farine fine ou grossière, comportant ou non du blé entier en mélange, trois sous forme de petits granulés comportant ou non du blé en mélange (entier ou broyé). Après une période d'adaptation aux aliments d'une semaine, les ingestions individuelles ont été mesurées après 30 minutes, 3h, 6h et 24 h de distribution, sur une période de quatre jours. Une analyse granulométrique des aliments ainsi que des refus a été réalisée.
La vitesse d'ingestion a été plus élevée durant les 30 premières minutes de distribution avec une forte variabilité individuelle : 24 et 23 g/h vs 7 et 6 g/h en moyenne le reste de la journée pour les farines et les granulés respectivement, quels que soient la forme et l'apport de blé. La présentation de l'aliment en mélange n'a pas eu d'impact sur l'ingestion quotidienne (118,1 et 112,9 g/j pour les farines et les granulés). Les poules pondeuses ont opéré un tri particulaire et ingéré préférentiellement les grosses particules (>2mm) et ont montré une préférence pour le blé entier. Ces différents phénomènes, observés à court terme, pourraient engendrer une hétérogénéité de production importante à l'échelle d'un élevage sur des cycles plus longs. Toutefois, la présentation de l'aliment complémentaire sous forme de granulés permet de limiter ce tri.

ABSTRACT

The aim of this study was to measure Isabrown laying hen feeding behaviour. The hens were housed individually and were fed equivalent diets differing in form and particle size profile. Seven diets were compared, four mashes (fine or coarse) eventually mixed with whole wheat and seven pellets eventually mixed with whole or ground wheat. After an adaptation week, feed intake was measured at 30minutes, 3h, 6h and 24h on a four day basis. The rate of feed intake was therefore calculated. Given feed and left-over feed particle profile were determined.
A higher rate of intake was observed 30mn after feeding: 24 and 23g/h *vs* 7 and 6g/h the remaining time for mash and pellets respectively. Feed form in loose-mix had no impact on daily feed intake (118 and 111g/d for mashes and pellets). Laying hens sorted feed particle and ingested preferentially particles bigger than 2mm. Moreover, they show a preference for whole wheat. These observations could lead to some heterogeneity during the production cycle of commercial hens. Nevertheless, balancer feed as pellets should allow reducing this sorting.

INTRODUCTION

L'aliment est le premier poste de charges en production de poules pondeuses, représentant selon les systèmes de 50 à 60% des coûts. En Europe du Nord, des élevages de poulets de chair apportent des céréales entières distribuées en mélange avec un aliment complémentaire. Ce mode d'alimentation permettrait de réduire les coûts en limitant le transport et la transformation des matières premières. Si de nombreuses études sur les performances zootechniques ont été menées en production de volailles de chair, peu de références sont disponibles en production de poules pondeuses.

Les conclusions d'études portant sur l'ingestion des poules pondeuses face à un mélange de céréales entières et d'un complémentaire sont divergentes. En comparaison avec des aliments complets, Robinson (1985) et Bennett et Classen (2003) observent une augmentation de l'ingestion tandis que Blair et al. (1973) et Scott et al. (2005) observent le contraire. Les poules auraient tendance à consommer les céréales en premier (Robinson et al., 1985), et par ailleurs, adaptent leur comportement alimentaire à la présentation de l'aliment. Le temps passé à manger est plus élevé avec un aliment présenté en farine qu'en granulé, et ce d'autant plus que l'aliment est dilué (Vilariño et al., 1996).

L'objectif de notre étude est de déterminer à court terme les cinétiques d'ingestion et le tri particulaire selon la forme de l'aliment et l'apport ou non de blé entier.

1. MATERIELS ET METHODES

1.1. Animaux

126 poules de souche Isa Brown ont été mises en place en cages individuelles à 19 semaines d'âge. 18 poules réparties dans le bâtiment ont ensuite été affectées par régime. Elles sont entrées en expérimentation à 24 ou 25 semaines d'âge selon les traitements. Le programme lumineux était composé de 16h de jour et 8h de nuit. La température d'ambiance était programmée à 20°C.

1.2. Régimes

Les poules pondeuses ont toutes reçu durant la semaine pré-expérimentale l'aliment témoin sous forme de farine grossière (FG). Elles ont ensuite reçu l'aliment expérimental durant 2 semaines. La quantité d'aliment distribuée a été fixée à deux fois l'ingestion théorique, soit 230g. Ces aliments ont été apportés en mangeoires individuelles. Les aliments, de caractéristiques nutritionnelles équivalentes, différaient par leur forme (farine ou granulés) et leur mode de distribution (mélangé ou non avec du blé). Le granulé avait un diamètre de 2,5 mm. L'aliment complémentaire, associé au blé en mélange, a été formulé à partir d'un aliment complet (EM=2750 kcal/kg, MAT = 17%) auquel ont été soustrait 20% de blé. La formulation des aliments est présentée en tableau 1.

Tableau 1. Formulation des aliments complets et complémentaires (en %)

Aliment (%)	Complet	Complémentaire
Maïs grain	33,45	41,81
Blé	30,00	10,00
Tourteau soja 48	21,50	26,88
Carbonate calcium	8,00	10,00
Son de blé	2,48	3,10
Gluten maïs 60	1,45	1,81
Phosphate bicalcique	1,28	1,60
Huile soja	0,80	1,00
Prémix	0,50	0,63
Bicarbonate sodium	0,20	0,25
Sel	0,20	0,25
DL-Méthionine	0,115	0,144
L-Lysine 78	0,025	0,031

Deux séries d'essais successives ont été réalisées avec différentes formes d'aliments complet et complémentaire :

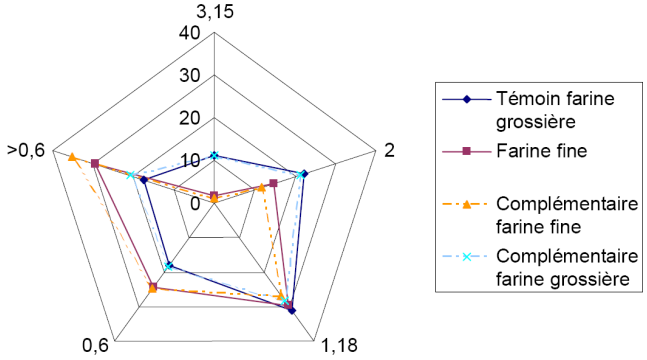
- sous forme de farine

Quatre aliments ont été testés sur des poules âgées de 24 semaines : deux aliments complets sous forme de farine fine (FF) et farine grossière (FG) ainsi que deux aliments comportant du blé entier (BE) en mélange avec un aliment complémentaire sous forme de farine fine (FF+BE) ou de farine grossière (FG+BE).

- sous forme de granulé

Trois aliments ont été testés sur des poules âgées de 25 semaines : un aliment complet sous forme de granulés (G) et deux aliments comportant un aliment complémentaire sous forme de granulés en mélange avec du blé entier (G+BE) ou broyé (G+BB).

Figure 1. Profil granulométrique des aliments testés (sous forme de farine) : % de particules en fonction de la taille des mailles (mm)



1.3. Mesures réalisées

Ingestion

L'ingestion quotidienne individuelle des animaux a été mesurée sur une base de quatre jours lors de la deuxième semaine de distribution. Des vitesses d'ingestion ont été calculées à partir de mesures d'ingestion réalisées 30minutes, 3h et 6h après introduction de l'aliment, à l'allumage.

Tri particulaire

Une analyse granulométrique des refus a été réalisée durant la deuxième semaine de distribution. Un échantillon de 100g a été prélevé de manière homogène sur la totalité des refus de la semaine. Il a ensuite été tamisé durant 3 minutes à l'aide d'un tamiseur (Retsch AS 200 digit). Les tamis utilisés avaient des ouvertures de maille de 3,15mm, 2mm, 1,18mm et 0,6mm de diamètre.

Pour les aliments FF+BE et G+BE, le pourcentage de blé présent dans les refus a également été mesuré.

Des analyses de variance ont été réalisées à l'aide du logiciel Statview®.

2. RESULTATS

Tableau 2. Ingestion des régimes apportés sous forme de farine ou de granulés

Série d'essai	Aliment	Ingestion g/j
Aliments sous forme de farine	FF	121,7 ±9,8
	FG	114,3 ±15,7
	FF+BE	117,8 ±14,0
	FG+BE	118,7 ±14,9
	Proba	*NS*
Aliments sous forme de granulé	G	114,4±10,2
	G+BE	112,2±10,9
	G+BB	112, 2±13,2
	Proba	*NS*

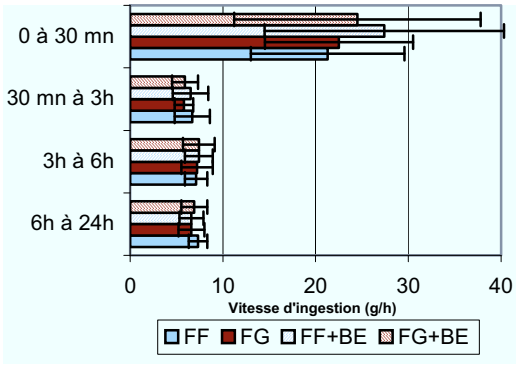
FF : farine fine, FG : farine grossière, FF+BE : farine fine en mélange avec blé entier, FG+BE : farine grossière en mélange avec blé entier. G : granulé, G+BE : granulé en mélange avec blé entier, G+BB : granulé en mélange avec blé broyé.

2.1. Première série : aliments sous forme de farine

Nous n'avons pas observé de différence de niveau d'ingestion quotidienne entre les aliments testés. Le niveau d'ingestion moyen était de 118g/j (Tableau 2).

Les vitesses d'ingestion à chaque période de la journée n'ont pas différé selon les aliments (Figure 1). La vitesse la plus élevée a été observée durant les trente premières minutes, avec une importante variabilité individuelle. La vitesse moyenne à 30 minutes a été de 23,9g/h.

Figure 2. Vitesses d'ingestion des régimes apportés sous forme de farine (Série 1)

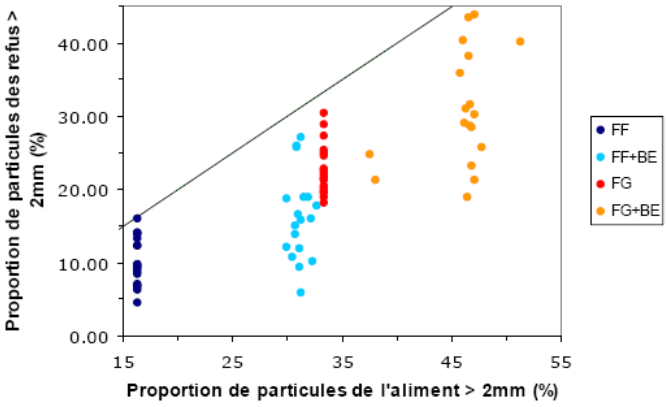


F

F : farine fine, FG : farine grossière, FF+BE : farine fine en mélange avec blé entier, FG+BE : farine grossière en mélange avec blé entier.

D'un point de vue qualitatif, nous observons que les animaux ont préférentiellement ingéré les particules dont la taille est supérieure à 2mm (Figure 3). La variabilité du tri entre les animaux est également plus élevée dans le cas du mélange. Les animaux ont préférentiellement ingéré le blé entier : le taux de blé entier retrouvé dans les refus était de 7,5% pour l'aliment FF+BE contre 20% théorique dans le régime.

Figure 3. Particules >2mm : proportion dans les aliments et les refus (régimes apportés sous forme de farine)



FF : farine fine, FG : farine grossière, FF+BE : farine fine en mélange avec blé entier, FG+BE : farine grossière en mélange avec blé entier.

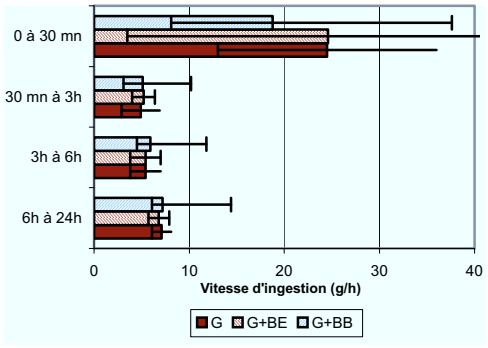
2.2. Deuxième série : aliments sous forme de granulés

Le niveau d'ingestion moyen a été de 112,9g/j, ce qui est légèrement inférieur à la consommation observée pour les aliments sous forme de farine. Comme pour la première série, il n'y a pas de différences de niveau d'ingestion quotidienne entre les aliments testés.

Les vitesses d'ingestion au cours de la journée n'ont pas différé selon les aliments (Figure 3). La vitesse la plus élevée, et la variabilité la plus importante ont été observées lors des 30 premières

minutes. La vitesse moyenne sur cet intervalle a été de 22,6g/h.

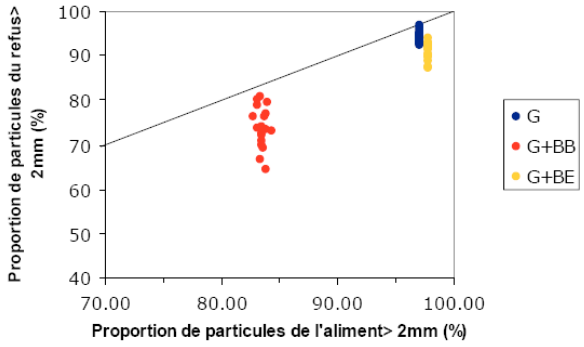
Figure 4. Vitesses d'ingestion selon les régimes apportés sous forme de granulés (Série 2)



G : granulé, G+BE : granulé en mélange avec blé entier, G+BB : granulé en mélange avec blé broyé.

Les animaux ont préférentiellement consommé les particules dont la taille était supérieure à 2mm (Figure 5). Les variations individuelles observées étaient moins importantes que pour la précédente série, les possibilités de tri étant également plus limitées avec les granulés. Les animaux ont préférentiellement ingéré le blé entier. Le niveau de blé entier retrouvé dans les refus était de 15% pour l'aliment G+BE pour 20% théorique dans le régime.

Figure 5. Particules > 2mm : proportion dans les aliments et les refus (régimes apportés sous forme de granulés)



G : granulé, G+BE : granulé en mélange avec blé entier, G+BB : granulé en mélange avec blé broyé.

3. DISCUSSION

Après une semaine d'adaptation et sur une courte période (4 jours), l'ingestion quotidienne moyenne de l'aliment n'a pas différé selon les régimes avec des vitesses d'ingestion équivalentes à chaque moment de la journée. Toutefois, que l'aliment complémentaire soit sous forme de farine ou de granulé, les poules ont consommé proportionnellement plus de blé entier que de complémentaire, avec une grande variabilité de comportement. Cette préférence semble accentuée avec l'aliment complémentaire en farine comparé au granulé, du fait d'une plus faible proportion de grosses particules, les animaux préférant les particules d'une diamètre supérieur à 2mm. Les volailles sélectionnent en effet leur prise alimentaire en fonction de la taille relative des particules au bec, quelle que soit la composition du régime (Portella et al., 1988, Nir et al., 1994, Wauters et al., 1997). Ces préférences peuvent ainsi induire un tri particulaire néfaste à l'ingestion d'une ration équilibrée pour tous les animaux et entraîner une baisse globale de production. De plus, un logement en grand groupe pourrait exacerber les différences d'ingestion des animaux.

CONCLUSION

A court terme, les poules pondeuses ont une consommation non modifiée par la forme d'apport mais montrent une préférence pour les grosses particules et ont ingéré préférentiellement le blé entier. En condition de production avec des animaux élevés en groupe et sur une longue période, un aliment comportant du blé en mélange pourrait entraîner un déséquilibre nutritionnel et une hétérogénéité de production. La présentation de l'aliment complémentaire sous forme de granulés doit limiter ce tri. Par ailleurs, l'apport de blé entier par séquence avec un aliment complémentaire devrait être envisagé afin de limiter la possibilité de tri des animaux.

Remerciements

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Annex 1b

LOOSE-MIX AND SEQUENTIAL FEEDING OF MASH DIET WITH WHOLE WHEAT: EFFECT ON FEED INTAKE IN LAYING HENS

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Summary

This work evaluates the feed intake of ISA Brown laying hens fed whole wheat in loose-mix or in sequential feeding with either fine or coarse mash protein balancer-diet. Four regimens were fed *ad libitum* on a 24h cycle in loose-mix with whole wheat, while four others sequentially. Wheat represents 20% of the total feed offered. Measurements include daily feed intake and feed particle profile determination. There was no regimen effect on the average feed intake. However, birds fed in loose mix demonstrated particle sorting phenomenon and consumed more of particles having sizes >1.18mm, eventually whole wheat. Conversely, all of the sequentially fed birds ingested less wheat than expected (7% instead of 20%). They however, compensated the quantity ingested by a higher balancer-protein diet intake. Since whole cereals in poultry feeding are to reduce feeding cost, the results suggest that in loose-mix feeding, it is necessary to provide at least a pellet balancer-protein diet in order to reduce particle sorting which could have a negative correlation with egg production. In sequential feeding, a period of learning is certainly required to obtain adequate wheat intake.

I. INTRODUCTION

Whole cereals grain in poultry feeding to reduce feeding cost associated to processing of raw materials are regaining interest in European countries (Noirot *et al.,* 1998). Transportation and processing of raw materials having an important place in the cost of feed production. This imposes on poultry nutritionist the need for an evaluation of different feeding methods that employ whole grain in poultry rations.

At present, feeding whole cereals in commercial poultry farms is limited to one of the three methods (split-feeding; loose-mix feeding; sequential feeding) (Noirot *et al.,* 1998). These methods are based on the principle of self-select feeding, where an animal is offered a choice between two or more

diets and left to compose his own diet according to his actual needs (Henuk and Dingle, 2002). In split-feeding the two dietary components (energy and protein) are fed in different feeding trough separately placed in different position in the poultry house. In loose-mix feeding the two components usually mixed on-farm are fed in a mixture in the same feeding trough. In sequential feeding the two components are fed in an alternating manner over the day. In sequential feeding, the cereals are usually offered in the morning and the protein concentrate is fed in the afternoon. In all the three methods, a source of calcium may be necessary especially so if laying hens are concerned.

For these systems to be applicable under commercial application, a consistent feed intake must be ensured. This work therefore studies the short term (2 weeks) feed intake of ISA Brown laying hens fed whole wheat in loose-mix or sequentially with a protein concentrate diet.

II. MATERIAL AND METHODS

a. Birds and housing

A total of 144 ISA-Brown laying hens were used. The birds were transferred to the laying house at 19th week of age. The animals were weighted before and after the experimental period. The duration of the experiments is 3 weeks, this including a pre experimental (adaptation) week. Data collection was done over two weeks. Experiment began when birds were between 26 and 27 weeks of age. The photoperiod was 14L:10D at 19th week and reached 16L:8D at 24th week of age. Temperature was maintained at 20 ± 2°C throughout the experimental period. Water was offered *ad libitum*.

b. Diets

Four regimens were fed on a 24 h cycle in loose-mix, while four others were fed sequentially with whole wheat representing 20% of the daily feed offered per bird. Each regimen was allocated 18 individual birds as replicates. All regimens were fed *ad libitum* (230g/bird/day). A control diet (Table 1) was fed during the adaptation period. During the experimental period, only 18 birds were fed the control diet. All the other regimens were fed a balancer diet sequentially or in loose-mix with wheat. The balancer diet is formulated from the control diet by subtracting 20% of wheat. The subtracted wheat was fed as whole or ground wheat sequentially or in loose-mix. During the second experimental week, feed left over was collected over a period of four days, and is subjected to particle size analysis. Diets were distributed once daily except for sequential diets, where the wheat is offered for a period of 3 h in the morning and the balancer diet for 13 h afterwards.

The regimens fed in loose-mix are a complete coarse mash and a complete fine mash (controls) without the addition of whole wheat. The other two are balancer coarse mash and a balancer

fine mash in loose-mix with 46g whole wheat. In sequential feeding, regimens include a balancer coarse mash diet fed sequentially with 25 g, 60 g or 150 g whole wheat. This diet is also offered sequentially with 60g ground wheat.

c. Measurements

In loose-mix, the average daily feed intake was measured per bird for four days per week using individual feeding trough. In sequential feeding, wheat and balancer diets intakes were measured separately and summed up to obtain daily feed intake. The percentage of feed particles in the feed left-over was determined by sieving method in dry condition adapted from Melcion (2000). A sample of 100 g of the left-over was passed for three minutes in a sieving machine (RETSCH AS 200 DIGIT[1]), having sieves diameters of 3.15 mm, 2.0 mm, 1.18 mm, 0.6 mm and the bottom plate was used. The weight of the feed retained in each sieve is taken and expressed as percentage in the sample weight. This data is then subtracted from the corresponding data of the offered feed to give the difference between the offered and the refused, thus determine which sizes are more ingested by the bird.

d. Statistical Analysis

Collected data were subjected to analysis of variance (Statview 5), and differences between treatments means were compared by Bonferroni test at 5% probability level.

III. RESULTS AND DISCUSSION

No mortality was observed throughout the experimental period. Table 2 shows no difference in average daily feed intake of birds fed in loose mix. The change in the feed form due to addition of wheat had no effect on intake. As the birds were never fed whole wheat during growing period, the result therefore suggests a quantitative adjustment of intake by the birds. The adjustment could be seen in Figure 1 with birds consuming particles of higher size (>1.18mm). This may also suggest a higher intake of wheat for regimens mixed with whole wheat grains. The result support reports by Picard *et al* (1997), reporting that birds shows a hierarchy in particles consumed, with the larger particles being consumed first.

Sequentially fed birds have equally no difference in feed intake. Although wheat intake is found to be inferior to the expected value of 20%/d, it was found to be different among regimens (table 3). The

[1] RETSCH GmbH, Rheinische Straße, 36 42781 Haan, Germany

highest wheat intake is recorded for regimens receiving the highest quantity of 150g, however this difference could not be associated to the quantity fed since 60 g of wheat fed is equally comparable to 25 g fed. The birds however, compensated the total quantity ingested with balancer diet intake. Sequentially fed birds consumed less wheat compared to loose-mix fed birds

From this work it could be concluded that loose-mix or sequential feeding methods have no effect on the quantity of diet consumed by the birds. However, the quality in terms of nutrient composition of the feed particle consumed must be investigated for an evaluation of the methods in terms of production performances.

TABLE 1: Composition (%) and calculated and analyzed nutrient content of experimental diets

Ingredient (%)	Complete mash diet	Balancer mash diet
Maize	33.5	33.6
Wheat	30	9.6
Added Whole Wheat	0	20
Soya bean meal (SBM 48)	21.5	21.5
Others	15	15.3
Calculated composition (%)		
ME (Kcal/kg)	2.750	
CP	17.3	
Calcium	3.63	
Phosphorus (total)	0.58	0.59
Analyzed composition (%)		
Dry matter	90.0	89.8
Crude Protein	17.1	17.2

[1] Vitamin and mineral premix provided per kilogram of diet : vitamin A, 8000 IU; vitamin D3, 2400 IU; vitamin E, 10mg; vitamin K3, 2mg; vitamin B1, 0.5mg; vitamin B2, 4.5mg; vitamin B12, 0.008mg; panthotenic acid, 7.5mg; nicotinic acid, 15mg; folic acid, 0.1mg; choline, 250mg ; Mn, 60 mg; Zn, 55 mg; Fe, 20 mg; Cu, 6 mg; I, 1 mg; Se 0.3 mg,

[2] ND : Not Determined

TABLE 2: Average total feed intake (TFI) g/d, of ISA Brown laying hens fed balancer mash diets in loose-mix with 46g (20%) whole wheat

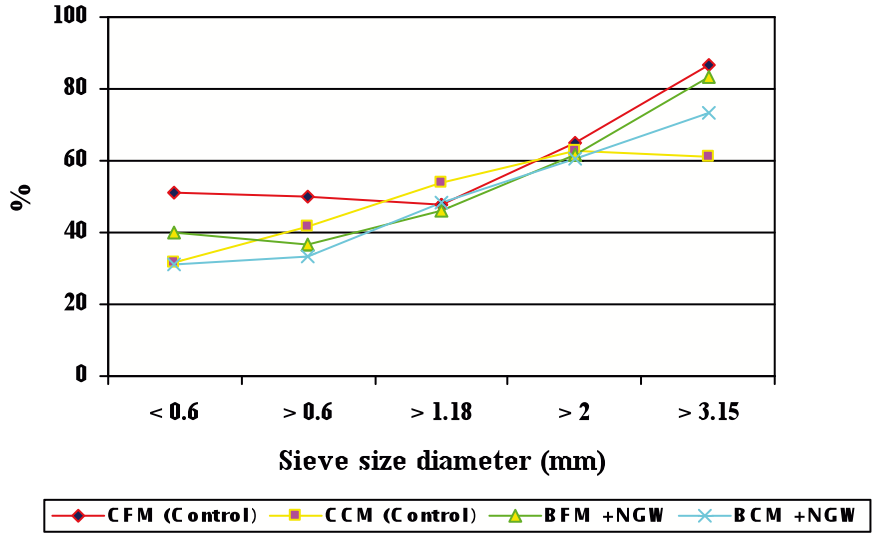
Regimen	TFI (g/day)
Complete coarse mash (control)	112.9±3.1
Complete fine mash (control)	120.0±2.2
Balancer coarse mash mixed with whole wheat	116.4±3.1
Balancer fine mash mixed with whole wheat	115.4±3.8
P	NS[1]

[1] NS : *P* >0.05
[a-d] Means ± SEM within the same column with no common superscript differ significantly (P<0.05)

TABLE 3: Average total feed intake (TFI) g/d, and wheat intake (WI) % of ISA Brown laying hens fed balancer mash diets sequentially with either 25 g, 60 g, 150 g whole wheat or 25 g ground wheat

Regimen	TFI (g/day)	WI (% Avg TFI)
Complete Coarse Mash (Control)	111.3±3.2	
Balancer Coarse Mash in sequential with 150g whole wheat	107.0±2.9	21.7±2.9[a]
Balancer Coarse Mash in sequential with 60g whole wheat	112.9±2.6	12.1±1.8[b]
Balancer Coarse Mash in sequential with 25g whole wheat	110.5±3.1	10.1±1.8[b]
Balancer Coarse Mash in sequential with 60g ground wheat	109.7±2.4	12.9±1.0[b]
P	NS[1]	<0.01

NS: *P* >0.05
[a-d]: Means ± SEM within the row with no common superscript differ significantly (P<0.05)



CFM = Complete Fine Mash, **CCM** = Complete Coarse Mash, **BFM + NGW** = Balancer Fine Mash mixed with non ground wheat, **BCM + NGW** = balancer Coarse Mash mixed with non ground wheat
Figure 1:Difference between the % sizes of particles fed to that ingested by the birds

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Annex 2

THE INFLUENCE OF SEQUENTIAL FEEDING ON BEHAVIOUR, FEED INTAKE AND FEATHER CONDITION IN LAYING HENS

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Abstract

Feeding of whole-wheat grains and a protein-mineral concentrate in sequence had been shown to modify behaviour in broilers and performance in laying hens. The objective of this study was to test whether sequential feeding with wheat would induce changes in laying hen's behaviour, feed intake, feather condition, and egg production. These parameters were measured on 320 non beak-trimmed ISA Brown laying hens from 30 to 37 week of age. The birds were placed in 64 standard cages (five birds/cage) and allotted to one of four treatments. The control (C) was fed a complete conventional diet. Three treatments were fed sequentially with whole wheat (SWW), ground wheat (SGW) or ground wheat with added vitamin premix+phosphorus+2% oil (SGWI). In sequential treatments, 50% of the ration was fed as wheat and the remaining 50% as a protein-mineral concentrate (balancer diet). All treatments received their daily ration in two distributions: 09:00 (4 h after light on) and 16:00 h (5 h before light off). During weeks 30, 32 and 34, hens' behaviour was recorded using scan sampling method (once per week during the light period), while focal sampling was used between the 32 and 34 weeks (two hours after each feeding, and two hours in between). Feather condition of individual hen was scored at 30 and 37 weeks, number of eggs and feed intake were recorded weekly.

Sequential feeding delayed the oviposition for almost one hour. When fed wheat-based diet (09:00 - 16:00 h) SWW birds spent less time feeding and stood still longer compared to birds in other treatments. Four hours after distribution of wheat diets, the occurrence of feather peaking was the highest in SWW and the lowest in the SGW treatment. The poorest feather condition was recorded in the SWW treatment. Total feed intake was the highest in the C treatment, while the intake of wheat diet and the ratio wheat diet intake/total feed intake was the highest in the SGWI treatment. We concluded that sequential feeding with whole wheat had detrimental effect on behaviour of laying hens probably due to long period of access to wheat used in this work. It is therefore suggested that wheat should be used either ground or presented on shorter time sequence. The time access should be reduced when whole wheat is used.

Key words: laying hens; sequential feeding; behaviour; feather condition; performance

1 Introduction

Increasing complexity of rearing conditions, also called “the enrichment”, has been often studied to find devices, which would increase the behavioural repertoire and thus improve the welfare of farm animals (Newberry, 1995). In poultry, devices such as perches, dust-bath, toys, strings etc. have been used to enhance general activity or reduce harmful behaviours such as feather pecking. However, very little attention has been paid to the complexity of the diet. In commercial poultry production, laying hens are fed with only one single complete diet, formulated to provide the nutrients requirements. This diet does not offer any heterogeneity, thus no possibility for the birds to choose. Possible enrichment may then consist in enhancing diet complexity by providing several diets instead of only one. Giving access to two diets at the same time often leads to unbalanced intake since birds prefer high to low energy diets (Picard et al., 1997). In an attempt to avoid this, birds have been given access to the diets at different times.

This feeding method is called sequential feeding, since various diets are given in sequence over time (Gous and Du Preez, 1975). Sequential feeding has been mainly used in broiler chickens with diets varying in energy or crude protein (Bouvarel et al., 2004; Bouvarel et al., 2008). However, in the past it has also been used in laying hens, with minerals being offered separately in the evening, so that calcium would be at birds disposal during the night, when the eggshell formation is in process. In broiler chickens it has been shown that commercial performance obtained with sequential feeding did not differ from standard feeding when a high-energy and a low-protein diet was fed to birds on one day and the following day was fed with a low-energy and a high-protein diet (Bouvarel et al., 2004; Bouvarel et al., 2008). In addition, sequential feeding modified time spent in eating and exploring (Bouvarel et al., 2008), and enhanced broilers general activity.

For these reasons it has been used to mitigate leg problems by reducing early growth without impairing body weight at slaughter, and thus improving the welfare of meat type chickens (Leterrier et al., 2008). Besides the welfare, sequential feeding is interesting from the nutritional point of view. Using two diets instead of only one allows different formulation of diets and use of various raw ingredients. For example, in sequential feeding it is possible to offer animals raw ingredients rich in protein in one diet and high-energy ingredient in the other diet. This of course is not possible in commonly used complete feed mixture since high levels of energy and protein are needed together in the same raw material. The use of raw ingredients has also economical benefits. Grinding,

mixing and other handling procedures associated with mash production are reduced to a great extent, which leads to substantial reduction of feed costs. Furthermore, the use of raw materials allows the use of locally grown cereals in the farm, which may additionally lower feed costs on account of transport reduction (Henuk and Dingle, 2002).

Due to the possibility of feeding birds with more than one diet, sequential feeding has been also used in feeding chickens with whole cereals. In this case a balanced nutrient intake was achieved with a protein concentrate (balancer diet), which provided additional necessary amounts of protein, vitamins and minerals (Bouvarel et al., 2004; Noirot et al., 1998; Rose, 1996). Sequential feeding with whole wheat has been shown to increase activity in broilers without impairing performance (Noirot, 1998), while in laying hens sequential feeding with wheat improved feed conversion (Umar Faruk et al., 2010). However, the possible influence of sequential feeding method on laying hens behaviour and consequently on their welfare is yet to be established. The present experiment was therefore designed to test whether sequential feeding with wheat would induce changes in laying hen's behaviour, feed intake, feather condition, and egg production.

2 Material and Methods

2.1 Animals and housing

The study included 320 non beak-trimmed ISA Brown laying hens obtained at the age of 15 weeks from a commercial supplier. They were distributed into 64 standard cages (five birds/cage). Live body weight was used as criterion such that there was no difference in weight among birds of the same cage and between the treatments. Cages were arranged into three-tier battery and were of following dimensions: length 60 cm, depth 56 cm (672 cm^2 area per hen), front height 41 cm and rear height 38 cm. Each cage was equipped with a feed trough (12 cm per hen) and two nipple drinkers. Temperature varied between 19 and 25 °C. Between 15 and 19 weeks of age photoperiod was gradually increased from 10 to 16 hours a day, with light on from 05:00 to 21:00 h. This lighting regime was maintained till the end of the experiment at 47 weeks of age.

During the experiment, eight hens died (1 C, 1 SGWI, 2 SGW, 4 SWW) and two from the SWW treatment had to be excluded because of their excessive feather pecking.

2.2 Experimental treatments

From 15 to 18 weeks of age all the hens were habituated to sequential feeding using whole wheat (3130 kcal/kg ME, 12.9% CP) in the morning followed by a balancer diet (2633 kcal/kg ME, 19% CP) after the wheat was removed from the feed trough. During this period, birds were phase fed to account for increase in feed intake with age. Thus, wheat offered was increased from 20% (week 15) to 50% (week 18). The duration of the period birds had access to wheat was increased from 3 hours (week 15) to 7 hours (week 18).

At 19 weeks of age, hens were allotted to one of four treatments (Fig. 1), which were randomised among cages. Each treatment contained 16 cages, with two neighbouring cages belonging to the same treatment. Each bird received a total of 121 g/hen/day of feed, corresponding to 105% of the estimated daily feed intake. All treatments, including the control one, were hand-fed in two distributions (09:00 and 16:00), with 50% of the daily ration in each distribution. The control treatment (C) received a complete conventional diet (2753 kcal/kg ME, 18% CP) throughout the two distributions. The remaining three treatments were fed sequentially. Two sequential treatments received either whole (SWW) or ground wheat (SGW) during the first distribution and balancer diet (B2) during the second distribution. Another sequential treatment (SGWI) received ground wheat with added vitamin premix+phosphorus+2% oil during the first distribution and the balancer diet (B1) during the second distribution. The composition of the experimental diets is presented in Table 1.

The balancer diets B1 and B2 were formulated such that ingesting equal proportion of wheat-based diet and balancer diet will provide on average the same nutrient intake as the control treatment. In sequential treatments, the previous diet was always removed from the feed trough by a vacuum cleaner before the next distribution, while in the control treatment it was removed only before the first distribution. Particle size distribution of the diets is shown on Fig. 2.

2.3 Measurements

2.3.1 Behaviour

Behaviour was monitored from 30 to 37 weeks of age, that is in the middle of experimental period, after the birds reached the peak of egg production. It was recorded directly by scan and focal sampling, with the observer standing in front of the cage at a distance of approximately 2 m. On account of the feeding regime birds

were used to frequent noise and people presence, therefore in order to avoid disturbing the hens, the observer had to wait only for a few moments before recording the behaviour. Scan sampling was conducted once a week by two observers at 30, 32 and 34 weeks of age. Hens' behaviour was recorded at one hour interval during the light period between 05:00 and 21:00 h. In the hours of feed distribution, the recording of behaviour started at the moment all the animals received feed. Each observer alternated sides and rows of the battery every hour. The number of hens standing up (number of all the animals that were not lying irrespective of what behavioural pattern they were performing) as well as performing the following behavioural patterns was recorded: feeding (pecking at the feed in the trough), drinking and standing still (standing without performing any other behavioural pattern). The number of eggs laid was also recorded.

Focal sampling was performed by one observer between 32 and 34 weeks of age using Observer 3.0 software (Noldus Information Technology, Wageningen, The Netherlands). Behaviour of hens was recorded over four days, six hours per day, that is two hours after feed delivers, starting at the moment when all the animals were fed (morning period: 09:00 to 11:00 and evening period: 16:00 to 18:00) and two hours in between (afternoon period: 12:30 to 14:30). In each cage, the behaviour of all birds was recorded simultaneously for 2 minutes within each of the three periods respectively. Each day we recorded the behaviour in 16 cages (4 cages per treatment) equally dispersed over the battery, making it possible to collect information on all the 64 cages in four days. We recorded number of animals feeding (defined as state) and occurrence of feather pecking (included gentle as well as strong pecks, where the feathers were plucked out), beak pecking (pecking at the beak of other hens; a behaviour usually observed during feeding), object pecking (pecking at the parts of the cage e.g. walls, trough) or aggressive pecking (vigorous, rapid pecks at another animal). These behavioural patterns were defined as events.

2.3.2 Feather condition

Feather condition of individual laying hen was scored twice at 30 and 37 weeks of age, using the scoring system of Tauson et al. (2005). Six body parts (back, wings, tail, vent/cloaca, neck and breast) were scored separately with scores from one to four with higher scores representing better plumage condition.

2.3.3 Performance

The number of all the eggs produced was recorded daily per cage and the percentage was calculated from the weekly data. Feed intake was measured weekly as the difference between total weekly feed offered and total weekly feed leftover. In sequential treatments, wheat and balancer diet intakes were measured separately and summed to obtain the total feed intake.

2.4 Statistical analysis

Data analyses were conducted using Statview 5.0 (SAS Institute Inc., USA). Average data from cages were analysed with the exception of data on feather condition, where individual hen represented the experimental unit. Data from scan sampling, except the number of eggs laid, were divided into four periods according to the daily rhythm of feeding (Fig. 3). Period 1 included scans before the first feed distribution (from 05:00 to 8:00 h), period 2 the scan just after feed distribution (09:00 h), period 3 scans between the first and the second feed distribution (from 10:00 to 15:00 h) and period 4 scans after the second feed distribution (from 16:00 to 20:00 h). Although hens had at their disposal greater amount of feed than the estimated daily feed intake, feed delivery represented an important stimulus with a great impact on hens' behaviour, which resulted in subordinating most of the observed behavioural patterns to these events. Therefore the analysis and consequently the data presentation were done separately for each of the period.

To investigate if hens in one treatment laid eggs earlier or later compared to hens in the other cages, the number of laid eggs obtained during scan sampling was transformed into index of laying according to the Eq. (1). The higher the value of index the earlier hens laid the eggs. However, the result of the index was transformed to hours to make understanding easier. This was done by defining an index of 16 to be 5:00 h, and adding one hour more to this for each subsequent, but smaller, index (i.e. an index of 15 would be 6:00 h etc.).

$$\text{Index of laying} = \sum_{i=16}^{1} (i \bullet N) \Big/ N_{SUM} \qquad (1)$$

where: i = value assigned to individual scan hour (value 16 is equivalent to the scan at 05:00 h and value one to the scan at 20:00 h); N = number of eggs laid in particular hour; N_{SUM} = sum of eggs laid during observation period (from 05:00 to 21:00 h).

Feeding (from scan and focal sampling), standing still and standing up were analysed with repeated measures ANOVA. The model included the effects of treatment (C, SGWI, SGW, SWW), period (scan sampling: period 1 – 4; focal sampling: morning, afternoon, evening period) and their interactions. The effect of treatment within individual period was tested with ANOVA, while differences between periods within individual treatment were assessed using repeated measures ANOVA. The effect of treatment on the index of laying, number of eggs produced corrected by hen number, total feed intake, intake of the individual diets and ratio between wheat-based diets and total feed intake was analysed using ANOVA. In the case the main effect (treatment or period) was significant, differences between means were tested by the Bonferroni test.

Drinking and feather condition were not normally distributed therefore the treatment effect within individual period or scoring was tested with nonparametric Kruskal-Wallis test followed by the Mann Whitney U test with Bonferroni correction for pairwise multiple comparison of means. Differences in drinking behaviour between periods were determined using Friedman test and differences between scores of the first and the second feather scoring with the Wilcoxon signed rank test. Differences in occurrence of feather, object or beak pecking and aggression between treatments were tested with χ^2 test. In the results occurrence of feather and object pecking is presented as the percentage of cages where these behavioural patterns were observed.

3 Results

3.1 Behaviour

Treatment influenced time of oviposition (ANOVA: $F_{3,60} = 7.878$, $P = 0.0002$, Fig. 4). In the C treatment hens reached the peak in laying approximately one hour and 40 minutes after light-on, while in sequential treatments, the majority of eggs were laid almost one hour later compared to the C treatment.

Repeated measures ANOVA revealed that interaction between treatment and period was significant only in feeding ($F_{9,180} = 11.099$, $P < 0.0001$) and standing still ($F_{9,180} = 4.178$, $P < 0.0001$). In both behavioural patterns the effect of treatment (feeding: $F_{3,60} = 16.545$, $P < 0.0001$; standing still: $F_{3,60} = 4.665$, $P = 0.0054$) and period was significant (feeding: $F_{3,60} = 469.135$, $P < 0.0001$; standing still: $F_{3,60} = 375.560$, $P < 0.0001$). Sequential feeding induced two peaks in feeding behaviour, each observed at the time of distribution of diets (Fig. 3). Similar daily rhythm was observed in all three observation days and in all treatments, even in the control one. The

ANOVA analysis showed that treatment significantly influenced time spent on feeding in all four periods (Table 2), but in standing still, which appeared to be inversely related to feeding, only in periods 2 and 3. In all treatments, the highest percentage of feeding was observed in periods 2 and 4, while hens stood still mostly during period 1. The Bonferroni pairwise comparisons revealed that in the first period of the observation day, hens in the C treatment fed longer than hens in the SGW and SWW treatment, while in period 4 they spent significantly less time feeding than birds in the other treatments. In the second and the third period the lowest percentage of feeding was observed in the SWW birds.

Time spent standing up was influenced only by period (repeated measures ANOVA: $F_{3,60} = 49.306$, $P < 0.0001$), but not by treatment (repeated measures ANOVA: $F_{3,60} = 1.670$, $P = 0.1829$). Interaction between treatment and period was not significant as well (repeated measures ANOVA: $F_{9,180} = 1.413$, $P = 0.1855$). The highest percentage of standing up (96.4%) was noticed in period 4, while the lowest (80.0 to 82.1%) was observed in period 1. Time spent drinking was similarly as time spent standing up influenced only by period (Friedman test: df = 3, $\chi^2 = 169.172$, $P < 0.0001$) and not by treatment (Kruskal-Wallis test: df = 3, $H = 4.797$, $P = 0.1873$). The highest percentage of drinking (10.7 to 11.2%) was noticed in the period 4, while the lowest (0.0%) was observed in period 2 (data not shown).

Duration of feeding (focal sampling) analysed with repeated measures ANOVA was significantly influenced by treatment ($F_{3,60} = 2.853$, $P = 0.0446$) and period ($F_{2,60} = 74.841$, $P < 0.0001$) as well as the interaction between treatment and period ($F_{6,120} = 5.963$, $P < 0.0001$). The Bonferroni pairwise treatment comparisons within each period (Fig. 5) confirmed the results obtained with scan sampling presented in Table 2. In the morning (Fig. 5), after the first feeding, hens in the SWW treatment spent less time feeding than hens in the other two sequential treatments and in the afternoon less than hens in the C treatment. In the evening, after the second feeding, the situation was just the opposite with the SWW hens feeding significantly longer than the C hens. Treatment significantly influenced the occurrence of feather pecking in the afternoon (χ^2 test: df = 3, $\chi^2 = 11.004$, $P = 0.0117$), which was more often observed in the SWW treatment than in the SGW (Fig. 6 a). Treatment significantly influenced also the occurrence of object pecking in the evening (χ^2 test: df = 3, $\chi^2 = 8.260$, $P = 0.0409$). The percentage of cages where object pecking was observed was higher in the SGWI treatment compared to the SGW and SWW treatment (Fig. 6 b). Treatment had no influence on the occurrence of beak (χ^2

test: df = 3, χ^2 = 0.722, P = 0.8680) and aggressive (χ^2 test: df = 3, χ^2 = 1.422, P = 0.7003) pecking (data not shown). The latter was observed very seldom, altogether in only 10 cages.

3.2 Feather condition

Sum of feather condition scores for all six evaluated body parts showed that with time, feather condition had been significantly impaired in the SWW treatment (Table 3). In the first scoring, when hens were 30 weeks old, there was no difference in feather condition between treatments, however in the second scoring, SWW hens had significantly lower sum of scores in comparison to hens in the C and SGW treatment.

Condition of feathers on the hen's back and vent/cloaca gave, according to our observation in the present experiment, the real insight into severity of feather pecking. Sum of these two scores showed significant impairment of feather condition with time regardless of treatment (Table 3), while at each scoring there was a significant difference between treatments. At 30 weeks of age, when the first scoring was performed, hens in the SGW treatment had higher sum of scores in comparison with SGWI and SWW hens. In the second scoring, in spite of impairment, feather condition on the back and vent/cloaca remained significantly better in the SGW compared to the SWW treatment.

3.3 Performance

ANOVA analysis showed that treatment had a significant influence on total feed intake ($F_{3,60}$ = 25.748, P < 0.0001; Table 4), the intake of individual diets (wheat or GWI: $F_{2,45}$ = 9.761, P = 0.0003; balancer diet: $F_{2,45}$ = 17.538, P < 0.0001; Table 4) as well as on the ratio between wheat-based diets and total feed intake ($F_{2,45}$ = 13.665, P < 0.0001; Table 4). The Bonferroni pairwise comparisons between treatments revealed that hens in the C treatment had higher total feed intake than hens in the three sequential treatments. Comparing the intake of individual diets, hens in SGWI treatment ate higher quantity of wheat and lower quantity of balancer diet in comparison to the SGW and SWW treatments. In these two treatments, wheat to total intake ratio was significantly lower than in the SGWI treatment.

The number of eggs per hen (Table 4) in the period studied (30 to 37 weeks) was significantly influenced by treatment ($F_{3,60}$ = 3.646, P = 0.0175). Hens in the SGW treatment laid less eggs compared to the C hens.

However, the production of eggs for the entire experimental period (week 19 to 44) was 91.1% and did not significantly differ between treatments (data not shown).

4 Discussion

Sequential feeding significantly delayed the mean time of oviposition for almost one hour compared to the control treatment. Some studies on broiler breeders reported delay in mean oviposition time when there was a delay in feeding time (Backhouse and Gous, 2005; Wilson and Keeling, 1991). On the contrary, in the cafeteria access to energy, protein and calcium diets, which gave hens the opportunity to consume nutrients parallel to their needs for egg formation, eggs were laid about two hours earlier compared to the complete feeding (Chah and Moran, 1985). According to the above mentioned findings, the explanation for delayed laying in treatments fed sequentially would therefore be a lack of essential nutrients necessary for the egg formation at the time needed. The other possible explanation for delayed oviposition in sequential treatments was the uneven supply of proteins. According to the findings of Keshavarz (1998a), for optimum performance hens need quality proteins available throughout the day. These findings may explain why in sequential treatments hens laid eggs later compared to the control, but they do not correspond to our results regarding the egg production. Within the studied period of 30 to 37 weeks of hens' age egg production was lower only in one treatment fed sequentially (SGW) and not in all three of them, therefore difficult to explain. Besides, this difference did not persist over longer period. The treatments did not result in differences in the number of eggs laid between 19 and 44 weeks.

Treatment significantly influenced feeding behaviour as well as feed intake. Observed daily rhythm of time spent feeding with two peaks, one in the morning and the other one in the afternoon, corresponds to the results of Bessei (1977) and Walser and Pfirter (2001). It is also comparable with the results of several authors studying the daily rhythm of feed intake (Choi et al., 2004; Savory, 1980). However, it seems that in our study the time spent in feeding was not regulated only by photoperiod (Lewis et al., 1995), oviposition and egg formation process (Morris and Taylor, 1967; Savory, 1977; Wood-Gush and Horne, 1970), but also by feed distribution itself. Well-marked peaks at feed distribution hours in the daily feeding activity are pointing out that stimulus of novel feed increased feeding motivation of all hens, even those in the C treatment, which had at their disposal only one diet. Results obtained with scan and focal sampling supplement each other and correspond to previous findings on the daily

rhythm of feed intake. Feed consumption was reported to be low prior to oviposition and increase immediately afterwards (Ballard and Biellier, 1969; Savory, 1977; Wood-Gush and Horne, 1970). In the middle of the light period hens usually eat less (Savory, 1980), while in the late afternoon, when the egg enters the uterus, another increment occurs, which is more pronounced and lasts longer (Ballard and Biellier, 1969; Savory, 1977).

According to the previous findings, laying hens consumed the greatest amount of feed in the afternoon (Hetland et al., 2003; Holcombe et al., 1976; Keshavarz, 1998a1998b), even regardless of dietary regimen (Holcombe et al., 1976; Keshavarz, 1998b). Since balancer diets were offered hens in the afternoon, this could be one of explanations why in our study the intake of balancer diets was greater compared to the wheat-based diets. Different intake of individual diets between treatments is difficult to explain, because the only remarkable difference in diet composition between SGWI and SGW and SWW treatments was in phosphorus content. Hens in the C treatment had significantly greater total feed intake compared to hens in sequential treatments, which supports earlier findings of Leeson and Summers (1978) and Reichmann and Connor (1979). Smaller feed intake in sequential treatments is indicating, that sequential treatments seem to offer hens sufficient opportunity to consume nutrients according to their daily cyclic requirements. When having this opportunity, hens used the nutrients more efficiently compared to the control treatment, where they had to consume also other nutrients and not only the one they needed at certain part of the day (Henuk and Dingle, 2002; Robinson, 1985), which consequently leads to a greater feed intake.

When given access to the wheat-based diets, hens in the SWW treatment spent less time feeding compared to the other treatments. This is related to the particle size, since hens fed fine structured diets need more time to consume the required amount of feed (Aerni et al., 2000; Savory and Mann, 1997; Vilarino et al., 1996; Walser and Pfirter, 2001). However, feeding large particle diets (El-Lethey et al., 2000; Lindberg and Nicol, 1994) and with this related shorter time spent feeding (Aerni et al., 2000; van Krimpen et al., 2005; Walser and Pfirter, 2001) was often reported to be related to a higher risk of feather pecking. The same connection appeared also in our study. In the afternoon, when hens in general spent the least time feeding, feather pecking was the most pronounced in the SWW treatment. Moreover, comparing the time hens spent feeding and standing still revealed a negative connection between these two behavioural patterns. The lower the percentage of time spent feeding the longer the time standing still. Increased time of standing still in the SWW treatment during the periods hens had at their disposal the wheat diets showed that the particle size influenced also this behavioural pattern

and not just the feeding and feather pecking. This result confirmed earlier findings of Savory and Mann (1997). Contrary to standing still, object pecking seemed to be unrelated to feeding or feather pecking whatever the period. In object pecking the difference between treatments occurred in the evening period after changing the diets. It is hard to explain why hens in the SGWI treatment pecked more parts of the cage than hens in the other two sequential treatments. Perhaps the reason for this was the change from preferred to a less-preferred diet as observed by Dixon (2006) with chicks from a laying strain, although it is hard to say why would hens preferred the B2 diet over the B1. The reason the occurrence of object pecking was unrelated to time spent feeding or occurrence of feather pecking was probably linked to the absence of objects of interest in the cage, since a previous study demonstrated that giving hens access to strands of string induced pecking at these strings and reduced feather pecking (McAdie et al., 2005).

In line with behavioural results, hens in the SWW treatment had the worst feather condition compared to the C or the SGW treatment. Feather scores appeared especially related to the variations in feather pecking observed in the afternoon. Although small, the differences in the sum of scores are very important, because of seriousness of the feather pecking problem present in laying hens. They are warning us about the possible negative consequences of feeding hens sequentially with the whole wheat. With time the feather condition got worse regardless the treatment. This has been clearly shown when only the sums of scores for the back and vent/cloaca, which in our opinion give real insight into severity of feather pecking, were compared. The negative impact of time on the feather condition supports previous findings of several authors (Blokhuis et al., 2001; Huber-Eicher and Sebö, 2001; Savory and Mann, 1997).

In the present study, the effect of sequential feeding with wheat on the behaviour, feed intake, feather condition, and egg production was tested on laying hens housed in standard cages, which will be banned in the EU in the near future. However, taking into account the experiments dealing with the sequential feeding in broilers housed in pens (e.g. Bouvarel et al., 2004; Bouvarel et al., 2008; Leterrier et al., 2008), we can be quite certain this feeding method is applicable also in alternative housing systems to cages, e.g. floor pens. Of course, we cannot state with certainty what would be the effect of sequential feeding with wheat on laying hens housed in e.g. enriched cages or floor pens. Nevertheless, we can expect that sequential feeding with ground wheat with or without additional ingredients would have no detrimental effect on the occurrence of feather pecking and consequently on laying hens' welfare. However, the negative effect of feeding hens with whole wheat might either

come to a greater expression or diminish on account of hens' spending more time exploring their environment when this is enriched.

5 Conclusions

Sequential treatments with wheat delayed the oviposition but otherwise had no detrimental effect on the behaviour of laying hens except when whole wheat was used. Large particle diet reduced the time spent feeding and increased the occurrence of feather pecking, which resulted in impaired feather condition. Therefore, when sequential feeding is to be employed in laying hen, wheat should be offered as ground or if whole wheat is to be fed, then perhaps it should be presented for shorter time periods. This may help to reduce its negative impact on the occurrence of feather pecking and consequent deterioration of feather condition.

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Table 1: Composition of experimental diets

Treatment[1]	Control	Sequential			
	C	SGWI		SGW, SWW	
Diet[2]		GWI	B1	Wheat	B2
Ingredient (%)					
Wheat	50.00	92.35		100.00	
Soya bean meal T48	17.00	0.34	41.36		41.06
Sunflower meal			3.00		
Maize	16.13		34.05		34.27
Calcium carbonate	7.96		14.93		15.23
Maize gluten 60	3.29	1.17			2.44
Wheat offal	2.54		3.55		
Bicalcium Phosphate	1.16	2.72			2.68
Soya bean oil	0.80	2.04	1.60		1.60
Sup 64 J02[3]	0.50	0.50	0.50		1.00
Sodium Bicarbonate	0.20	0.19	0.18		0.40
Refined salt	0.20	0.20	0.22		0.43
DL-Methionine	0.11		0.34		0.27
L-Lysine 78 Pou	0.11		0.05		0.07
Ucx Super Jaunis[4]		0.48	0.22		0.55
Calculated composition					
ME (kcal/kg)	2753	3130	2400	3130	2400
CP (%)	18.0	12.9	23.0	12.9	23.0
Ca (%)	3.64	0.97	6.20	0.03	7.20
P (%)	0.53	0.76	0.39	0.29	0.81

[1] C: control; SGWI: sequential with ground wheat having additional ingredients; SGW: sequential with ground wheat; SWW: sequential with whole wheat

[2] GWI: ground wheat having additional ingredients; B1, B2: balancer diet (protein concentrate)

[3] Vitamin and mineral premix supplied the following amounts per kilogramme of diet: Vitamin A 1600000 IU, Vitamin D3 480000 IU, Vitamin E 2000 mg, Vitamin K3 400mg, Vitamin B1 109 mg, Zn 11000 mg, Mn 12000 mg, Cu (sulphate) 1200 mg, Fe 4000 mg, I 200 mg, Se 60 mg, DL-Methionine 120 g;, Canthaxanthine 200 mg.

[4] Yolk pigment contains per kilogramme of diet: Cantaxanthine E 161g 300 mg, Luteine E 161b 1633 mg, Zeaxanthine E 161h 91 mg, Cryptoxqnthine E 161c 36 mg.

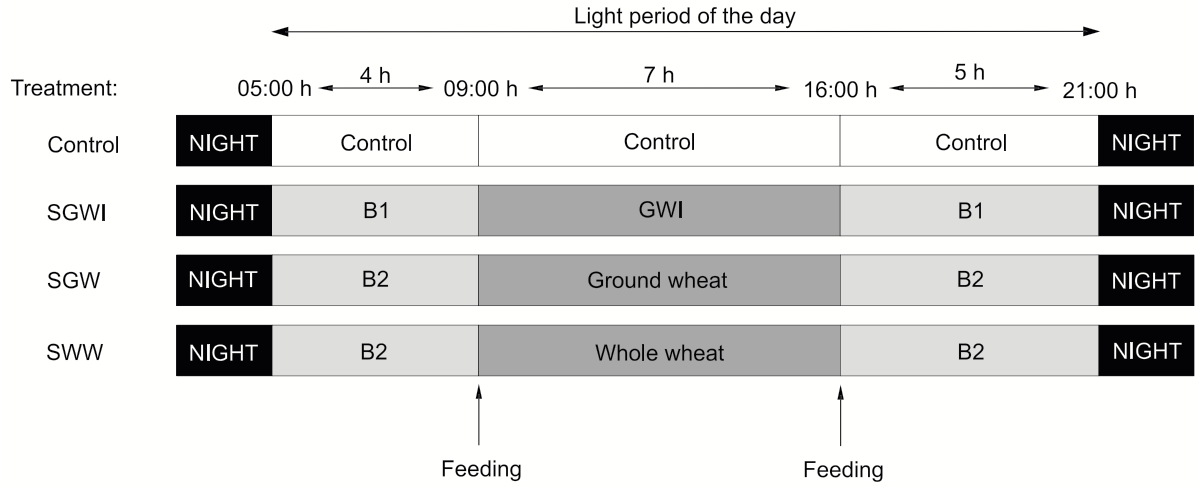


Fig. 1: Time schedule of diets distribution (treatment: SGWI: sequential with ground wheat having additional ingredients; SGW: sequential with ground wheat; SWW: sequential with whole wheat; diet: B1, B2: balancer diet; GWI: ground wheat having additional ingredients)

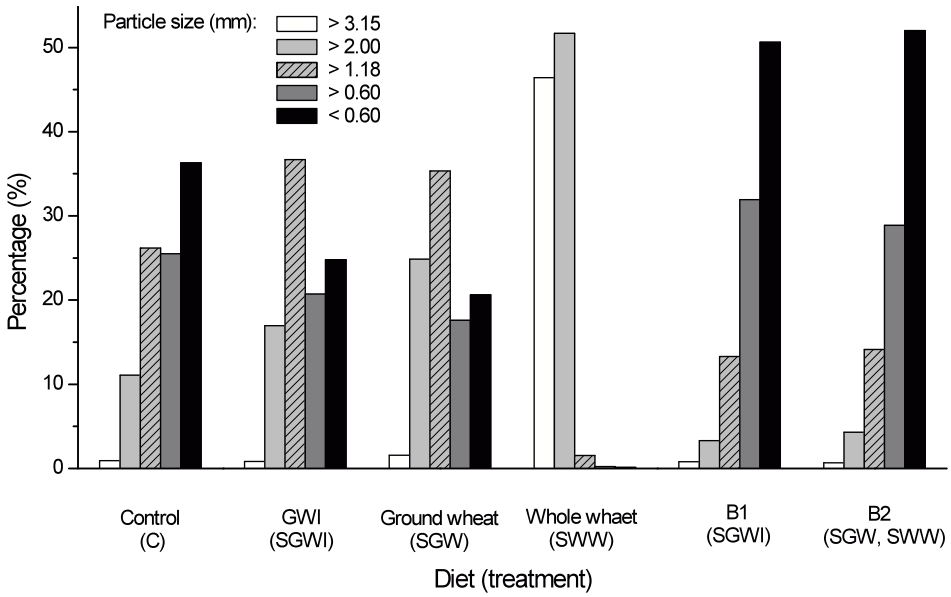


Fig. 2: Particle size distribution of the diets with the treatment (in parenthesis) the individual diet belongs to (treatment: C: control; SGWI: sequential with ground wheat having additional ingredients; SGW: sequential with ground wheat; SWW: sequential with whole wheat; diets: GWI: ground wheat having additional ingredients; B1, B2: balancer diet)

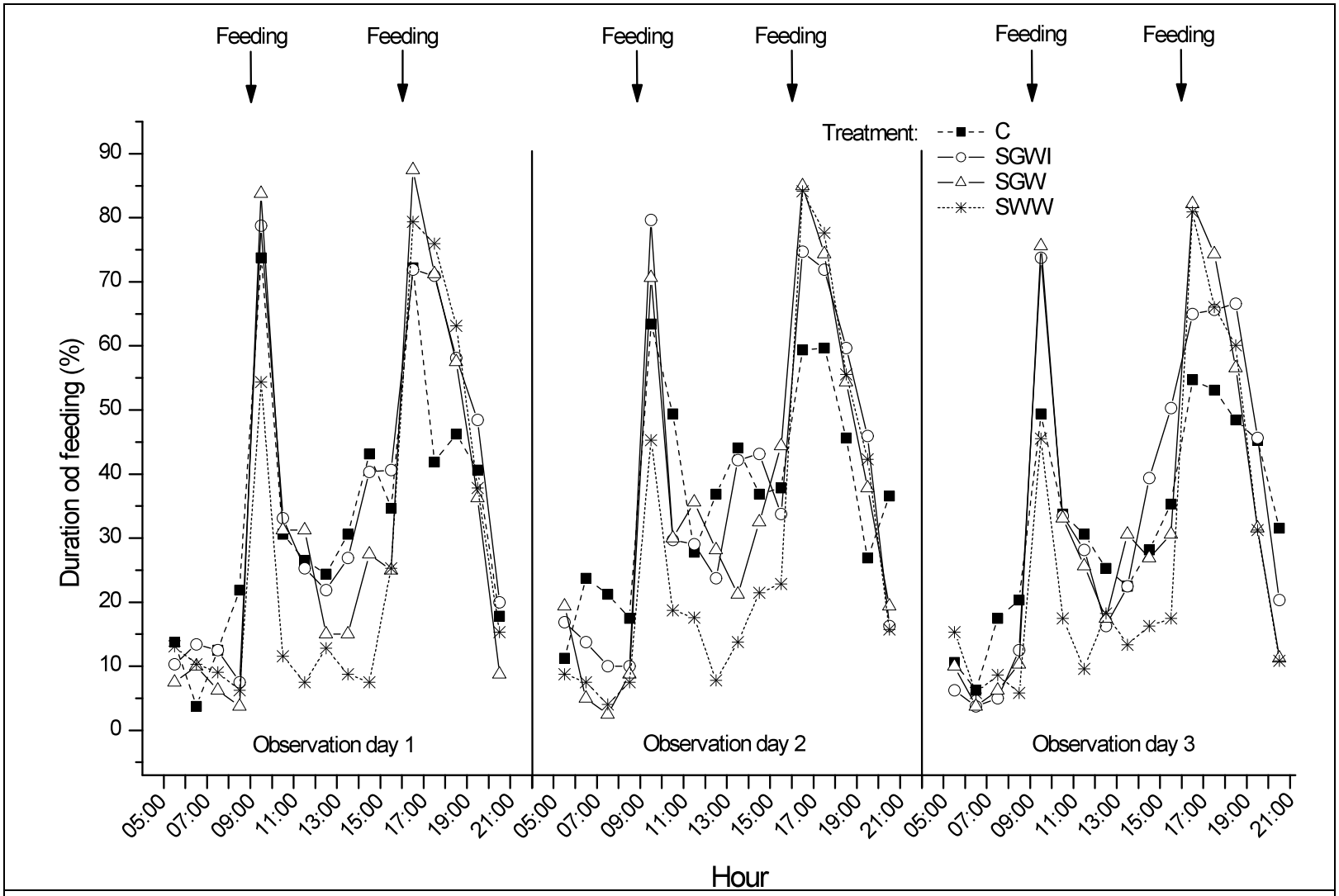


Fig. 3: Daily rhythm of feeding duration by treatment and observation day (C: control; SGWI: sequential with ground wheat having additional ingredients; SGW: sequential with ground wheat; SWW: sequential with whole wheat)

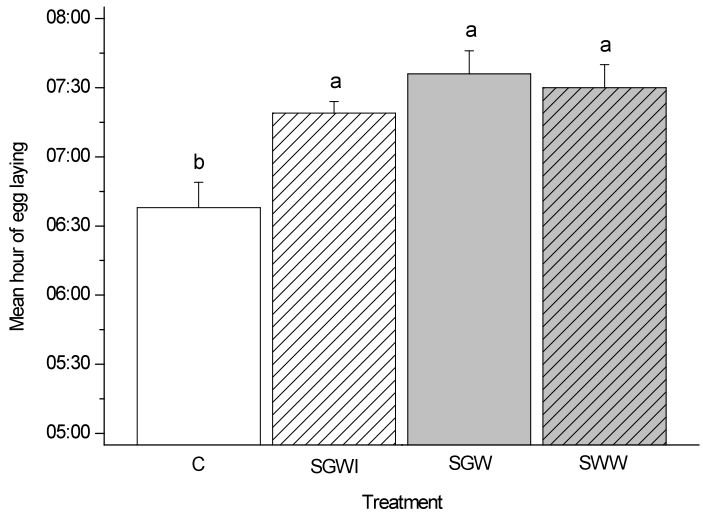


Fig. 4: Mean hour of oviposition (mean ± S.E.M.; C: control; SGWI: sequential with ground wheat having additional ingredients; SGW: sequential with ground wheat; SWW: sequential with whole wheat). Significant differences (Bonferroni test $P < 0.008$) are indicated by different letters (a, b).

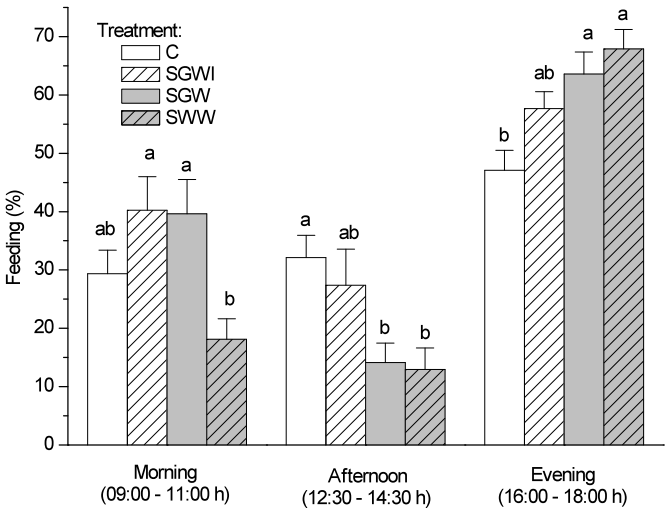


Fig. 5: Duration of time spent for feeding (mean ± S.E.M.) by period of the day recorded with focal sampling (C: control; SGWI: sequential with ground wheat having additional ingredients; SGW: sequential with ground wheat; SWW: sequential with whole wheat). Significant differences (Bonferroni test $P < 0.008$) are indicated by different letters (a, b).

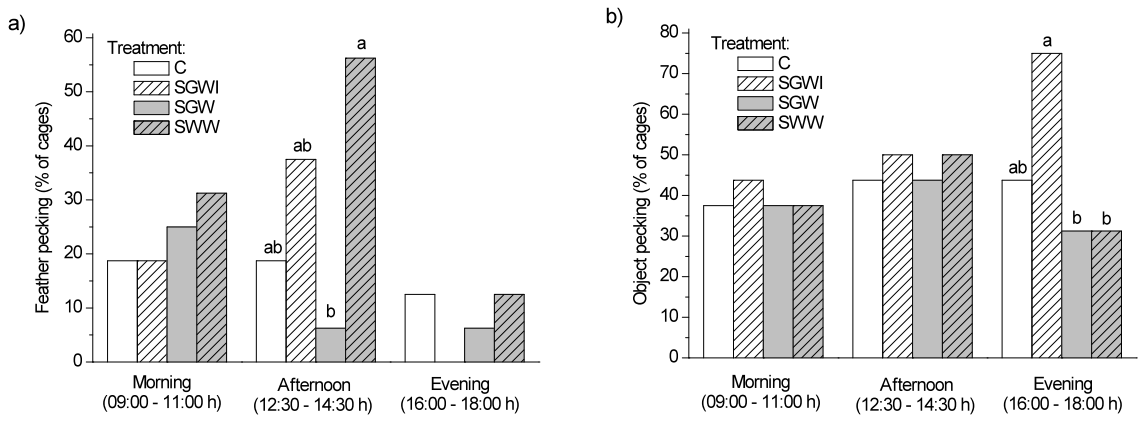


Fig. 6: Percentage of cages where feather pecking (a) and object pecking (b) were recorded with focal sampling in certain period of the day (C: control; SGWI: sequential with ground wheat having additional ingredients; SGW: sequential with ground wheat; SWW: sequential with whole wheat). Significant differences (χ^2 test $P < 0.05$) are indicated by different letters (a, b).

Table 2: Duration of feeding and standing still (± S.E) by treatment and period of the day (scan sampling)

	Treatment[1]					
	C	SGWI	SGW	SWW	*F*-value[3] df (3, 60)	*P*-value[3]
Feeding (%)[2]						
Period 1	15.0 ± 1.9 $^{a, Z}$	10.2 ± 2.0 $^{ab, Z}$	7.8 ± 1.5 $^{b, Z}$	8.5 ± 0.9 $^{b, Y}$	4.031	0.0112
Period 2	62.2 ± 3.8 $^{bc, W}$	77.4 ± 3.2 $^{a, W}$	76.7 ± 3.7 $^{ab, W}$	48.4 ± 4.9 $^{c, W}$	12.321	0.0001
Period 3	33.2 ± 2.0 $^{a, Y}$	32.2 ± 1.8 $^{a, Y}$	27.8 ± 1.6 $^{a, Y}$	14.9 ± 1.5 $^{b, X}$	23.942	0.0001
Period 4	45.3 ± 1.4 $^{b, X}$	53.4± 1.1 $^{a, X}$	52.5 ± 1.3 $^{a, X}$	53.1 ± 1.1 $^{a, W}$	9.931	0.0001
F-value; df (3,15)[4]	85.668	185.213	185.655	73,162		
Period effect (*P*-value)[4]	0.0001	0.0001	0.0001	0.0001		
Standing still (%)[2]						
Period 1	43.3 ± 1.9 ^{W}	45.8 ± 2.6 ^{W}	51.7 ± 2.3 ^{W}	49.5 ± 2.7 ^{W}	2.371	0.0794
Period 2	9.9 ± 2.3 $^{ab, Z}$	7.7 ± 2.1 $^{ab, Z}$	4.3 ± 1.6 $^{b, Z}$	14.4 ± 3.0 $^{a, Y}$	3.365	0.0243
Period 3	21.5 ± 1.8 $^{b, X}$	27.4 ± 2.2 $^{b, X}$	26.4 ± 1.5 $^{b, X}$	36.2 ± 2.3 $^{a, X}$	9.685	0.0001
Period 4	13.3 ± 1.5 ^{YZ}	9.8 ± 1.0 ^{YZ}	11.1 ± 1.3 ^{YZ}	11.8 ± 0.6 ^{YZ}	1.686	0.1797
F-value; df (3,15)[4]	114.650	93.353	149.964	62.741		
Period effect (*P*-value)[4]	0.0001	0.0001	0.0001	0.0001		

[1] C: control; SGWI: sequential with ground wheat having additional ingredients; SGW: sequential with ground wheat; SWW: sequential with whole wheat

[2] Period 1: 05:00-09:00 h; period 2: 09:00-10:00 h; period 3: 10:00-16:00 h; period 4: 16:00-21:00 h

[3] Analysis data were obtained by ANOVA.

[4] Analysis data were obtained by repeated measures ANOVA.

$^{a, b, c}$ Means in the same row with a different superscript differ significantly (Bonferroni test $P < 0.008$).

$^{W, X, Y, Z}$ Means in the same column with a different superscript differ significantly (Bonferroni test $P < 0.008$).

Table 3: Feather condition by treatment and time of scoring

	Treatment[1]					
	C	SGWI	SGW	SWW	*H*-value (df=3)[3]	*P*-value[3]
Sum of scores for all six body parts[2]						
Scoring 1	20.8 ± 0.2	20.5 ± 0.2	21.1 ± 0.1	20.4 ± 0.2	3.834	0.2800
Scoring 2	20.8 ± 0.2 [a]	20.1 ± 0.3 [ab]	21.0 ± 0.2 [a]	19.7 ± 0.3 [b]	13.169	0.0043
Z-value[4]	-0.133	-1.773	-0.518	-3.774		
Time effect (*P*-value)[4]	0.8946	0.0762	0.6043	0.0002		
Sum of scores for back and vent/cloaca[2]						
Scoring 1	7.8 ± 0.1 [ab]	7.6 ± 0.1 [b]	7.9 ± 0.0 [a]	7.5 ± 0.1 [b]	10.100	0.0177
Scoring 2	7.5 ± 0.1 [ab]	7.3 ± 0.1 [ab]	7.6 ± 0.1 [a]	7.2 ± 0.1 [b]	8.739	0.0330
Z-value[4]	-3.541	-2.822	-4.147	-4.056		
Time effect (*P*-value)[4]	0.0004	0.0048	0.0001	0.0001		

[1] C: control; SGWI: sequential with ground wheat having additional ingredients; SGW: sequential with ground wheat; SWW: sequential with whole wheat

[2] Scoring 1 was performed at 30 and scoring 2 at 37 weeks of age. The higher the sum of scores the better the feather condition.

[3] The treatment effect within each scoring was evaluated with the Kruskal-Wallis test.

[4] Differences between scoring 1 and scoring 2 were evaluated with the Wilcoxon signed rank test.

[a, b] Means in the same row with a different superscript differ significantly (Mann Whitney U test with Bonferroni correction $P < 0.008$).

Table 4: Feed intake and egg production from 30 to 37 week of age

	Treatment[1]					
	C	SGWI	SGW	SWW	*F*-value	*P*-value
Total feed intake						
Intake (g)	112.6 ± 0.9[a]	106.4 ± 0.6 [b]	103.5 ± 0.7 [b]	105.4 ± 0.9 [b]	25.748	<0.0001
Intake of the individual diets						
Wheat or GWI[2] (g)	-	49.6 ± 1.0 [a]	44.4 ± 0.7 [b]	46.2 ± 0.9 [b]	9.761	0.0003
Balancer diet (B1, B2) (g)	-	56.8 ± 0.5 [b]	59.1 ± 0.2 [a]	59.2 ± 0.3 [a]	17.538	<0.0001
Ratio (%): Wheat (or GWI[2])/total intake	-	46.6 ± 0.7 [a]	42.8 ± 0.4 [b]	43.7 ± 0.5 [b]	13.665	<0.0001
Number of egg corrected by hen number (%)						
	97.8 ± 0.4[a]	97.0 ± 0.4 [ab]	95.3 ± 0.7 [b]	96.4 ± 0.5 [ab]	3.646	0.0175

[1] C: control; SGWI: sequential with ground wheat with additional ingredients; SGW: sequential with ground wheat; SWW: sequential with whole wheat

[2] GWI: ground wheat having additional ingredients (see Table 1)

[a, b] Means in the same row with a different superscript differ significantly (Bonferroni test $P < 0.008$).

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Murtala UMAR FARUK

Evaluation of the Impact of Loose-Mix and Sequential Feeding Using Locally Available Feed Ingredients on Performance in Layer Hen

Résumé

L'objectif de cette thèse est d'évaluer l'impact de deux systèmes d'alimentation (mélange et séquentiel) sur les performances de production chez la poule pondeuse en France et au Nigéria. En France avec 50% de blé entier, l'alimentation séquentielle provoque une baisse significative de l'ingestion comparée au mélange et témoin. Le nombre et la masse d'œufs restent identiques entre les trois modes, conduisant ainsi à une amélioration importante de l'indice de consommation en alimentation séquentielle par rapport au mélange (-10%) ou au témoin (-5%). Au Nigéria avec 33% du millet, l'ingestion en séquentielle a été aussi plus faible que pour le mélange et témoin. Le nombre et le poids de l'œuf ont été supérieurs en mode séquentiel, conduisant à une amélioration de l'indice de consommation par rapport au mélange (-20%) ou au témoin (-10%). L'alimentation séquentielle permet d'utiliser des graines entières avec une amélioration de l'efficacité alimentaire. Le modèle se présent donc comme une innovation importante pour améliorer la durabilité de la production d'œufs en France et au Nigeria, contribuant dans ce dernier cas à une amélioration de la sécurité alimentaire.

Mots-clés : Alimentation séquentielle, alimentation mélangée, durabilité, sécurité alimentaire, poule pondeuse,

Abstract

The objective of this thesis was to evaluate the impact of sequential and loose-mix feeding of whole cereal grain on the production performance in laying hens in France and in Nigeria. Using 50% whole wheat in France, sequential feeding resulted to a significant decrease in feed intake compared to loose-mix and control. Egg number and mass were however, identical between the three systems, thus, leading to a significant improvement in the efficiency of feed utilisation in sequential compared to loose-mix (-10%) and control (-5%). Using 33% millet in Nigeria, sequential feeding also reduced feed intake compared to the two other systems. Egg number and egg weight were higher in sequential feeding system. This largely improved feed efficiency compared to loose-mix (-20%) and control (-10%). Sequential feeding allows the use of whole cereal grains with improved feed efficiency. It is therefore an innovation that can be used to sustain durable egg production in France and in Nigeria. It is also a solution to further food security in Nigeria.

Key words: Sequential feeding, loose-mix feeding, sustainable production, food security, laying hen, egg