FEEDING METHIONINE TO LAYING HENS IN DRINKING WATER

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ABSTRACT

The present study intended to demonstrate the appetite of laying hens for methionine when offered as a choice in drinking water. Methionine, the first limiting amino acid in practical layer diets is currently provided in the feed in constant amounts. In contrast to this arguable practice, its addition to the drinking water would enable the birds to consume amounts according to their actual methionine requirements. Ten experiments were performed to investigate the conditions of this way of delivery. For the first 5 experiments ISA Brown, for the second 5 Lohmann layers were used.

1) Two preliminary tests determined that a minimum of 140 g/kg dietary crude protein (CP) is necessary for normal egg production of at least 90% of commercial target values, and that the typical feed intake / water intake ratio is 1:1.44.

2) Initially, the appetite of layers for methionine in drinking water was investigated by subjecting them to the combinations of feed adequate or deficient in methionine, and plain or methionine-treated water. Neither cues nor training were used. In the choice situation, the birds could not express an appetite for treated water.

4) Colour cues and training of the birds were introduced to enable birds to express an appetite for methionine-treated water. The choices made in favour of treated water were 98.9% in a period of 5 days, and 93.9% when the position of the bottles was swapped.

5) Methionine deficiency resulted in decreased feed and water intake which were restored when methionine was added to the diet. The way of delivery (in water or feed) was insignificant in this effect, indicating that the source of methionine does not influence normal appetite.

6) Next, the birds' ability to regulate their intake of methionine was investigated. The introduction of an additional colour was expected to avoid the association of plain water with the effects of methionine deficiency, thus in the choice situation, neither the treated nor the plain water had a "history" of causing

discomfort to the birds previously. Yet, the birds did not stop the consumption of the treated water even when they had satisfied their methionine requirements, indicating that they can not regulate methionine intake.

7) The detection threshold for methionine was investigated by using different levels of methionine in the drinking water in combination with methionine-deficient feed. It was found that the minimum level of methionine in drinking water for which birds can express appetite is at least 0.025%.

8) The investigations on the metabolic effects of methionine deficiency showed that the birds respond to the deficiency by reducing their feed intake, which decrease becomes significant after eight hours.

9) The hypothesis was examined that giving a methionine-deficient diet in the last 5 hours of the feeding period, the birds will reduce their feed intake in the following morning even if they are fed on an adequate diet that time. It is indicated that the time of receiving a methionine-deficient diet is closely associated with the time of feed intake depression.

10) Determination of the threshold period of training of the birds showed that the using of an 8-hours exposure time resulted in a more than 90% of choices for methionine-treated water.

11) The memory of the birds was tested once after a period of 15 days and again after a period of 45 days on commercial diet. In the subsequent choice situation, birds receiving the 8-hours treatment showed a strong preference for methionine indicating that birds memory last at least 45 days.

12) It was shown that 2.1 g/kg methionine in the feed was deficient for almost all the birds. Birds receiving adequate or nearly adequate amounts of methionine do not feel the physiological effects of deficiency. These birds do not select methionine treated water in a choice situation.

The main conclusions of the study are that the source of methionine is insignificant in terms of feed intake, and that layers can express an appetite for methionine in drinking water with the aid of a cue and adequate training.

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LIST OF ABBREVIATIONS

AME	Apparent metabolizable energy
ARC	Agricultural Research Council
%	Percent
CP	Crude protein
°C	Degree Celsius
EAA	Essential amino acid
FCR	Feed conversion ratio
HD	Hen-day
%HD	Hen-day percent
IU	International Unit (i.e. the amount of enzyme causing conversion of 1 μmol substrate per minute under specified conditions)
LSD	Least-significant difference
NRC	National research council
r	Pearson's correlation coefficient
SAA	Sulphur amino acid
SEM	Standard error of the mean
TSAA	Total sulphur amino acid
w/v	Weight for volume

DECLARATION

No part of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Apart from the rearing of the main stocks, the designing and management of experiments, sample analysis and statistical evaluation were my own work. Sources of information have been acknowledged in the form of references.

Signed: Date: March-2001

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For my parents

1.0 Introduction

One of the main aims of nutritional research is to reduce the cost of poultry production and increase profit by increasing the utilisation of feeds and nutrients. A new way of delivering methionine to poultry could partly help to achieve this goal. Methionine is the first limiting amino acid in the conventional corn-soybean and wheat-soybean diets, and synthetic methionine has been used for over five decades as a supplement to these diets. However, the present practice of adding methionine to the feed is not fully satisfactory. In contrast to this, delivering methionine in the drinking water could offer a number of potential advantages.

It is well established that birds' individual methionine requirements vary greatly (Fisher *et al.*, 1970), the range of their intake requirements being between 220 and 470 mg/day (Tolan and Morris, 1969). Yet, the current practice is to provide this amino acid in constant amounts in the feed. However, if a diet is formulated to meet the requirements of 95% of the birds in a given flock, it is possible that it will contain varying excessive amounts for 94% of the flock. Such a level may be economically wasteful. The ideal level should be near to the percentage where the marginal cost of supplying the limiting nutrient is equal to the marginal increase in product value output. This approach is the basis of the Reading Model (Fisher *et al.*, 1973). Consequently, presently, some birds are under supplied and others are over supplied with methionine. It is now suggested that if the birds take amino acids provided in water when they need them, this problem would partly be eliminated. If birds were able to consume amounts of the limiting nutrient exactly to meet their requirements then the total level of consumption would be the average requirement of the flock. The average requirement of the flock may be less than the percentage where the marginal costs and returns are equal. Thus savings could be made.

It is also well known that small particles in a mixture settle to the bottom while the larger particles work to the surface. The same is true for mash feeds; dry ingredients separate out, especially if the feed is handled often. As feed is handled a great deal before being consumed, it is difficult to maintain a good, even mixture of ingredients. In addition, birds have a preference for larger particles: there is evidence that domestic chicks prefer a particle size of 2 to 3 mm (Bessei, 1973; Perry *et al.*, 1976). Dry DL-Methionine particles are very small (300-600 microns), that is much smaller than most ingredients used in commercial feeds, thus segregation of methionine is a potential problem. Indeed, when standard particle size screens were used on typical layer rations, it was found that almost 95% of the dry DL-methionine was in the half portion of the feed containing the smallest particles (Anonymous, 1985), instead of the ideal 50%. As a consequence of separation, methionine is probably consumed in irregular quantities. If a bird is undersupplied for a day, the average rate of supply will decrease and thereby output will decrease. For a partial solution, the use of the fluid ALIMET had been advised (Anonymous, 1985). This product coats and adheres to all feed particles - both large and small - thus resulting a much more uniform distribution (65%-35%) of methionine. A complete solution, however, would be the supplementation of methionine in the water.

A further advantage would be the provision of a more consistent supply of methionine to the birds which would ensure that they all receive the balance of nutrients they require and consequently improve the feed efficiency.

In addition, birds under stressful conditions (hot temperature, rapid temperature changes, crowding, etc.) would also benefit from the delivering methionine in the drinking water. It is known that under suboptimal conditions, birds often are unable to eat but are able to drink. The use of methionine in drinking water may allow the producers to supply this nutrient rapidly and efficiently, when birds have a reduced food intake.

Finally, nutritional manipulation of egg component composition by methionine may offer economic and production advantages for the liquid egg industry (Shafer *et al.*, 1996, 1998). Methionine is currently supplemented to most commercial layer diet formulations and fed to flocks to influence egg size. Increasing methionine level of the layer diet should not require additional labour, equipment, or time, therefore, application to flocks dedicated to liquid egg production requires no investment other than the cost of the methionine. Delivering methionine in drinking water can provide an opportunity to manipulate the diet without changing the feed.

In summary, if a system is used where amino acids (methionine) can be delivered through water as well as feed, there could be a major efficiency gain.

The existence of a specific appetite for amino acids (lysine and methionine) has been studied in broilers (Newman and Sands, 1983; Steinruck *et al.*, 1990a, b) and sparrows (Murphy and King, 1987), however, these investigators did not provide cues to allow birds to associate each food with its metabolic consequences. Methionine had been given to broilers in drinking water, and, compared with the practical diet, this practice had no adverse effect on production (Damron and Goodson-Williams, 1987; Damron and Flunker, 1992). However, a specific appetite of layers for any amino acid in drinking water has not been investigated at all.

In the present project, a series of experiments have been carried out to investigate the delivery of methionine in drinking water for the domestic fowl, and its consequences in terms of the opportunity to improve efficiency of production.

The aim was to investigate the birds' ability to express an appetite for methionine in drinking water and to correct a methionine deficiency. The experiments during the course of this study examined the feed and water intake of laying hens when receiving a methionine-deficient feed and methionine is added to the feed or drinking water. The minimum level of methionine in drinking water and the level of methionine deficiency in feed which enables birds to express an appetite for methionine-treated water, moreover, the minimum training time necessary for the birds to learn to associate colours with methionine deficiency or adequacy were determined.

We chose the adult laying hen as the experimental animal, and not the growing broiler where, potentially, the economic impact would be even greater.

2.0 REVIEW OF THE LITERATURE

2.1 Methionine in poultry diet

Methionine is an essential amino acid (EAA) which means it can not be synthesised by poultry at all. In contrast, non-essential amino acids can be synthesised from essential amino acids that are in excess of requirements, and other non-essential amino acids. Often, the literature discusses methionine requirement together with requirements for other sulphur amino acids, mainly cysteine. This is partly because methionine and cysteine are metabolically related to each other: methionine is readily converted to cysteine (Ensminger, 1992). The other reason is the difficulty of assessing the true requirement for methionine due its complicated role in different metabolic pathways. It is needed for maintenance and egg production, for the building of body proteins and feather proteins (Pack, 1996). Methionine is also the first limiting amino acid in commercially prepared corn-soybean and wheat-soybean meal layer rations (Leong and McGinnis, 1952; Harms and Damron, 1969; Fisher and Morris, 1970; Schutte and Van Weerden, 1978; Schutte *et al.*, 1983, 1984, 1994; Waldroup and Hellwig, 1995). Thus, methionine must be included in corn-soybean or wheat-soybean diets to meet requirements. For this a synthetic source is commonly used. There are basically four different sources of methionine, as shown in Table 2.1. Methionine is usually supplied in the food either in solid form or, because it is easy to incorporate at the feed mill, sprayed over dry products (Leeson and Summers, 1997). Methionine can also be supplied in the drinking water for poultry, although unpublished results by Baker (1977) indicated that small amount of methionine in drinking water could cause severe problems due to its oxidative degradation into aldehyde, namely a 50% reduction of water intake of growing chicks was reported (cited by Damron and Flunker, 1992) was concerned by the use of small (not detailed) amount. The reference did not give details of the amounts or source of the methionine used. In contrast to this, works by Damron and Goodson-Williams (1987) and by Damron and Flunker (1992) found no evidence for such adverse effect of methionine supplementation in water even though the typical cabbage-like odour of the treated water (containing 0.05% methionine) was detected at the end of a 21-days experiment with growing chicks (Damron and Goodson-Williams, 1987). According to these publications, both the DL form (Damron and Goodson-Williams, 1987) and the liquid methionine analogue (Damron and Flunker, 1992) are suitable for supplementation in water without adverse effects on water and feed intake, and production or mortality in broilers. What is more, mortality and stress were reported to be even reduced as a result of this practice (Anonymous, 1984).

Table 2.1 Methionine sources.

<table>
<tr><th>DL-Methionine</th><th>DL-Methionine-Na</th><th>Methionine hydroxy analogue</th><th>Methionine hydroxy analogue-Ca</th></tr>
<tr><td>Powder form</td><td>Liquid form</td><td>Liquid form</td><td>Powder form</td></tr>
<tr>
<td>CH3
|
S
|
CH2
|
CH2
|
H — C — NH2
|
COOH</td>
<td>CH3
|
S
|
CH2
|
CH2
|
H — C — NH2
|
COONa+</td>
<td>CH3
|
S
|
CH2
|
CH2
|
H — C — OH
|
COOH</td>
<td>CH3
|
S
|
CH2
|
CH2
|
H — C — OH
|
COOCa+</td>
</tr>
</table>

2.1.1 Methionine requirement of laying hens

The several reports (Table 2.2) on the requirement of laying hens for methionine and sulphur amino acids (SAA) suggest a wide range of needs; many of the estimations agree well, however some suggest considerably lower requirements. A possible explanation for these low values is that they were estimated by using mainly egg production criteria, rather than feed conversion efficiency. It has been reported by a number of authors (Janssen, 1974; Schutte and Van Weerden, 1978; Schutte *et al.*, 1983, 1984, 1994) that the SAA requirement of laying hens for maximum efficiency of feed utilisation is distinctly higher than that for obtaining maximum egg yield. This phenomenon has also been observed with respect to weight gain and feed conversion efficiency in broiler chicks (Adams *et al.*, 1962; Bornstein and Lipstein, 1964, 1966; Van Weerden *et al.*, 1976; Pack and Schutte, 1992). A partial explanation for this can be the feed intake regulation action of methionine (see sections 2.1.2.1 and 2.5.1).

Table 2.2 Recommendations for methionine, sulphur amino acid and/or protein levels in diets.

Author	**Methionine requirement [mg/HD]**	**SAA requirement [mg/HD]**
Novacek and Carlson, 1969	320	TSAA 460
ARC, 1975	350	SAA 470
Schutte *et al.*, 1985	375	TSAA 750
Calderon and Jensen, 1990	381 (with 130 g/kg CP) 388 (with 160 g/kg CP) 414 (with 190 g/kg CP)	TSAA between 659 and 773
Cao *et al.*, 1992	424 364	TSAA 785 TSAA 670
NRC, 1984, 1994	350	TSAA 600
Harms and Damron, 1969	250-280	TSAA 530
Fisher and Morris, 1970	275	n.a.

ARC = Agricultural Research Council
CP = crude protein
TSAA = total sulphur amino acid
n.a. = not available

The variation amongst the majority of the values is due to the major changes in feeding practices and management of hens which have been used, and to the genetic factors influencing their methionine/sulphur amino acid requirements. These include production and body weight, which are affected by food consumption and age. The requirement of layers may increase in the middle or last quarter of their egg production period (Waldroup and Hellwig, 1995).

The requirement for sulphur amino acids is also influenced by the concentration of dietary protein both in broilers (Rosenberg and Baldini, 1957; Nelson *et al.*, 1960; Hartel, 1970; Mendonca and Jensen, 1989), and layers (Calderon and Jensen, 1990). Depending on the range of protein levels used and the methionine source involved, the requirement has been reported as increasing in direct proportion to the concentration of dietary protein, or to increase, but at a slower rate than the rise in dietary protein. In contrast, when Fisher and Morris (1970) used a procedure in which they diluted a high-protein diet with an isocaloric, protein-free diet in order to investigate the requirement for total sulphur amino acid (TSAA) by hens, they found that the response to methionine obtained is virtually independent of the protein level.

2.1.2 Effects of dietary methionine

2.1.2.1 Effects of methionine supplementation on feed intake

Calderon and Jensen (1990) conducted an experiment using three diets containing 130 g/kg, 160 g/kg, and 190 g/kg CP of which the methionine content was 255 mg/kg, 290 mg/kg and 325 mg/kg, respectively. Each of these diets were supplemented with 0 mg/kg (control), 50 mg/kg, 100 mg/kg and 150 mg/kg of methionine. They found that overall feed intake increased with increasing protein concentration and methionine supplementation. On the other hand, Harms *et al.* (1962, 1967) reported increased feed intake by birds even on low methionine diet. They gave egg production type pullets five different levels of SAA (80%, 95%, 100%, 115% and 130% of the estimated requirement of the laying hen), and showed that when they receive the diets most deficient in methionine there is a trend to over consume on energy. This was indicated by a higher feed intake of pullets on the two lower levels of amino acids than those on the higher levels of amino acids. The authors suggested that this was in an attempt to meet the amino acid requirement. The contrast between the two observations might be due to differences in the egg production rate: Calderon and Jensen (1990) reported very high (~90%) production, whereas production of the birds 35 years previously in the trial of Harms and co-workers (1962, 1967) was considerably lower (~70%).

Gous and Kleyn (1989) pointed out in a review that as the concentration of dietary methionine and other amino acids decrease slightly, feed intake increases. That is, by eating more the birds try to compensate for marginal deficiency of the first limiting amino acid. This proposal is supported by the findings of Schutte *et al.* (1994). They fed a group of layers with a basal diet containing 230 mg/kg methionine while others received additional methionine of 50 mg/kg, 70 mg/kg, 95 mg/kg, 125 mg/kg, and 165 mg/kg. Daily feed intake increased significantly when 50 mg/kg methionine was added. While compared to this daily feed intake was not changed significantly at higher supplemental levels. That is, the highest feed intake was observed at the lowest methionine supplementation. Similarly to others (e.g. Schutte and Van Weerden, 1978;

Schutte *et al.*, 1983, 1984; Waldroup and Hellwig, 1995), these authors concluded that birds have the ability to compensate for a marginal deficiency of methionine by consuming more feed.

Finally, if the food is severely deficient in methionine, feed intake declines (e.g. Gous and Kleyn, 1989; Harms and Russel, 1998). Chee and Polin (1978) found that feed intake of chickens on methionine deficient diet was only 68% of the amount of adequate methionine diet consumed.

2.1.2.2 Effects on body weight

Body weight gains of laying hens is altered significantly by the level of SAAs in the diet. Harms *et al.* (1967) demonstrated that body weight gain of laying hens increased significantly with increasing the level of methionine in the diet. Moreover, a body weight loss was recorded at the lowest level of methionine, i.e. when 80% of the required amount was supplied. In support of this, later investigations (e.g. Harms and Damron, 1968; Calderon and Jensen, 1990) also found that body weight was significantly increased by adding methionine and by increasing the concentration of protein. On the other hand, Penz and Jensen (1991) reported that when a low protein diet (130 g/kg CP) was supplemented with methionine at 20% above NRC (1994) requirement, body weight gain declined in comparison to that of birds fed a high protein diet (160 g/kg CP) but with methionine levels no higher than the NRC (1994) recommendation.

Growing chickens of laying strains also demonstrate that reductions in weight are linked to a decrease in feed intake of a methionine-deficient feed. For example, Chee and Polin (1978) fed 130 g/kg CP diet to White Leghorn male chicks and supplemented it with three levels of methionine: 160 mg/kg (deficient), 480 mg/kg (adequate) and 1160 mg/kg (excess). They observed a reduction in body weight gain by the deficient and excess diets. Further, their experiments indicated that weight loss from a deficient diet is due to its effect on the feed intake regulating mechanism, while excess methionine does not appear to have the same role.

2.1.2.3 Effect on protein utilisation, feed efficiency and egg production

The efficiency of protein utilisation depends to a large extent upon the amino acid composition of the diet. The closer the amino acid composition of the diet matches the hen's requirement, the more efficient the protein of the diet is utilised. As methionine is generally the first limiting amino acid in practical i.e. corn-soybean or wheat-soybean meal diets for laying hens (Harms *et al.*, 1962; Harms, 1992), supplementation of laying hen diets with methionine provides a means of increasing the efficiency of protein utilisation (Sell and Johnson, 1974; Schutte and Van Weerden, 1978; Schutte *et al.*, 1983, 1984). It has been found that the protein content in layer diets can be reduced to 140 g/kg throughout a laying period provided methionine and lysine were added to the diet (Sell and Hodgson, 1966; Fernandez *et al.*, 1973; Sell and Johnson 1974; Schutte and Van Weerden, 1978). Schutte *et al.* (1983) also demonstrated that satisfactory laying performance can be achieved using a low protein diet provided it is supplemented with methionine. They fed layers 140 g/kg CP corn-soybean diet containing 500 mg/kg SAA, and supplemented with three levels of methionine: 500 mg/kg, 1000 mg/kg and 1500 mg/kg. Overall egg production was 85% during the 52 weeks experimental period, at all levels of supplementation. As the unsupplemented diet was already adequate for high performance, the addition of 500 mg, 1000 mg and 1500 mg methionine did not increase egg production, but, 500 mg methionine significantly improved feed efficiency. However, these workers also noted that in the early stage of laying, when daily food intake is low, more than 140 g/kg CP diet might be required even when concentrations of SAA and lysine are adequate.

Feed efficiency was reported to improve significantly with increasing amounts of SAA in the diet (Moran, 1969; Schutte and Van Weerden, 1978; Schutte *et al.*, 1983, 1984). Maximum efficiency of food utilisation was obtained with the intake of 775 to 800 mg methionine+cystine /hen-day (HD), of which about 390 to 440 mg is methionine. Shafer *et al.* (1998) also found improved feed efficiency for birds fed a 130 g/kg CP diet supplemented with lysine, methionine, and tryptophan at 20% above the NRC (1994) requirement based on both per gram egg and per dozen eggs, in comparison with that of hens fed either the 160 g/kg CP diet alone or the 130 g/kg diet supplemented with individual amino acids.

Ingram *et al.* (1951) were the first to show that methionine is required for egg production, and they concluded that the methionine requirement of the laying hen was not more than 380 mg/kg of feed. Maximum rate of egg production was obtained with a level of 268 mg/kg methionine and 533 mg/kg TSAAs when corn and soybean meals served as the sources of protein. The requirement for maximum egg production and maximum egg weight parallels very closely the requirement for body weight gain. There is a significant linear response in egg production from increasing the level of methionine and sulphur amino acids in the diet, but, the daily intake that is necessary to support maximum egg production is slightly lower than the required amount of SAAs (methionine) for maximum egg weight and body weight (Harms *et al.*, 1966, 1968; Schutte and Van Weerden, 1978; Schutte *et al.*, 1983, 1984). Several experiments have indicated that the protein content of the diet strongly influences the effect of EAAs on egg production. The addition of methionine to a corn-soybean meal diet of low (140 g/kg) CP improved egg production, while no such effect was seen when the CP content was high (160 g/kg) (Carlson and Guenthner, 1969; Jensen *et al.*, 1974). But when corn-pea diet was used, the addition of methionine to the 160 g/kg CP diet both egg production and egg weight were improved (Jensen *et al.*, 1974). On the other hand, Harms *et al.* (1962) observed a marked increase in the rate of egg production with methionine supplementation to corn-soybean meal diets containing 147 g/kg, 157 g/kg or 167 g/kg CP. In general, however, if the diet is low in protein, EAA supplementation might support only good but not maximum performance (Johnson and Fisher, 1959; Novacek and Carlson, 1969; Fisher and Morris, 1970). The performance (egg production and egg weight) of hens, both young and old, fed a 130 g/kg or a 140 g/kg CP diet containing adequate levels of EAAs was found to be not as satisfactory as that of hens fed 160 g/kg protein (Morris and Gous, 1988; Calderon and Jensen, 1990; Jensen *et al.*, 1990).

Several attempts failed to achieve improved egg production when using diets of various CP levels when supplemented with a combination of amino acids. Thus, in a trial where birds were fed 130 g/kg or 160 g/kg CP diet, Penz and Jensen (1991) could not improve egg production by increasing the level of lysine, methionine or tryptophan individually or in combination. Also, Keshavarz and Jackson (1992) failed to get an increase in egg mass output when they supplemented diets containing 130 g/kg, 140 g/kg, and 150 g/kg protein with methionine, lysine, tryptophan, and isoleucine. The choice of the lowest level to supplement obviously has a bearing on the ability of hens to respond. For example, Summers *et al.* (1991), reported that hens fed a diet containing 17 g/kg CP with supplemental lysine, methionine, arginine, and tryptophan produced 11% more egg mass than hens fed a diet containing 10 g/kg CP.

In addition, investigations into the fortification of laying hen diets with methionine have shown increases in egg weight (Martin *et al.*, 1969; Calderon and Jensen 1990). Dietary methionine intakes of approximately 450 vs. 330 mg/day produced significant differences in egg weight without significantly affecting other production statistics (Carey *et al.*, 1991).

2.1.2.4 Effects on egg composition

Several experiments showed that methionine content of the diet affects egg composition. Hens were fed diets containing methionine well over the NRC (1994) recommendation of 326 mg/HD: levels of 507 and 556 mg/HD were fed by Martin *et al.* (1969), a level of 512 mg/HD was fed by Carey *et al.* (1991), and the same level (512 mg/HD) was fed by Shafer *et al.* (1996), and the increase of egg component weight (shell, albumen, yolk) was observed. Shafer *et al.* (1996) further noted that these findings indicated that the increase in egg size was due to an increase in both liquid components, i.e. the extra methionine increased the absolute amount of liquid mass produced but did not alter the proportion of albumen to yolk in the liquid. Further analyses also showed that hens fed the higher methionine level produced albumen and yolk with significantly greater solids content than those consuming 326 mg/HD (Gardener and Young, 1972; Shafer *et al.*, 1996). Additionally, Shafer *et al.* (1996, 1998) reported increases in albumen and yolk protein (CP) at higher methionine intakes. They therefore suggested that higher levels of methionine supplementation may influence egg component yield, solids, and CP content without adversely affecting egg production, weight, functionality, or mortality rate; moreover, that changes in total solids and CP can occur while egg size remains unchanged. Thus egg component composition can be manipulated by amino acids.

2.1.2.5 Effects of excess methionine

Adding methionine in excess levels to poultry diets can be the result of supplementation or feed-mixing errors. Although the possibility of this increases as methionine supplementation is becoming more common, very little has been reported on the effects of feeding excess methionine to chickens. Young chickens have been reported to suffer distinct negative effect (retardation in growth) at excesses greater than 10 g methionine/kg (Boorman and Fisher, 1966; Tamimie, 1967; Griminger and Fisher, 1968; Katz and Baker, 1975; Hafez *et al.*, 1978; Ekperigin and Vohra, 1981). Koelkebeck *et al.* (1991) conducted an experiment on high-producing laying hens in peak production. When feeding a 1% excess level of DL-methionine in a practical corn-soybean meal diet on short term (4 weeks) performance of the birds, feed intake, feed efficiency, hen-day egg production, egg weight and egg yield (grams of egg/HD) was not affected. They thus concluded that a considerable excess of methionine would not affect adversely the production performance of layers in peak production. Similarly, Schutte *et al.* (1983) using a low protein diet (140 g/kg CP), found no adverse effects on egg production, weight gain and food intake when methionine was added at 0.5 to 3.5 g/kg in excess of the requirement, however, they observed a tendency of declining feed efficiency. In addition, feed intake might be reduced as a result of excess methionine in the diet. Chee and Polin (1978) fed male chickens a low protein diet (130 g/kg CP) and an excess of 680 mg/kg methionine, and found a 17% reduction feed intake as compared to the amount of adequate methionine diet consumed.

For a better understanding of hens expressing an appetite for methionine, some aspects of diet selection and amino acid imbalance will be described next.

2.2 Evidence for diet selection

The basis of a specific appetite for various nutrients (e.g. methionine) is the ability of diet selection. Under natural conditions, animals face many foodstuffs, and as not all of these are balanced nutritionally, animals need to be able to select appropriate amounts of each food, in order to ingest an adequate diet. In the case of hens, Kempster (1916) and Rugg (1925) found evidence for diet selection. They observed that hens given a choice between foods could balance their own diets and produce more eggs than those fed a single food. Most experiments (e.g. by Funk, 1932; Graham, 1932; Forbes and Shariatmadari, 1994) demonstrate that broilers and laying hens are able to select an adequate diet from a choice of two foods which are individually inadequate (e.g. one food is higher in protein content than required and the other one is lower). However, Ahmed, 1984 (cited by Rose and Kyriazakis, 1991) showed that broilers selected a nutritionally balanced diet from as many as nine different foodstuffs. This diet provided nutrients in similar proportions to those normally recommended (NRC, 1994; see Table 2.3).

Similarly, when Banta (1932) gave Rhode Island Red yearling hens access to 13 feedstuffs, the birds did not eat at random but they selected a diet similar to the recommendation at that time, and their performance was satisfactory. The composition of the diet selected by the birds is shown in Table 2.4.

Table 2.3 Diet selection of broilers (6-9 weeks) given a choice of nine food stuffs.

Feeding stuff*	**[g/kg]**
Ground wheat	625.0
Ground barley	192.0
Maize oil (plus sawdust)	49.0
White-fish meal	117.0
Meat-and-bone meal	15.0
Soya-bean meal	9.0
Dicalcium phosphate	0.7
Salt	0.2
Vitamin and mineral premix	0.2

	Calculated composition	**NRC (1994)****
Metabolizable energy [MJ/kg]	12.5	13.4
Crude protein [g/kg]	17.7	18.0
Lysine [g/kg]	0.87	0.85
Methionine+cystine [g/kg]	0.61	0.60
Calcium [g/kg]	1.05	0.80
Phosphorus [g/kg]	0.80	0.50

*Values adapted from Ahmed (1984), cited by Rose and Kyriazakis, (1991).
**NRC (1994) recommendation.

Table 2.4 Diet selection of Rhode Island Red yearling hens from a choice of thirteen foodstuffs.

Feeding stuff	Percentage of total diet
Yellow corn meal	34.40
Whole wheat	19.92
Cracked yellow corn	15.58
Wheat bran	15.14
Standard wheat middlings	3.63
Oyster shell	3.21
Ground oats	3.01
Mica grit	1.97
Fish meal	1.37
Meat scraps	0.84
Dried skimmilk	0.79
Alfalfa leaf meal	0.07
Sodium chloride	0.05

Source: Banta (1932).

2.3 Prerequisites for diet selection

2.3.1. Sensory discrimination

In order for animals to differentiate between foods to make up an appropriate diet, sensory cues are very important. These can be, for instance, colour, smell, the taste or texture of the food. Birds rely primarily on their vision to identify foods, but they also use their sense of taste and "post-ingestional" factors, and, possibly, both olfaction and temperature when making the correct choice of food (Gentle, 1972). With the use of sensory cues it is possible to envisage a learned appetite for an essential nutrient (e.g. methionine), thus if the hens can be taught an appetite then this can be used in a choice-feeding situation to improve the balance between their nutrient requirements and intake.

2.3.1.1 Vision

In common with most birds, both young and mature chickens have an acute sense of vision, therefore the look of the food is a very strong signal for them (Kilham *et al.*, 1968). The most important parameters are colour, shape and size. It has been established that turkeys have an overall preference for green followed by red, yellow, blue and white in that order (Cooper, 1971). The literature on the colour preference of chickens agree in that they like natural, reddish colours. Hess and Gogel (1954) found that chickens prefer light-coloured foods, particularly pink, while Van Prooije (1978) concluded that chickens prefer yellow-white seed, followed by yellow, orange and finally orange-red. The red, red-blue and blue seeds were only chosen in exceptional circumstances (severe hunger). Kennedy (1980) also observed that adult chickens show a preference for red and natural coloured diets over black and green. Studies by Hess (1956) indicate that chickens actually have a bimodal preference for colour with one peak occurring in the orange of the spectrum and a second in the blue region. An explanation for what induces colour preference is offered by Kennedy (1980). He demonstrated that the colour of the food offered just after hatching determined the later colour preference of hatchling chicks. In addition, Hurnik *et al.* (1971) observed that the preferred food colour is not necessarily the preferred trough colour. The order of preference by adult White Leghorn hens for the feeder was red 29%, blue 27%, green 23% and yellow 21%, therefore red seems to be the most preferred feeder colour.

Responses to different patterns were also investigated (Hurnik *et al.*, 1971), and the highest food intake was observed with the most complex pattern (blue, green, yellow and red), with green/yellow next whereas yellow alone resulted the lowest intake.

Using transparent drinkers Wilcoxon *et al.* (1971) showed that colour can be a cue for drink as well. However, as chickens usually spend very little time in contact with the visual properties of water, taste of the drink seems to have a primary importance, although colour cues are also noticed and attended to (Gillette *et al.*, 1980).

Newly hatched chickens have an innate preference for round objects (Frantz, 1957). In addition, they also have an innate preference for solidity: they peck more at a solid hemisphere than to a flat disk, whether real or on a photograph (Dawkins, 1968). In contrast, the preference for size is learnt by experience. Hogan-Warburg and Hogan (1981) observed that young chicks given a mixture of feed and sand learn to ingest primarily feed but still ingest some sand. They suggest that an increase in feed ingestion is probably the result of an association between the visual-tactile-gustatory stimuli from the feed and the positive long-term effects of the feed ingestion. In addition, chickens' preference for feed particle size has also been demonstrated. Portella *et al.* (1988) noted that feed particles were selected by broilers according to size. When offered one large and one small corn seed, chickens selected the larger seed (Frantz, 1957; Schreck *et al.*, 1963; Dawkins, 1968; Van Prooije, 1978). Moreover, as chickens age their preferred particle size increases. When a mixture of particles of different sizes were offered to broilers, larger than 1.18 mm particles were selected by all ages of birds, while at 8 and 16 days old they favoured particles between 1.18 and 2.36 mm, and as they aged they preferred particles larger than 2.36 mm (Portella *et al.*, 1988). The importance of preference for size on food consumption was demonstrated by Schreck *et al.* (1963). Reducing the size of the feed granules were associated with decreased body weight and even with increasing mortality.

Thus it appears that colour is a property which may be particularly useful in feeding practice. For instance by using coloured food or feeder or cage, food consumption could be increased (Hurnik *et al.*, 1971, 1974), also trace amounts of nutrient supplementation can be associated with colour cue (Kutlu and Forbes, 1993). However, it has been indicated that visual cues are not always necessary. For example, when a calcium-deficient diet is offered with calcite, the cues are obvious. But when the choice is between two mash diets differing only in the presence or absence of calcium, the cues are more subtle. The addition of calcium carbonate results in a paler diet, probably with a different taste. But if white flour is added to the deficient diet to give the same visual aspect, birds still exhibit a significantly greater preference for the calcium-enriched diet (Hughes and Wood-Gush, 1971b).

2.3.1.2 Taste

The sense of taste helps animals to select among feeds, to choose that which is palatable and to avoid those that are unpalatable or toxic. It also encourages the ingestion of nutrients. It has been demonstrated that chickens have taste buds (Lindermaier and Kare, 1959; Saito, 1966; Gentle, 1971a), and that they have a good sense of taste (Kare *et al.*, 1957; Kare and Medway, 1959; Kare and Pick, 1960; Gentle, 1971a, 1972). The ability to taste, however, is not uniformly present in all chickens. Williamson (1964) found significant sex differences indicating a genetic difference in the ability of chicks to taste ferric chloride, and Gentle (1972) reported that some of them are 'taste blind'.

Taste plays a major role in the initial selection of feed and possibly in the motivation to eat (Gentle, 1971b). Therefore many flavours have been studied to improve feed consumption, weight gain and feed conversion (Berkhoudt, 1985). It has been shown (Jacobs and Scott, 1957; Williamson, 1964; Kare and Mason, 1986; Yang and Kare, 1968) that birds can differentiate between the taste qualities of sweet, salt, sour and bitter. They have very strong preferences for some flavours; e.g. they will not drink solutions of saccharin, salt or quinine (El Boushy and Van der Poel, 1994), but like citric acid (Balog and Millar, 1989). Interestingly, in common with most avian species tested, chickens do not avidly select sugar solutions, when fed on an energy-balanced diet (Jukes, 1938; Kare and Medwat, 1959; Kare and Pick, 1960; Kare and Rogers, 1976). It has also been shown that even an unpleasant flavour, such as lactate in the case of a calcium source, can assist chickens in making the appropriate choice. But if the diet containing calcium is made less palatable by the addition of quinine, the aversion is so strong that the diet will be rejected even if the bird is deficient in calcium (Hughes and Wood-Gush, 1971b).

Changes in taste preferences of chickens readily occur following experimental manipulation (Gentle, 1975). They also quickly become accustomed to aversive chemicals such as dimethyl anthraniline, and many times the natural concentration of bitter tasting substances in the food is required to depress food intake over long periods compared with the amount which is selected against when choice is given (Kare and Pick, 1960).

2.3.1.3 Olfaction

Smell takes place in the olfactory organ, which consists of the nostrils, the taste buds which lie in the olfactory epithelium, and the olfactory bulbs in the brain (Bang, 1971). That birds lack the behaviour of sniffing indicates that they need moving air to effect contact between odour stimuli and receptors. Many birds have a well developed olfactory system; pigeons, for instance, use olfactory cues for navigation over long distances (Kare and Mason, 1986). There is no direct evidence for chickens using this olfaction in food selection, but Tucker (1965) has shown by electrical recording from the olfactory nerves -innervating the nasal cavity of birds- that they respond to amyl acetate. The chicken therefore appears to have a functional olfactory system and it seems likely that it is used. In addition, it has been suggested that they may regulate their behaviour in response to olfactory factors (Jones and Gentle, 1985).

2.3.1.4 Texture of food

The texture of food partly means a visual effect, partly a factor in the palatability of the food. The effects of the shape of the food (round/solid/granulated) have been mentioned above (section 2.3.1.1). When the food is swallowed, the texture is sensed by the mouth/tongue. Forbes, (1995) proposed that texture is a dynamic feature, as foods give changing sensations during grinding and swallowing. Not only the texture of the food changes but also its temperature, and as metabolic processes already begin in the mouth, its taste and smell changes too. Hyde and Witherly (1993) propose that all these changes during a meal, or even a swallow, have a big impact on a food's palatability. Thus Forbes (1995) suggests that texture should be considered as an additional cue in characterising a food, in conjunction with its sight, smell and taste.

2.3.1.5 Experience and nutritional needs

The experience and nutritional needs of the animal can alter its natural preferences and thus food consumption. Research of the past years have made it clear that animals learn to associate the sensory properties of foods with the metabolic consequences of eating those foods. They are sensitive to a number of nutrients and can make appropriate choices, according to how they feel. Therefore, for instance, if a food is deficient or imbalanced for one or more essential nutrients, the animal is malnourished and feels ill. This influences how much it eats.

Colour has been shown to be a strong cue for learned aversions (e.g. Martin *et al.*, 1977) and preferences (e.g. Kutlu and Forbes, 1993) in birds. Although chickens prefer light-coloured foods, particularly pink, preferences for other colours can be induced simply by prior exposure to them (Hess and Gogel, 1954; Taylor *et al.*, 1969). Thus, for example, Capretta (1969) has managed to increase the birds' consumption of red-coloured food. Also, the innate preference of newly hatched chickens for round objects can be increased or decreased (Frantz, 1957).

Memories of grinding pressures and the number of swallows help to recall how much food to eat for satiety (Miller and Teastes, 1986). Memorable foods are more easily learned with regard to their eventual metabolic properties, compared with bland foods. Adding spices to foods enhances palatability, even if not at the first exposure, by making the food subsequently more identifiable.

Post-ingestional effects also add to the animal's experience in choosing food. Capretta (1961) found that preferences for different coloured foods could be altered by noxious stimulation of the crop. Flavours, though initially able to influence intake and preference, soon lose this ability (Balog and Millar, 1989) if the birds learn that there is no nutritional implication of the different flavours.

The nutritional state of the birds can also change the preference behaviour. Kare and Maller (1967) observed that although chickens do not naturally exhibit a marked preference for a sucrose solution, when fed on a diet low in energy their sucrose intake increased to balance the calorie intake. When a calorie-enriched diet was again given, the consumption of sucrose was not reduced.

The importance of experience and learning in the mechanism of choosing the appropriate diet by poultry is described in more detail in the next section (2.3.2), whereas the effects of nutritional needs of the birds are detailed in section 2.4.

2.3.2 The role of learning in diet selection by poultry

Birds quickly learn to associate the sensory properties of a food with the metabolic consequences of eating it. The fowl, for example, often initially rejects the unfamiliar feed by recognition. This is because the chicks of the domestic hen are not fed directly by the parents, therefore there is an elaborate system of innate behavioural patterns which protect the birds from ingesting noxious diets. However, new experiences or the influence of conspecifics subsequently modify these innate reflexes thus allowing the birds to exploit a variety of valuable feed sources. Therefore, not merely innate preferences/aversions, but also the bird's own experience and social factors play an important part when selecting from a choice of foods.

2.3.2.1 Prior experience

Only a limited number of experiments have been carried out to study the feeding behaviour of chickens on choice feeding, i.e. when they have the opportunity for diet selection. It is now understood that chickens are capable of rapidly modifying their feeding behaviour by experience. When their preferred grains were stuck to the floor, newly hatched feral, commercial layer and broiler chickens quickly learned to avoid them (Adret-Hausberger and Cumming, 1985).

However, previous observations (Dun, 1977) showed that introducing choice feeding to laying birds previously given complete foods causes a 5% decrease in rate of lay over the next four weeks. Also, the sudden change from one feeding system to another largely reduced the birds' feed intake and growth performance (Scholtyssek, 1982). These, and additional observations (Kennedy, 1980; Mastika and Cumming, 1987; Covasa and Forbes, 1993b) have made it clear that the characteristics of the previous diet affect feed intake and performance of choice-fed birds. Therefore, prior experience is very important for birds on choice feeding (Cumming, 1987), and it is necessary for birds to have been given the opportunity to learn the difference between the two (or more) feeds on offer and hence to learn their nutritional characteristics. Mastika and Cumming (1981) noted that once imprinted, chickens can be introduced to choice feeding at any age. This observation implies that imprinted chickens have an effective memory for food type. It appears that for chickens the optimum age for imprinting is the second week after hatching (Covasa and Forbes, 1993a). Cumming (1987) noted that, whatever the age of introduction to the whole grain, chickens need a learning period of at least seven to ten days.

In summary, training the birds by accustoming them to whole grains at an early age improves their ability to select foods to meet nutrient requirements at later stages of growth.

2.3.2.2 Training

In many cases birds will learn about two foods if they are introduced simultaneously but they may learn more quickly if each food is given in turn for a few days. During the learning period, an alternating method can be used if the birds are to distinguish between, for instance, the properties of different types of mash (Shariatmadari and Forbes, 1993). However, Forbes and Covasa (1995) noted that the same method in case of choice feeding and the use of whole grains is not useful because, although there are obvious visual differences between the foods offered, the digestive tract of birds fed whole grains has to adapt and it undergoes physical changes in order to facilitate digestion. Moreover, the bird can avoid eating grain by learning when to eat in relation to the time of day (Pinchasov *et al.*, 1985) and wait until the normal food is on offer (Rose *et al.*, 1994). In addition, Covasa and Forbes (1994b) reported that choice-fed birds exhibit better dietary selection than those fed alternately.

2.3.2.3 Social interactions

Animals living together in a group tend to copy from each other and they are more likely to learn about foods when they are in groups than in individual cages. There is also usually a leader which guides the others to the desired food. To peck at food, newly hatched chickens stimulated by the sight and sound of the hen pecking (Savory *et al.*, 1978), i.e. social facilitation plays an important role in the initiation of pecking (Strobel and McDonald, 1974). Also, visual cues are important in the synchronisation of feeding in individually caged birds (Hughes, 1971). Joshua and Mueller (1979) found that within five days of being given a choice between a calcium-deficient food and calcite, broilers consumed enough calcium when kept in groups, however, individual caging inhibited this ability even when there was visual contact between the birds. When the birds were then caged individually after learning to eat calcium in a group, they took an adequate amount of calcium. Similar observations were made by Covasa and Forbes (1994c) who compared wheat consumption of pairs of birds to that of single-caged birds, and found a significant improvement despite the fact that individually caged birds could see each other.

It has been suggested (Mastika, 1987) that for the best result in selection birds need to be in groups of at least eight. A larger number of birds seem to make no further difference in diet selection (Rose *et al.*, 1986). It is now commonly accepted that group-housed animals are more successful in selecting a diet which meets their requirements than those caged singly (McDonald *et al.*, 1963; Adret-Hausberger and Cumming, 1987).

As learning is influenced by the presence and behaviour of conspecifics (Nicol and Pope, 1993), it seems likely that the process of learning could be accelerated by using experienced birds as 'teachers' (Mastika and Cumming, 1987). However, Covasa and Forbes, (1994a) demonstrated that simply putting birds together encourages wheat intake, therefore it is not necessary to use experienced birds as teachers.

2.4 Specific appetite

In order to demonstrate a specific appetite for an individual nutrient, rather than a particular type of food, deficiency of that nutrient is induced in the test animals and they are placed in a two-choice situation, with one food supplemented with the nutrient in question, whereas the other is not. A significant preference for the supplemented food demonstrates a specific appetite for the nutrient. As energy and proteins are the most important diet components in commercial practice, most investigations in choice feeding have focused on feeds with protein contents higher or lower than that required for optimum performance. Also, protein is expensive, therefore it is important to optimise its dietary concentration. However, there are also other targets of the birds' selection, namely: minerals and vitamins. Some of these appetites may be innate but mostly they are learned. When deprived birds learn to increase their intake of a supplemented diet, they are probably responding to the effect of the nutrient in producing a generalised improvement in their metabolism. Fowls also appear able to select an appropriate diet without ever experiencing a severe deficiency - in such cases they must learn to respond to more subtle internal signals (Hughes, 1979).

In order to develop an appetite for a nutrient it is necessary for animals to be able to differentiate between foods with different nutrient compositions by sensory cues, and they need to be taught to associate these sensory properties with the metabolic consequences of eating the food. Therefore, if the nutrient in question is only required in trace amounts, and especially if it is colourless (e.g. in the case of individual amino acids) it is necessary to give a cue by means, for example, of artificial flavouring or colour. Murphy and Pearcy (1993) reported, for instance, that sparrows which were offered foods deficient in valine and lysine or threonine and lysine, did not select a balanced diet. It may well be that there were no obvious differences in appearance or taste between the foods.

2.4.1 Specific appetite for minerals

2.4.1.1 Calcium

A specific appetite for calcium was first demonstrated by Wood-Gush and Kare (1966). Chickens discriminated in favour of calcium-rich diet against a calcium-deficient food when the choice was given after 21 days of deprivation. In addition, the form in which calcium is presented is also important; calcium-lactate is well accepted in a mash but rejected in solution (Wood-Gush and Kare, 1966). Experiments by Hughes and Wood-Gush (1971b) showed that calcium carbonate is the most suitable source of calcium (in comparison with calcium acetate and calcium borogluconate).

Laying hens need only 0.5 hour to develop appetite for calcium suggesting that it is innate in chickens (Hughes, 1979). On the other hand, there are several observations suggest that, the preference for calcium must be learned. Growing chickens need a longer time, two to four days, to develop calcium-appetite (Hughes and Wood-Gush, 1971b, 1972). Holcombe *et al.* (1975) had also found that growing pullets self-regulated their calcium intake well. When they switched containers between left and right, the birds gradually realised this and changed their selection appropriately within about three days. The fact that this change was not immediate suggested that the birds were using positional cues which they had learnt to associate with the calcium levels of the two foods. Joshua (1976) also observed a group effect: birds deprived of calcium and without visual or physical contact with their congeners do not develop a preference for calcium. However, if they have learned to consume the calcium supplement whilst in a group, they retain the ability to adjust the calcium intake when they are isolated. In addition, that most calcium salts are insoluble, and, therefore have little taste, also suggests that the preference for calcium is learned.

The reinforcement which leads to preference for a calcium-rich diet is likely to be that its ingestion gives a feeling of well-being which becomes associated with that particular food.

2.4.1.2 Phosphorus

The literature on appetite for phosphorus is controversial. Holcombe *et al.* (1976a) showed that laying hens would reject a diet of either low (1.9 g/kg) or high (24.3 g/kg) phosphorus content in favour of diets of intermediate (4.6 g/kg or 10.0 g/kg) contents. They also found that raising the dietary calcium content from 30 g/kg to 60 g/kg increased the proportion of high-phosphorus diet selected from 20% to 73%. This suggests a homeostatic choice by the hens, and that factors (such as increasing the dietary Ca:P ratio) which tend to ameliorate the adverse effects of a high-phosphorus diet, cause the hen to relax the stringency of her selection against phosphorus. There was also some evidence of a diurnal rhythm, in that birds choosing between diets of 1.9 and 24.3 g phosphorus/kg showed their greatest preference for phosphorus around the middle of the photoperiod. In contrast, Shannon *et al.* (unpublished, cited by Hughes, 1979) found no evidence of regulation of phosphorus intake in laying hens. The birds were offered a choice between a deficient diet and a control diet and consumed almost equal quantities of each. It was suggested (Hughes, 1979) that differences in the source of phosphorus might be involved in the difference between these findings.

2.4.1.3 Sodium

Hughes and Whitehead (unpublished, cited by Hughes, 1979) and Hughes *et al.* (unpublished, cited by Hughes, 1979) found a weak appetite for sodium in laying hens, but it was not enough to prevent a reduction in egg production. Others (Hughes and Wood-Gush, 1971a) failed to increase the preference for a diet of high sodium content in deprived birds. Instead, they found that a high sodium chloride content is aversive for the birds. These studies show that even when the sodium status of a fowl is very low, it is difficult to demonstrate that it positively selects sodium chloride.

2.4.1.4 Zinc

The specific appetite for zinc was reported by Hughes and Dewar (1971). They observed that zinc-depleted birds showed an immediate preference for zinc-supplemented diet (containing 65 mg/kg), and within a week they consumed it at greater than twice the level of the low zinc diet (containing 5 mg/kg). Meanwhile, control birds ate about 40% of the zinc rich food but became marginally deficient. This was gradually overcome by eating more of the supplemented diet. The immediate preference for the supplemented diet shown by the deprived birds was suggested to represent an aversion to the deficient diet which developed during the period of deprivation.

2.4.2 Specific appetite for vitamins

2.4.2.1 Vitamin A

There is very little evidence for vitamin A appetite in chickens. One experiment (Price, 1929) found that chicks select butter high in vitamin A in preference to that low in vitamin A. However, another (Jukes, 1938) showed that the birds do not consume alfalfa meal, which contains vitamin A but has a bitter flavour.

2.4.2.2 Vitamin B_1 (Thiamine)

One study has been published on thiamine selection (Hughes and Wood-Gush, 1971a). The workers induced deprivation by intramuscular injections of oxythiamine hydrochloride, a metabolic antagonist of thiamine. When thiamine-deficient and supplemented foods were offered, the deprived group's intake of supplemented diet was consistently higher than that of the control group's, demonstrating a specific appetite for thiamine in the chicken.

2.4.2.3 Vitamin B_6

A specific appetite for vitamin B_6 in broilers was reported by Steinruck *et al.* (1991). The birds first ate too little of the B_6-sufficient diet when given a choice of sufficient and deficient foods, and showed signs of deficiency of the vitamin. They then increased their relative intake of the supplemented food to re-establish normal growth and continued to eat proportions of the two foods which provided them with a balanced diet. Thus, it has been demonstrated that specific appetite for vitamin B_6 is based on the learned consequences of eating the two foods.

2.4.2.4 Vitamin C (Ascorbic acid)

It has been generally assumed that chickens can synthesise sufficient ascorbic acid and that dietary supplementation was unnecessary. However, the amount of ascorbic acid might be inadequate in situations of stress such as high environmental temperature. Using colour cues, Kutlu and Forbes (1993) trained young chicks to differentiate a supplemented food from an unsupplemented one, and showed that chicks express their desire for an intake of ascorbic acid appropriate to their needs. If the two foods were offered without colour, the birds ate at random. Thus, they demonstrated that the different requirements for ascorbic acids, under temperate and hot conditions, would be expressed as an appetite for ascorbic acid.

2.4.3 Specific appetite for proteins

In order to demonstrate specific appetite for proteins, a choice between diets of higher and lower than the required (NRC, 1994) level are used. An appetite for protein has been demonstrated both in growing chickens and layers.

2.4.3.1 Appetite for protein in growing chicks

A clear demonstration of an appetite for protein was provided by Tobin and Boorman (unpublished, cited by Boorman, 1979). Young chicks consumed more of a carefully balanced low protein content diet (10%) than of a similarly balanced high protein diet (20%), even though the ingredients of the two diets were similar. Growth rates were such that the demand for food should have been less in birds receiving the low-protein diet. The authors reasoned that, by the compensation in intake, the chicks receiving the low-protein diet attempted to increase their protein intake. In addition, Kaufman *et al.* (1978) noted that when young chicks had free access to high-protein and low-protein diets they selected proportions which gave the same growth as controls but with a lower protein content in the selected diet. Similarly, using single foods of five different levels of protein, Shariatmadari and Forbes (1993) demonstrated that both growing layers and broilers can approximate their protein requirements by taking appropriate amounts of high-protein and low-protein diets. They have also found that the birds selected a protein content somewhat lower than that which gives maximum growth, thus it is possible that they may utilise the protein more efficiently when choice-fed. When pairs of diets differing in protein content were offered to broilers from four to nine weeks of age, where possible, they chose proportions of the two to give themselves a diet containing 180-220 g protein/kg (Shariatmadari and Forbes, 1993). When both diets had protein contents below this range the birds ate predominantly from the higher one, and when both foods had protein contents higher than this range they ate mostly from the lower. Thus, they were selecting a diet close to the optimum while still tasting the more extreme food from time to time to ensure that it was still unsuitable. When high-protein and low-protein diets were offered in alternate periods, broilers still selected a diet of about 180 g protein/kg (Shariatmadari and Forbes 1991).

2.4.3.2 Appetite for protein in laying hens

There is a marked increase in protein demand, and thus intake in choice-feeding situations, of growing pullets at about two weeks before the onset of lay, i.e. at the time of the rapid development of the ovaries and oviduct (Scott and Balnave, 1989). However, once in adult age, the requirement of layers for protein is less than that of growing chicks and broilers (NRC, 1994). Nevertheless, Holcombe *et al.* (1976b) demonstrated that layers are also capable of choosing an appropriate protein intake. However, when birds had to choose between two foods which both had a protein content lower than that required, the initial increase in preference for the higher protein food was followed by random choice. In addition, their egg production decreased from 51 to 21 eggs /100 HD although, despite the random food selection, the birds should have had no difficulty in maintaining half the rate of egg production. Reviewing these results, Hughes (1979) suggested three possible reasons. Firstly, a flock laying at half-maximal production is composed of many individuals laying at rates which may vary from 0 to maximal production. A low protein intake would have little effect on hens laying at less than half-rate, but would have a rapid deleterious effect on those laying well. Secondly, it is not clear whether a bird needs a higher protein intake on egg-forming days, or whether protein can be stored on a preceding non-egg-forming day for later use. Finally, it is likely that the diets were inadequate in some other respect, possibly an imbalance in essential amino acids. Hughes (1979) also offered an explanation to why the birds stopped selecting for protein and reverted to a random choice. The suggestion is that because homeostasis was not achieved, they received insufficient beneficial feedback, and therefore their adaptive behaviour was not adequately reinforced or rewarded. Gradually, therefore, initial response of selecting the diet higher in protein waned, and they reverted to random choice.

Steinruck and Kirchgessner (1992) also attempted to demonstrate the ability of laying hens to select a protein intake corresponding to their requirements for optimal egg production. Pairs of foods containing 80/170, 110/170, 80/230 or 110/230 g protein/kg feed were offered as choices to laying hens. There were no differences in egg output between any of the choice-fed groups compared with those on single foods of 170 or 230 g CP/kg, nor any significant differences in body weight gain. The dietary protein concentrations selected on the four choice treatments were 153, 152, 188 and 179 g/kg respectively. The authors noted that the birds' protein selection was not very accurate.

Protein selection has also been investigated by Savory (unpublished, cited by Boorman, 1979). He used two groups of laying hens: one was fed on a low-protein (70 g/kg) diet for 2 days before selection began, while the other group received an adequate diet (170 g/kg) up to the start of dietary choice. Both groups were then offered a choice between two diets, one containing 100 g protein/kg and another 140 g/kg. During the selection period the two groups behaved similarly. Initially the intake of the two diets was approximately equal, then gradually over a period of days the proportion of the 140 g/kg protein diet eaten increased, until after about ten days both groups were taking about 65% of their intake from this feed (140 g/kg protein). Then the proportion became more or less constant. It was also observed that the pre-deprived group did not show a more marked preference than the controls, indeed, if anything, the contrary was the case.

2.4.4 Specific appetite for amino acids

In a choice feeding situation, chicks fed an unsupplemented low (10%) and high (60%)-protein diet were reported to over-consume protein (40%) (Elkin *et al.*, 1985). However, the precise nature of the deficiency was uncertain, i.e. it is not known which amino acid is limiting, or even whether the diet is deficient in several. Therefore, the over consumption of protein observed in their unsupplemented group was attributed to the attempt made by birds to compensate for the deficiency in amino acids, and the authors suggested that regulation of protein intake in choice feeding situation is only possible when the diet offered has adequate sulphur amino acids.

It has been shown (Gous and DuPreez, 1975) that short periods of amino acid imbalance can be compensated for by growing birds. Layer strain cockerels were given, in alternating periods of 6 or 12 hours, two foods which were individually poorly balanced but complementary in their amino acid composition. There were no significant differences in food intake or weight gain, either between the two alternating treatments or compared with controls given the two foods mixed together. However, it is known that, over a longer period, diets which are imbalanced in the amino acids absorbed from the digestive tract, lead to metabolic disturbances and a reduction in food intake which is directly proportional to the degree of amino acid deficiency or imbalance (Harper *et al.*, 1970; Boorman, 1979). The effect on intake may be due to the metabolic cost of deaminating the excess of those amino acids which cannot be utilised because of the deficiency, relative or absolute, of others (for details see section 2.5.1). Sparrows even preferred a protein-free food to one with a severe imbalance of amino acids, even though the total protein content of the latter was quite high (Murphy and King, 1989).

The few investigations made on feed preferences of chickens involving specific amino acids (e.g. Newman and Sands, 1983; Edmonds and Baker, 1987; Roth *et al.*, 1990; Steinruck *et al.*, 1990a,b) have demonstrated that growing chicks and laying hens select a diet supplemented with the amino acid when offered a choice. Most of these studies involved lysine and methionine, as these are the two amino acids most likely to be limiting in poultry feeds.

Picard *et al.* (1993) found that both in choice and non-choice situations, young broiler chickens discriminated between a balanced diet and one deficient in lysine, methionine, and tryptophan. They also observed that the intensity of the animals' response was modified by age, prior experience, genetic line and the type of amino acid. Responses were reported to be slower for older birds and faster with experienced animals (Picard *et al.*, 1993). As genetic stocks are known to differ in food intake (Boa-Amponsem *et al.*, 1991), a genetic variance in discriminating feeding behaviour was also expected (Picard *et al.*, 1993; Noble *et al.*, 1993a). Indeed, after a period of deficiency in dietary methionine, lysine and tryptophan, a White Plymouth Rock line but not a White Leghorn line was observed to discriminate between a balanced diet and diet deficient in one specific amino acid (Noble *et al.*, 1993a). The only indication of dietary deficiencies in the slower growing White Leghorn line was reduced food consumption when fed diets moderately deficient in lysine and tryptophan. Reduced growth was most evident for a lysine deficiency with an effect on diet preference present at the greater deficiency. In contrast, methionine deficiencies influenced dietary preferences with growth reductions only at the greater deficiency. Tryptophan deficiencies had no effect on growth. It was suggested by the authors that these chicks failed to discriminate between the diets because the amino acid content of the so-called deficient feeds was adequate for them. Earlier reports also indicate that, compared to White Rock laying hens (Cherry and Siegel, 1981), light hybrid laying hens fed diets deficient in methionine needed a longer period before they chose the diet higher in methionine (Roth *et al.*, 1990). A second experiment on the same genetic lines by Noble *et al.*(1993b) showed that there is consistency within and among genetic stocks in exhibiting preferences for diets differing in methionine content. The results also indicated that chicks may identify a preferred diet and its location.

In addition, the degree of deficiency or imbalance of an amino acid may result in variable reactions. Whereas most major deficiencies depress food intake (Boorman, 1979), marginal deficiencies of amino acids such as methionine can stimulate an increase in energy intake (Boorman, 1979; Cherry and Siegel, 1981). Also, major excesses of some essential amino acids in the diet are irregularly rejected by chicks when offered a choice (Edmonds and Baker, 1987).

2.4.4.1 Appetite for lysine

Shannon *et al.* (unpublished, cited by Hughes, 1979) formulated diets deficient in only one amino acid and containing adequate quantities of all other essential ones. In the case of lysine deficiency, normal egg output was sustained even when only the deficient diet was offered. Apparently, this diet contained about adequate amounts of lysine, so this part of the experiment provided no information as to the hen's ability to select lysine. However, it was clear that the birds did not discriminate against the higher lysine diet.

Newman and Sands (1983) demonstrated that broiler chicks could select a diet adequate in L-lysine in preference to low-lysine, lysine-free food, or one that contains D-lysine, which is not metabolised and thus nutritionally worthless. However, they still chose a D-lysine diet rather than a lysine-free one, indicating that the D-form might trigger a receptor mechanism even though it is biologically unavailable. Although selection for lysine has occurred in all feeding regimes, it was not enough to maintain a growth rate as high as the control group which were given a single adequate food. Hughes (1984), and Forbes and Shariatmadari (1994) therefore postulated that there is some evidence for an innate sensory recognition for lysine, and the selection is supported by post-ingestional feedback, however this nutritional wisdom is not sufficient to permit the bird to consume a properly balanced diet.

2.4.4.2 Appetite for methionine

Broilers given a choice between a complete food and one with half the recommended methionine chose predominantly the former, especially after they had been made methionine-deficient by prior feeding on the low-methionine food (Steinruck *et al.*, 1990a). In a separate experiment, it has also been demonstrated that birds learn the difference between the two foods primarily by their positions in the cage (Steinruck *et al.*, 1990b). Moving the positions confuse them and necessitates their re-learning the relationship between position and methionine content whenever the position of the foods was changed.

It has been recognised that during productive processes, such as egg laying or moult, requirements for amino acids become particularly high. Murphy and King (1987) evaluated the ability of moulting sparrows to discriminate between diets differing only in SAA concentration, and found that birds selected an adequate diet on the basis of SAA adequacy. Deficiency caused reduced body mass, longer moulting and many malformed feathers. The tests also indicated that the birds could sense the diet quality; they responded to an altered diet location after one day of feeding.

Shannon *et al.* (unpublished, cited Hughes, 1979), experimenting with laying hens, found that a methionine deficiency caused a pronounced decrease in egg output. When offered a choice between deficient and supplemented diets, the hens were showing a positive discrimination for methionine, although it was not pronounced enough to result in a methionine intake sufficient to allow maximum egg production. In support, Hughes (1979) reported that layers select for methionine, but it is not enough to prevent a decline in egg production.

2.5 Amino acid imbalance

A food is said to be imbalanced (Harper, 1964) if the essential amino acids in it are available in a ratio which is widely different from the animals' requirements, thus bringing on depressions in food intake and growth/egg production, which can be completely alleviated by supplementation with the first-limiting amino acid (or by infusion of this amino acid into the digestive tract or circulation). These effects of the imbalanced diet are comparable to those of a low protein food (Forbes and Shariatmadari 1994). Abnormal feeding behaviour following amino acid imbalance has been observed in both mammals and avians. When offered a choice, rats consumed a balanced diet in preference to an imbalanced one, but more remarkably, in such situations they selected a protein-free diet incapable of supporting growth instead of an imbalanced diet which allows growth, albeit at a low level (Sanahuja and Harper, 1962; Leung and Rogers, 1987). Sparrows also preferred a protein-free food to one with a severe imbalance of amino acids, even though the total protein content of the latter was quite high (Murphy and King, 1989).

2.5.1 Regulatory mechanisms

Association between changes in the tissue patterns of amino acids and food intake depression has been demonstrated in mammals (Harper and Rogers, 1965; see Figure 2.1.) as well as in poultry (Boorman, 1979). After the consumption of an imbalanced diet, surplus amino acids arriving in the portal circulation stimulates synthesis or suppresses breakdown of protein in the liver, and this leads to an increased retention of the limiting amino acid. Therefore, peripheral tissues such as muscle are supplied with a reduced amount of the limiting amino acid, although protein synthesis in these tissues continues unimpeded. Eventually, however, in both muscle and plasma, concentrations of the limiting amino acid decline whereas there is an accumulation of those amino acids added to precipitate the imbalance. Thus their free amino acid patterns become so deranged that an appetite-regulating system intervenes to reduce food intake. Growth is reduced as a consequence of the depressed appetite and the intake of nutrients.

Figure 2.1. Effects of amino acid imbalance in the rat.*

Liver

Surplus amino acids stimulate synthesis or suppress breakdown of proteins; efficient utilisation of limiting amino acids

↓

Muscle

Protein synthesis continues normally; greater retention of limiting amino acid; deranged free amino acid pattern

↓

Plasma

Deranged free amino acid pattern

↓

Brain

Abnormal pattern monitored by appetite-regulating regions

↓

Whole animal

Depressed food intake

↓

Reduced nutrient intake

↓

Reduced growth

* Based on the hypothesis of Harper and Rogers (1965).

Thus, it is clear that the primary effect of an imbalanced diet is on voluntary food intake with a secondary effect on growth. In poultry, the feed intake control mechanisms operate in a manner similar to those in mammals (e.g. Wilson, 1949; Hill and Dansky, 1954; Sharma *et al.*, 1961; Jensen *et al.*, 1962; Fisher and Shapiro, 1966; Morris and Taylor, 1967; Lepkovsky *et al.*, 1968; Gleaves *et al.*, 1968, 1977; Duncan *et al.*, 1970; Hughes, 1971, 1972; Kuenzel, 1972; Tweeton *et al.*, 1973; Miller and Sunde, 1975), i.e. by the hypothalamic region of the brain (e.g. Cannon and Washburn, 1912; Bailey *et al.*, 1921; Brobeck, 1946, 1948; Grossman *et al.*, 1947; Anand and Brobeck, 1951; Kennedy, 1952; Mayer, 1953; Mellinkoff *et al.*, 1956; Anand, 1961; Lepkovsky and Yasuda, 1966), which responds to various regulatory mechanisms such as the glucostatic, the thermostatic and the lipostatic mechanism, the distention of the gastrointestinal tract, behavioural influences, circulating amino acids and protein intake. Thus, increasing food intake by force-feeding (Leung *et al.*, 1964), insulin injections (Kumta and Harper, 1962), adjustment of dietary protein to energy ratios (Fisher and Shapiro, 1961) or by exposure to cold environmental temperatures (Klain *et al.*, 1962), had improved growth. Also, a depression in appetite was prevented in rats (Leung and Rogers, 1969) and in cockerels (Tobin and Boorman, 1979) fed on an imbalanced diet when they received an infusion of a small quantity of the first limiting amino acid via the carotid artery, while administration through the jugular vein, was ineffective. These observations indicated that the pattern of amino acids passing to the brain cause the animal to respond as if the dietary amino acid pattern was balanced, i.e. to increase its food intake. For more comprehensive reviews on feed intake control in avians, see

Boorman and Freeman (1979), Denbow (1985, 1994), Barbato (1994) Kuenzel (1994), Forbes (1995), and Savory (1999).

Although the hypothalamus was the candidate for being the regulating centre, specific investigations into its role in responses in food intake to various alterations of dietary amino acid patterns showed that mediation of these responses did not involve the satiety centre in the ventromedial hypothalamus (Leung and Rogers, 1970; Scharrer *et al.*, 1970). Further research in rats defined those areas of the central nervous system which are directly sensitive to amino acid imbalance, and it appears that specific neural sites are associated with the depression in food intake (Leung and Rogers, 1969). These include the anterior prepyriform cortex, the medial amygdala of the brain and certain regions of the hippocampus and septum (Leung and Rogers, 1971, 1973, 1987; Rogers and Leung, 1973). These areas are connected to the lateral hypothalamus enclosing a centre that is active in feeding responses; hence there are such pathways by which an inhibitory effect on feeding could occur (Leung and Rogers, 1987).

That reduction of feed intake occurs within a few hours of ingestion imbalanced diets (e.g. within three to six hours in rats (Harper and Rogers, 1965)) implied that changes in plasma amino acid patterns may provide the metabolic signal which ultimately results in anorexia and abnormal feeding behaviour. It has been demonstrated (e.g. Peng *et al.*, 1972) that the concentration of first limiting amino acid declines more rapidly in cerebral tissues than in plasma, and it is now clear that the fall in the brain concentration of the limiting amino acid initiates the signal which causes the changes in food intake and dietary choice (Leung and Rogers, 1987). Although the precise mechanism is still not fully understood, it has been revealed (Gietzen *et al.*, 1986; Beverly *et al.*, 1990a,b, 1991a) that the presence of the limiting amino acid in the prepyriform cortex elicits separate effects on dietary selection and on intake of an imbalanced diet, depending upon dose level. Infusion of the dietary limiting factor directly in the prepyriform cortex of rats influenced dietary selection and intake separately (Beverly *et al.*, 1991b). By isolating the same area of the brain in chicks, Firman and Kuenzel (1988) observed that capability to select against a tryptophan-deficient diet fed free choice was lost.

The monitoring process of blood amino acids has not yet been explained, but the existence of specific sensors for individual amino acids seems unlikely (Boorman, 1979). There are, however, a number of candidates for the sensory mechanism (as listed by Boorman, 1979). Namely, that

1) impaired synthesis of a protein critical in a neural pathway causes interference with the transport of the limiting amino acid into the sensitive area of the brain (Harper *et al.*, (1970), or

2) impaired synthesis of an amino acid into the brain causes impaired synthesis of its metabolite, a neurotransmitter (Wurtman and Fernstrom, 1975), or

3) ammonia, a common metabolite arising from amino acids would have some role in the responses in food intake to amino acids (Russek, 1971; Simson and Booth, 1973; Noda and Chikamori, 1976), or

4) insulin might be required for entry of nutrients into sensitive monitoring areas (Stevenson, 1969).

Furthermore, specific observations on monitoring systems of rat (Leung and Rogers, 1971) and chicken (Firman and Kuenzel, 1988), in relation to choice of diets suggest that it is possible that the prepyriform cortex monitors amino acid levels, or that its separation disrupted the sense of smell (it is part of the olfactory system), or both of these.

Summarising the behavioural aspects of feeding, Boorman (1979) noted that although the monitoring of the signals arising from the dietary protein and the following adjustment to feeding may be initially fully autonomic, the repetition of this cycle of events *in toto* is unnecessary once it has occurred once or a few times, because the animal will respond virtually immediately to that diet in the same way on subsequent encounters. Feeding from a nutritionally beneficial diet will reinforce the animal's initial reaction. As also shown by choice experiments, these types of responses allow animals to choose proportions of alternative diets or foodstuffs such that the mixture chosen approximates to the best that can be obtained from the available alternatives (Summers and Leeson, 1979). However, the situation is different when only a single diet is provided. Then, the diet must be eaten and, therefore, re-tested for nutritional quality to a greater degree.

In summary, the feeding response of animals is the result of the re-testing of diets and the re-dressing of the associations between sensory and nutritional characteristics as circumstances change; it "arises from a constant re-adjustment of behavioural responses acquired to sensory characteristics in previous encounters with the diet and current physiological responses due to its nutritional characteristics" (Boorman, 1979).

2.6 Practical implications of diet selection

The practical importance of the ability to select an adequate diet from a choice of two or more foods which are individually inadequate is, that if a single food is available for the hens, their intake is determined predominantly by its energy content, however, by offering two (or more) foods, the intake of two (or more) nutrients can be controlled independently (Kaufman *et al.*, 1978; Elkin *et al.*, 1985).

2.6.1 The composition of feed

Because of their price, whole grains are popular ingredients, and they are also of a suitable size for poultry to eat. Feeding studies using wheat, barley, sorghum, pelleted oats, cracked corn, corticated cotton seed meal, millet, paddy rice (Forbes and Covasa, 1995) suggest that certain cereals are more acceptable for poultry than others, depending on the combination of form of presentation, palatability and metabolic consequences. Broilers, for instance, eat more sorghum than wheat when both are on offer (Cumming, 1983). Moreover, studies by Rose *et al.* (1986), Amar-Sahbi (1987) showed that birds prefer whole grain or pellet to mash, presumably because they stimulate gizzard activity (Hijikuro and Takewasa, 1981) thus increasing the ability to grind food and ease digestion. The types and form of cereals eaten, however, have very little or no effect on the birds' ability to compose a suitable diet and on their production performance. Performance of chickens given a choice between concentrate pellets and sorghum grains was about equal to that of pellet-fed controls, but the choice-fed birds ate less protein and converted it more efficiently (Matsika and Cumming, 1981).

As cereals are low in protein and have an imbalanced amino acid composition, they must be supplemented with high-protein pellet. In practice, the concentrate is made by removing the cereal from a conventional formulation with added premix and calcium. As standard commercial foods over-provide the great majority of the birds with protein, it is not necessary to formulate a special high protein food to balance the grain.

2.6.2 Performance of birds in relation to choice feeding systems

Although the pattern of their food selection might be different due to differences in nutrient requirements, the principles of the diet selection of broilers and layers is the same. Therefore offering a choice of feeds can be effective for both types of birds. Essentially there are three ways of providing choice diets under commercial conditions, namely: alternate (sequential, or split-time) feeding, semi-choice and choice feeding.

2.6.2.1 Alternate feeding

It is known that if one food is eaten to satiety and another, contrasting, food is then offered animals usually eat again, and varying the type of food given at certain times of the day has been practised with good results (e.g. Martin and Insko, 1929). However, if alternate feeding is to be used, the birds must learn when to eat in order to select a balanced diet. Also, as there is only limited storage for many nutrients in the animal's body, the length of the feeding periods is important. Experiments with broilers and layers (Gous and DuPreez, 1975; Rovee-Collier *et al.*, 1982; Shariatmadari and Forbes, 1991; Rose *et al.*, 1993; Covasa and Forbes, 1994c) suggest that provided the periods are not longer than half a day, alternate feeding can be an effective way of choice feeding with performance equal to that achieved with birds on a complete diet.

2.6.2.2 Semi-choice feeding and choice feeding

When semi-choice feeding is used, the foods are offered together but they are not mixed. They can be offered in separate troughs or layered one on top of another, for instance. In contrast, the feed can be also fully mixed when offered to the birds, in which case mixing takes place during the normal handling of the food through the augers, bins and feeders.

Lee *et al.* (1949) and Leeson and Summers (1983) reported reduced performance by laying hens with choice feeding. However, others (Cumming, 1984; Elwinger and Nilsson, 1984; Robinson, 1985) found that choice-fed layers performed at least as well as those fed conventionally, with higher egg weights, although there was a tendency for poorer shell strength and plumage with choice feeding (Al Bustany and Elwinger, 1988). In addition, Cumming (1984) observed that water consumption was lower in choice-fed layers, and droppings have tended to be dryer and to cone more readily under the cages. He suggested this was probably due to the better gizzard development in choice-fed birds.

The large-scale application of choice feeding of caged hens was studied by Tauson and Elwinger (1986) using two narrow flat-chain feeders, one distributing a mash concentrate, the other whole grain. In a semi-choice treatment, the mash was given as a layer on top of the grain which was provided *ad libitum.* Two experiments with over 5000 birds showed greater egg size with choice and semi-choice feeding than conventionally fed controls, with no difference in the number of eggs laid. Egg shell quality and cracks tended to be worse in the choice treatments. Profit margin was higher over the two production cycles for both choice-fed groups than control and these authors concluded that choice feeding from flat feeders is feasible but that further studies are necessary before similar systems are used in practice.

A choice of whole grain and crushed peas, and a concentrate was given to caged hens in a chain feeder and a mechanised device for feeding restricted amounts of concentrates by Tauson *et al.* (1991). Choice- or semi-choice-fed hens produced significantly heavier eggs than those on a conventional mash diet and which also ate more food. The authors concluded that choice feeding may be economically beneficial to farmers with access to inexpensive cereals and peas.

Given a choice of pellets and wheat, turkey hens ate 46% from wheat whereas males chose only 38% (McDonald and Emmans, 1980). There was no significant effect on body weight, but the early maturing choice-fed turkey hens produced significantly fewer eggs; however, this was redressed to a large extent later in the laying period. Egg weights and hatchability were marginally lower on choice feeding. Emmerson *et al.* (1991) reported reduced broodines but increased fertility and hatchability of choice-fed turkeys. Moreover, they consumed protein considerably below NRC (1984) recommendations.

In the case of broilers, choice feeding trials were not very promising, however, large scale applications of choice feeding gave good results. Summers and Lesson (1979) found that choice-fed birds performed less well than those on a normal dietary programme and deposited a large amount of fat, thus they concluded that broilers lacks the ability to select the nutrients for their needs. On the other hand, in Scandinavia and other European countries choice feeding of broilers is now a common practice. They feed a starter food containing 240 g protein/kg throughout and add increasing amounts of wheat, up to 40% as the broilers grow. Results in terms of growth rate and carcass quality are reported to be at least as good as with the commercial grower food fed on its own (Jensen, 1994).

In summary, under commercial conditions, choice-fed birds usually perform well although efficiency is sometimes reduced (Rose and Kyriazakis, 1991). Interest is growing especially in the combined use of compound feed and whole cereals. There is uncertainty about the optimum methods of training and feeding, however (Farrell *et al.*, 1989).

2.6.3 Advantages of choice feeding

Choice feeding is a flexible feeding technique because after a period of adjustment, choice-fed individual birds in a flock are able to select from various feed ingredients, and thus they can compose their own diet according to their actual needs and production capacity. Choice feeding is able to meet the wide variety of needs of individual birds within flocks of various types of stock under different climatic conditions, while having both practical and economic advantages (Cumming, 1984, 1994 -in Forbes and Covasa, 1995).

Its potential benefits have been summarised by Forbes (1995):

firstly, allowing the animals to make nutritionally wise choices between foods would cut the expenses and labour on determining and calculating nutrient requirements for use in food formulations;

secondly, separate sex feeding will be unnecessary, as within a mixed-sex flock the males and the females will be able to select different diets which reflect the different requirements of the sexes. There would be also compensation for individual birds and strains with different growth potentials;

thirdly, food changes will not be needed. Two foods, offered as a choice, could be used throughout the growing and finishing periods. In addition, nutrient undersupply (with consequent loss in output) or nutrient oversupply (with no resulting benefit but increased cost) will be avoided since the diet selected by each individual will precisely meet its requirements. Changes in environmental temperature will be accommodated by the animals without the need for re-formulation of the foods;

fourthly, excretion of nitrogenous and other waste will be reduced as individual animals select diets to meet their nutrient requirements. There is potential for a significant reduction in the pollutants generated by intensive animals units, and the birds are less susceptible to coccidosis;

and finally, choice diets are cheaper than conventional feed. If whole grain and high protein pellets are offered in free choice, expenses can be cut by not having to grind and pellet as much food, and because grain stores better whole, especially in hot, humid areas.

A disadvantage of the method, however, is the expense of installing a comprehensive, computer-monitored and -controlled complete housing and feeding system, such as the Flockman system (Filmer, 1991), for controlling the proportion of whole grain more accurately to mach the birds' potential and actual growth.

2.7 Objectives of the project

Methionine must be added to corn-soybean and wheat-soybean meal layer rations because it is the first limiting amino acid in these diets. Its delivery to conventional poultry diets via drinking water may offer many potential advantages in contrast to the current supplementation of constant amounts to the feed. Crucial to this method is the ability of the animals to express a specific appetite for this amino acid. The basis of developing an appetite for a nutrient is, however, the ability to differentiate between foods, to select a diet. Sensory discrimination and learning the associations between the properties and metabolic effects of the food are prerequisites of this ability.

For the experiments in this project adult laying hens had been chosen. A specific appetite for amino acids (lysine and methionine) exists in the domestic fowl, however, a specific appetite of layers for any amino acid in drinking water has not been investigated at all.

The central aim of this project was to assess the ability of laying hens to express an appetite for methionine in drinking water and to correct a methionine deficiency. A series of experiments have been carried out to investigate the delivery of methionine in drinking water for these birds, and its consequences in terms of the opportunity to improve efficiency of production. The following sequence of investigations was planned:

1) to determine the crude protein content of the diet that is sufficient to support a high egg production rate, and can be used in the subsequent experiments to create diets marginally deficient in methionine;

2) to determine the water intake of hens at high laying level, using one nipple per bird drinking system;

3) to investigate the expression of appetite for methionine in water with and without the aid of a sensory cue (colour);

4) to investigate whether the birds are able to regulate their methionine intake from water, i.e. if they would stop or reduce the intake of methionine-treated water once their methionine requirements have been satisfied;

5) to determine if a detection threshold for methionine is existent in layers, i.e. to determine the minimum methionine level in drinking water which enables birds to express an appetite for it;

6) to investigate the effects of the ways of methionine delivery (via feed or water) on the birds' feed and water consumption patterns; this would provide some information about the length of time hens need to respond to a methionine deficiency;

7) to examine whether there is a threshold period that is necessary for the birds to learn the associations between the sensory cue and metabolic effects of the deficient and sufficient diets; on the basis on this, to investigate the birds' ability to remember the colour-treatment associations;

8) to determine the level of methionine deficiency in feed which enables birds to express an appetite for methionine-treated water.

3.0 MATERIALS AND METHODS

This chapter outlines the materials, equipment, the handling of the birds and the general experimental procedures used in this research. Specific details relating to individual experiments are presented in subsequent chapters relating to each or groups of experiments.

3.1 Birds

For the first four experiments, 1000 commercial ISA Brown, and for the second four experiments 1000 Lohmann laying hens were the main stocks. The pullets were purchased from a commercial rearer at 18 weeks of age. For each experiment, fully feathered adequate birds were taken from these main stocks. After each experiment, birds were returned to the main stock and were fed commercial diet. They were labelled and not re-used.

3.1.1 Housing and cages

3.1.1.1 Main stocks

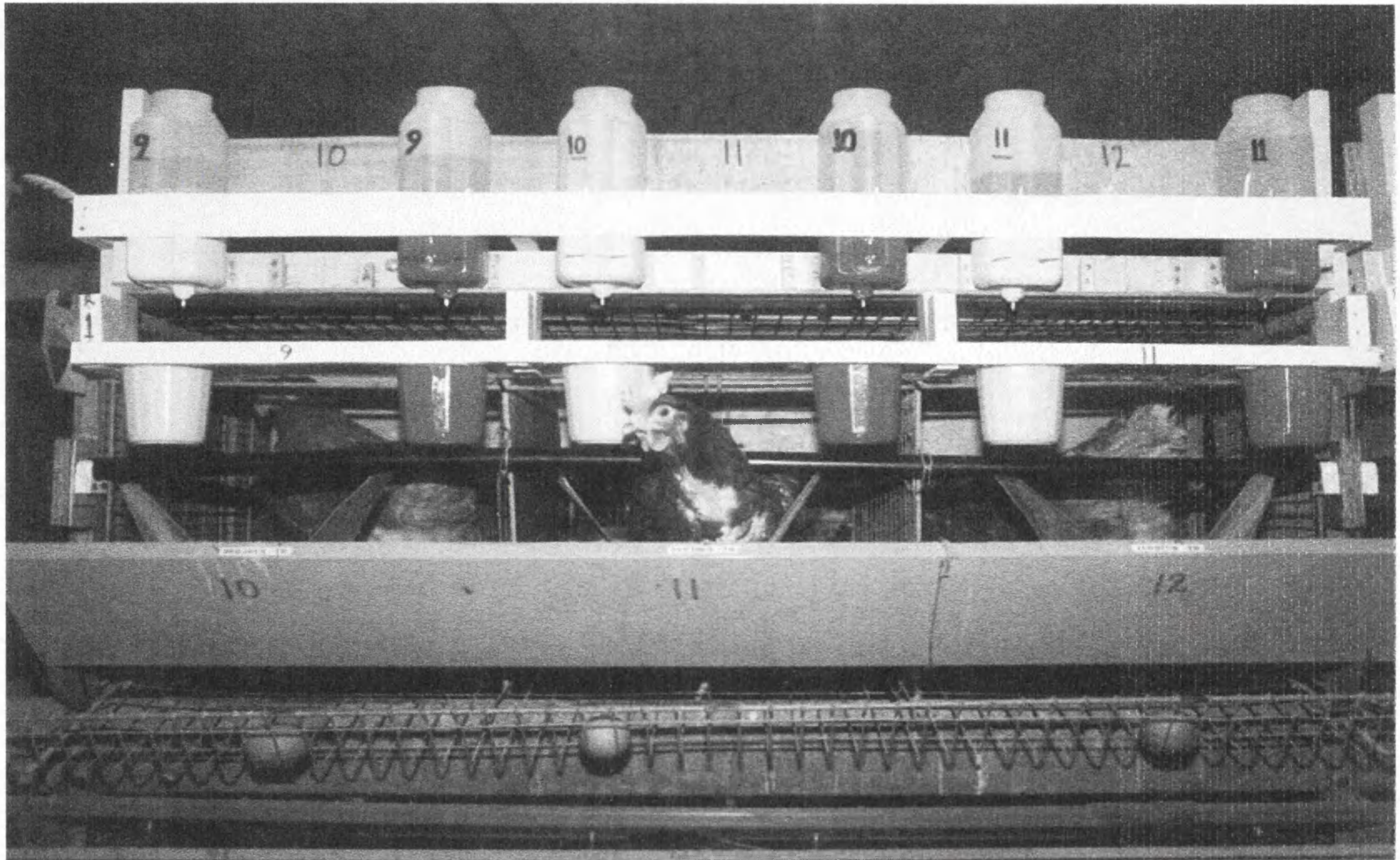
All birds were housed at the Scottish Agricultural College, in the same building using the same type of cages. The house was a conventional, windowless, fan-ventilated laying house.

The main stocks were held in two banks of three tier back-to-back cages. Each cage had mesh sides and back, fitted with a nipple drinker shared between a pair of cages, on the back partition. Each cage measured 43 cm wide, 43 cm high and 46 cm deep. Four birds were housed in each cage.

3.1.1.2 Experimental units

A separate small bank of three tier back-to-back cages were used to hold birds in the experiments. For the experimental units 18 top and 18 bottom tiers were redesigned in such way that the sides of the cages were closed with 3-ply wood so that the birds could not see their neighbours, and thus could not influence each other's behaviour. In addition, the middle tier's cages were kept empty. Water was supplied from 1000 ml plastic water bottles which were fitted with nipples (Val Watering System, 2599 Old Philadelphia Pike, Bird-In-Hand, PA-USA) at the base. The bottom and side of the bottles were painted in one colour: red, yellow or blue. To collect and measure water waste, red, yellow or blue plastic cups were used and were matched with the bottle colour. For each cage, one trough, two water bottles, and two waste-water collector cups were located so that the birds could only see the painted part of the bottles (see Plate 3.1). The bottle-cup pairs were located at the cage front nearest the cage side with a 20 cm gap between the nipples.

Plate 3.1. Experimental units fitted with painted water suppliers and waste-water collector cups.



3.2 House environment

The house temperature control system was set to maintain a daily minimum of 21°C at the middle tier level of the main banks of cages by controlling the ventilation rate. Temperature was measured at the middle tier level using maximum-minimum thermometers. Table 3.1 indicates the mean temperature reading for the middle tiers for each experiment.

Day length was increased from 8 hours by 60 minutes per week to 13 hours starting when the birds were housed at 18 weeks of age. In subsequent weeks, day length was increased by 20 minutes per week to 16 hours, and then was held constant.

Light was supplied by 40 Watt tungsten bulbs in all experiments.

Table 3.1 The temperatures over the duration of the eight experiments.

	Temperature [°C]	
Experiment	**Maximum**	**Minimum**
1a,b	19.9 ± 0.21	15.8 ± 0.31
2	20.8 ± 0.13	16.8 ± 0.22
3	21.8 ± 0.30	17.6 ± 0.22
4	22.1 ± 0.24	20.4 ± 0.15
5	18.5 ± 0.33	14.2 ± 0.65
6a,b	22.5 ± 0.33	19.7 ± 0.34
7	19.7 ± 0.41	15.8 ± 0.33
8	21.1 ± 0.23	17.5 ± 0.21

Data are means ± SEM.

3.3 Feed preparations

Commercial feed and water was provided *ad libitum* for the main stock until the beginning of each experiment. All diets were mixed at the Scottish Agricultural College farm, using a 100 kg mixer. The feed ingredients were mixed for 30 minutes and then were stored for up to a maximum of two months. There was no visual evidence of deterioration during the storage time. All diets were based on wheat and soybean meal.

In experiment 1a, 140 g/kg, 150 g/kg and 160 g/kg CP feeds, adequate in all nutrients, were used (Table 3.2). The 140 g/kg and 150 g/kg CP feeds contained the minimum level of methionine recommended by the ISA Brown Guide Book (1996). The 160 g/kg CP feed was used as a positive control in which the methionine level was above the other two feeds. In experiment 1b, a 155 g/kg CP commercial feed was used to determine the water intake of birds.

For all other experiments, a 140 g/kg CP feed adequate in all nutrients was reformulated and used (Table 3.3): it contained methionine according to NRC (1994) recommendation. Methionine-deficient feeds were made up using only soyabean and wheat as the source of methionine.

All nutrient values are based on calculated book values (NRC, 1994), and not measurements.

In all diets, the energy content was calculated to be 12.14 MJ/kg apparent metabolizable energy (AME).

In all experiments feed intake was used as criteria of methionine adequacy.

In all experiments, one cage and one bird were used as the experimental unit except in experiment 1a, in which two birds were used. Each day, the hens were given enough feed to just exceed their expected daily food intake.

3.4 Water preparations

According to the design of each experiment, birds received plain water (i.e. normal tap water) or a water solution of methionine. For this, commercially available, water soluble crystalline DL-methionine (*dl*-2-Amino-4(methylthio)-butanoic acid) was used (Weast, 1975).

The water and water-methionine mixture remaining in the bottles was discarded daily and replaced with fresh.

Table 3.2 The composition of 140, 150, and 160 g/kg crude protein feeds.

Ingredient composition	**Crude Protein level [g/kg]**		
	140	**150**	**160**
Wheat (10.4 % CP)	708.8	664.6	621.3
H.P. Soya (46.2 % CP)	159.9	191.8	222.0
Limestone	77.6	83.8	90.0
Maize Oil	33.7	40.9	47.9
Dicalcium phosphate	11.9	11.6	11.3
NaCl	3.1	3.1	3.3
Vit/Min. Premix[1]	2.5	2.5	2.5
Yolk Colour A[2]	1.0	1.0	1.0
DL-Methionine	0.9	0.7	0.7
L-Lysine HCl	0.5	-	-
Calculated nutrient composition			
Crude protein	140	150	160
Calcium	32.8	35.2	37.5
Total Phosphorus	5.6	5.6	5.6
Sodium	1.7	1.7	1.7
Arginine	8.4	9.3	10.0
Isoleucine	5.4	6.0	6.5
Leucine	10.1	11.0	11.8
Lysine	6.9	7.3	8.0
Methionine	3.1	3.1	3.3
Methionine + cystine	5.7	5.7	6.1
Threonine	4.9	5.3	5.8
Tryptophan	1.7	1.9	2.0
Apparent Metabolizable Energy (AME) [MJ/kg]	12.14	12.14	12.14

[1] The composition of vitamins and minerals in the premix provided the following amounts per kilogram of diet: vitamin A, 2400000 IU; vitamin D_3, 1200000 ICU; vitamin E (α-tocopherol acetate), 1600 IU; nicotinic acid, 4000 mg; pantothenic acid, 1600 mg; vitamin B_2 1000 mg; hetrazeen, 800 mg; iron ($FeSO_4$), 0.40%; cobalt ($CoSO_4$), 100 mg; manganese (MnO), 3.20%; copper ($CuSO_4$), 0.20 %; zinc (ZnO), 2.00%; iodine (CaI_2), 400 mg; selenium (Na_2SeO_3), 60 mg.
[2] Contains: canthoxanthin, ethyl ester of β-apo-8-carotenoic acid, citronaxanthin.

H.P. = high protein

Table 3.3 The ingredient- and estimated nutrient composition of Feeds 1 and 2.

Ingredient composition	**Feed 1 [g/kg]**	**Feed 2 [g/kg]**
Wheat (10.4 % CP)	714.0	712.8
H.P. Soya (46.2 % CP)	137.3	139.8
Limestone	90.3	90.3
Maize Oil	36.7	37.1
Dicalcium phosphate	11.4	11.4
NaCl	3.7	3.7
Vit/Min. Premix[1]	2.5	2.5
Yolk Colour A[2]	1.0	1.0
DL-Methionine	1.6	-
L-Lysine HCl	1.5	1.4
Calculated nutrient composition		
Crude protein	140	140
Calcium	37.5	37.5
Total Phosphorus	5.5	5.5
Sodium	1.8	1.8
Arginine	8.3	8.3
Isoleucine	5.3	5.3
Leucine	9.9	10.0
Lysine	7.2	7.2
Methionine	3.7	2.1
Methionine + cystine	6.4	4.8
Threonine	4.7	4.7
Tryptophan	1.7	1.7
AME [MJ/kg]	12.14	12.14

[1] The composition of vitamins and minerals in the premix provided the following amounts per kilogram of diet: vitamin A, 2400000 IU; vitamin D_3, 1200000 ICU; vitamin E (α-tocopherol acetate), 1600 IU; nicotinic acid, 4000 mg; pantothenic acid, 1600 mg; vitamin B_2 1000 mg; hetrazeen, 800 mg; iron ($FeSO_4$), 0.40%; cobalt ($CoSO_4$), 100 mg; manganese (MnO), 3.20%; copper ($CuSO_4$), 0.20 %; zinc (ZnO), 2.00%; iodine (CaI_2), 400 mg; selenium (Na_2SeO_3), 60 mg.
[2] Contains: canthoxanthin, ethyl ester of β-apo-8-carotenoic acid, citronaxanthin.

H.P. = high protein

3.5 Recording procedures

Daily feed intake, water consumption per replicate were measured gravimetrically every 24 hours. For all measurements of weight, an Oertling NB 33 balance (Brash, Glasgow) accurate to ± 0.1g was used.

Methionine intake values were calculated from consumption of feed and treated water. In Experiments 6, and 7, feed and water intakes were measured hourly.

3.6 Statistical analysis

The results of all experiments were analysed statistically using the analysis of variance procedures of the statistical programme Genstat-5 (release 4.2), copyright 1994, Lawes Agricultural Trust (Rothamsted Experimental Station). Significant differences were tested further using Least-significant difference multiple range test to determine the differences among treatments.

For each experiment, feed intake to water intake ratio frequency distribution was applied to look for presence of polydipsia. Three cases were found: in Experiment 1b bird Number 5, in Experiment 2, bird Number 18, and in Experiment 6b one bird in group 2. These birds were rejected from the experiments.

Proportional water intake (treated and untreated) data were subjected to angular (arc sinus) transformation, then both these transformed data and the original (untransformed) data were statistically analysed. There were no differences between the variance analysis results obtained from the two sources, therefore, percentage values were used in the text and tables to express preference for methionine-treated water.

Correlation and regression analyses were used for the water and feed intake data in Experiment 1b. For this, the statistical facilities of Microsoft Excel (v. 6.0) were used.

Graphs were constructed using the graphic functions of Microsoft Excel (v. 6.0). Error bars indicate the standard error of the mean.

3.7 Protocol approval by the Animal Experiment Committee

Prior to the commencement of all experiments, the protocol was prepared and submitted to the Animal Experiment Committee of S.A.C., Auchincruive for approval and permission to proceed. Criteria for approval included adequacy of experimental design to achieve planned objectives following statistical analyses, provision for welfare protection in the event of ill-health or food deprivation, and the credibility of the statements in terms of the biology of the domestic fowl.

4.0 DETERMINATION OF THE MINIMUM PROTEIN REQUIREMENT AND WATER CONSUMPTION OF LAYING HENS

4.1 Experiment 1a

4.1.1 Introduction

The purpose of the whole of this project was to assess the appetite of birds for methionine in drinking water. Previous investigations indicated that birds do not show appetite for a specific nutrient before deficiency symptoms occur (Kirchgessner and Paulicks, 1994). In the case of amino acids, the symptoms are intensified by lower protein intake because the intensity of nitrogen metabolism is relatively low, and the ability to catabolise imbalancing amino acids is less (Peng *et al.*, 1969; Harper *et al.*, 1970). Thus, the effects of imbalance or deficiency are usually studied largely with experimental, low-protein diets.

Therefore, in order to reliably assess a specific appetite for methionine in drinking water, the minimum amount of crude protein in the diet required for normal egg production of at least 90% of commercial target values was determined. This protein level was then used in the subsequent experiments to create feeds that were also marginally deficient in methionine.

The aim was:

to establish the protein content of a diet formulated to meet NRC (1994) nutrient requirements that would allow the hens to achieve egg production near to their commercial target values. A feed nutrient content was considered to be on the margin of being inadequate if egg weight or egg production was less than the control; or, if food intake was slightly higher than the control indicating a marginal deficiency of one or more amino acids.

4.1.2 Materials and methods

4.1.2.1 Stock

Seventy-two birds were taken randomly from a 1000-hen flock of 23 weeks old ISA Brown layers. The birds were placed in pairs into the three levels of the experimental bank of cages. Each cage was an experimental unit. Three experimental diets were fed to 12 replicates of birds, four replicates in each tier. The replicates were randomly assigned in the tiers.

4.1.2.2 Diets

Three feed formulations were used in this experiment, the ingredients and nutrient compositions are shown in Table 3.2. The birds were fed *ad libitum* but in a way that birds from neighbouring cages had no access to each other's feed. A two-week period was allowed for the birds to adapt to the new diets. This was necessary in order to reduce the possible effect of the previously fed commercial diet.

4.1.2.3 Measurements

From week three, the feed intake for each cage was measured in every 24 hour for three consecutive weeks. The body weight of each bird was measured at the beginning and at the end of each week. Additionally, the daily egg production and egg weights were recorded for each cage. Methionine and CP intake values were calculated from feed compositions and consumption data.

4.1.3 Results

Table 4.1 summarises the comparison of the effect of crude protein content in the diet on the measured performance traits. A dietary CP level of 160 g/kg compared to 140 and 150 g/kg did not have an effect on the rate of egg production. The increased intake of CP resulted in the production of heavier eggs by hens on the positive control diet ($p<0.05$). There was no significant difference between body weights ($p>0.05$). Feed intake of the hens fed the diet containing 160 g/kg CP was lower than of those fed the other diets, moreover, the feed conversion ratio (FCR) improved with increasing protein content. However, in both cases, the difference was significant ($p<0.05$) only between the effect of 140 and 160 g/kg crude protein content.

Table 4.1 The effect of different dietary protein levels on body weight, egg weight, feed conversion ratio, daily feed intake, and percentage of egg production.

CP [g/kg]	Bird weight [kg]	Egg weight [g]	FCR	Feed intake [g/day/bird]	Egg production [HD]
140	1.841 $\pm 0.0149^{a}$	58.41 $\pm 0.381^{a}$	2.127 $\pm 0.0332^{a}$	120.16 $\pm 1.536^{a}$	96.69 $\pm 0.812^{a}$
150	1.855 $\pm 0.0202^{a}$	58.89 $\pm 0.399^{a}$	2.059 $\pm 0.0330^{ab}$	116.82 $\pm 1.537^{ab}$	96.31 $\pm 0.859^{a}$
160	1.863 $\pm 0.0214^{a}$	60.42 $\pm 0.603^{b}$	2.032 $\pm 0.0306^{b}$	115.40 $\pm 1.705^{b}$	93.97 $\pm 0.972^{a}$

^{a,b} Values within a column with different superscripts differ significantly ($p<0.05$).
Values are mean ± SEM, n=12.

In the diet containing 150 g/kg CP, based on calculated feed compositions and consumption data, the methionine intake was significantly ($p<0.05$) lower compared to the positive control (160 g/kg CP) diet. In the case of protein intake, significant differences ($p<0.05$) were observed amongst the three diets. Table 4.2 shows the effect of increasing crude protein content of the diet on the daily protein and methionine intake.

Table 4.2 Intake of protein and amino acids as calculated from the daily feed intake/bird.

CP [g/kg]	Protein Intake [g/day/bird]	Methionine Intake [mg/day/bird]
140	16.82 ± 0.214^{a}	372.6 ± 4.82^{ab}
150	17.52 ± 0.231^{b}	362.1 ± 4.84^{a}
160	18.46 ± 0.273^{c}	380.9 ± 5.63^{b}

^{a,b,c} Values within a column with different superscripts differ significantly ($p<0.05$).
Values are mean ± SEM, n=12.

4.1.4 Discussion

The results of Experiment 1a indicate that the 150 g/kg CP diet contained the minimum amount of crude protein required in terms of normal egg production, FCR, and minimum feed intake. However, the birds' feed intake at 140 g/kg CP level was the highest. This is because the birds might have been compensating low protein level of the diet by consuming more food. Such response is, however, only likely if the protein is well balanced (Boorman, 1979).

The high level of feed intake, in turn, increased FCR value, which is in agreement with the findings of several authors (e.g. Deaton and Quisenberry, 1964; Carlson and Guenthner, 1969; Nivas and Sunde, 1969; Novacek and Carlson, 1969). Moreover, the 140 g/kg CP diet was sufficient to support a high rate of egg production; the rate of production of hens receiving the 140 g/kg diet was as high as that on the higher CP diets (i.e. above 90 HD%). It has been shown by Lillie and Denton (1967) that a low-protein content diet, i.e. one of 140 g/kg is adequate for maximum egg production, whereas suboptimal dietary protein levels or intake levels decrease egg production (Harms and Waldroup, 1963; Deaton and Quisenberry, 1964; Carlson and Guenthner, 1969; Dewan and Gleaves, 1969; Harms and Damron, 1969; Novacek and Carlson, 1969). The other measured parameters in this experiment (body weight and egg size) showed gradual decrease as dietary CP level decreased. In support, body weight was shown to decrease with lower dietary protein levels by Smith (1967), and Harms and Damron (1969), whereas Thornton *et al.* (1957), March and Biely (1963), Biely and March (1964) reported on lowering egg size in response to decreasing protein intake. They suggested that egg size is more sensitive than rate of production to a dietary protein or amino acid deficiency.

Based on the above, in order to show an appetite for methionine, a 140 g/kg CP (i.e. marginally deficient in protein) diet was used in the subsequent experiments. If the birds' response for the three diets (149 g/kg, 150 g/kg, 160 g/kg CP) were not different from one another, a next experiment using lower CP levels (e.g. 130 g/kg, 120 g/kg, etc. CP) would have been necessary to find out the level of marginal protein deficiency.

4.2 Experiment 1b

4.2.1 Introduction

Because in later experiments methionine is supplied in the water, it was important to determine the amount of water that hens normally consume while laying at high level.

The aim was:

to obtain the typical water intake of laying hens in the husbandry system being used for the experiments.

4.2.2 Materials and methods

4.2.2.1 Stock

Eighteen, 29 weeks old ISA Brown layers were randomly selected from the pool flock. The birds were caged individually, and each cage was fitted with two sets of water bottles. The cages were also provided with cups under each nipple waterer for the collection of any waste water.

4.2.2.2 Diets

Feed and water was provided for *ad libitum* consumption. The birds were fed with a commercial diet containing 155 g/kg CP and 12.14 MJ/kg AME. Water consumption and feed intake was measured gravimetrically every 24 hours for seven days.

4.2.2.3 Measurements

Individual body weights were measured on the first and seventh day.

4.2.3 Results

Table 4.3 summarises the average body weight, feed intake and water intake of individual birds. Within the same flock, body weight, feed intake and water intake showed great variation between the birds. There was a high correlation (r=0.534) between water consumption and feed intake.

Table 4.3 Individual body weight, feed and water intake.

Bird No	**Average Body Weight [g]**	**Average Feed Intake [g]**	**Average Water Intake [ml]**
1	1677	115.0 ± 7.17	143.0 ± 10.41
2	1973	127.8 ± 5.15	157.9 ± 7.33
3	1703	114.9 ± 7.28	152.2 ± 8.74
4	1755	105.7 ± 2.32	137.0 ± 6.55
5	-	-	-
6	2159	124.0 ± 2.94	193.3 ± 8.93
7	1829	116.4 ± 3.23	143.3 ± 8.07
8	1948	111.2 ± 2.46	151.3 ± 9.04
9	2184	121.2 ± 1.78	160.8 ± 9.13
10	1568	113.3 ± 3.83	179.2 ± 8.15
11	2391	125.9 ± 4.45	168.9 ± 11.87
12	1686	117.4 ± 3.72	174.0 ± 9.68
13	1806	101.5 ± 2.57	133.3 ± 3.63
14	1679	116.6 ± 3.93	165.2 ± 11.02
15	1899	107.6 ± 3.44	133.3 ± 11.14
16	1834	117.5 ± 4.63	192.8 ± 8.42
17	2231	116.0 ± 3.25	165.1 ± 9.34
18	1968	121.3 ± 3.04	148.3 ± 8.41

Values are mean ± SEM.

The following equation describes the relationship between feed intake and water consumption:

$y = -20.1 + 1.54x$ where y = Water intake

x = Feed intake

The total average of body weight, feed and water intake were 1916.1 g, 116.7 g/day and 168.5 g/day, respectively. The feed:water ratio was 1:1.44.

4.2.4 Discussion

Experiment 1b determined the feed and water intake of caged birds using one nipple per bird. Previously, Dun and Emmans (1971) found that, when four hens shared a nipple, their feed and water consumption were 124.9 g and 166 ml. Hearn and Hill (1978) also compared feed and water consumption of hens on a nipple watering system, but with varying numbers of birds per nipple, and measured an average feed consumption of 108.6g and water intake of 172.0 ml. In addition, examining individually caged laying hens (i.e. one hen per nipple), Gardiner (1982) found that the mean feed consumption of birds was 109 g and their daily water intake was 183 ml. In agreement with these results, the present measurements showed that the water intake of birds were higher than their feed intake, the feed:water ratio being 1.44. When compared to feed intake, individual water intakes varied considerably, which was indicated by the fact that the SEM value was 6.1% of the mean water intake, whereas it was only 3.2% in case of feed intake. This high variation was not unexpected as Hill *et al.* (1979) reported similar phenomenon.

5.0 Determination of the Appetite of Laying Hens for Methionine in Drinking Water

The experiments described in this chapter were carried out to gain some initial information about birds' appetite for methionine in water without and with the use of colour cues.

5.1 Experiment 2

5.1.1 Introduction

Kutlu and Forbes (1993) used colour cue successfully with ascorbic acid in feed, and Wilcoxon *et al.* (1971) showed that colour can be a good cue for drinks as well. However, there is virtually no literature on offering a choice of amino acid in drinking water, or on the using of cues when amino acid (methionine) is supplied in drinking water.

In Experiment 2, the appetite of birds for methionine in drinking water was investigated while feeding them with either an adequate or a methionine-deficient feed.

The aims were:

1. to determine the pattern of choice between drinking bottles (containing plain water) in relation to their position;

2. to determine if the feed intake, the pattern of water intake, and the egg production of laying hens is different when they are subjected to a combination of two different types of feed (normal or deficient in methionine) and drinking water (treated and untreated).

5.1.2. Materials and methods

5.1.2.1 Stock

Eighteen birds were taken randomly from a 1000-hen flock of 49 weeks old ISA Brown layers. They were placed singly in cages. One trough, two water bottles, and two waste-water collector cups were located for each cage. The bottle-cup pairs were located at the cage front, nearest the cage side with a 20 cm gap between the nipples from which hens were to select water. All bottles and cups were the same colour throughout the regimens of the experiment.

5.1.2.2 Diets

Two feed formulations were used in this experiment. For Feed 1 the nutrient specifications were set to meet or exceed NRC (1994) requirements; Feed 2 was essentially Feed 1 without supplemental methionine (see Table 3.3). Feed and water was provided *ad libitum*. Before starting the experiment, a one-week period was allowed for the birds to adapt to the new diets. All birds were subjected to three feeding regimens; each regimen period was 15 days. In regimen A, birds were fed Feed 1 and plain water was provided from both bottles. In regimen B, birds were fed Feed 1 and one bottle had plain water, while the other one contained 0.1% methionine-treated water. In regimen C, birds were fed Feed 2 and one bottle had plain water, the other one contained 0.1% methionine-treated water. The sufficiency of methionine in the diets throughout the regimens of the experiment are shown in Table 5.1.

Table 5.1. Feeding regimens of birds in Experiment 2.

Regimens	Methionine in Feed	Methionine in Water bottle 1	Methionine in Water bottle 2	Methionine in diet when drinking from bottle 1	Methionine in diet when drinking from bottle 2
A	adequate	-	-	adequate	adequate
B	adequate	-	0.1%	adequate	excessive
C	deficient	-	0.1%	deficient	adequate

Methionine content of the drinking water in this experiment was based on the results of Experiments 1a and 1b (Chapter 3). When using the re-formulated Feed 1 (140 g/kg CP and 3.7 mg/kg methionine), the average daily feed intake (115 g/hen) provides 425 mg methionine, whereas consuming the same amount from reformulated Feed 2 (140 g/kg CP and 2.1 mg/kg methionine) the birds receive 241 mg methionine a day, i.e. there is a difference of 184 mg/day of methionine intake. The daily water intake of birds was 168.5 g, thus adding 0.1% (w/v) methionine to it provides approximately the difference (168.5 mg/day) between Feed 1 and 2.

Bottles containing methionine-treated water were positioned so that one half of the hens received treated water on their right and the other half on their left. This positioning was to ensure that location did not affect the water selection.

5.1.2.3 Measurements

Feed intake and water intake (treated and plain) for each cage was measured in every 24 hour through the three consecutive regimens. Daily egg production and egg weights were recorded for each cage. Body weights were recorded at the beginning and at the end of each regimens.

5.1.3 Results

Table 5.2 shows the effects of feeding regimens on feed-, water- and methionine intake, the average egg mass and body weight of laying hens. Feed intake of the birds did not change ($p>0.05$) when their methionine requirement was met by the diet (regimens A and B). However, during regimen C feed intake was depressed with an average of 9% compared to regimens A ($p<0.01$) and B ($p<0.01$). A similar change in the total water intake was not found; it remained unaffected throughout the three regimens of the experiment ($p>0.05$). In contrast, total methionine intake was significantly ($p<0.05$) different between the regimens (increased in regimen B and decreased in regimen C compared to regimen A). Average egg mass was affected by the feeding regimens. In regimen B, when an excess of 0.1% methionine was given to the birds, a 1.1 g decrease was found compared to regimen A, a not significant difference. However, the reduction became significant ($p<0.001$) in regimen C, where the difference from the control value was 9.2 g. Body weight of the birds did not change significantly during the experiment ($p>0.05$), although an average of 40 g decrease was found in regimen C when compared to regimens A and B.

Table 5.2. Feed-, water-, and methionine intake, average egg mass, and body weight during the regimens of the experiment.

	Regimens		
	A (15 days)	**B (15 days)**	**C (15 days)**
Feed intake [g/hen/day]	108.2^{b}	107.7^{b}	98.2^{a}
Water intake [ml/hen/day]	146.8	144.0	139.1
Methionine intake [mg/hen/day]	400.3^{b}	486.2^{c}	288.0^{a}
Average egg mass [g/hen/day]	55.4^{b}	54.3^{b}	46.2^{a}
Body weight [g]	1931	1930	1887

^{abc} Values within a row with different superscripts differ significantly ($p<0.01$ for Feed intake; $p<0.001$ for Methionine intake and Average egg mass).

Values are mean of $n=17$.

Proportions of treated water consumed during the choice period of the experiment are shown in Table 5.3. The observed proportions or the overall value did not differ significantly ($p>0.05$) from 50% (i.e. random choice). In addition, there was no significant difference ($p>0.05$) in preferences between the two regimens.

Table 5.3. Intake proportions of treated water during the choice period of the experiment.

Regimens		
^{A}B (15 days)	**^{A}C (15 days)**	**^{B}Mean water intake proportions**
61.0 n.s.	56.7 n.s.	**58.9** n.s.

Water intakes are expressed as percentage of total (treated + untreated) water intake.
n.s. Difference from 50% is not significant ($p<0.05$).
Values are mean of $^{A}n=17$, and $^{B}n=34$.
LSD of $^{A}=32.6$, and $^{B}=31.0$.

Data from individual birds (Table 5.4) confirm that the hens displayed some form of position preference, consistently drinking more from the drinker on one side of the cage than the other. This preference was almost consistent throughout the regimens (No 1, 2, 9, 11, 12, 16).

It is also shown by the individual data during the choice phase that when birds (No 1, 2, 6, 11) preferred the bottle containing methionine-treated water, feed intake did not change substantially. Moreover, there was a positive correlation ($r=0.49$) between feed intake and methionine-treated water consumption of these hens. However, there were also birds (No 4, 5, 7, 8, 9, 10, 13, 14, 15 and 17) which did not exclusively drink the methionine-treated water, but still consumed a considerable amount of it.

Table 5.4. Water-, feed-, methionine intake, egg production, egg weight and body weight of individual birds during the feeding regimens of the experiment.

Feeding regimens		Water Intake [g/day] Untreated water	Water Intake [g/day] Methionine treated water	Feed Intake [g/day]	Methionine Intake [g/day]	Number of eggs	Egg weight [g]	Body weight [g]
Bird 1	RA	3.3 ± 0.7	126.4 ± 8.3	102.6 ± 5.8	3.8 ± 0.3	12	65.6 ± 0.6	1782
	RB	2.9 ± 0.4	124.5 ± 6.9	101.4 ± 3.7	5.0 ± 0.2	12	67.0 ± 0.7	1786
	RC	2.6 ± 0.2	125.0 ± 7.0	105.2 ± 3.0	3.5 ± 0.1	12	67.3 ± 1.1	1753
Bird 2	RA	49.6 ± 7.2	114.4 ± 6.8	126.5 ± 2.2	4.7 ± 0.1	15	70.7 ± 0.6	2368
	RB	39.7 ± 4.2	119.0 ± 4.2	126.5 ± 1.3	5.9 ± 0.1	15	67.3 ± 0.4	2353
	RC	46.5 ± 8.3	105.9 ± 11.1	122.5 ± 5.1	3.6 ± 0.2	15	67.0 ± 1.1	2324
Bird 3	RA	70.4 ± 9.9	89.2 ± 7.8	103.9 ± 4.2	3.6 ± 0.2	13	68.1 ± 0.9	1995
	RB	56.3 ± 6.1	80.7 ± 6.5	85.2 ± 4.4	4.0 ± 0.2	11	69.5 ± 1.1	1907
	RC	75.7 ± 7.4	3.3 ± 1.4	37.4 ± 4.5	0.8 ± 0.1	6	57.6 ± 1.5	1685
Bird 4	RA	114.4 ± 6.2	35.0 ± 7.5	108.3 ± 2.6	4.0 ± 0.1	14	66.4 ± 0.5	1994
	RB	139.4 ± 3.6	5.9 ± 1.6	109.9 ± 1.4	4.1 ± 0.1	14	66.6 ± 0.9	1982
	RC	55.2 ± 11.9	72.6 ± 13.9	89.3 ± 3.1	2.6 ± 0.2	10	62.7 ± 1.4	1887
Bird 5	RA	65.5 ± 10.4	73.4 ± 10.6	91.6 ± 6.0	3.4 ± 0.3	9	62.5 ± 3.0	2106
	RB	107.8 ± 9.3	34.0 ± 8.1	106.8 ± 3.0	4.3 ± 0.2	12	67.9 ± 1.2	2069
	RC	84.7 ± 10.0	60.0 ± 8.9	106.3 ± 3.3	2.8 ± 0.1	9	63.6 ± 1.5	2086
Bird 6	RA	53.1 ± 18.0	78.1 ± 14.9	89.4 ± 2.9	3.3 ± 0.1	12	61.0 ± 0.8	1633
	RB	24.7 ± 6.0	110.3 ± 7.4	97.0 ± 1.8	4.7 ± 0.1	12	63.5 ± 0.6	1653
	RC	6.7 ± 1.9	162.6 ± 10.8	99.3 ± 2.2	3.7 ± 0.1	12	60.7 ± 0.8	1622
Bird 7	RA	76.3 ± 4.7	66.8 ± 6.1	101.5 ± 2.7	3.8 ± 0.1	14	56.0 ± 0.4	1711
	RB	74.9 ± 8.2	64.4 ± 9.0	101.9 ± 2.4	4.4 ± 0.2	13	58.3 ± 0.5	1723
	RC	105.2 ± 4.8	18.3 ± 2.3	88.9 ± 3.4	2.0 ± 0.1	11	55.4 ± 0.8	1660
Bird 8	RA	74.2 ± 7.0	78.9 ± 7.6	109.1 ± 3.4	4.0 ± 0.2	13	63.2 ± 0.8	1744
	RB	53.0 ± 3.9	92.1 ± 3.9	108.8 ± 2.7	4.9 ± 0.2	13	63.8 ± 0.6	1715
	RC	60.3 ± 5.4	91.4 ± 7.5	112.1 ± 1.8	3.3 ± 0.1	11	64.1 ± 1.2	1690
Bird 9	RA	137.3 ± 6.0	23.9 ± 4.9	117.8 ± 3.5	4.4 ± 0.2	12	63.2 ± 0.6	1960
	RB	134.2 ± 7.5	28.1 ± 6.0	124.0 ± 3.2	4.9 ± 0.2	14	67.6 ± 0.5	2040
	RC	126.9 ± 4.8	34.0 ± 2.9	111.0 ± 2.0	2.7 ± 0.1	14	63.7 ± 0.7	2042

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Table 5.4 continued from previous page

Feeding regimens		Water Intake [g/day] Untreated water	Methionine treated water	Feed Intake [g/day]	Methionine Intake [g/day]	Number of eggs	Egg weight [g]	Body weight [g]
Bird 10	RA	30.4 ± 5.0	97.5 ± 5.6	110.6 ± 2.3	4.1 ± 0.1	12	67.3 ± 0.6	1841
	RB	36.6 ± 7.0	93.0 ± 6.9	107.3 ± 2.6	4.9 ± 0.1	11	70.6 ± 1.3	1846
	RC	50.7 ± 3.6	77.5 ± 4.3	96.5 ± 4.4	2.8 ± 0.1	9	67.9 ± 1.6	1827
Bird 11	RA	4.1 ± 0.4	161.4 ± 4.9	116.4 ± 2.8	4.3 ± 0.1	15	58.8 ± 0.2	1975
	RB	4.4 ± 0.5	152.0 ± 4.1	106.9 ± 2.0	5.5 ± 0.1	13	59.2 ± 0.3	1994
	RC	7.9 ± 1.6	160.0 ± 6.5	119.4 ± 3.5	4.1 ± 0.1	12	61.6 ± 1.2	1990
Bird 12	RA	1.2 ± 0.1	137.7 ± 4.6	101.5 ± 2.6	3.8 ± 0.1	14	59.5 ± 0.9	1848
	RB	0.6 ± 0.1	144.2 ± 4.9	103.0 ± 3.0	5.3 ± 0.2	13	59.2 ± 0.6	1864
	RC	0.5 ± 0.1	142.1 ± 5.3	92.2 ± 3.0	3.4 ± 0.1	13	56.9 ± 0.5	1831
Bird 13	RA	32.3 ± 6.7	100.9 ± 8.1	105.4 ± 1.9	3.9 ± 0.1	14	62.9 ± 0.9	1896
	RB	12.5 ± 5.4	127.8 ± 6.2	111.7 ± 3.1	5.4 ± 0.2	14	65.1 ± 1.1	1869
	RC	48.9 ± 6.4	74.5 ± 8.3	99.5 ± 3.6	2.8 ± 0.2	11	63.0 ± 0.9	1802
Bird 14	RA	54.1 ± 5.6	116.0 ± 6.4	124.4 ± 2.1	4.6 ± 0.1	15	50.7 ± 0.3	2321
	RB	54.4 ± 6.2	109.5 ± 4.4	116.4 ± 2.0	5.4 ± 0.1	15	48.6 ± 0.2	2373
	RC	92.8 ± 6.1	69.2 ± 6.6	117.0 ± 1.5	3.1 ± 0.1	15	49.2 ± 0.5	2371
Bird 15	RA	64.5 ± 11.2	75.7 ± 10.8	113.5 ± 2.2	4.2 ± 0.1	13	64.5 ± 0.3	1934
	RB	113.0 ± 6.2	30.7 ± 3.5	109.9 ± 2.6	4.4 ± 0.1	11	67.1 ± 1.1	1948
	RC	101.3 ± 8.8	37.6 ± 7.0	101.1 ± 2.6	2.5 ± 0.1	12	62.4 ± 1.4	1925
Bird 16	RA	14.2 ± 3.0	136.1 ± 3.1	112.2 ± 2.1	4.2 ± 0.1	14	63.6 ± 0.5	1906
	RB	7.3 ± 1.1	137.6 ± 4.2	111.0 ± 2.4	5.5 ± 0.2	13	65.7 ± 1.0	1877
	RC	4.3 ± 0.4	134.6 ± 6.1	91.9 ± 5.6	3.3 ± 0.2	9	61.7 ± 1.5	1797
Bird 17	RA	84.7 ± 5.2	55.3 ± 5.4	104.3 ± 2.1	3.9 ± 0.1	15	62.2 ± 0.6	1964
	RB	98.3 ± 6.6	34.8 ± 5.4	103.6 ± 2.2	4.2 ± 0.1	13	62.0 ± 0.7	1952
	RC	101.4 ± 6.5	23.9 ± 5.5	79.2 ± 3.6	1.9 ± 0.1	11	59.6 ± 1.5	1932
Bird 18*	RA	-	-	-	-	-	-	-
	RB	-	-	-	-	-	-	-
	RC	-	-	-	-	-	-	-

* Polydipsic bird rejected from the experiment.

5.2 Experiment 3

5.2.1 Introduction

In this experiment, colour cue was introduced to study the expression of appetite for methionine in the drinking water.

The aim was:

to train laying hens when given a feed deficient in methionine, to recognise by colour cue the water supply bottles containing methionine treated water.

5.2.2 Materials and methods

5.2.2.1 Stock

Fifty-four 55 weeks old ISA Brown laying hens were taken randomly from a 1000 hen commercial laying flock and placed singly in cages. They were monitored for egg production for two weeks, then the ten best egg producers of similar body weight (2067 ± 46 g; mean and SEM) were chosen for the experiment. The birds were caged individually with one empty cage between neighbouring birds. The sides of the cages were closed with 3-ply wood so that the birds could not see their neighbours. This separation was to ensure that social influence did not affect the bottle selection. The birds were housed in cages of the top tier of a three-tier battery system. One trough, two water bottles, and two waste-water collector cups were located in front of each cage in such way that the birds could only see the painted part of the bottles. The bottle-cup pairs were located at the cage front nearest the cage side with a 20 cm gap between the nipples from which hens were to select water. Altogether twenty water bottles were used, ten were red, ten were yellow.

5.2.2.2 Diets

The feed formulations (Feed 1 and Feed 2) used in this experiment were the same as in Experiment 2. Treated water contained 0.15% (w/v) methionine. In Experiment 2, some birds consumed a considerable amount of the methionine-treated water, and in anticipation of birds exhibiting discrimination of water supply, the methionine level was increased in an attempt to shorten their response time to treated water.

The ten birds were divided into two groups of five. For group 1, plain water was given in yellow bottles and treated water in red bottles. For group 2, plain water was given in red bottles and treated water in yellow bottles. The grouping of birds was necessary in order to eliminate the colour effect on their choice. The experiment consisted of five regimens (A-E) which differed in the feed and water offered to the birds.

To determine normal feed intake, methionine intake and water consumption, hens received Feed 1 and plain water for the first seven days (regimen A). Then followed a four-day cycle where birds received Feed 2 and plain water for two days (regimen B), then Feed 2 and treated water for two days (regimen C). The cycle (regimens B and C) was repeated once. Subsequently, birds received Feed 2 and plain water for an additional two days (regimen B). During this training period (a total of ten days) the two types of feed were given to the hens alternately every two days in order to allow them to become accustomed to the colour cue and physiological effect of the feeds adequate and deficient in methionine.

Then followed a "free choice" part of the experiment during which the birds were fed Feed 2 and were offered both plain and treated water (in the red and yellow bottles) for five days (regimen D). The position of the bottles was then swapped and the drinks were offered for another five days (regimen E).

Each day, the hens were allocated just enough feed to exceed their expected daily food intake.

5.2.2.3 Measurements

Daily feed intake and water consumption were measured gravimetrically every 24 hours. The plain water and water-methionine mixture remaining in the bottles was discarded daily and replaced with fresh.

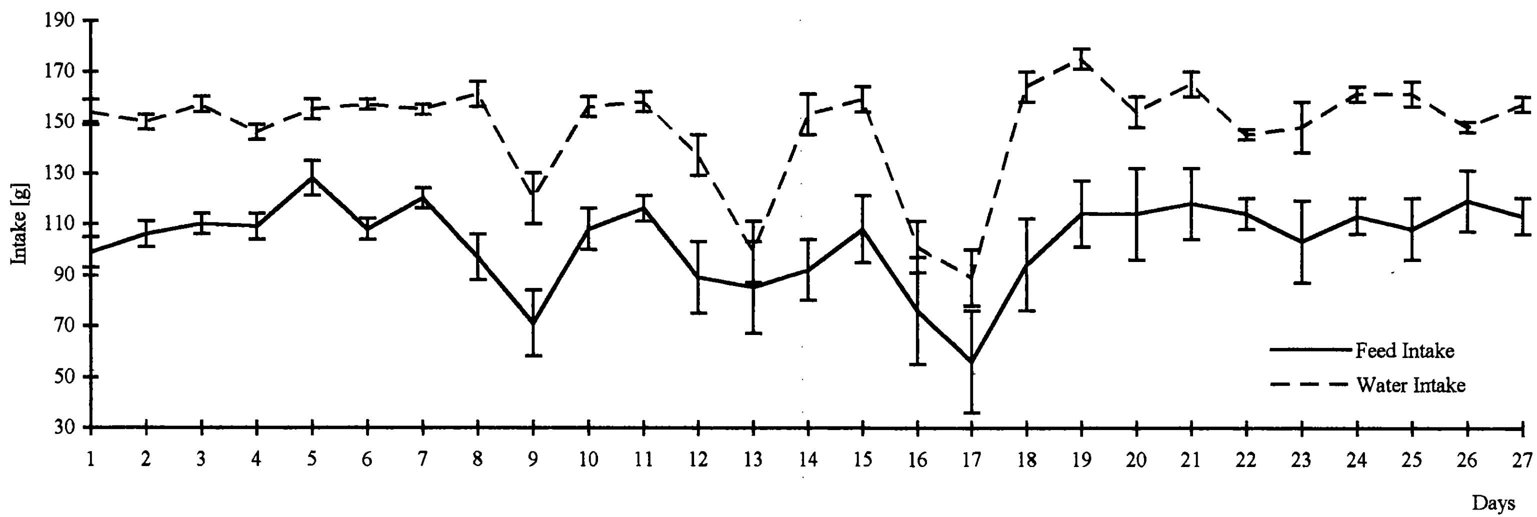
Every effort was made to create an experimental condition where the methionine-treated water was the only variable.

The body weights (mean and SEM) at the beginning and at the end of the experiment were 2067 ± 46 g and 1995 ± 57 g, respectively, the difference was not significant. The average rate of egg production and egg weight during regimens A and E was 94.00 %HD and 88.00 %HD, and, 62.79 g and 65.18 g, respectively.

5.2.3 Results

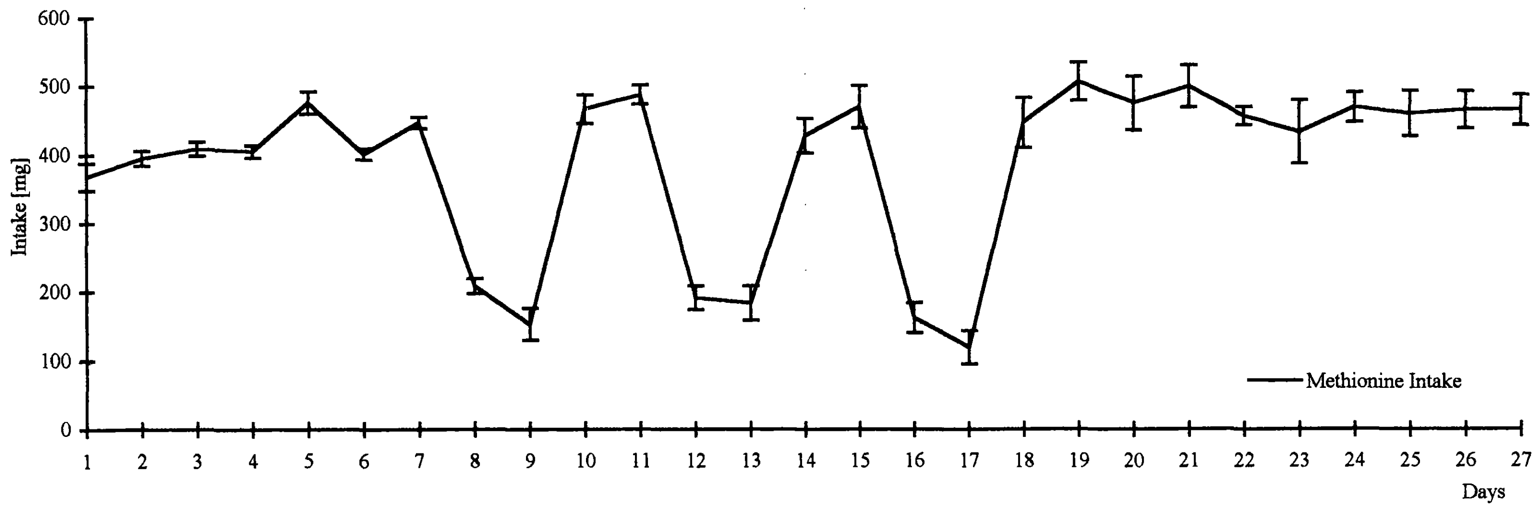
The daily feed- and water intake of layers is presented in Figure 5.1, and the estimated methionine intake during each of the 27 days is shown in Figure 5.2. Each point is the mean ± SEM of the results from ten birds.

Figure 5.1. Daily changes in feed intake and water consumption of laying hens in association with the five feeding regimens.



Regimens	A	B	C	B	C	B	D	E
Days	7	2	2	2	2	2	5	5

Figure 5.2. Daily changes in methionine intake of laying hens in association with the five feeding regimens.



Regimens	A	B	C	B	C	B	D	E
Days	7	2	2	2	2	2	5	5

It is apparent from the figures that the daily intakes followed a similar pattern, that is there were five distinct phases corresponding to the regimens of the feeding. In addition, the correlation between feed intake and water consumption was found to be significant (r=0.7755).

During the first phase of the experiment (regimen A), i.e. when the birds received feed containing adequate methionine and plain water, the standard errors were small, and the birds' appetite for water and feed was without a dramatic change. On days eight and nine (regimen B), i.e. when plain water and feed with inadequate methionine content was given to the hens, they lost their appetite for food and water. This decrease of appetite became more apparent by the second day of this regimen. Additionally, birds were more aggressive than usual and appeared hungry even though they had access to food. In the subsequent regimen (regimen C), i.e. when the methionine-deficient feed was accompanied with methionine-treated water (0.15%, w/v), the birds' appetite for feed and water increased up to the level in regimen A. The same responses were observed when the treatments were repeated. Moreover, during the days when the hens were on regimen B, feed intake had a greater variability which diminished when they proceeded to regimen C, that is, when they received methionine-treated water. The last two phases of the experiment (regimens D and E) tested the birds for their ability to choose water supplemented with methionine. By this time (day 18), it appeared that all birds had become trained for the colour cues. When offering both supplemented and unsupplemented water with the deficient feed, the birds always preferred the treated water even when the position of the bottles was changed.

In addition to the daily changes presented above, Table 5.5 shows the feed-, water-, and methionine intakes during the five regimens. The effect of regimens was significant in all three parameters measured. Feed intake of birds decreased significantly ($p<0.001$) when progressing from regimen A to B, then returned to the control level in regimen C, and did not change in the remaining of the experiment. Changes in water intake during the experiment were similar to those in feed intake, i.e. there was a significant difference ($p<0.01$) between the values of regimen B and the others. The finding that there were no significant differences in feed and water intakes between regimens C and A ($p>0.05$) confirms that the birds' appetite was restored during regimen C. This indicates that the source of methionine is insignificant for maintaining a normal appetite. In methionine intake, significant difference ($p<0.001$) between regimens B and A, moreover, methionine intake in these two regimens was significantly lower ($p<0.001$) than in the rest of the regimens. The data show that the average of 30% depression of appetite observed in regimen B resulted a methionine deficiency, the magnitude of which was 60% of the methionine intake during regimen A. The overconsumption of methionine during the choice phase compared to regimen A further confirms that the birds were able to recognise and choose methionine-treated water.

Table 5.5. Feed-, water-, and methionine intakes during the feeding regimens of the experiment.

	Regimens					
	A (7 days)	**B (6 days)**	**C (4 days)**	**D (5 days)**	**E (5 days)**	**LSD**
Feed intake [g/day]	111.3^{b}	79.1^{a}	106.0^{b}	110.6^{b}	111.0^{b}	**10.3**
Water intake [ml/day]	153.5^{b}	117.7^{a}	156.5^{b}	160.6^{b}	154.8^{b}	**23.2**
Methionine intake [mg/day]	414.6^{b}	170.0^{a}	462.3^{c}	475.9^{c}	457.7^{c}	**41.9**

^{abc} Values within a row with different superscript differ significantly ($p<0.001$ for Feed intake and Methionine intake; $p<0.01$ for Water intake).
Values are mean of $n=10$.

Proportions of water intakes during regimens D and E are shown in Table 5.6. After the training period, when the hens were able to chose between plain and treated water, they practically ignored the plain water, and consumed almost exclusively from the supplemented water. Comparison of the birds' preference for treated water to 50% (i.e. random choice) showed consistently significant difference ($p<0.001$) in the period examined. Regimen had no significant effect on the values ($p>0.05$), indicating that the position of bottles had no effect on water preference. Overall proportions for treated water were significantly different from 50% ($p<0.001$).

Table 5.6. Intake proportions of treated water during the choice period of the experiment.

Regimens		
^{A}**D (5 days)**	^{A}**E (5 days)**	^{B}**Mean water intake proportions**
98.9 s.	93.9 s.	**96.4** s.

Water intakes are expressed as percentage of total (treated + untreated) water intake.
s. Difference from 50% is significant ($p<0.001$).
Values are mean of $^{A}n=10$, and $^{B}n=20$.
LSD of $^{A}=17.0$, and $^{B}=14.1$.

5.3 Discussion

In order to obtain some preliminary information about the selection of layers for methionine when supplied in water, the birds were subjected to the combinations of two types of feed (adequate or deficient in methionine) and plain-or methionine-treated (0.1%) water in Experiment 2. Thus, in the regimens of the experiment they received methionine either in adequate (regimens A, B and C), or excess (in regimen B), or deficient amount (regimen C). It became apparent that the birds could not express their appetite for methionine under the conditions of the experiment, therefore a cue (colour) was introduced in Experiment 3 to assist self-selection. It is known (Kutlu and Forbes, 1993) that hens can be trained to express their appetite for individual nutrients (Vitamin C) if there is some discernible sensory difference between two foods, one of which contains too little of it for the birds' requirements and the other too much of it. However, such visualisation of methionine appetite, when methionine is offered in drinking water, has not been previously reported. Experiment 3 has demonstrated that, after a sufficient training period, adult layers are able to choose between plain water and methionine-treated water. If the methionine requirement of the hens could not be obtained from the food alone, the hens voluntarily selected water containing methionine to meet their requirements.

Several pieces of additional information were also gained from the two experiments. Both experiments showed that methionine deficiency results decrease in feed intake. Previously, several investigators (e.g. Almquist 1954; Gous and Kleyn 1989; Roth *et al.*, 1990; Uzu *et al.*, 1993) noted that as the deficiency in methionine or other amino acids becomes severe, feed intake declines. In Experiment 2, feed intake was depressed when the diet did not meet the birds' methionine requirement. Similarly, in Experiment 3 feed intake declined already at the first day of supplying the deficient feed, and became more apparent by the next day of the regimen. When the option of restoring methionine level through supplemented drinking water was offered, the birds responded quickly, and the feed intake returned to normal within a day. A similar observation was reported by Almquist (1954) when withdrawing and restoring crystalline indispensable amino acids to the feed.

The results of Experiment 2 suggest that when the birds receive a diet containing adequate methionine (NRC, 1994), the intake from water containing an additional 0.1% methionine has no adverse effect on egg production and the body weight of birds. These findings are in agreement with earlier observations by Koelkebeck *et al.* (1991) and Schutte *et al.* (1983). Similarly, in agreement with the literature (Shafer *et al.*, 1996, 1998), it was observed that this additional methionine might have a beneficial effect on egg weight. However, both Experiments 2 and 3 showed that during the period of consuming methionine-deficient feed (15 days in Experiment 2, six days in Experiment 3), both egg production and body weight of the birds decreased. These responses to methionine deficiency are the consequence of a reduced feed intake which, in turn, is the primary result of the amino acid imbalance (Harper and Rogers, 1965).

The mean water intake of the birds followed closely the changes in feed intake in Experiment 3. This phenomenon can be explained by the strong correlation between food and water intake (Hill *et al.*, 1979). In contrast, total water intake of the hens was affected neither by the excess nor the deficiency of methionine, in Experiment 2. This apparent disagreement in the results could be explained by the different magnitudes of methionine deficiency that birds experienced in the two experiments; in Experiment 2, methionine intake during regimen C was reduced by 28% from regimen A, whereas in Experiment 3, the intake of methionine was depressed by 60% during regimen B when compared with regimen A.

A further observation in Experiment 3 was that once the methionine intake of the birds had exceeded that achieved in regimen A, they did not stop drinking the treated water, thus increasing the intake of methionine by 40 to 60 mg more than that achieved when consuming the methionine supplemented feed. This might be because the birds associated the plain water with methionine deficiency (i.e. an adverse effect) as plain water was previously always paired with deficient feed. This explanation is supported by El Boushy and Kennedy (1987) who reported that birds will not usually like a feed the second time once it has previously caused digestive disturbance or discomfort. If the birds could be trained in a way that they do not associate plain water with deficiency, they may start drinking it once their methionine appetite (requirement) from treated water was satisfied.

The results of both experiments suggest that the source of methionine does not influence its metabolic effect. In Experiment 2, those birds which choose mainly methionine-treated water while fed deficient feed, did not reduce their feed intake (i.e. birds 1, 2, 6, and 11). In Experiment 3, the uptake of methionine via drinking (in regimens C, D and E) resulted normal feed- and water intake. Thus it seems that methionine from the water is "as good as" when supplied wholly from the feed. In addition, Experiment 3 showed that the addition of methionine to drinking water, rather than to the feed, does not adversely effect water or feed intake. Similar observations were reported by Damron and Goodson-Williams (1987) and Damron and Flunker (1992). However, Baker (1977, cited by Damron and Goodson-Williams 1987) reported that water consumption of growing chicks was reduced by 50% when a low level of methionine was added in the drinking water.

The results of Experiment 2 suggest that, without a cue, feeding regimens have no effect on choice of water supplier, i.e. birds follow a pattern of choice associated to the position rather than the metabolic effect of the diet. On the other hand, when colour cue assisted the selection (in Experiment 3), the birds were able to express their appetite for methionine. This was indicated by the observation that when the position of the bottles was changed, the proportioned choice for treated water was the same in both regimens, moreover, they were significantly different from 50% in both regimens.

The individual results in Experiment 2 indicate that the specific appetite for this amino acid is not innate but has to be learned, with the help of a cue. There were birds (No. 4, 5, 7, 8, 9, 10, 13, 14, 15 and 17) which had consumed a considerable amount of the treated water, and thus had experienced the beneficial effect of methionine supplement, but did not change the drinking pattern. Without a cue, these birds were unable to express their appetite for methionine. The amount of methionine consumed perhaps did not give an immediate feeling of well being, therefore, the birds did not respond quickly enough to the treated water.

The main conclusions of the experiments were:

1. the birds can not express their appetite for methionine without a discernible cue;

2. with the help of colour cues, birds can be trained to choose between two types of nutrient supply, one deficient and another supplemented with methionine;

3. methionine deficiency disturbs the birds' feed intake which can be fully restored by the addition of methionine;

4. the source of methionine (in feed or water) is indifferent in terms of its metabolic effect;

5. an 0.1% excess of methionine does not effect adversely egg production and body weight of the birds.

6.0 REGULATION OF METHIONINE INTAKE FROM DRINKING WATER

6.1 Experiment 4

6.1.1 Introduction

In the previous experiment (Experiment 3), birds consumed methionine from drinking water well above their requirements. A possible reason for this could be that the total effect of the deficient feed plus plain water and the deficient feed plus supplemented water were associated with different colours, thus the colour of plain water supply bottle also meant methionine deficiency, which the birds might have associated with metabolic discomfort (El Boushy and Kennedy, 1987).

To avoid this possible association, a new colour had been introduced during the training period in Experiment 4. In this way this new colour would "mean" the adverse effects of methionine deficiency (arising from the combination of deficient feed plus plain water), instead of the colour designating plain water (when paired with adequate feed). Thus in the choice situation, neither the treated, nor the plain water would have a "history" of causing discomfort to the birds previously. Therefore, it was expected that if the birds can regulate methionine intake from water, they might not drink more from the treated water than what satisfies their requirements, but they would quench their thirst from the plain water (which now would not be associated with adverse effects).

The aims were:

1. to determine if hens can correct a feed deficient in methionine by choosing the methionine-treated water, while maintaining normal egg production and body weight;

2. to determine if the hens can regulate consumption of methionine-treated water in order to satisfy their optimum methionine requirement.

6.1.2 Materials and methods

6.1.2.1 Stock

Eighteen fully feathered 68 weeks old ISA Brown laying hens were taken randomly from a 1000-hen commercial laying stock. The birds were distributed into two groups (group 1 and group 2) of equal number, and placed singly in cages. The grouping of birds was necessary in order to eliminate the colour effect on their choice. The body weights (mean and SEM) of the two groups at the beginning of the experiment (2143.9 ± 92.23 g and 2087.8 ± 79.82 g) were not significantly different ($p>0.05$). According to the plan of the feeding regimens, three different coloured (yellow, red and blue) bottles and waste water collector cups were provided for each cage. Also, one individual feeding trough was located for each cage, placed in the usual feed trough used for flock-based feeding. The side of the cages were closed with 3-ply wood.

6.1.2.2 Diets

Two feed formulations (Feed 1, Feed 2) were used in this experiment, the ingredients and estimated nutrient contents of which are shown in Table 3.3.

Hens were given water in bottles coloured accordingly to the treatment and the birds were trained to recognise which bottle has methionine supplemented water or plain water.

For group 1: plain water was given

in yellow bottles with a 140 g/kg protein feed supplemented with methionine (F1), or

in blue bottles with the same feed without methionine supplementation (F2);

treated water was given

in red bottles with feed without methionine supplementation (F2).

For group 2: plain water was given

in red bottles with a 140 g/kg protein feed supplemented with methionine (F1), or

in blue bottles with the same feed without methionine supplementation (F2);

treated water was given

in yellow bottles with feed without methionine supplementation (F2).

The experiment consisted of six regimens (A-F). To determine normal feed-, water and methionine intake, birds were fed an adequate feed (Feed 1) for 7 days (regimen A). During this time each hen received plain water.

To induce a methionine deficiency, the birds were subsequently transferred to a methionine-deficient feed (Feed 2), and were given plain water for one day (regimen B). In order to avoid the possible association of the future "choice"-colours (red and yellow) with the feeling of discomfort, the water bottles were blue in this regimen.

The following day, the birds were given the same, deficient, feed and water containing methionine, thereby training the hens to recognise and correct the deficiency through drinking water (regimen C).

The following day, the birds were returned to the adequate feed (Feed 1), and received plain water in order to train them that once their methionine need is satisfied there is no need for a supplement from the drinking water (regimen D).

Subsequently, the birds were returned again to the deficient feed (Feed 2) and water containing methionine (regimen C). This was in order to emphasise that the methionine deficiency of the feed can be corrected from the (treated) drinking water.

The above four-day cycle was repeated three more times. After the last cycle, there was one additional day when feed without methionine supplementation and plain water was given from blue bottles (regimen B).

The response to training was then tested for ten days by offering the hens a choice of plain and methionine supplemented water from the appropriately coloured bottles containing treated water (regimen E). Until the end of regimen E, treated water always contained 0.15% methionine.

To test the birds' response to methionine concentration in the drinking water, the above ten-days testing period was repeated with methionine content increased to 0.3% (regimen F). The regimens of the experiment are summarised in Table 6.1.

Table 6.1. Training regimen of birds in Experiment 4.

	Regimens	Diet		Bottle colour	
		Feed	Water	Group 1	Group 2
	A (7 days)	F1	plain	yellow	red
4-day	B (1 day)	F2	plain	blue	blue
cycle	C (1 day)	F2	treated*	red	yellow
repeated	D (1 day)	F1	plain	yellow	red
three times	C (1 day)	F2	treated*	red	yellow
	B (1 day)	F2	plain	blue	blue
	E (10 days)	F2	plain and	yellow	red
			treated*	red	yellow
	F (10 days)	F2	plain and	yellow	red
			treated**	red	yellow

F1 = 140 g/kg CP feed supplemented with methionine.
F2 = 140 g/kg CP feed without methionine supplementation.
*treated water contains 0.15% methionine.
**treated water contains 0.3% methionine.

6.1.2.3 Measurements

Food intakes and water intakes were recorded daily. Eggs laid each day were weighed. Body weights were measured at the beginning and end of the experiment.

6.1.3 Results

The body weights (mean and SEM) at the beginning and end of the experiment were 2113.9 ± 58.74 g and 1989.2 ± 54.62 g, respectively the difference was not significant (p>0.05). The average rate of egg production and egg weight during regimens A and F was 95.00 %HD and 80.00 %HD, and 63.9 g and 62.8 g, respectively.

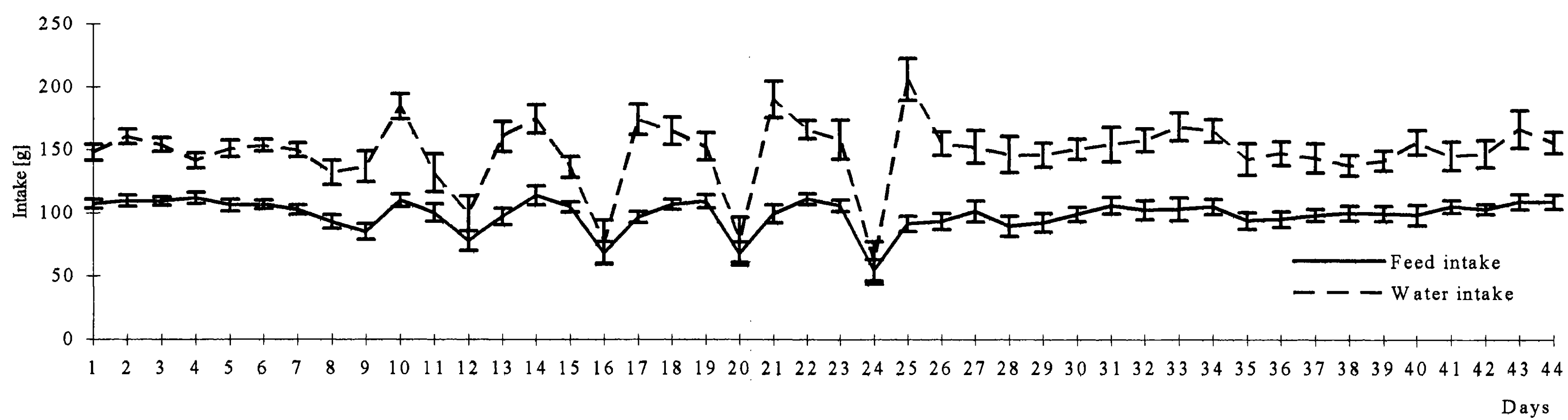
The daily feed and water intakes during each of the 44 days are presented in Figure 6.1, and the estimated methionine intake in the same period is shown in Figure 6.2. Each point is the mean ± SEM of the results from eighteen birds.

Common in both figures that four distinct phases can be observed, corresponding to the regimens of the feeding. During the first seven days, when the birds received plain water and Feed 1 (adequate methionine content), the standard errors were small and the birds' appetite for water and feed was without dramatic changes. At day 8, when the birds received plain water and Feed 2 (inadequate methionine content), they lost appetite for food and water. The decrease was more apparent by the following days of regimen B (days 12, 16, 20, and 24). When the birds received methionine-treated water (0.15%, w/v), or Feed 1 (regimens C and D) their appetite for feed and water increased up to the level of that in regimen A. When repeating the treatment, the same responses were observed. During the days when hens were on regimen B, feed intake had a greater variability which diminished when they proceeded to regimens C and D, that is when they received Feed 2 and methionine-treated water or Feed 1. At the end of the training period, birds increased their water intake more apparently than at the beginning. This, perhaps, indicates that they have learnt the benefit of treated water and colour cue. It has to be noted, however, that the birds were probably responding to thirst, as indicated by the water intake depression which gradually increased each time they progressed onto regimen B.

By day 25, it appeared that all birds had become trained to recognise, with the help of colour cues, which bottle has methionine-supplemented water or plain water, and it was decided to test the birds for their ability to regulate the consumption of methionine-treated water to satisfy their methionine requirement.

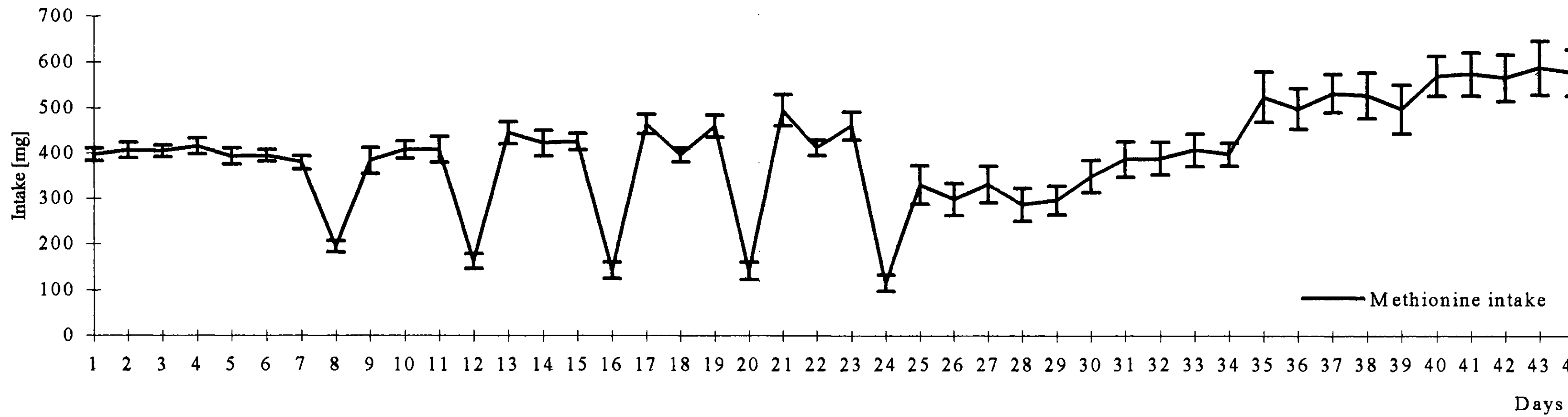
The pattern of methionine intake (Figure 6.2) follows the pattern of food and water intake (Figure 6.1) until the beginning of regimen F, when, increasing the methionine concentration in treated water resulted in a slight decrease of water intake, and initially a slight decrease in feed intakes but an increase of methionine intake. There is a significant correlation (r=0.761) between feed intake and water consumption.

Figure 6.1. Daily changes in feed intake and water consumption in association with the six feeding regimens.



Regimens	A	B	C	D	C	B	C	D	C	B	C	D	C	B	C	D	C	B	E	F
Days	7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	10	10

Figure 6.2. Daily changes in methionine intake in association with the six feeding regimens.



Regimens	A	B	C	D	C	B	C	D	C	B	C	D	C	B	C	D	C	B	E	F
Days	7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	10	10

Feed-, total water-, and methionine intakes during the regimens of the experiment are shown in Table 6.2. The comparison of values shows that regimen had significant ($p<0.001$) effect on intake of feed, water and methionine. When receiving deficient diet (regimen B), all three intakes of the birds reached their lowest in the experiment. Additionally, other feed intakes did not show significant differences from the control value during the experiment ($p>0.05$). Water intake also returned to the control level in regimen C, showed a significant increase in regimen D, and returned gradually again to the value of regimen A ($p>0.05$). The feed and water intake data indicate that the source of methionine did not influence normal appetite, and birds are able to recover their appetite within a day. Similarly to feed and water intakes, methionine intake also showed normal values (as in regimen A) once methionine was supplemented to the diet (regimens C and D) ($p>0.05$). Subsequently, methionine intake decreased when the birds moved to the first choice period (regimen E), however, giving the birds 0.3% methionine treated water during the second choice period (regimen F) resulted in an average of 145 mg/day more methionine consumption which was significantly greater ($p<0.001$) than the methionine intake during regimen A and the rest.

Comparing regimen B to A, it is apparent that the size of methionine deficiency during regimen B was 62% of the intake in regimen A. Moreover, the appetite for food was depressed by an average 32% during regimen B. However, excess methionine was not paired with a similarly substantial change in feed intake. Comparing regimens A and F, an average of 145 mg over-consumption of methionine can be observed, a significant difference ($p<0.001$), but this results only an average of 6 g decrease in feed intake, a not significant difference ($p>0.05$).

Table 6.2. Feed-, water-, and methionine intakes during the regimens of the experiment.

	Regimens						
	A (7 days)	**B (5 days)**	**C(8 days)**	**D (4 days)**	**E (10 days)**	**F (10 days)**	**LSD**
Feed intake [g/day]	107.7^{b}	72.3^{a}	99.8^{b}	110.8^{b}	98.6^{b}	101.2^{b}	**12.7**
Water intake [ml/day]	151.4^{b}	89.7^{a}	155.3^{bc}	172.6^{c}	160.7^{bc}	148.6^{b}	**20.1**
Methionine intake [mg/day]	398.5^{bc}	151.8^{a}	442.4^{c}	409.8^{bc}	348.3^{b}	544.2^{d}	**64.5**

^{abcd} Values within a row with no common superscript differ significantly ($p<0.001$).
Values are mean of n=16.

Water intake proportions during the choice period are presented in Table 6.3. The birds' proportional consumption from treated water was random (i.e. not significantly different from 50%; $p>0.05$) in regimen E (first choice period). In contrast, during regimen F (second choice period) the difference from 50% was significant ($p<0.05$). In addition, the high preference for treated water in regimen F resulted in an overall proportion of choices made in favour of methionine-treated water significantly different ($p<0.05$) from 50%.

Table 6.3. Intake proportions of treated water during the choice period of the experiment.

Regimens		
^{A}E (5 days)	**^{A}F (5 days)**	**^{B}Mean water intake proportions**
57.1 n.s.	73.5 s.	**65.3** s.

Water intakes are expressed as percentage of total (treated + untreated) water intake.
s. Difference from 50% is significant ($p<0.05$).
n.s. Difference from 50% is not significant ($p>0.05$).
Values are mean of $^{A}n=16$, and $^{B}n=32$.
LSD of $^{A}=18.7$, and $^{B}=13.2$.

6.1.4 Discussion

In the previous experiment (Experiment 3) more than 90% of the choices were made in favour of methionine-treated water. In contrast, in Experiment 4, although the birds clearly have learned to recognise the supplemented water by the end of the training period, the choices made in favour of methionine-treated water were only an average 57.1% in the first choice phase (regimen E). When progressing to the next choice period (F), this figure increased to 73.5%. A possible explanation can be that as neither bottles (red or yellow) were previously associated with the adverse effect of methionine deficiency, the birds drank at random from both at the beginning of the first choice phase. However, after a while (towards the end of regimen E), birds began to feel the difference between the two drinks in their metabolic effects, and began to discriminate in favour of the treated water. It might be that at this time, treated water was chosen already in a much higher proportion than the average figure, but the full effect on the result has only become apparent by the second choice period (regimen F). Further evidence for this explanation is provided by the daily methionine intake results. It is clear that from the middle of the first choice period, birds increased their methionine consumption so that by the last two days of the regimen, they have reached the level of methionine intake during the control period (regimen A). Feed and water intakes behaved in a similar manner. The reason behind this is that the correcting of methionine deficiency resulted in the "repair" of feed intake and, consequently, water intake.

A further observation in this experiment was that, increasing methionine concentration from 0.15% to 0.3% resulted in an increase of the intake from methionine-treated water, and decrease from plain water. The overall outcome was a decreased total water intake. The increase of concentration plus the increasing choice from treated water resulted in increasing total methionine consumption indicating that, below the harmful levels, there is no upper limit of methionine consumption by the birds. Thus, in practice, birds seem unable to regulate their methionine intake once the requirement is satisfied. This presumption is supported in the review by Hughes (1979) in which he noted the lack of evidence available that the birds will keep their intake below upper limits.

In theory, the intake of a nutrient is kept within lower and upper limits set by metabolic needs and maximal nutritional requirements. The nutrient deficit caused by the decreased intake to well below the lower limit has harmful physiological effects, which can be reversed by an inflow of the nutrient. As a result, well-being improves, which reinforces the animal's behaviour in selecting the appropriate food (Hughes and Wood-Gush, 1972; McFarland, 1973). This phenomenon is called "positive post-ingestional feedback". Indeed, there is evidence (reviewed by Hughes, 1979) that, in the case of several nutrients, the domestic fowl is able to keep nutrient intake above the lower limits necessary for growth, maintenance and production. In contrast, it appears that in practice, the final limit on consumption is not set by metabolic requirements but by palatability and, eventually, by adverse physiological effects (Hughes, 1979). Active rejection of a nutrient at high dietary levels was only showed in the case of phosphorus (Holcombe *et al.*, 1976a). Methionine intake seems to be governed by the same mechanisms since the results of Experiment 4 suggest that there seems to be no upper limit for methionine intake, at least within the range of concentrations used here which were below the harmful concentration of 10g/kg (Katz and Baker, 1975). In this experiment, the birds have carried on drinking from the methionine-treated water even after having satisfied their requirement.

A reduction of average body weight, egg production and egg weight was also observed in Experiment 4. Feed intake over the 37 days of the training and choice periods (regimens B-F) was an average of 97 g, i.e. 10 g less than in regimen A. Thus, it might be expected that a whole range of nutrients were in undersupply, and the above changes were a consequence of this. It is likely that there were birds which were always deficient in methionine because of not choosing treated water at all, as suggested by the fact that the choices (averaged over all birds) made in favour of methionine-treated water have reached a maximum of only 73.5%. It is likely that these birds contributed more to the decrease in performance than those choosing methionine-treated water during the choice period. In these birds, body protein turnover would have been used to supply the amino acids. This assumption is based on the report of Boorman (1979) that a mechanism exists in birds which temporarily prevents the distortion of the plasma and tissue amino acid levels. Thus, an amino acid deficiency results in the net catabolism of body proteins in order to supply the amino acids to prevent a distortion in the plasma and tissue amino acid levels. In support, Harms and Ivey (1992) and Harms and Russell (1995), for example, reported that hens receiving an amino acid deficient diet reduce their performance. It was also demonstrated (Harms and Russell, 1998) that, after the methionine depletion period, at least three weeks are needed to return to normal egg production.

The main conclusions of the experiments were:

1. when using 3 colours (red, yellow, blue) during the training of layers, birds show an appetite for the methionine-treated water in 73.5%;

2. the birds are unable to regulate their consumption of treated water.

6.2 Experiment 5

6.2.1 Introduction

Experiment 5 investigated the detection threshold of the birds for methionine. It has been shown by Rensch and Neunzig (1925) that a rejection threshold (i.e. a lowest concentration at which solutions are rejected) exists in birds for sodium chloride. They studied 60 species of birds and found that threshold levels range from as low as 0.35% (in parrots) to the extremely high 20% (in partridge). Our hypothesis was that a detection threshold also exists for methionine, i.e. that there is a minimum level of methionine delivered in water for which birds can express appetite when receiving a methionine-deficient diet. In order to investigate this threshold of the birds, different levels of methionine in the drinking water were used in combination with a methionine-deficient feed. The level of deficiency was the same in each case. In the previous experiments (Experiments 3 and 4) 0.15% methionine were used and hens were able to express an appetite for methionine treated water.

The aims were:

1. to determine the minimum level of methionine in drinking water for which birds can express appetite;

2. to determine the birds' ability to correct a methionine deficiency by increasing methionine water consumption, while maintaining normal egg production and body weight.

6.2.2 Materials and methods

6.2.2.1 Stock

Twenty-two weeks old Lohmann layers were used for the experiment. They were chosen at random from a flock of 1000 hens in the same house. A total of 32 laying hens were distributed into four groups of eight, then each group was further divided into two subgroups (A and B) of four hens in order to eliminate the effect of colour preference. The body weights (mean and SEM) of the four groups were 1856.4 ± 54.03 g for group 1, 1876.1 ± 50.85 g for group 2, 1814.4 ± 42.72 g for group 3, and 1865.8 ± 58.07 g for group 4; the differences between them were not significant ($p>0.05$). The birds were placed singly in cages. According to feeding regimens, two different coloured water suppliers and two waste-water collector cups (yellow, red), and one trough were located for each cage. The sides of the wire cages were made solid with 3-ply wood.

6.2.2.2 Diets

Two feed formulations (Feed 1, Feed 2) were used in this experiment, as shown in Table 3.3. Water was given in bottles coloured accordingly to the treatment and the birds were trained to recognise which bottle had methionine supplemented water or plain water. The treated water contained four concentrations of methionine (0.025% for group 1; 0.05% for group 2; 0.075% for group 3; 0.1% for group 4).

To determine normal feed intake, methionine intake and water consumption, the four groups of hens received Feed 1 and plain water for seven days (regimen A). During this time each hen received plain water supplied in red (subgroup A) or yellow (subgroup B) plastic bottles. Subsequently, all birds were transferred to the same feed without methionine supplementation (Feed 2) and given plain water for two days to induce a methionine deficiency. The untreated water was supplied in yellow (subgroup A) or red (subgroup B) plastic bottles (regimen B). In this way the hens were exposed to the metabolic effects of a methionine deficiency and allowed to associate it with a colour cue. Over the following two days the birds were offered the same feed (Feed 2), and water containing methionine (0.025% for group 1; 0.05% for group 2; 0.075% for group 3; 0.1% for group 4) was given from red (subgroup A) or yellow (subgroup B) bottles (regimen C). Thus the four groups of hens became accustomed to the effects of plain water and the four types of methionine-treated drinking water when consuming the deficient feed. The above four-day cycle (regimens B and C) was repeated once more. Thereafter, the birds received feed without methionine supplementation (Feed 2) and plain water from yellow (subgroup A) or red (subgroup B) bottles for an additional two days. During this training period (a total of 10 days), the two types of feed were given to the hens alternately every two days in order to allow them to become accustomed to the colour cue and physiological effect of the diets adequate or deficient in methionine. During the following five days, the birds, while still on Feed 2, were offered a choice of both plain water and one of the four types of methionine-treated water, from red and yellow bottles (regimen D). Thereafter, the position of the bottles was swapped and the choice offered for another five days (regimen E).

Each day the hens were allocated enough feed to just exceed their expected daily food intake. The plain water and the four levels of water-methionine mixture remaining in the bottles was discarded daily and replaced with fresh.

6.2.2.3 Measurements

Daily feed intake and water consumption was measured gravimetrically every 24 hours. Body weights were recorded at the beginning and end of the experiment. Eggs were collected daily and weighed individually during regimens A and E.

6.2.3 Results

6.2.3.1 Egg production and body weight

The body weights (mean and SEM) at the beginning and end of the experiment were 1856.4 ± 54.07 g and 1800.4 ± 67.55 g for group 1, 1876.1 ± 50.83 g and 1910.6 ± 62.14 g for group 2, 1814.4 ± 42.76 g and 1843.9 ± 51.72 g for group 3, 1865.8 ± 58.04 g and 1907.5 ± 41.92 g for group 4. Except for group 1, body weights had increased by the end of the experiment, although not significantly ($p>0.05$). The average rate of egg production (mean and SEM) during A and E were 98.2 ± 1.70 %HD and 95.0 ± 3.20 %HD for group 1; 98.2 ± 1.70 %HD and 100.0 ± 0.00 %HD for group 2; 94.6 ± 2.60 %HD and 92.5 ± 3.60 %HD for group 3; 94.6 ± 5.30 %HD and 97.5 ± 2.50 %HD for group 4, respectively. Egg weights (mean and SEM) during A and E were 54.9 ± 1.20 g and 53.3 ± 1.30 g for group 1; 54.6 ± 1.80 g and 58.8 ± 1.40 g for group 2; 54.1 ±

1.20 g and 57.7 ± 1.40 g for group 3; 56.0 ± 1.70 g and 58.7 ± 1.00 g for group 4, respectively. Group 1 showed decrease in egg weight while the other three groups showed an increase in egg weight, repeating the pattern of body weight changes.

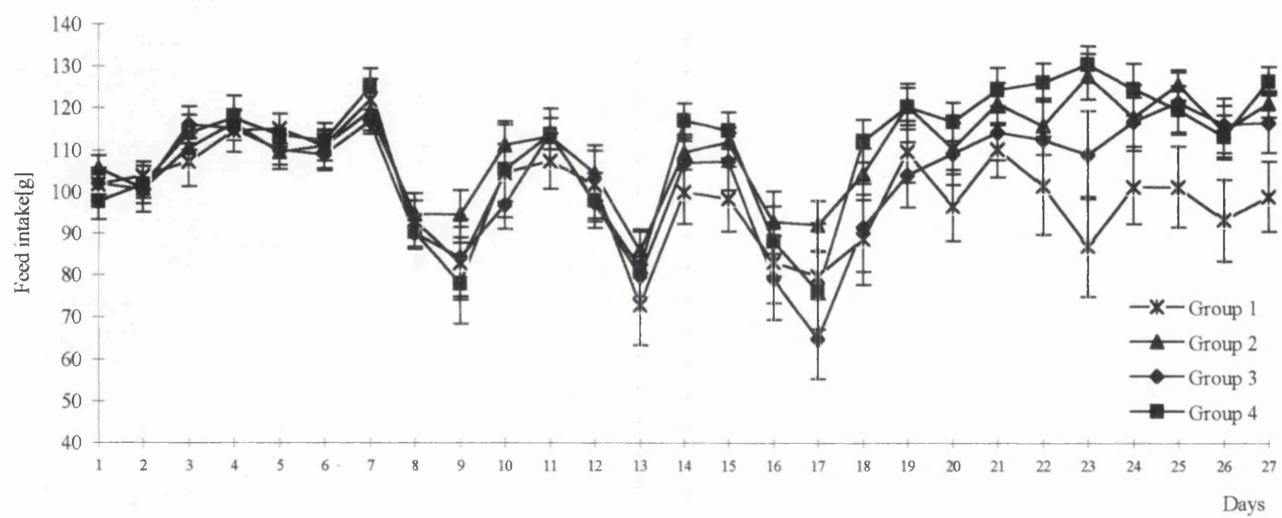
6.2.3.2 Daily feed-, water-, and estimated methionine intake

The daily feed and water intake, and the estimated methionine intake during each of the 27 days is presented in Figures 6.3, 6.4 and 6.5. Each point represents the mean ± SEM of the results from eight birds. Five distinct phases can be observed, corresponding to the regimens of the feeding. During the first seven days, when the birds received Feed 1 (adequate methionine content) and plain water, the standard errors are small and the birds' appetite for feed and water was without dramatic changes. At days 8 and 9, when the birds received plain water and Feed 2 (inadequate methionine content), all groups lost appetite for food and water; the decrease was more apparent by the second day of this regimen. When the four groups of birds received methionine-treated water (0.025%, w/v for group 1; 0.05%, w/v for group 2; 0.075%, w/v for group 3; 0.1%, w/v for group 4), their appetite for feed and water increased up to the level of that in regimen A, except for group 1. When repeating the treatment, the same responses were observed. During those days when the hens were on regimen B, the standard error of means were high which indicates that feed intake had a greater variability within and between the groups. Variability diminished when the groups proceeded to regimen C, except for group 1, that is when they received methionine-treated water (0.025%, w/v for group 1; 0.05%, w/v for group 2; 0.075%, w/v for group 3; 0.1%, w/v for group 4). By day 18, it appeared that all groups of birds had become trained to the colour cues and it was decided to test the birds for their ability to choose water supplemented with different levels of methionine. In regimen D, the choices made in favour of treated water was average 90.4% for group 1, 92.0% for group 2, 89.0% for group 3, 87.3% for group 4, while in regimen E the figure was 94.2% for group 1, 91.9% for group 2, 93.7% for group 3, 85.6% for group 4 (see also Table 6.6 in section 6.2.3.4).

In regimen A, feed:water ratios were 1:1.46 for group 1; 1:1.51 for group 2; 1:1.48 for group 3 and 1:1.43 for group 4. In regimen D, these ratios decreased in all groups when compared to regimen A (1:1.93 for group 1; 1:1.65 for group 2; 1:1.88 for group 3 and 1:1.82 for group 4). On the other hand, feed:water ratios in regimen E returned to values similar to those seen in regimen A, except for group 1. The feed:water ratios were 1:1.92 for group 1; 1:1.46 for group 2; 1:1.55 for group 3 and 1:1.43 for group 4.

The pattern of methionine intake (Figure 6.5) followed almost the feed intake and water intake pattern (Figures 6.3 and 6.4). Moreover, in contrast to feed intake, water intake pattern showed no dramatic differences between the four groups.

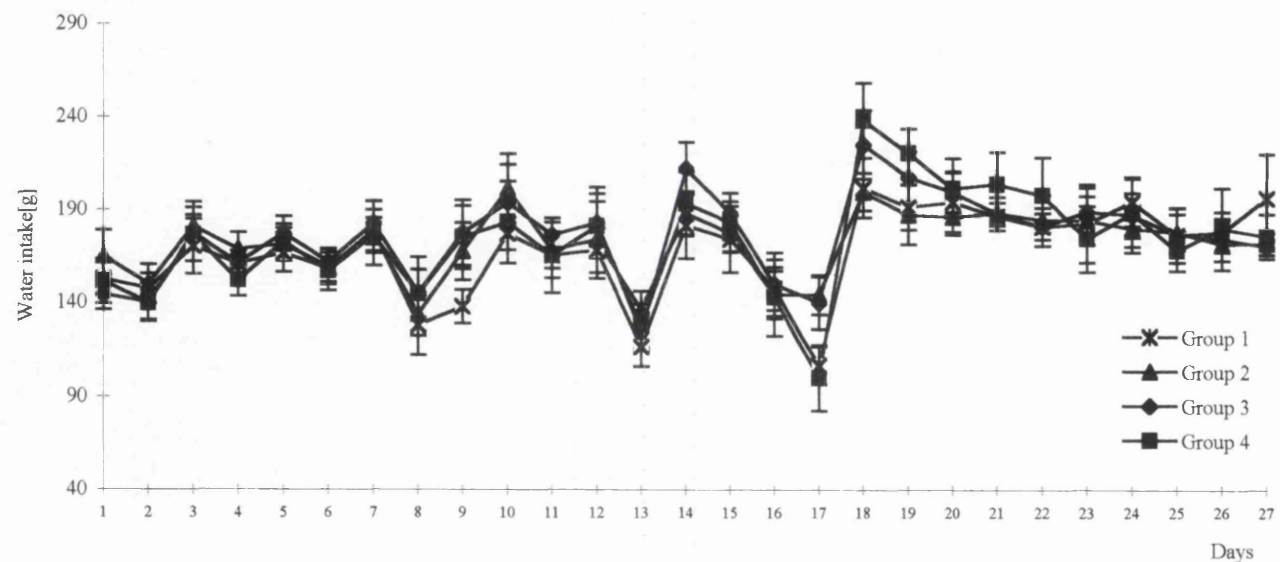
Figure 6.3. Daily changes in feed intake of birds receiving different amounts of methionine in drinking water during the five regimens of the experiment.



Regimens	A	B	C	B	C	B	D	E
Days	7	2	2	2	2	2	5	5

Methionine content of the drinking water was 0.025% for group 1, 0.05% for group 2, 0.075% for group 3, 0.1% for group 4.

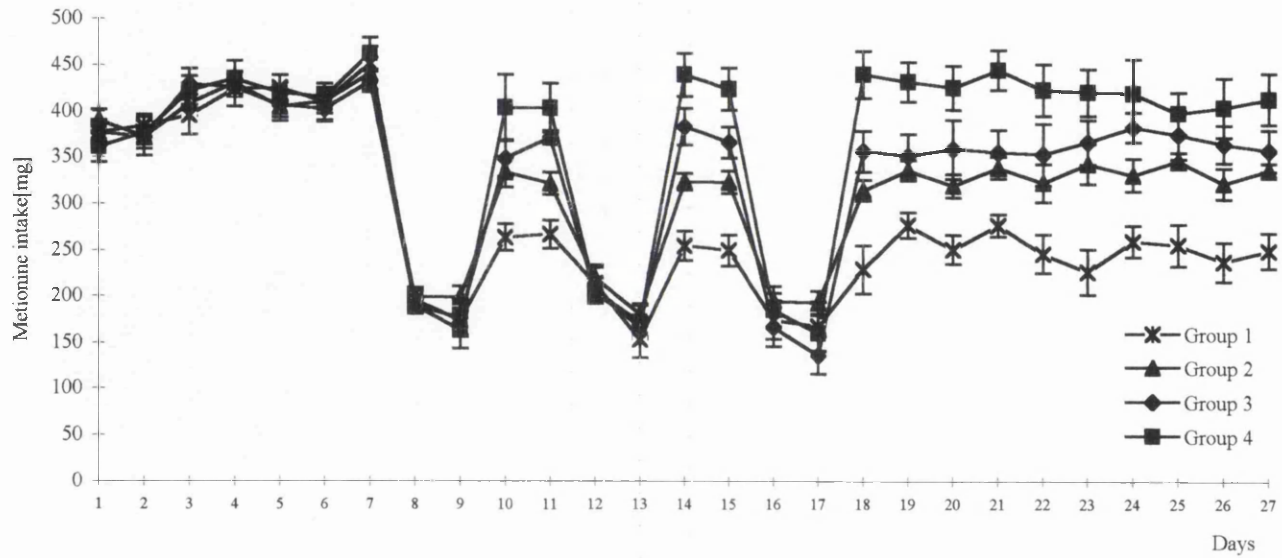
Figure 6.4. Daily changes in water intake of birds receiving different amounts of methionine in drinking water during the five regimens of the experiment.



Regimens	A	B	C	B	C	B	D	E
Days	7	2	2	2	2	2	5	5

Methionine content of the drinking water was 0.025% for group 1, 0.05% for group 2, 0.075% for group 3, 0.1% for group 4.

Figure 6.5. Daily changes in estimated methionine intake of birds receiving different amounts of methionine in drinking water during the five regimens of the experiment.



Regimens	A	B	C	B	C	B	D	E
Days	7	2	2	2	2	2	5	5

Methionine content of the drinking water was 0.025% for group 1, 0.05% for group 2, 0.075% for group 3, 0.1% for group 4.

6.2.3.3 Feed intake

Feed intakes during the regimens of the experiment are presented in Table 6.4. Different level of methionine in the drinking water had no significant effect on mean feed intakes. When the effect of regimen was examined significant differences ($p<0.001$) were found. The mean feed intake value in regimen B was significantly the lowest during the entire experiment. The highest intake mean was found in regimen E, significantly different from corresponding values in regimens B and C. The differences between regimens in response to different level of methionine in the drinking water were confirmed by a significant interaction between the regimens and the effects of treatment ($p<0.05$).

When the birds received adequate diet, there were no significant differences ($p>0.05$) in feed intakes between the four groups in regimen A. During regimen B when on deficient diet, the resulted reduction of feed intake was significant in all groups, moreover, there were no significant differences ($p>0.05$) between the groups. When they received only methionine-treated water (0.025%, w/v for group 1; 0.05%, w/v for group 2; 0.075%, w/v for group 3; 0.1%, w/v for group 4) all groups recovered their feed intake in regimen C and there were no significant differences ($p>0.05$) compared to regimen A. In addition, significant differences ($p>0.05$) were not observed between the groups within regimen C. After the training period, when the hens were allowed to choose between plain and treated water (regimen D), the feed intake of group 1 was significantly different from group 4 ($p<0.05$). In regimen E, the feed intake of groups 2, 3 and 4 were significantly different ($p<0.05$) from group 1. None of the groups in regimen D and E showed a significant difference ($p>0.05$) from the values in regimen A.

Table 6.4. Feed intakes during the regimens of the experiment in relation to the level of methionine in drinking water, and significance of effects of treatment, regimen, and their interaction.

Treatment	Regimens					
Methionine level in drinking water [%]	**A (7 days)**	**B (6 days)**	**C (4 days)**	**D (5 days)**	**E (5 days)**	**^{C}Mean feed intakes**
0.025 (group 1) ^{A}	110.7^{defgh}	85.6^{ab}	102.5^{cdef}	100.1^{bcde}	98.2^{abcd}	**99.4**
0.050 (group 2) ^{A}	110.3^{defgh}	94.1^{abc}	111.4^{defgh}	114.4^{efgh}	120.7^{gh}	**110.2**
0.075 (group 3) ^{A}	109.8^{cdefgh}	82.8^{a}	106.1^{cdefg}	106.0^{cdefg}	116.1^{fgh}	**104.2**
0.100 (group 4) ^{A}	111.7^{defgh}	85.4^{ab}	112.3^{defgh}	120.3^{gh}	123.5^{h}	**110.6**
^{B}Mean feed intakes	**$110.6^{2,3}$**	**87.0^{1}**	**108.1^{2}**	**$110.2^{2,3}$**	**114.6^{3}**	

	Probability	LSD
Effect of treatment	0.228	13.5
Effect of regimens	0.001	4.9
Interaction between the effect of treatment and regimens	0.012	15.9

^{abcdefgh} Values within a column with no common superscripts differ significantly ($p < 0.05$).
Feed intakes are expressed as g/day.
Values are mean of ^{A}n=8, ^{B}n=32, and ^{C}n=40.

6.2.3.4 Total water intake and water- (treated and untreated) intake proportions

Total water (treated and untreated) intakes during the regimens of the experiment are shown in Table 6.5. The level of methionine in drinking water did not but regimens affected significantly mean water intake values ($p>0.05$ and $p<0.001$, respectively). The values of regimens C and E were not significantly different, however, all other comparisons between regimen means showed significant differences. There was no significant interaction between level of methionine in the drinking water and regimens.

The overall preference of birds for treated and untreated water was 90.5% and 9.5% respectively, a significant difference from 50% ($p<0.001$). Table 6.6 shows the proportional intakes of treated water during the choice period. Proportions of treated water were significantly ($p<0.05$) greater than 50% (i.e. random choice) regardless of treatment or regimen. Consistently with this, overall values were also significantly ($p<0.05$) above 50%. In addition, no significant effects of treatment, regimen or their interaction was observed, indicating that methionine concentration of the treated water, and the position of bottles had no effect on water preference.

Table 6.5. Water (treated and untreated) intakes during the regimens of the experiment in relation to the level of methionine in drinking water, and significance of effects of treatment, regimen and their interaction.

	Regimens					
Methionine in drinking water [%]	**A (7 days)**	**B (6 days)**	**C (4 days)**	**D (5 days)**	**E (5 days)**	**^{C}Mean water intakes**
0.025 (group 1)^{A}	162.1	134.2	174.6	193.4	189.4	**170.7**
0.050 (group 2)^{A}	167.6	150.1	183.9	189.3	176.4	**173.5**
0.075 (group 3)^{A}	163.6	153.6	193.3	202.6	180.8	**178.8**
0.100 (group 4)^{A}	160.7	145.4	184.7	217.0	177.0	**177.0**
^{B}Mean water intakes	**163.5^{2}**	**145.8^{1}**	**184.1^{3}**	**200.0^{4}**	**180.9^{3}**	

	Probability	**LSD**
Effect of treatment	0.910	24.7
Effect of regimens	0.001	10.8
Interaction between the effect of treatment and regimen	0.371	31.0

Water intakes are expressed as ml/day.
Values are mean of ^{A}n=8, ^{B}n=32, and ^{C}n=40.

Table 6.6. Intake proportions of treated water during the choice period of the experiment in relation to the level of methionine in the drinking water, significance of treatment, regimen, and their interaction.

	Regimens		
Methionine in drinking water [%]	**D (5 days)**	**E (5 days)**	^{C}**Mean intake proportions**
0.025 (group 1)^{A}	90.4 s.	94.2 s.	**92.3** s.
0.050 (group 2)^{A}	92.0 s.	91.9 s.	**91.9** s.
0.075 (group 3)^{A}	89.0 s.	93.7 s.	**91.3** s.
0.100 (group 4)^{A}	87.3 s.	85.6 s.	**86.4** s.
^{B}**Mean intake proportions**	**89.7** s.	**91.4** s.	

	Probability	**LSD**
Effect of treatment	0.758	17.9
Effect of regimens	0.439	9.9
Interaction between the effect of treatment and regimens	0.679	19.7

Water intakes are expressed as percentage of total (treated + untreated) water intake.
s. Difference from 50% is significant ($p<0.05$).
Values are mean of ^{A}n=8, ^{B}n=32, and ^{C}n=16.

6.2.3.5 Methionine Intake

Methionine intakes during the regimens of the experiment are shown in Table 6.7. Comparison of the mean methionine intakes showed significant effect ($p<0.001$) of the different levels of methionine in the drinking water. Values increased with increasing amount of methionine supplementation, however, this change was not significant between groups 2 and 3. When the effect of regimens on mean methionine intakes was examined, significant differences were found ($p<0.001$). In regimen B, methionine intake decreased significantly compared to regimen A, then increased significantly in regimen C to a level significantly below of that in regimen A. Intake means did not change significantly during the remaining period of the experiment (regimens D and E). There was also a significant interaction between regimen and the level of methionine in the drinking water ($p<0.001$).

In regimen A, when the birds received the adequate diet (Feed 1), there were no significant differences ($p>0.05$) in methionine intake between the four groups. When the birds received the deficient diet (Feed 2), the mean methionine intake decreased significantly ($p<0.001$) in all groups during regimen B compared to regimen A. However, there were no significant differences ($p>0.05$) between the groups within regimen B. When the birds received only methionine-treated water during regimen C (0.025%, w/v for group 1; 0.05%, w/v for group 2; 0.075%, w/v for group 3; 0.1%, w/v for group 4), the comparison of methionine intakes showed significant differences ($p<0.05$) between the groups with the exception of groups 2 and 3; between these groups methionine consumption was not significantly different between them. This pattern stayed the same when the birds were on free-choice, in regimens D and E. When regimens A and C were compared, there was no significant difference ($p>0.05$) of methionine consumption between groups 3 and 4, but a significant difference ($p<0.001$) was observed between groups 1 and 2. Methionine consumption in groups 1, 2 and 3 significantly decreased after the training period (regimen D) when compared to regimen A. However, in regimen E only groups 1 and 2 were significantly different ($p<0.001$) from the corresponding values in regimen A.

Table 6.7. Methionine intakes during the regimens of the experiment in relation to the level of methionine in drinking water, and significance of effects of treatment, regimen, and their interaction.

Treatment			**Regimens**			
Methionine in drinking water [%]	**A (7 days)**	**B (6 days)**	**C (4 days)**	**D (5 days)**	**E (5 days)**	**^{C}Mean methionine intakes**
0.025 (group 1)^{A}	409.6^{def}	179.7^{a}	258.8^{b}	254.2^{b}	250.8^{b}	**270.6^{A}**
0.050 (group 2)^{A}	408.2^{def}	197.5^{a}	326.0^{c}	326.7^{c}	334.7^{c}	**318.6^{B}**
0.075 (group 3)^{A}	406.4^{def}	173.9^{a}	367.7^{cd}	356.7^{c}	370.8^{cde}	**335.1^{B}**
0.100 (group 4)^{A}	413.3^{def}	179.4^{a}	420.5^{f}	438.3^{f}	412.0^{def}	**372.7^{C}**
^{B}Mean methionine intakes	**409.4^{3}**	**182.6^{1}**	**343.3^{2}**	**344.0^{2}**	**342.1^{2}**	

	Probability	**LSD**
Effect of treatment	0.001	35.5
Effect of regimen	0.001	15.9
Interaction between the effect of treatment and regimen	0.001	44.9

^{abcdef} Values within a column with no common superscripts differ significantly ($p < 0.05$).
Methionine intakes are expressed as mg/day.
Values are mean of ^{A}n=8, ^{B}n=32, and ^{C}n=40.

6.2 4 Discussion

Whereas the feed intake of birds is determined primarily by the energy content of the feed, their water intake can increase more readily under certain circumstances (e.g. an increase of temperature) (Hill *et al.*, 1979). Using this feature, Experiment 5 tested the birds' ability to regulate the methionine intake, by giving them different levels of methionine in the water.

It is clearly shown by the results that birds fed on a methionine deficient feed are able to select the water supplemented with methionine, as even the lowest amount of methionine in the water proved to be enough to improve the birds' well-being, and was, therefore, selected for.

That there were no substantial differences between the feed:water ratios of the four treatment groups in regimen A reflected that the groups had similar feed and water intakes before training had started. In regimen D, however, decreased feed:water ratios were observed in all groups; that is water intakes increased considerably while feed intakes remained virtually unchanged. The reason for this can be that by increasing their water intake, the birds were compensating the effects of the previous, methionine-depriving period (regimen B). Progressing to regimen E, the feed:water ratio of groups 2, 3 and 4 increased, that is their water intake decreased below the levels observed in regimen D. This might be because during the five days of regimen D, these birds have satisfied their methionine requirements. Methionine intake results support this explanation as the consumption by the three groups in question exceeded the 300 mg/day recommended by NRC (1994). By contrast, water intake by group 1 remained unchanged from regimen D to E indicating that these birds still showed an appetite for methionine. Indeed, their methionine intake was below the requirements.

It is known that drinking is initiated whenever there is an actual or potential deficit in the body water pool (Hill *et al.*, 1979), thus the control of drinking helps to maintain the water balance. The present results suggest that, except from polydipsic ones, birds under the methionine deficient conditions can only drink an average of 30-40 g/day more than normally. Therefore, it is suggested that the consumable amount of water should contain the amount of methionine necessary for the full compensation of the deficiency in drinking water.

In this experiment, on average, 90% of the choices were made in favour of treated water, regardless of the concentration of methionine in it. Thus, it can be stated that the level of methionine in drinking water for which birds can express their appetite is at least 0.025 %. However, the minimum level is probably much lower. Because the optimum intake of the first limiting amino acid will influence the optimum intakes of all other amino acids (Gous and Kleyn, 1988), birds will show an appetite for any amount of methionine in drinking water that gives well being.

Feed intake oscillated when hens were fed diets adequate and deficient in methionine alternately because the consumption of balanced and deficient diets increased and decreased, respectively. Response was rapid regardless of the different methionine concentrations in the water.

The balanced diet (Feed 1) permitted a high level of production of egg mass (90 %HD). Supplementation of DL-methionine in drinking water increased feed intake after the methionine-depletion period. Where the methionine consumption rose above the NRC 1994 requirement (groups 2, 3, 4), egg weight increased. For a particular hen, age is the primary factor in determining the egg weight: egg weight increases with age with progressively smaller increments, but environment and diet can change it (Rose, 1997). Indeed, in this experiment, egg weights were higher at the end than at the beginning of the experiment. Birds were 22 weeks old when the experiment started and were 26 weeks old by the end. This period is at the beginning, i.e. the steepest part, of the egg weight-layer age curve. In contrast, egg weights decreased in group 1, because the amount of methionine was not enough to maintain normal egg production.

These results are consistent with previous reports on methionine requirements of laying hens (Harms and Waldroup, 1963; Carlson and Guenthner, 1969; Schutte *et al.*, 1984).

The main conclusions of the experiments were:

1. when receiving a methionine-deficient feed, hens are able to express an appetite for water containing methionine in a concentration as low as 0.025%;
2. when supplying the hens a methionine-deficient feed and methionine-treated drinking water, in an attempt to meet their methionine requirement the birds can drink 30-40 g/day more than when kept on a normal diet (i.e. adequate feed plus plain water);
3. 90% of the choices were made in favour of the treated water regardless of its methionine concentration.

7.0 THE EFFECT OF THE WAY OF METHIONINE DELIVERY ON FEED- AND WATER INTAKE

7.1 Experiment 6a

7.1.1 Introduction

Previous experiments (Experiments 3, 4, and 5) indicated that in response to the deficiency of methionine, feed intake decreased on the first day and became even more apparent by the second day. The results also suggested that, in its metabolic effects, methionine taken with water is as effective as when taken with feed. When introducing methionine in drinking water, feed intake increased on the first day and was usually fully recovered by the second day. However, a similar study has not been performed with feed. Therefore, Experiment 6a has been designed to investigate whether or not the method of delivery of supplementary methionine (in water or feed), affects the time needed for feed intake recovery.

The aims were:

1. to determine the daily feed and water intake patterns when birds receive methionine deficient feed and methionine is supplemented in the feed or drinking water;

2. to determine more exactly how long it takes (in hours rather than days) for the hens to begin to show the feed intake depressing effect of methionine deficiency.

7.1.2 Materials and methods

7.1.2.1 Stock

A total of 36 Lohmann laying hens (36 weeks old) were caged individually. They were chosen at random from a flock of 1000 hens in the same house, and had not been previously used in an experiment. The birds were distributed into two groups (group 1 and group 2) of equal number and placed singly in cages. The average body weights (mean ± SEM) of the two groups (2038.0 ± 46.34 g for group 1 and 2187.8 ± 46.37 g for group 2) were not significantly different ($p>0.05$). One water bottle, one waste-water collector cup, and one trough was allocated to each cage.

7.1.2.2 Diets

Two feed formulations (Feed 1, Feed 2) were used in this experiment, as shown in Table 3.3. The experiment consisted of three feeding regimens (regimens A, B and C). During the first 4 days (regimen A), group 1 received untreated water plus Feed 1 with 140 g/kg CP and 3.7 g/kg methionine (i.e. an adequate amount), while group 2 received 0.1% methionine-treated water plus Feed 2 containing 140 g/kg CP and 2.1 g/kg methionine (i.e. a deficient amount). After regimen A, all birds were transferred to Feed 2 without methionine supplementation and given plain water for two days to induce a methionine deficiency (regimen B). Over the following 4 days, both groups were returned to their previous diets (regimen C).

7.1.2.3 Measurements

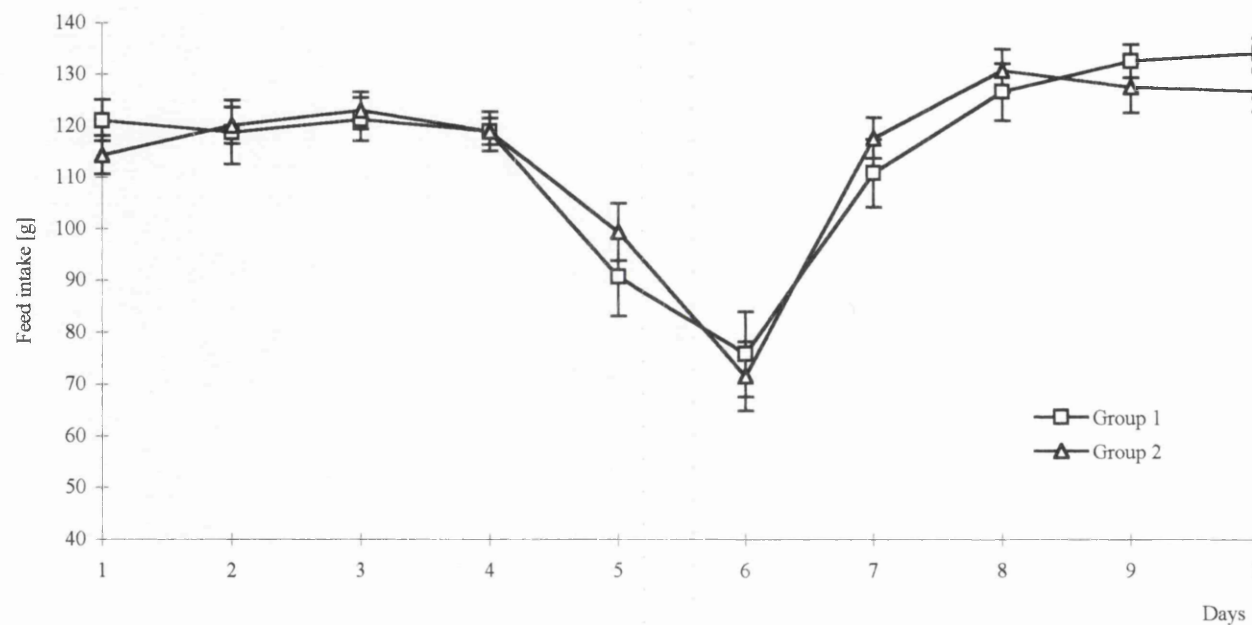
Food intake and water intake was recorded daily. Methionine intake was calculated from the amounts consumed via feed and water. Additionally, feed and water intakes were recorded hourly on day 4 (in regimen A), days 5 and 6 (in regimen B), days 7 and 8 (in regimen C). All data were obtained on an individual hen basis.

7.1.3 Results

7.1.3.1 Daily feed- and water intake

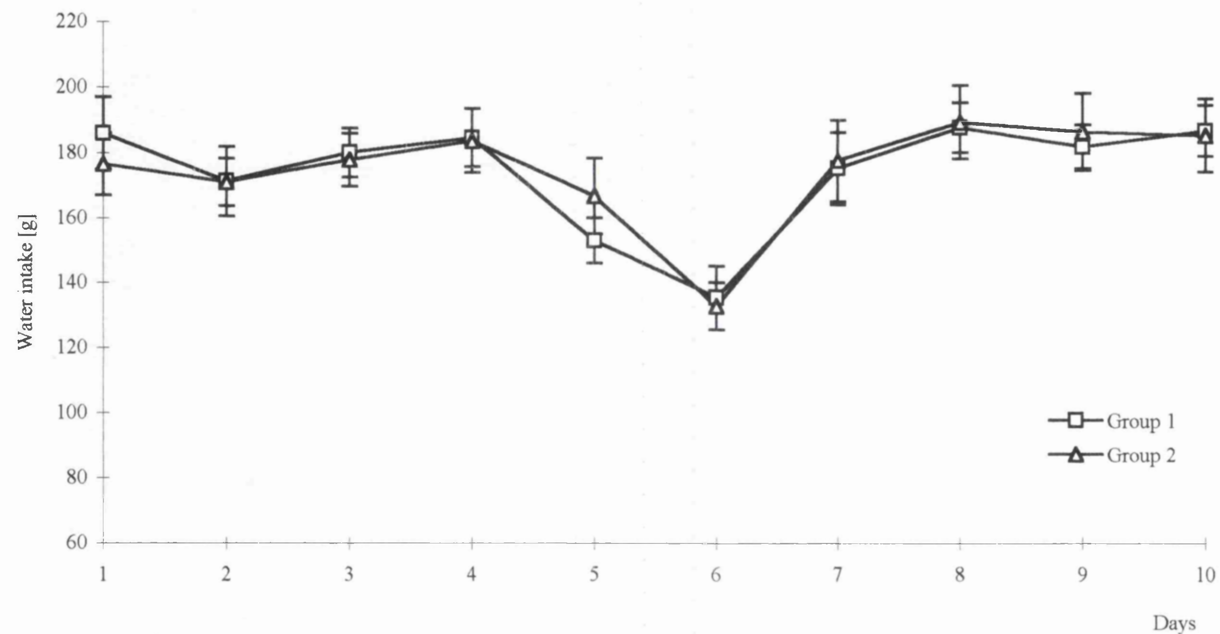
Daily feed and water intake data during each of the 10 days are presented in Figures 7.1 and 7.2, respectively. Each point is the mean ± SEM of the results from eighteen birds. Three distinct phases can be observed, corresponding to the feeding regimens. During the first four days of the experiment (regimen A), when both groups of birds received adequate amount of methionine, the standard errors were small and the birds' appetite for water and feed was almost the same, regardless of the way of supplementation. In the next two days when the birds received inadequate amount of methionine (regimen B), both groups lost appetite for food and water, and the decrease was more apparent by the second day. Finally, when the birds received adequate amount of methionine again, their appetite for feed and water increased up to the level of that in regimen A.

Figure 7.1. Daily changes in feed intake when methionine is supplemented in feed (group 2) or added in drinking water (group 1).



Regimens	A	B	C
Days	4	2	4

Figure 7.2. Daily changes in water intake when methionine is supplemented in feed (group 2) or added in drinking water (group 1).



Regimens	A	B	C
Days	4	2	4

Feed-, water-, and methionine intakes during the regimens of the experiment are shown in Table 7.1. The effect of regimens was significant ($p<0.001$) on the means of feed-, water-, and methionine intake. All three intakes decreased when methionine was withdrawn from the diet (regimen B), then increased again to the control level when methionine was supplemented in the diet (regimen C). There were no other significant effects ($p>0.05$) of either treatment or treatment x regimen interaction. This indicates that the source of methionine does not influence normal appetite.

Table 7.1. Feed-, water-, and methionine intakes during the regimens of the experiment in relation to the way of methionine delivery, and significance of effects of treatment, regimen, and their interaction.

Treatment		**Regimens**			
Source of methionine		**A (4 days)**	**B (2 days)**	**C (4 days)**	^{C}**Mean intakes**
Feed (group 1)	^{A}Feed Intake	120.1	83.3	126.1	**109.8**
Water (group 2)	^{A}Feed Intake	119.1	85.6	125.7	**110.1**
	^{B}**Mean feed intakes**	**119.6**^{2}	**84.4**^{1}	**125.9**^{2}	
Feed (group 1)	^{A}Water Intake	180.5	144.3	183.2	**169.3**
Water (group 2)	^{A}Water Intake	177.3	149.9	184.9	**170.7**
	^{B}**Mean water intakes**	**178.9**^{2}	**147.1**^{1}	**184.0**^{2}	
Feed (group 1)	^{A}Methionine Intake	432.7	319.3	448.0	**400.0**
Water (group 2)	^{A}Methionine Intake	440.8	316.6	465.1	**407.5**
	^{B}**Mean methionine intakes**	**436.7**^{2}	**317.9**^{1}	**456.6**^{2}	

	Probability		
	Feed Intake	**Water Intake**	**Methionine Intake**
Effect of treatment	0.960	0.906	0.697
Effect of regimen	0.001	0.001	0.001
Interaction between the effect of treatment and regimen	0.871	0.542	0.636

Feed intakes are expressed as g/day, methionine intakes are expressed as mg/day, water intakes are expressed as ml/day.
Values are mean of ^{A}n=18, ^{B}n=36, and ^{C}n=54.

7.1.3.2 Hourly feed- and water intake

In general, when the feed and water intakes by the two groups were measured hourly, similar patterns were observed. The feed intake was steady during the first 11-12 hours, followed by a substantial increase for 3-4 hours, then a sharp decrease in the last hour before the lights were turned off. The pattern of water intake followed a similar sequence, that is, there was a steady, slight increase in the first two-thirds of the day, followed by a sharp increase then a drop. The intake patterns obtained on days 5, 6, 7 and 8 were compared to those of day 4.

Figures 7.3 and 7.4 show the comparison of hourly feed and water intake between day 4 and 5, respectively. On day 5, when the methionine-deficient diet was introduced to the hens, both feed and water intake showed a pattern similar to that on day 4 for the first 6 hours. Then, however, both feed and water intakes decreased which became the most prominent by the 10th hour. Thereafter, both feed and water intake increased until the last 1-2 hours when it decreased.

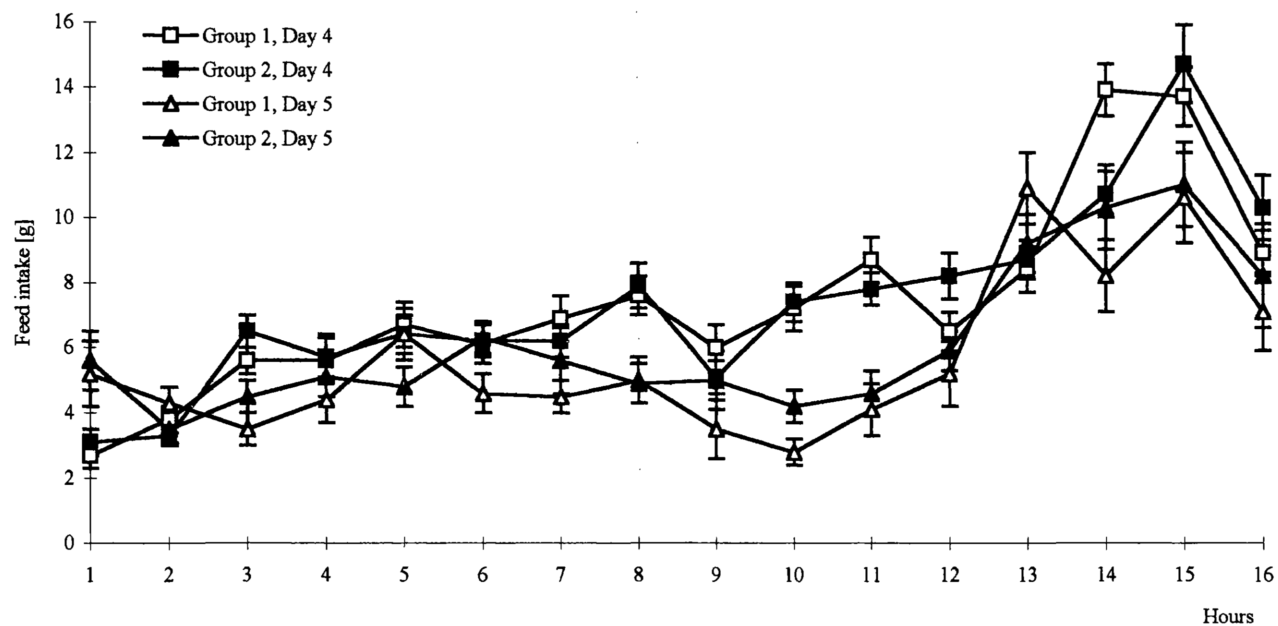
Figures 7.5 and 7.6 show the comparison of hourly feed and water intake between day 4 and 6, respectively. On day 6, i.e. on the second day of methionine deficiency, the general pattern of both the feed and water intake was similar to that observed on day 4, however, the values were significantly lower up to the last quarter of the observation period. Nevertheless, by the 12th hour, the feed and water intake of the hens on day 6 had caught up with the 4th-day (hourly) levels, and continued to increase until two hours before the lights went off. During the last two hours, both intakes decreased.

Figures 7.7 and 7.8 show the comparison of hourly feed and water intake between day 4 and 7, respectively. When the birds were returned to diets containing the adequate amount of methionine, the feed intake of group 1, but not group 2, was initially below the values on day 4 (when diets were also adequate in methionine). However, after the first 4 hours, the additional methionine (in the feed or in drinking water) has shown an effect, and the feed intake was similar to that of day 4. In contrast, a similar pattern was not found in water intake in the first hours, i.e. the values on days 4 and 7 were very similar from the start.

Figures 7.9 and 7.10 show the comparison of hourly feed and water intake between days 4 and 8, respectively. The pattern of feed intake and water intake were almost the same on day 8, moreover, they were not different from the patterns on day 4. This indicates that the birds can recover their appetite within two days after the methionine deficiency.

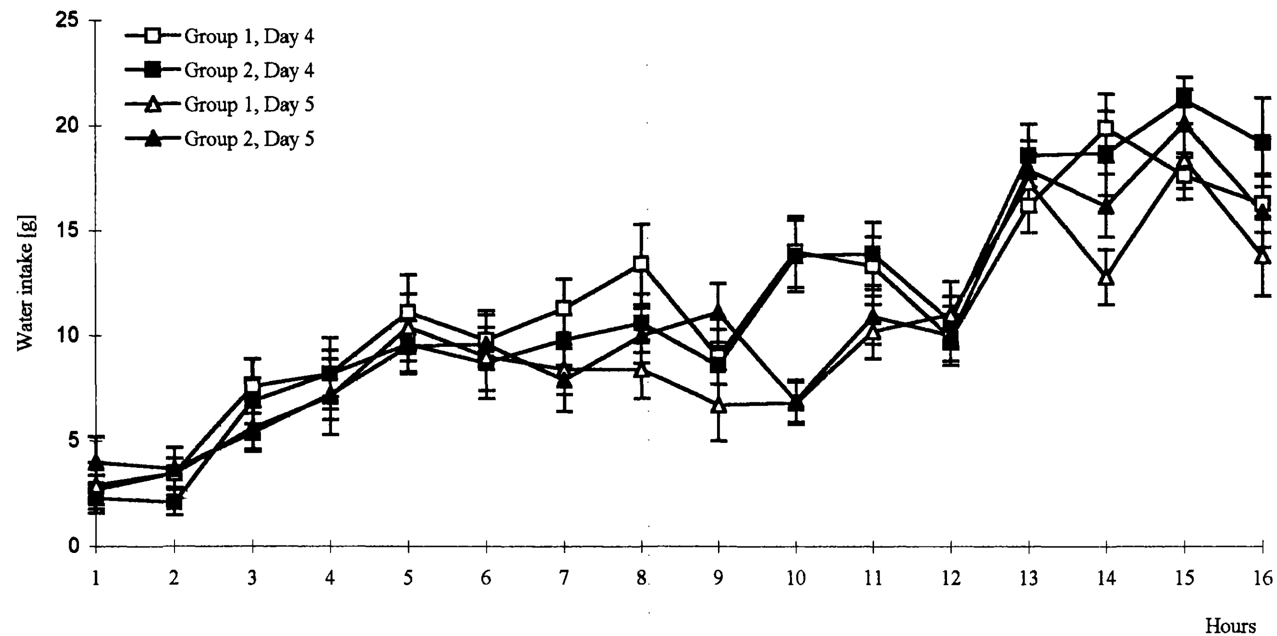
Figures 7.11 and 7.12 show the comparison of hourly cumulative feed and water intake patterns between day 4 and 5, respectively. It is clear, that the metabolic effects of methionine deficiency showed only after 6 hours, when the birds began to reduce their feed and water intake. However, this decrease became significant ($p<0.05$) only after 8 hours.

Figure 7.3. Comparison of hourly feed intake of hens between day four and five.



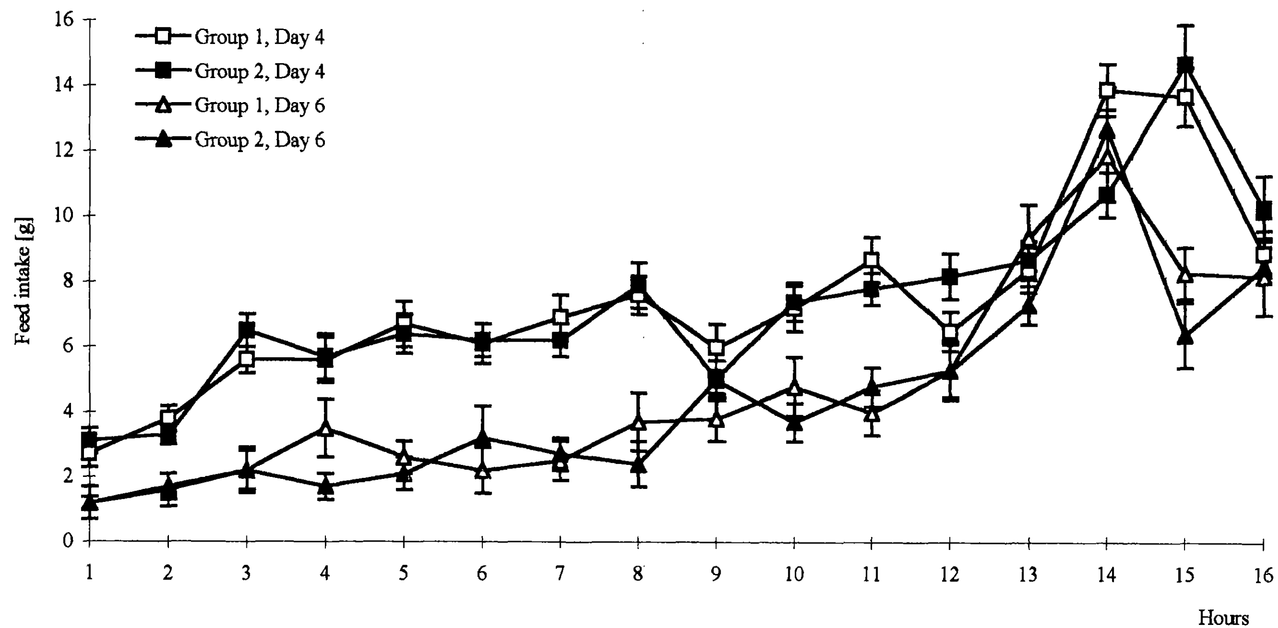
Light Off	Light On	Light Off
On day 4	Group 1 fed methionine adequate diet and plain water	
On day 4	Group 2 fed methionine deficient diet and methionine treated water	
On day 5	Group 1 fed methionine deficient diet	
On day 5	Group 2 fed methionine deficient diet	

Figure 7.4. Comparison of hourly water intake of hens between day four and five.



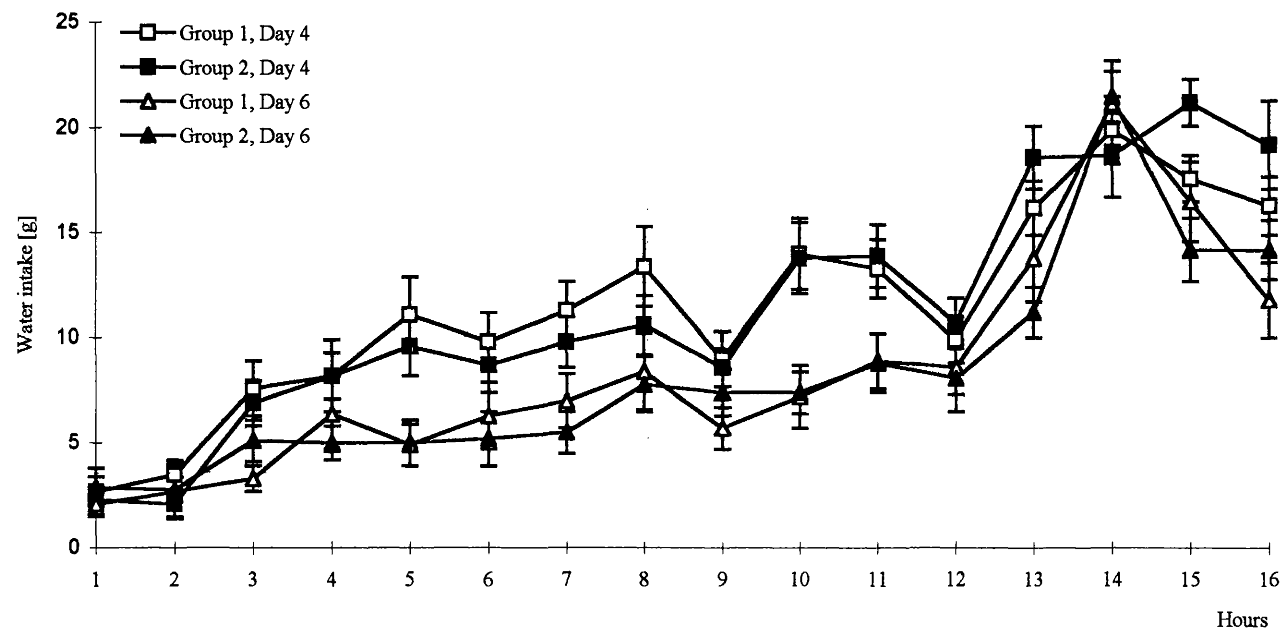
Light Off	Light On	Light Off
On day 4	Group 1 fed methionine adequate diet and plain water	
On day 4	Group 2 fed methionine deficient diet and methionine treated water	
On day 5	Group 1 fed methionine deficient diet	
On day 5	Group 2 fed methionine deficient diet	

Figure 7.5. Comparison of hourly feed intake of hens between day four and six.



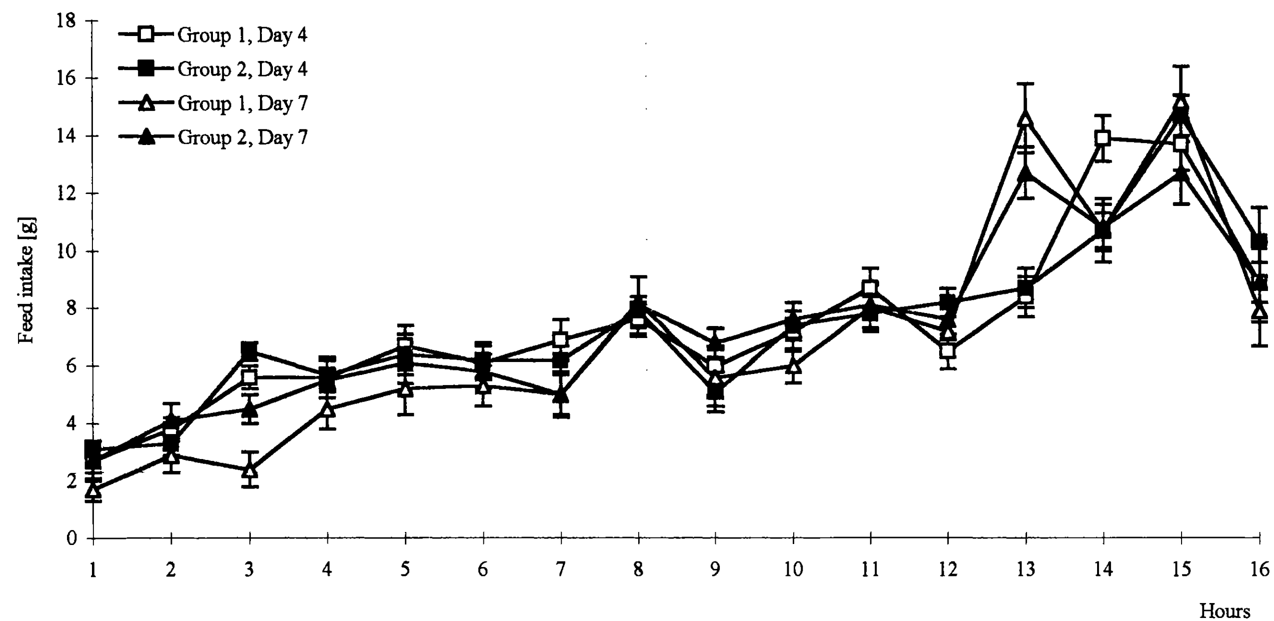
Light Off	Light On	Light Off
On day 4	Group 1 fed methionine adequate diet and plain water	
On day 4	Group 2 fed methionine deficient diet and methionine treated water	
On day 6	Group 1 fed methionine deficient diet	
On day 6	Group 2 fed methionine deficient diet	

Figure 7.6. Comparison of hourly water intake of hens between day four and six.



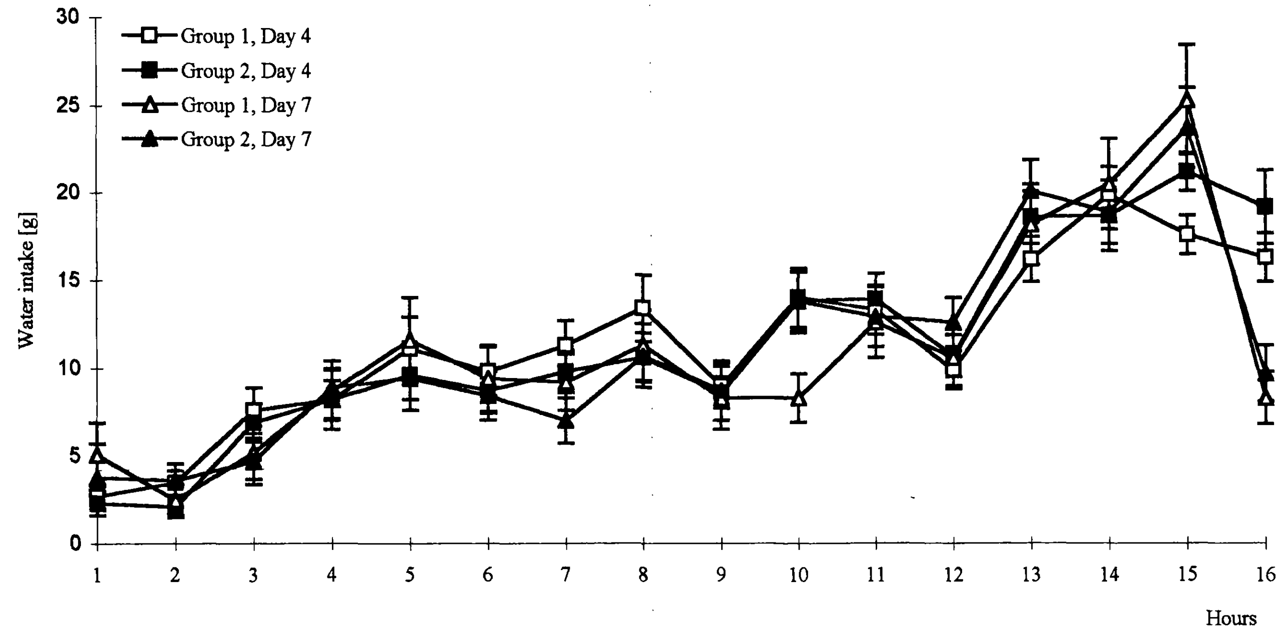
Light Off	Light On	Light Off
On day 4	Group 1 fed methionine adequate diet and plain water	
On day 4	Group 2 fed methionine deficient diet and methionine treated water	
On day 6	Group 1 fed methionine deficient diet	
On day 6	Group 2 fed methionine deficient diet	

Figure 7.7. Comparison of hourly feed intake of hens between day four and seven.



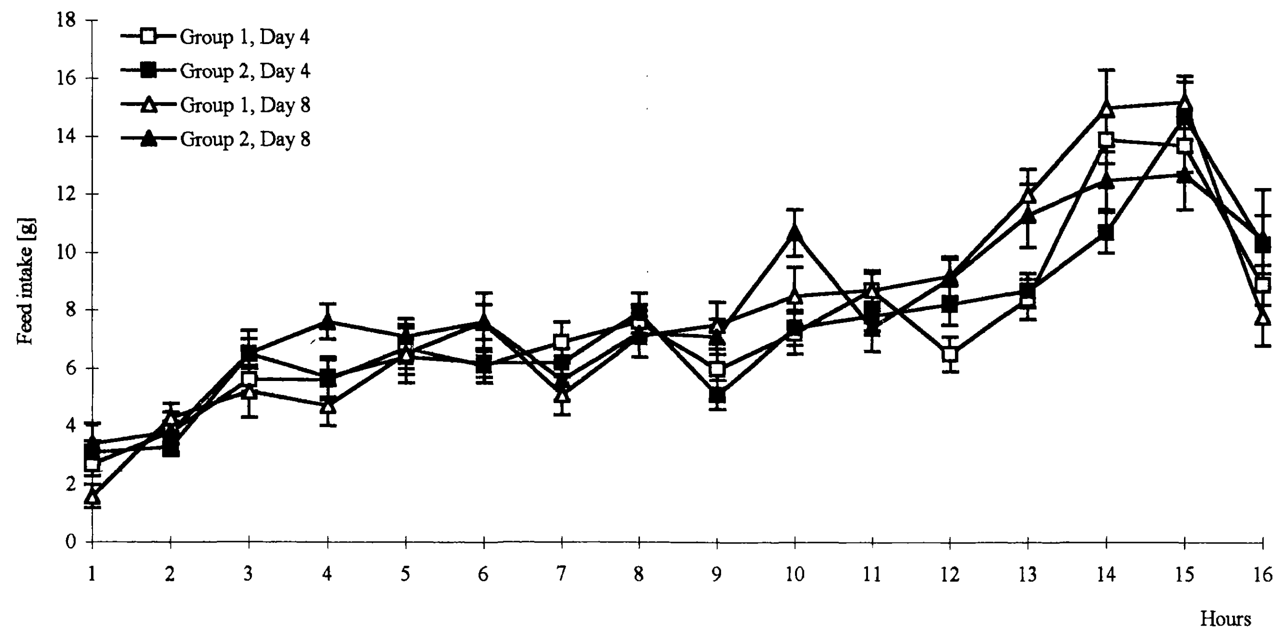
Light Off	Light On	Light Off
On day 4	Group 1 fed methionine adequate diet and plain water	
On day 4	Group 2 fed methionine deficient diet and methionine treated water	
On day 7	Group 1 fed methionine adequate diet and plain water	
On day 7	Group 2 fed methionine deficient diet and methionine treated water	

Figure 7.8. Comparison of hourly water intake of hens between day four and seven.



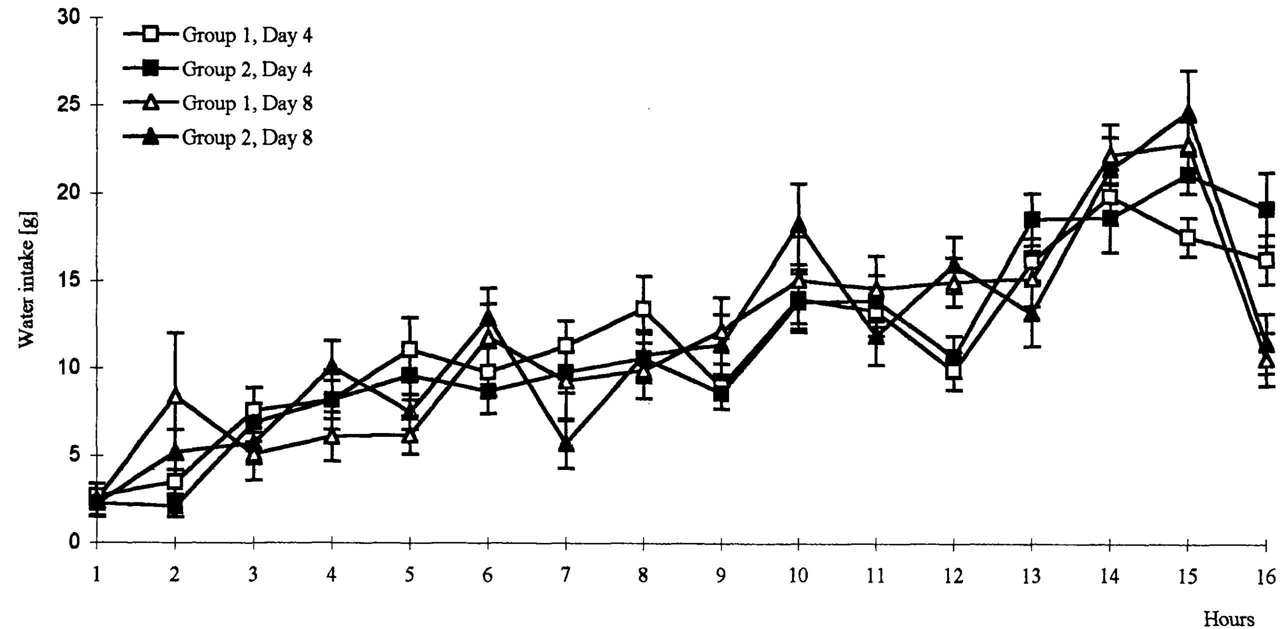
Light Off	Light On	Light Off
On day 4	Group 1 fed methionine adequate diet and plain water	
On day 4	Group 2 fed methionine deficient diet and methionine treated water	
On day 7	Group 1 fed methionine adequate diet and plain water	
On day 7	Group 2 fed methionine deficient diet and methionine treated water	

Figure 7.9. Comparison of hourly feed intake of hens between day four and eight.



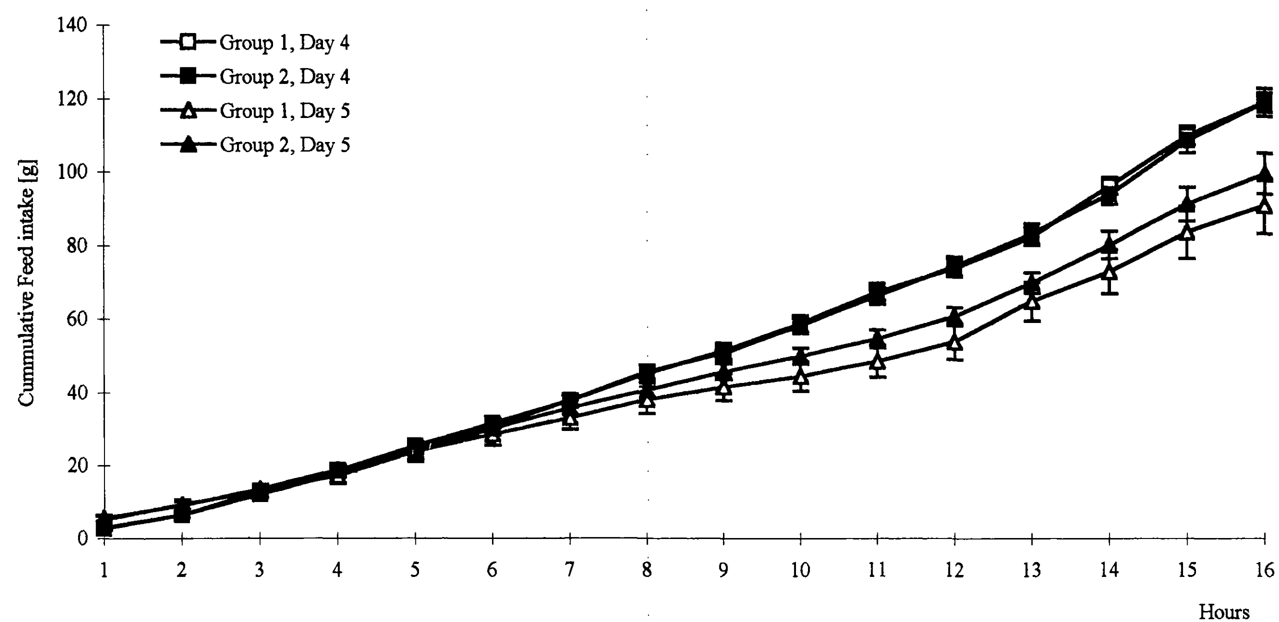
Light Off	Light On	Light Off
On day 4	Group 1 fed methionine adequate diet and plain water	
On day 4	Group 2 fed methionine deficient diet and methionine treated water	
On day 8	Group 1 fed methionine adequate diet and plain water	
On day 8	Group 2 fed methionine deficient diet and methionine treated water	

Figure 7.10. Comparison of hourly water intake of hens between day four and eight.



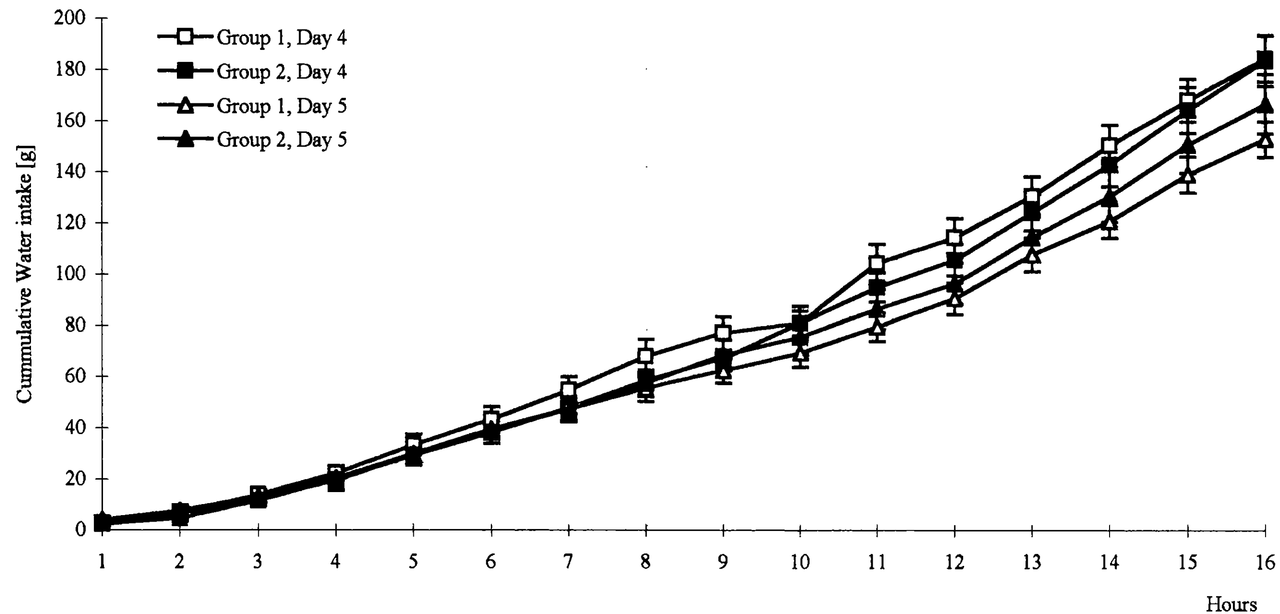
Light Off	Light On	Light Off
On day 4	Group 1 fed methionine adequate diet and plain water	
On day 4	Group 2 fed methionine deficient diet and methionine treated water	
On day 8	Group 1 fed methionine adequate diet and plain water	
On day 8	Group 2 fed methionine deficient diet and methionine treated water	

Figure 7.11. Comparison of hourly cumulative feed intake of hens between day four and five.



Light Off	Light On	Light Off
On day 4	Group 1 fed methionine adequate diet and plain water	
On day 4	Group 2 fed methionine deficient diet and methionine treated water	
On day 5	Group 1 fed methionine deficient diet	
On day 5	Group 2 fed methionine deficient diet	

Figure 7.12. Comparison of hourly cumulative water intake of hens between day four and five.



Light Off	Light On	Light Off
On day 4	Group 1 fed methionine adequate diet and plain water	
On day 4	Group 2 fed methionine deficient diet and methionine treated water	
On day 5	Group 1 fed methionine deficient diet	
On day 5	Group 2 fed methionine deficient diet	

7.1.4 Discussion

The daily feed, water and methionine intake results of Experiment 6a have confirmed that the way of delivering methionine to layers does not influence their normal appetite. It has also been indicated that, the feed intake of birds experiencing a methionine deficiency returns to normal following the addition of methionine, regardless of whether it is supplied in feed or water.

Hourly measurements of feed intake revealed that the hens' feeding activity increased in the afternoon, and peaked in the evening, then food consumption declined in the last hour of the photoperiod. This pattern is common in layers (Savory, 1980) and, in general, in fowls living in natural lighting, irrespective of whether they are laying or not (Wood-Gush, 1959; Savory *et al.*, 1978). Observations made on wild birds in Alaska by Irving *et al.* (1967) showed that birds eat more at the end of the day, particularly in winter when nights are long, to store food in their crops to last the night. Therefore, it was assumed (Savory 1980) that this could be the main function of the evening peak observed in hens. In addition, the evening feeding peak could be associated with the timing of oviposition or egg formation (Savory 1980; Morris and Taylor, 1967). The present experiment has also demonstrated that this pattern as well as the amount of feed intake is statistically, and therefore metabolically, the same when methionine is added to the feed or the water.

The hourly measurements have also confirmed that feed intake recovery is independent of the way of methionine supplementation.

Any effect on the results of oxidation of methionine to aldehyde was assumed to be minimal because: (1) the methionine-treated water was changed every day; (2) where consumption of methionine-treated water was measured hourly there was no consistent decline in that consumption from hour to hour (Figures 7.4 and 7.12).

The main conclusions of the experiment were:

1. deficiency symptoms (i.e. reduced feed intake) occur 8 hours after consuming a methionine-deficient diet;

2. feed intake recovery is independent of the way of methionine supplementation;

3. the way of methionine delivery does not affect the patterns of the daily or hourly feed- and water intake;

4. the hens' feeding activity increases in the afternoon, peaks in the evening, and declines in the last hour of the photoperiod.

7.2 Experiment 6b

7.2.1 Introduction

Based on the conclusions of Experiment 6a, it was expected that, if the birds are given a methionine-deficient diet during the period of increased feed intake (i.e. during the last five hours of the lighting period), their feed intake will be reduced in the following morning even if receiving normal diet. The rationale behind this is that during the eight hours of darkness, the plasma amino acid pattern becomes deranged as a consequence of digesting the deficient diet (Harper and Rogers, 1965). Experiment 6b was therefore performed to test this hypothesis.

The aims were:

1. to determine the feed and water intakes of birds on three consecutive days, when receiving a methionine-deficient feed and methionine is added to the feed or drinking water;

2. to determine the hourly feed and water intake of birds on the same three days.

7.2.2 Materials and methods

7.2.2.1 Stock

A total of 32 Lohmann laying hens (54 weeks old) were caged individually. They were chosen at random from a flock of 1000 hens in the same house, and had not been previously used in an experiment. The birds were distributed into two groups (group 1 and group 2) of equal number and placed singly in cages. The average body weight of the two groups (2046.9 ± 44.72 and 2177.8 ± 56.86) was not significantly different ($p>0.05$). One water, one waste-water collector cup, and one trough were allocated to each cage.

7.2.2.2 Diets

Two feed formulations (Feed 1, Feed 2) were used in this experiment (Table 3.3). The experiment consisted of three feeding regimens (regimen A, B and C). On the first day (regimen A), group 1 received untreated water plus Feed 1 with 140 g/kg CP and 3.7 g/kg methionine (i.e. an adequate amount), while group 2 received 0.1% methionine treated water plus Feed 2 containing 140 g/kg CP and 2.1 g/kg methionine (i.e. a deficient amount). During regimen B, all the birds were fed as in regimen A for the first 11 hours, then they were transferred to Feed 2 without methionine supplementation and given plain water for 5 hours to induce a methionine deficiency. The following day (regimen C), both groups were returned to their previous diets (Feed 1 and plain water or Feed 2 and methionine treated water) when the lights came on.

7.2.2.3 Measurements

Feed intakes and water intakes were recorded hourly. All data were obtained on an individual hen basis.

7.2.3 Results

Feed and water intakes during the experiment are shown in Table 7.2. The way of methionine delivery did not have significant effect on feed or water intakes ($p>0.05$) indicating that the source of methionine does not influence normal appetite. In contrast, there was a significant ($p<0.001$) effect of regimens on both mean intake values. Feed and water intakes reduced in regimen C despite the methionine supplementation (in feed or water), reflecting the effect of regimen B when methionine was withdrawn from the diet 5 hours before the light went off.

There was no significant interaction between source of methionine and regimens.

Table 7.2. Feed- and water intakes during the regimens of the experiment in relation to the way of methionine delivery and the introducing time of deficiency, and significance of effects, regimen, and their interaction.

Treatment		Regimens							
Source of methionine		**A (4 days)**		**B (2 days)**		**C (4 days)**		**Mean intakes**	
Feed (group 1)	Feed Intake	124.6	n=16	118.8	n=16	88.6	n=16	**110.7**	**n=48**
Water (group 2)	Feed Intake	129.6	n=15	125.3	n=15	96.3	n=15	**117.1**	**n=45**
	Mean feed intakes	**127.0**[2]	**n=31**	**122.0**[2]	**n=31**	**92.3**[1]	**n=31**		
Feed (group 1)	Water Intake	183.5	n=16	177.3	n=16	142.0	n=16	**167.6**	**n=48**
Water (group 2)	Water Intake	200.2	n=15	189.3	n=15	141.8	n=15	**177.1**	**n=45**
	Mean water intakes	**191.6**[2]	**n=31**	**183.1**[2]	**n=31**	**141.9**[1]	**n=31**		

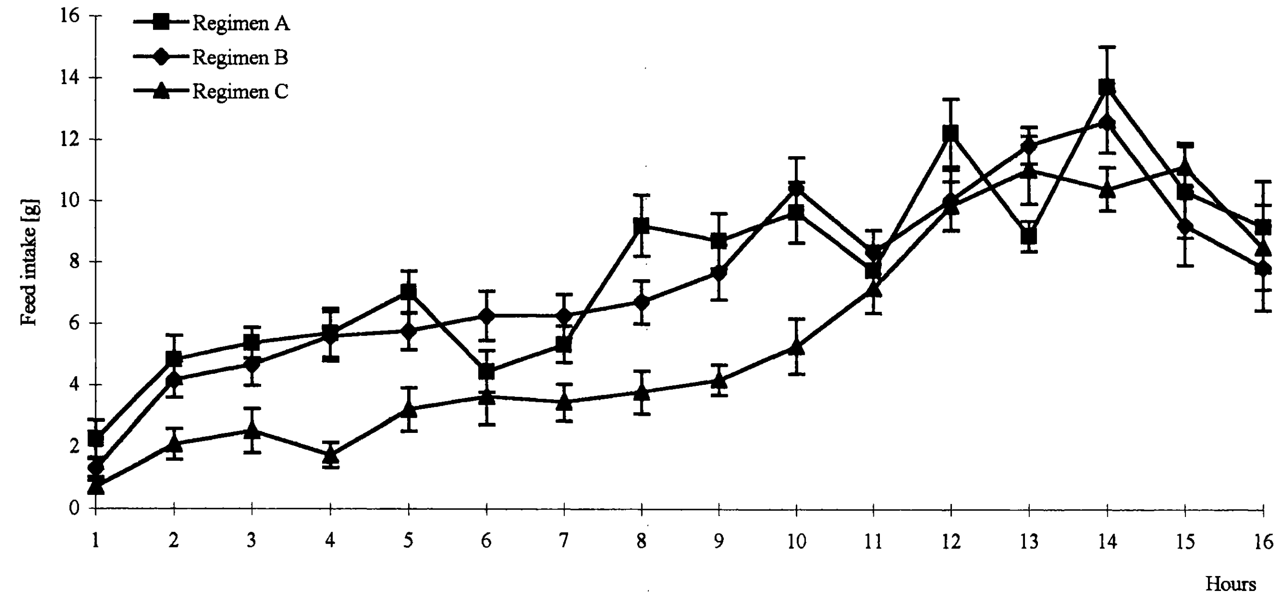
	Probability	
	Feed Intake	**Water Intake**
Effect of treatment	0.001	0.001
Effect of regimen	0.306	0.355
Interaction between the effect of treatment and regimen	0.951	0.453

Feed intakes are expressed as g/day, water intakes are expressed as ml/day.
Values are mean of n=x.

The comparison of hourly feed and water intake patterns throughout the three regimens of the experiment are shown for group 1 in Figures 7.13 and 7.14, and for group 2 in Figures 7.15 and 7.16, respectively. The hourly consumption of feed and water was steady in regimens A and B, in spite of introducing the deficient diet in the last 5 hours of regimen B. The following day (regimen C), however, both feed and water intake showed a slow increase.

The hourly cumulative feed and water intake pattern throughout the three regimens of the experiment are shown for group 1 in Figures 7.17 and 7.18, and for group 2 in Figures 7.19 and 7,20, respectively. In regimens A and B, the two groups exhibited similar feed and water intake patterns, however, from the 13th hour i.e. two hours after the withdrawal of methionine, a trend of lowering intakes were seen. In addition, the values in regimen C were always below the values in regimens A and B, indicating that if the bird experiences a methionine deficiency, it will be reflected in its later feed and water intake.

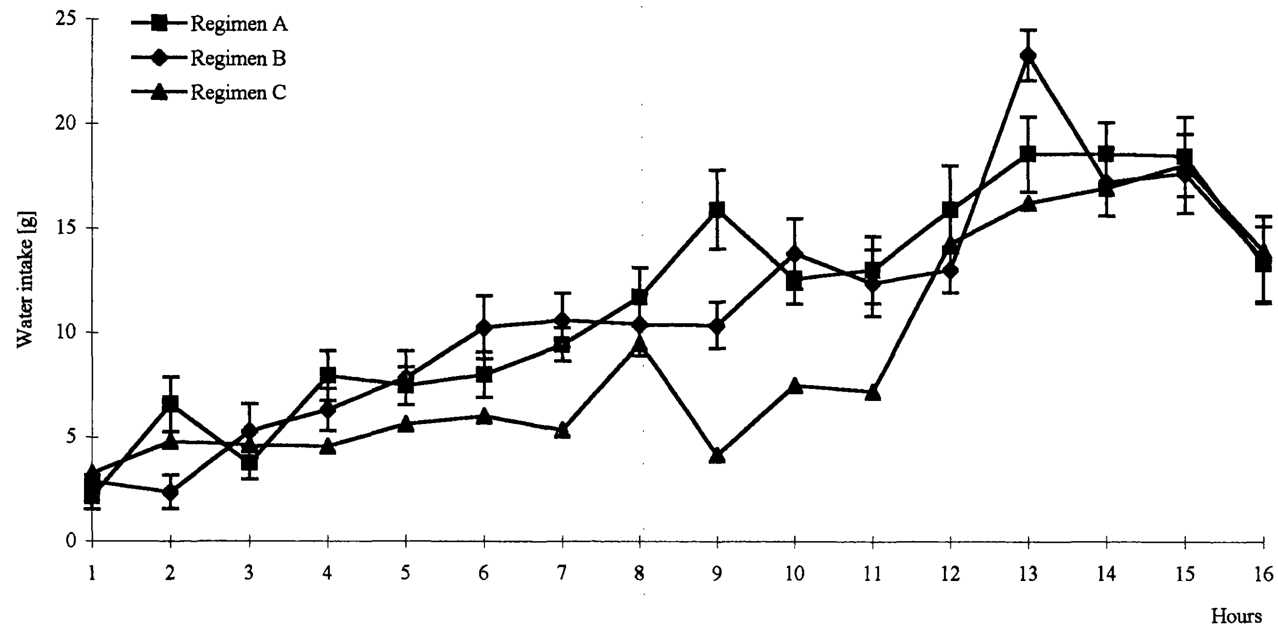
Figure 7.13. Comparison of hourly feed intake of group 1 between regimen A, B and C.



Light Off	Light On		Light Off
R A	Fed methionine adequate diet and plain water		
R B	Fed methionine adequate diet and plain water until 11 hours	Fed methionine deficient diet	
R C	Fed methionine adequate diet and plain water		

R=regimen

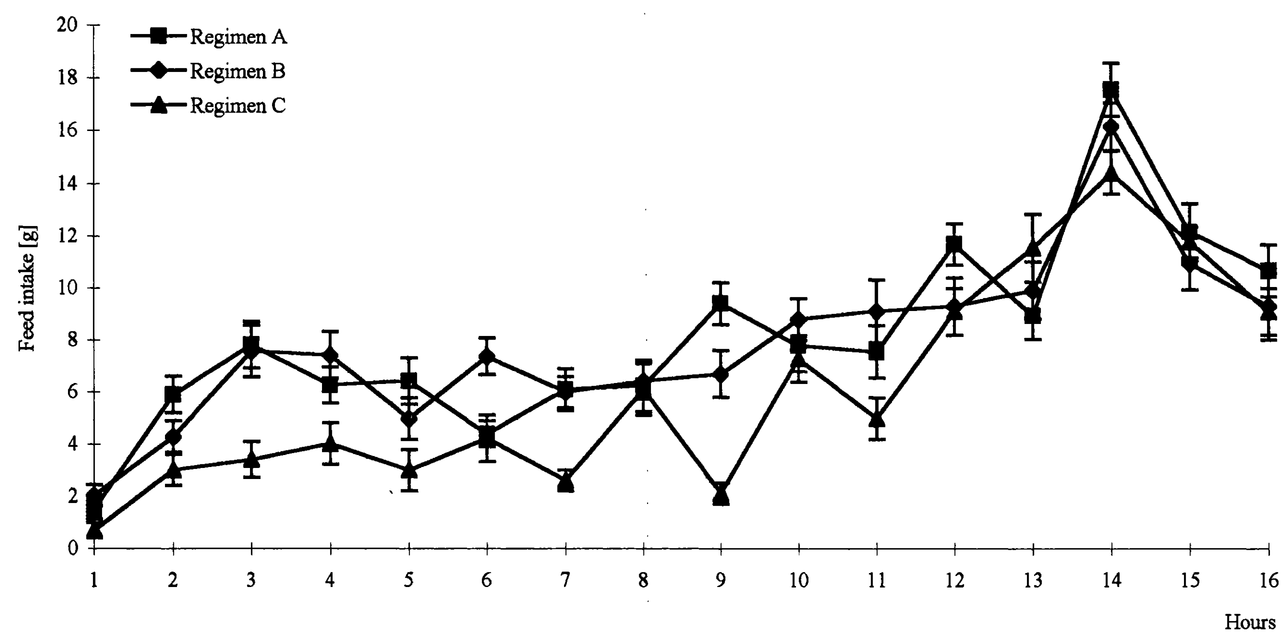
Figure 7.14. Comparison of hourly water intake of group 1 between regimen A, B and C.



Light Off	Light On		Light Off
R A	Fed methionine adequate diet and plain water		
R B	Fed methionine adequate diet and plain water until 11 hours	Fed methionine deficient diet	
R C	Fed methionine adequate diet and plain water		

R=regimen

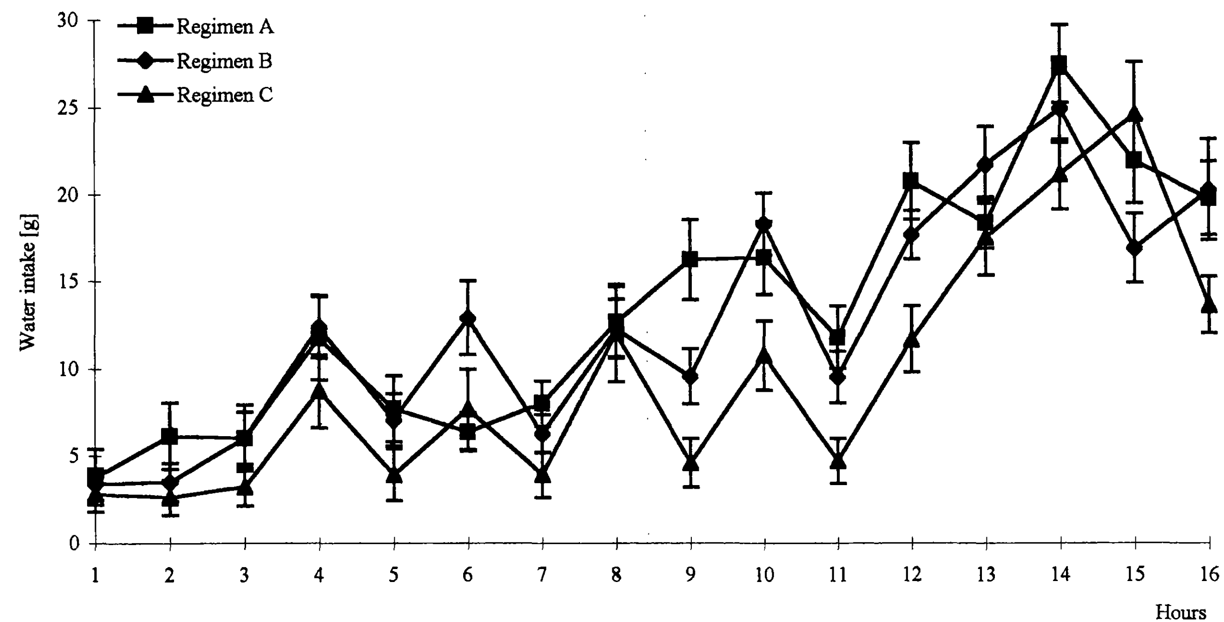
Figure 7.15. Comparison of hourly feed intake of group 2 between regimen A, B and C.



Light Off	Light On		Light Off
R A	Fed methionine deficient diet and methionine treated water		
R B	Fed methionine deficient diet and methionine treated water until 11 hours	Fed methionine deficient diet	
R C	Fed methionine deficient diet and methionine treated water		

R=regimen

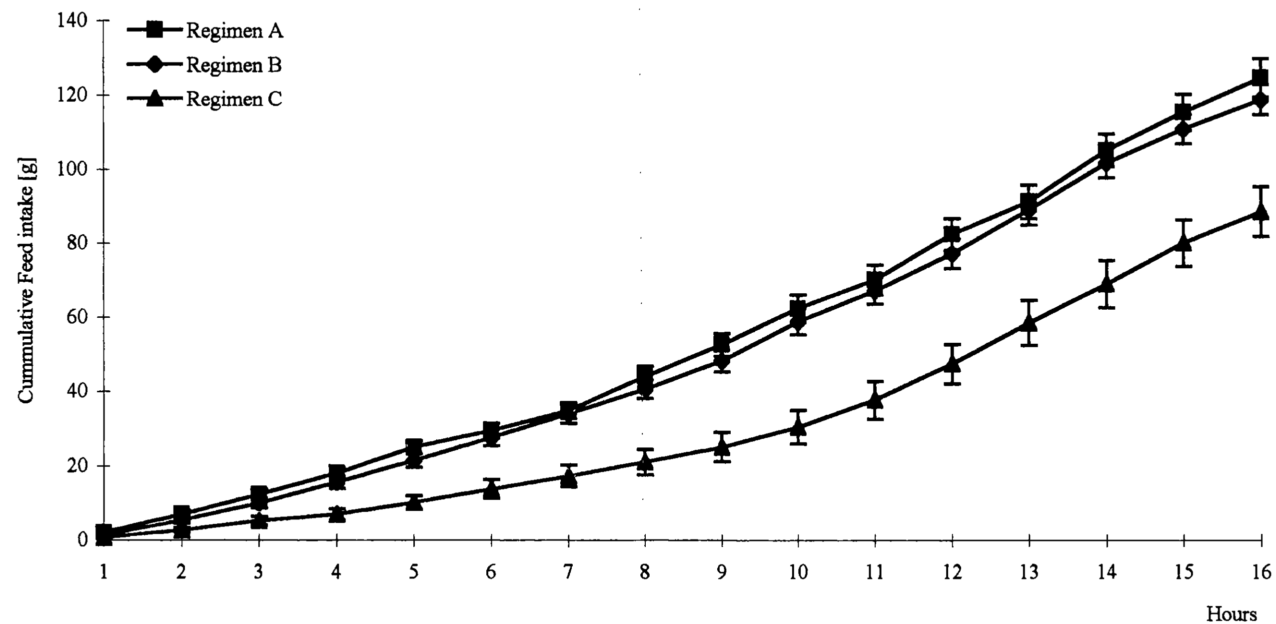
Figure 7.16. Comparison of hourly water intake of group 2 between regimen A, B and C.



<table>
<tr><td>Light Off</td><td>Light On</td><td></td><td>Light Off</td></tr>
<tr><td>R A</td><td colspan="2">Fed methionine deficient diet and methionine treated water</td><td></td></tr>
<tr><td>R B</td><td>Fed methionine deficient diet and methionine treated water until 11 hours</td><td>Fed methionine deficient diet</td><td></td></tr>
<tr><td>R C</td><td colspan="2">Fed methionine deficient diet and methionine treated water</td><td></td></tr>
</table>

R=regimen

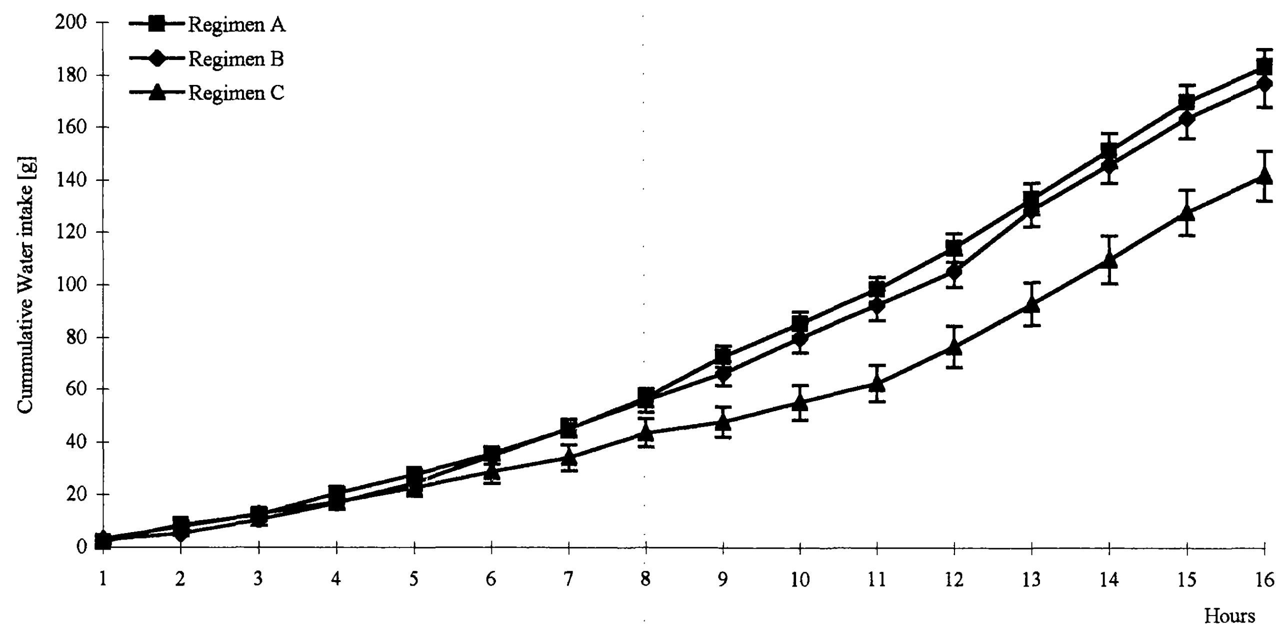
Figure 7.17. Comparison of hourly cumulative feed intake of group 1 between regimen A, B and C.



Light Off	Light On		Light Off
R A	Fed methionine adequate diet and plain water		
R B	Fed methionine adequate diet and plain water until 11 hours	Fed methionine deficient diet	
R C	Fed methionine adequate diet and plain water		

R=regimen

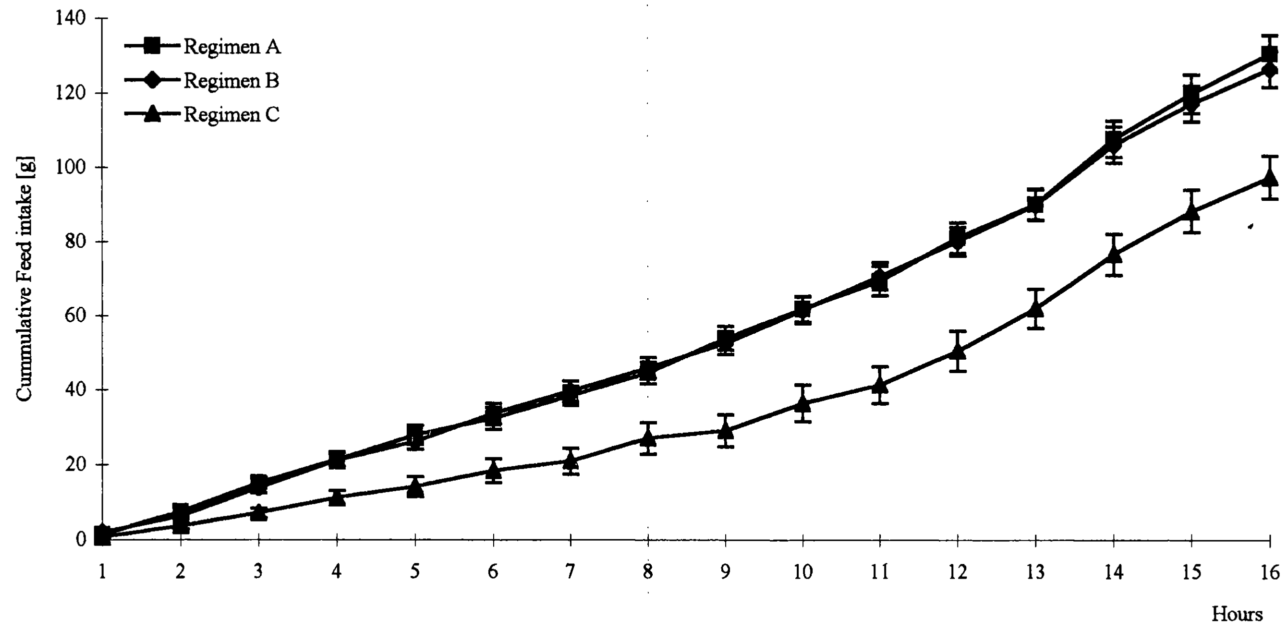
Figure 7.18. Comparison of hourly cumulative water intake of group 1 between regimen A, B and C.



Light Off	Light On		Light Off
R A	Fed methionine adequate diet and plain water		
R B	Fed methionine adequate diet and plain water until 11 hours	Fed methionine deficient diet	
R C	Fed methionine adequate diet and plain water		

R=regimen

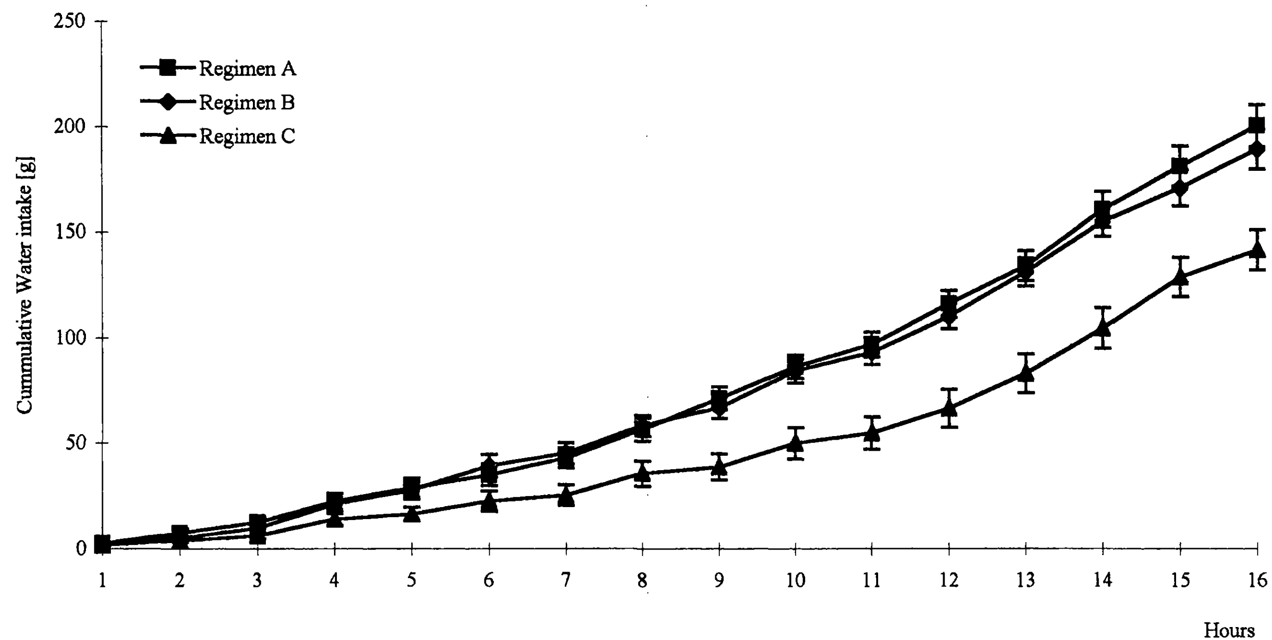
Figure 7.19. Comparison of hourly cumulative feed intake of group 2 between regimen A, B and C.



Light Off	Light On		Light Off
R A	Fed methionine deficient diet and methionine treated water		
R B	Fed methionine deficient diet and methionine treated water until 11 hours	Fed methionine deficient diet	
R C	Fed methionine deficient diet and methionine treated water		

R=regimen

Figure 7.20. Comparison of hourly cumulative water intake of group 2 between regimen A, B and C.



Light Off	Light On		Light Off
R A	Fed methionine deficient diet and methionine treated water		
R B	Fed methionine deficient diet and methionine treated water until 11 hours	Fed methionine deficient diet	
R C	Fed methionine deficient diet and methionine treated water		

R=regimen

7.2.4 Discussion

Experiments 6a and 6b both aimed to give some information about how long it takes for the hens to respond to a methionine deficiency. There are no reports on the precise determination of when the hens begin to decrease their feed intake in response to methionine deficiency, but it is known that, under normal temperature conditions (20°C), food passage through the digestive tract of hens takes approximately 4.5 hours (Savory, 1986). The effects of an amino acid deficient feed could be expected to start influencing the amino acid pool within 2-3 hours of consumption. Taking a dynamic view of the process, all of the food consumed during the first 4 hours of the day could be expected to have passed through to the duodenum and jejunum by the 6th or 7th hour of the day. The reduced level of methionine being absorbed gradually affects the plasma and intracellular pool of the amino acid. The depression of appetite is seen by the 8th hour, and thus 4 hours after the hens had consumed approximately 20 g of feed, the quantities of absorbed amino acids, with the incumbent methionine deficiency, is sufficient to depress appetite. The quantity of methionine deficiency can be expressed as below:

X = methionine content in normal feed (in 20 g of 140 g/kg CP) = 74 mg

Y = methionine content in deficient feed (in 20 g of 140 g/kg CP) = 42 mg

Thus the deficiency is X-Y = 32 mg

A cumulative deficiency of this magnitude upsets the birds substantially. This result is also consistent with the observation that hens recognise a change in the protein content of the food within 12 hours (Chah and Moran, 1985), and that in response to lysine deficient diet, 4 weeks old chicks reduce feed intake within 6 to 8 hours (March and Walker, 1970). As it has been detailed in the literature review (section 2.5.1), observations on rats (Harper and Rogers, 1965) and poultry (Almquist, 1954; Boorman, 1979) suggest that food intake regulation may be influenced by plasma amino acid concentrations or patterns. Experiment 6b seems to offer an evidence for this theory; the animals received a deficient diet for a 5-hour period, during which the deficiency had not yet affected significantly their feed intake. After the 8-hour period of digestion (dark hours), the birds exhibited a reduced appetite, even though they were now given normal (adequate) diet. This indicates that by now, the previous day's deficient diet has deranged the plasma amino acid pattern which, in turn, caused a feed intake depression. In support, experiments on rats suggested that the time of depression in food intake is closely associated with the time of feeding amino acid imbalanced diets (Harper *et al.*, 1970).

The main conclusion of the experiment was:

if the birds consume a diet deficient in methionine for a short period (i.e. 5 hours), the feed intake of even those birds which have been transferred to an adequate diet is adversely effected.

8.0 DETERMINATION OF THE MINIMUM TRAINING TIME OF LAYERS TO SHOW AN APPETITE FOR METHIONINE IN THE DRINKING WATER

8.1 Experiment 7

8.1.1 Introduction

Experiment 7 examined the hypothesis that there is a threshold period of training that is necessary for the birds to become accustomed to the "deficiency-colour" and "supplement-colour" associations. It is clear from both the literature and the previous experiments (Experiments 3, 4 and 5) that, after a training period, the birds exhibit an appetite for methionine-treated water. Various authors trained birds for different lengths of time before the self-selection period. Thus, Kutlu and Forbes (1993) used an eight-day training period while a six-day period was used by Shariatmadari and Forbes (1993). Cumming (1994) reported that chickens learn to select their nutrient requirements for optimum production during seven to ten days, In addition, from a study on female broiler chickens fed alternatively a starter diet and whole wheat, Covasa and Forbes (1995) concluded that, as long as the birds are offered both feeds from an early age, they do not need a training period before introducing choice feeding. Despite the number of experiments which have involved training birds, the optimal training period has not been determined.

Kirchgessner and Paulicks (1994) reported that animals do not start to select a nutrient before deficiency symptoms occur, but it is not known, how quickly a deficiency of an amino acid leads to a metabolic reaction to stimulate the appetite for sources of the nutrient. The present experiment intended to determine the minimum exposure time to a methionine deficiency or adequacy during the training period that will stimulate or satiate an appetite.

It is known (El Boushy and Kennedy, 1987) that a feedstuff or a formula feed that has once caused digestive disturbances or discomfort are remembered by the birds and are not eaten a second time. Based on this knowledge, the memory of the birds was assessed using the colour cue associations with the physiological needs.

The aims were:

1. to determine the minimum training time necessary for the birds to learn to associate colours with methionine deficiency or adequacy;

2. to determine the birds' ability to correct a methionine deficiency;

3. to determine the birds' ability to remember with the colour cue to the colour associations with the physiological needs.

8.1.2 Materials and methods

8.1.2.1 Stock

Thirty-seven weeks old Lohmann layers, reared under the conditions of the current commercial practice, were used for the experiment. These were chosen at random from a flock of 1000 hens in the same house. A total of 32 laying hens were distributed into four groups of eight, then each group was further divided into two subgroups (A and B) of four hens in order to eliminate the effect of colour preference. The body weights (mean ± SEM) of the four groups were 2160.3 ± 62.25 g for group 1, 2113.3 ± 55.72 g for group 2, 2119.6 ± 71.71 g for group 3, and 2113.6 ± 106.47 g for group 4; the differences between them were not significant ($p>0.05$). The birds were placed singly in cages. According to the plan of the feeding regimens, two different coloured water suppliers and waste-water collector cups (yellow, red), and one feed trough was located for each cage. The sides of the wire cages were made solid with 3-ply wood.

8.1.2.2 Diets

Two feed formulations (Feed 1, Feed 2) were used in this experiment, as shown in Table 3.3. Feed 1 (adequate) contained 3.7 g/kg methionine, while Feed 2 (deficient) contained 2.1 g/kg methionine. Treated water contained 0.1% methionine.

The experiment consisted of six feeding regimens (regimens A-F). The hens were maintained on a commercial layer diet, and, to begin the experiment, they were transferred to a 140 g/kg protein feed supplemented with methionine (Feed 1) and fed this for 7 days (regimen A). During this time, each hen received plain water supplied in red (A) or yellow (B) plastic bottles. After this pretraining phase, the birds were transferred to a training regimen (regimen B) which allowed them to become accustomed to the effects of the treatments and associate them with a colour. Each group of birds was exposed to two types of treatments alternately, for the 16-hour lighting (and feeding) period during each of three consecutive days. The exposure regimens for each group are shown in Table 8.1. Diet 1 contained 140 g/kg CP feed without methionine supplementation and plain water which was supplied in yellow plastic bottles for subgroups A, or in red bottles for subgroups B. Diet 2 contained the same feed and water containing 0.1% methionine which was given in red bottles for subgroups A or in yellow bottles for subgroups B (Table 8.1).

Table 8.1. Training regimen of birds in Experiment 7.

Group	**Exposure time [hours]**		**Repetitions in a day (16 hrs)**
	Diet 1	Diet 2	
1	1	1	8x
2	2	2	4x
3	4	4	2x
4	8	8	1x
Subgroup	**Bottle colour**		
A	yellow	red	
B	red	yellow	

Diet 1 contains 140 g/kg CP feed without methionine supplementation and plain water.

Diet 2 contains 140 g/kg CP feed and water containing 0.1% methionine.

Over the following five days, the response to training was tested (first assessment phase, regimen C). The birds, while on Feed 2, were offered a choice of plain water in yellow (for subgroups A) or red (for subgroups B) bottles, and methionine-supplemented water in red (for subgroups A) or yellow (for subgroups B) bottles. Subsequently, a second assessment phase followed (regimen D), in which the birds were in a choice situation for five days, similar to regimen C, but the position of the bottles was swapped.

The memory of the birds was assessed in regimens E and F. In regimen E, the birds were returned to the main flock for 15 days. During this period they were on commercial diet (155 g/kg CP feed and plain water). After this, in order to test their memory of the training, the birds were put in the same choice situation as in regimen C for three days. Subsequently, the birds were again returned to the main flock (on commercial diet) for another 45 days (regimen F), then their memory was tested again in the same choice situation for three days.

8.1.2.3 Measurements

Food intakes and water intakes were recorded daily. Methionine intake was calculated from the amounts consumed via feed and water. Additionally, feed and water intakes were measured according to four different exposure times, on day seven (in regimen A), and on days eight, nine and ten (in regimen B). All data were obtained on an individual hen basis.

Body weights were recorded at the beginning and end of the experiment. Eggs were collected daily and weighed individually during regimens A and D.

8.1.3 Results

8.1.3.1 Egg production, egg weight, and body weight

The body weights (mean ± SEM) at the beginning and end of the experiment were 2160.3 ± 62.25 g and 2113.8 ± 71.64 g for group 1, 2113.3 ± 55.72 g and 2082.5 ± 57.63 g for group 2, 2119.6± 71.71 g and 2014.9 ± 62.76 g for group 3, 2113.6 ± 106.47 g and 2087.3 ± 105.11 g for group 4, respectively. Body weights of all groups had decreased by the end of the experiment, although not significantly (p>0.05). The average rate of egg production (mean ± SEM) during A and D were 100.0 ± 0.00 %HD and 92.5 ± 5.20 %HD for group 1; 100.0 ± 0.00 %HD and 95.0 ± 3.20 %HD for group 2; 98.0 ± 1.70 %HD and 87.5 ± 7.50 %HD for group 3; 96.0 ± 2.30 %HD and 92.5 ± 3.60 %HD for group 4, respectively. Egg weights (mean ± SEM) during A and D were 65.8 ± 1.40 g and 61.5 ± 1.00 g for group 1; 63.6 ± 0.70 g and 63.0 ± 1.40 g for group 2; 66.5 ± 1.30 g and 63.1 ± 1.50 g for group 3; 64.7 ± 0.80 g and 62.0 ± 0.90 g for group 4, respectively. All groups showed a decrease in egg production and egg weight, thus repeating the pattern of body weight changes.

8.1.3.2 Daily feed-, water-, and estimated methionine intake

The daily feed and water intake, and the estimated methionine intake during each of the 26 days is presented in Figures 8.2, 8.3 and 8.4. Each point represents the mean ± SEM of the results from eight birds. During the first seven days, when the birds received Feed 1 (adequate methionine content) and plain water, the standard errors were small and the birds' appetite for feed and water was without dramatic changes. During the training phase (regimen B), groups 3 and 4 responded to the regimen with a decrease in feed intake while the appetite of groups 1 and 2 increased. However, by day ten, this difference between the groups had disappeared, and they all showed an appetite similar to that during regimen A. When the choice situation was introduced, all birds reduced their feed intake to a level slightly below of that during days five, six and seven in regimen A, and it remained steady throughout the whole of the choice regimens. Moreover, there was no distinct difference between the appetite of the four groups. In regimen E, when the birds were returned to the choice situation after 15 days in the commercial conditions, the appetite of all groups was as good as in regimen A. In regimen F, when the birds were returned after 45 days, initially the birds showed the lowest appetite in the whole of the experiment, but in the following two days they gradually increased their appetite to the level of regimen A.

In general, water intake pattern paralleled the pattern of feed intake, except during the three days of training. At the beginning of the training phase, water intake of all groups decreased, then was restored. During regimen B, when the birds alternately received treated and untreated water, the intake from treated water averaged 66.7% in group 1, 51.5% in group 2, 62.6% in group 3, and 75.1% in group 4. In the subsequent choice period (regimens C-F), the birds showed preference for treated water. The choices made were (in addition see Table 8.4 in section 8.1.3.4):

in regimen C, 61.7% in group 1, 73.4% in group 2, 47.3% in group 3, 93.2% in group 4;

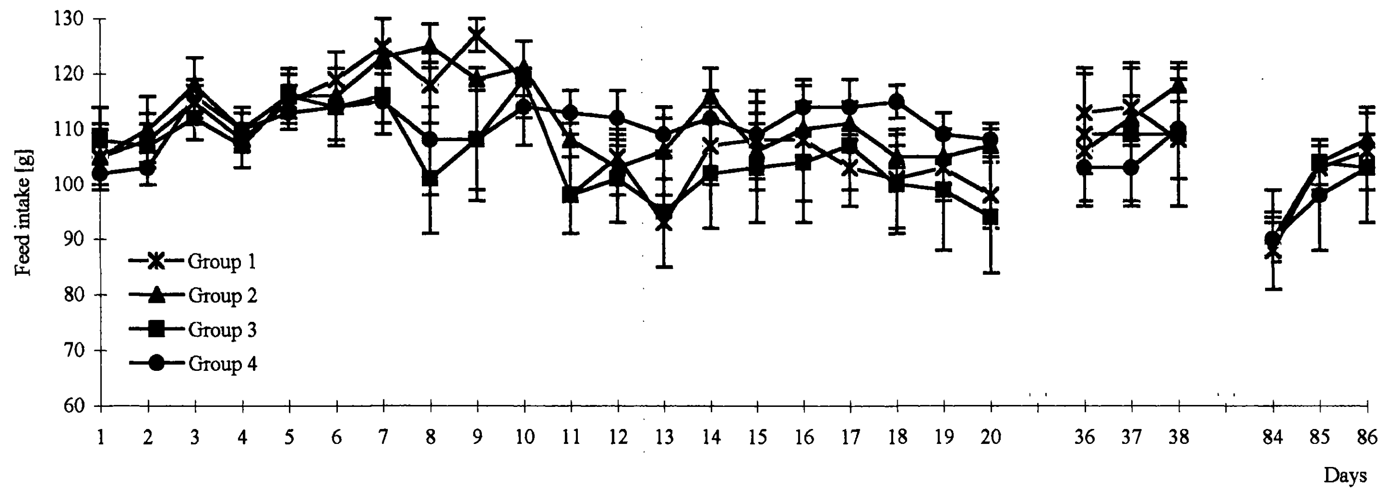
in regimen D, 59.9% in group 1, 83.8% in group 2, 70.8% in group 3, 94.6% in group 4;

in regimen E, 58.9% in group 1, 75.8% in group 2, 67.9% in group 3, 87.3% in group 4;

in regimen F, 71.5% in group 1, 83.3% in group 2, 76.5% in group 3, 80.7% in group 4.

There was a sharp decrease in methionine intakes of all groups at the beginning of the training phase, and they stayed on this lower level throughout the whole of the experiment. Nevertheless, group 4 receiving the 8-hour exposures showed always the highest intake for methionine.

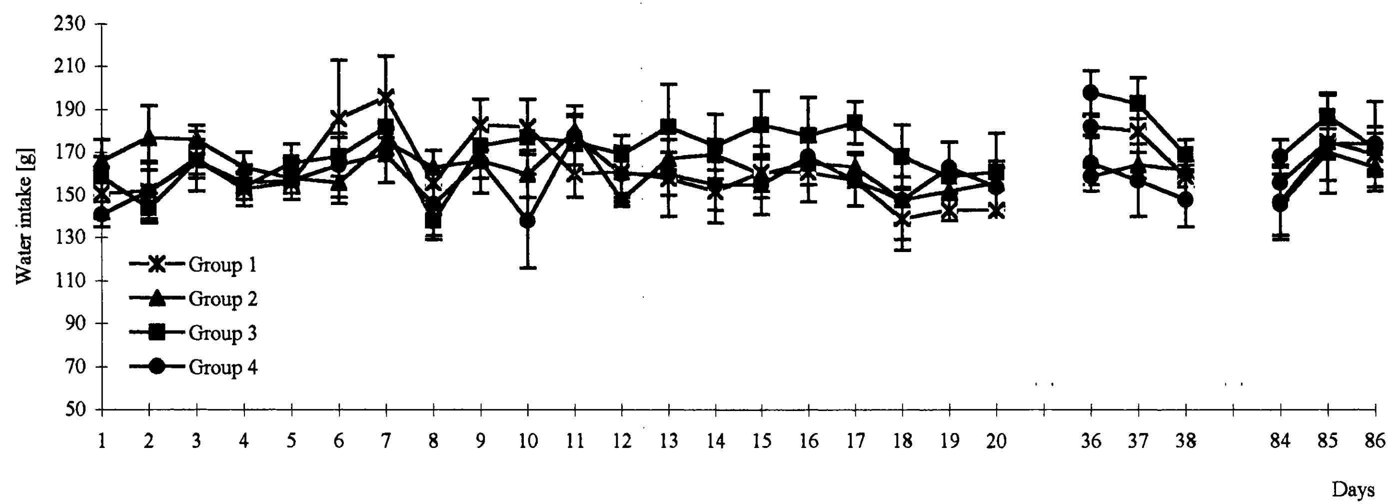
Figure 8.1. Comparison of daily feed intakes in relation to the length of exposure to methionine in drinking water during the six regimens of the experiment.



Regimens	A	B	C	D	E	F
Days	7	1 1 1	5	5	3	3

Exposures were 1 hour for group 1; 2 hours for group 2; 4 hours for group 3 and 8 hours for group 4. Birds were on commercial diet for 15 days prior to regimen E and for 45 days prior to regimen F.

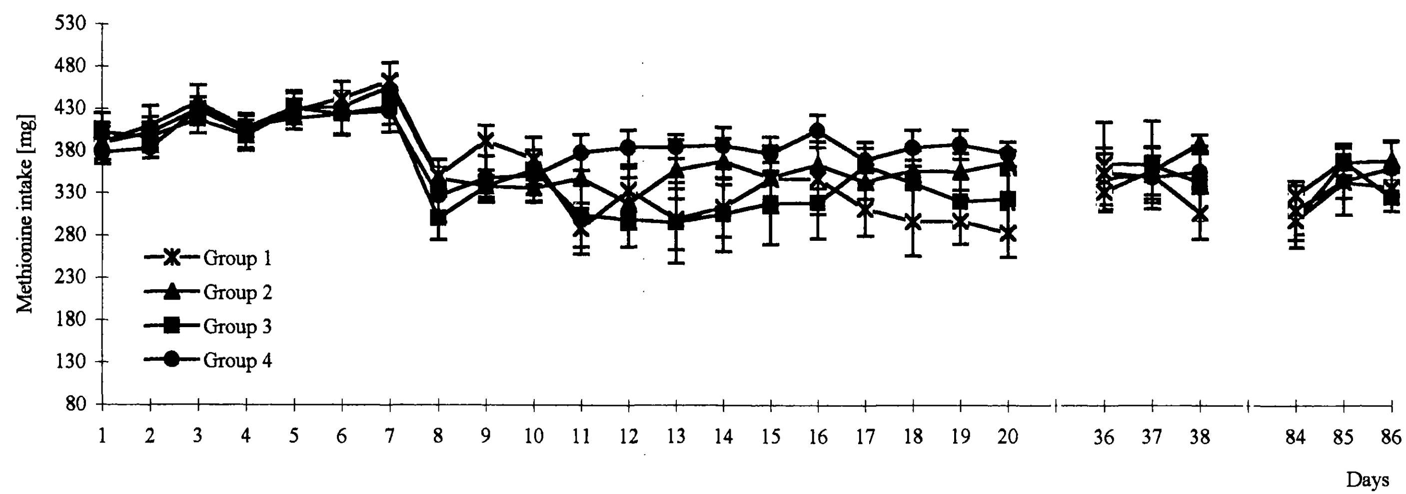
Figure 8.2. Comparison of daily water- (treated and untreated) intakes in relation to the length of exposure to methionine in drinking water during the six regimens of the experiment.



Regimens	A	B	C	D	E	F
Days	7	1 1 1	5	5	3	3

Exposures were 1 hour for group 1; 2 hours for group 2; 4 hours for group 3 and 8 hours for group 4.
Birds were on commercial diet for 15 days prior to regimen E and for 45 days prior to regimen F.

Figure 8.3. Comparison of estimated daily methionine intakes in relation to the length of exposure to methionine in drinking water during the six regimens of the experiment.



Regimens	A	B	C	D	E	F
Days	7	1 1 1	5	5	3	3

Exposures were 1 hour for group 1; 2 hours for group 2; 4 hours for group 3 and 8 hours for group 4. Birds were on commercial diet for 15 days prior to regimen E and for 45 days prior to regimen F.

8.1.3.3 Feed intake

Feed intakes during the regimens of the experiment are shown in Table 8.2. Different lengths of exposure time had no significant effect on mean feed intake ($p>0.05$). In contrast, the effect of regimens on mean feed intake was significant ($p<0.001$) once the birds progressed to the choice periods. Mean feed intakes during the choice phase i.e. in regimens C and D were lower than during the previous phase (in regimens A and B), but were not different significantly from one another. In regimen E, when the birds were returned to choice situation after 15 days under commercial condition, the mean feed intake did not differ significantly ($p>0.05$) from that of regimen A, C and D but it was significantly different from the value in regimen B. In regimen F, when the birds were again returned to choice situation after 45 days under commercial condition, significantly the mean feed intake was significantly ($p<0.001$) lower than in any of the other regimens. There was no interaction between the effects of regimens and length of exposure time.

Table 8.2. Daily feed intakes during the regimens of the experiment in relation to the length of exposure time to methionine in drinking water, and significance of effects of treatment, regimen, and their interaction.

Treatment	Regimens						
Exposure Time [Hour]	**A (7 days)**	**B (3 days)**	**C (5 days)**	**D (5 days)**	**E (3 days)**	**F (3 days)**	**[C]Mean feed intakes**
1 (group 1)[A]	114.1	121.8	102.9	103.0	112.2	100.1	**109.0**
2 (group 2)[A]	114.4	122.4	108.3	108.0	112.7	100.7	**111.1**
4 (group 3)[A]	112.1	109.6	100.8	101.2	109.7	99.4	**105.5**
8 (group 4)[A]	112.7	110.4	111.6	112.3	105.6	97.4	**108.4**
[B]Mean feed intakes	**113.3**[3,4]	**116.0**[4]	**105.9**[2]	**106.2**[2]	**110.0**[2,3]	**99.4**[1]	

	Probability	LSD
Effect of treatment	0.820	12.2
Effect of regimen	0.001	5.9
Interaction between the effect of treatment and regimen	0.548	16.0

Feed intakes are expressed as g/day.
Values are mean of [A]n=8, [B]n=32, and [C]n=48.

8.1.3.4 Total water intake and water- (treated and untreated) intake proportions

Daily water intakes (total) during the regimens of the experiment are shown in Table 8.3. There were no significant effects of treatment or regimens, and treatment x regimen interaction.

During the choice period, the birds' overall preference for treated and untreated water was 72.0% and 28.0%, respectively, a significant difference from 50% ($p<0.001$). Proportional water intakes during this period are shown in Table 8.4. When the effect of lengths of exposure time on percentage of treated water intake was examined, the value at the shortest exposure (group 1) was found significantly lower ($p<0.05$) than at the longest exposure (group 4) time. In case of effect of the regimens, the mean intake values were in all regimens higher than in regimen B, however, the difference was significant ($p<0.05$) only between regimens B and F. There was no interaction between length of exposure time and regimens on the percentage of treated water intakes.

Birds consumed treated water in significantly ($p<0.05$) greater proportions than 50% (i.e. random choice) in group 2 in regimens D and F, and in group 4 during regimens C, D, and E. The overall values were consistently above 50% ($p<0.05$) in all regimens, however, examining the treatment groups, overall proportion of choices made in favour of treated water were significantly different from 50% only in groups 2 and 4 ($p<0.05$).

It is clear from the proportional data that the birds had sustained their ability to select for methionine after a 15-, and even after a 45-days period on commercial (i.e. not methionine deficient) diet.

Table 8.3. Daily water- (treated and untreated) intakes during the regimens of the experiment in relation to the length of exposure time to methionine in drinking water, and significance of effects of treatment, regimen, and their interaction.

Treatment	Regimens						
Exposure Time [Hour]	**A (7 days)**	**B (3 days)**	**C (5 days)**	**D (5 days)**	**E (3 days)**	**F (3 days)**	**^{C}Mean water intakes**
1 (group 1)^{A}	166.3	174.0	158.8	148.9	173.9	167.2	**164.8**
2 (group 2)^{A}	166.9	163.3	165.3	157.2	162.1	159.8	**162.4**
4 (group 3)^{A}	163.1	163.3	177.0	170.4	187.3	175.9	**172.8**
8 (group 4)^{A}	161.9	150.7	162.1	158.8	157.3	165.7	**159.4**
^{B}Mean water intakes	**164.6**	**162.8**	**165.8**	**158.8**	**170.2**	**167.2**	

	Probability	LSD
Effect of treatment	0.708	24.6
Effect of regimen	0.299	9.8
Interaction between the effect of treatment and regimen	0.377	29.9

Water intakes are expressed as ml/day.
Values are mean of ^{A}n=8, ^{B}n=32, and ^{C}n=48.

Table 8.4. Intake proportions of treated water during the choice period of the experiment in relation to the length of exposure time to methionine in drinking water, and significance of effects of treatment, regimen, and their interaction.

Treatment	**Regimens**					
Exposure Time [Hour]	**B (3 days)**	**C (5 days)**	**D (5 days)**	**E (3 days)**	**F (3 days)**	^{C}**Mean intake proportions**
1 (group 1)^{A}	66.6 n.s.	61.7 n.s.	59.8 n.s.	58.9 n.s.	71.5 n.s.	**63.7*** n.s.
2 (group 2)^{A}	51.5 n.s.	73.2 n.s.	83.7 s.	75.7 n.s.	83.2 s.	**73.5* **** s.
4 (group 3)^{A}	62.5 n.s.	47.2 n.s.	70.6 n.s.	67.8 n.s.	76.5 n.s.	**64.9* **** n.s.
8 (group 4)^{A}	74.9 n.s.	93.1 s.	94.5 s.	87.2 s.	80.7 n.s.	**86.1**** s.
^{B}**Mean intake proportions**	**63.9**^{1} s.	**68.8**^{1,2} s.	**77.2**^{1,2} s.	**72.4**^{1,2} s.	**78.0**^{2} s.	

	Probability	**LSD**
Effect of treatment	0.013	20.4
Effect of regimen	0.038	11.4
Interaction between the effect of treatment and regimen	0.102	32.4

Water intakes are expressed as percentage of total (treated + untreated) water intake.
s.; n.s. Difference from 50% is significant ($p<0.05$), or not significant ($p>0.05$), respectively.
Values are mean of $^{A}n=8$ $^{B}n=32$ $^{C}n=48$.

8.1.3.5 Mean methionine intake

The mean methionine intakes during the regimens of the experiment are shown in Table 8.5. Different lengths of exposure time had no significant effect on the methionine intake. When the effect of regimens was examined, significant difference was found only between regimen A and the others ($p<0.001$); when the birds were on training or on choice (Regimens B to F), mean methionine intakes were low compare to the period when they were on adequate diet (Regimen A). There was no significant effect of length of exposure time x regimen interaction.

Table 8.5. Daily estimated methionine intakes during the regimens of the experiment in relation to the length of exposure time to methionine in drinking water, and significance of effects of treatment, regimen, and their interaction.

Treatment	**Regimens**						
Exposure Time [Hour]	**A (7 days)**	**B (3 days)**	**C (5 days)**	**D (5 days)**	**E (3 days)**	**F (3 days)**	**^{C}Mean methionine intakes**
1 (group 1)^{A}	422.3	371.9	316.8	307.6	336.1	326.9	**346.9**
2 (group 2)^{A}	423.1	341.1	348.7	357.9	359.9	344.8	**362.6**
4 (group 3)^{A}	414.8	332.2	304.6	333.8	358.1	341.5	**347.5**
8 (group 4)^{A}	417.0	342.6	382.7	385.2	353.8	338.7	**370.0**
^{B}Mean methionine intakes	**419.3^{2}**	**347.0^{1}**	**338.2^{1}**	**346.2^{1}**	**352.0^{1}**	**338.0^{1}**	

	Probability	**LSD**
Effect of treatment	0.629	43.4
Effect of regimen	0.001	27.0
Interaction between the effect of treatment and regimen	0.365	64.7

Methionine intakes are expressed as mg/day.
Values are mean of ^{A}n=8, ^{B}n=32, and ^{C}n=48.

8.1.3.6 Hourly feed-, and water intake

In general, the water intake of each group in Experiment 7 parallels the feed intakes. On each of the four days, both intakes showed a trend of gradual increase during the 16-hour lighting (feeding) period, followed by a more pronounced increase then a drop in the last two hours (this drop, of course, would have been observed in groups 3 and 4, if the measurements had been taken frequently enough). A closer examination of the patterns, however, reveals differences according to the different exposure times used. Thus, it can be observed that in group 1, although feed intakes on days 8, 9 and 10 showed slight fluctuations, their patterns more or less followed that of day 7 (i.e. the last day of the pretraining phase), when the birds were fed a 140 g/kg CP feed supplemented with methionine (Figure 8.4). Similarly, in the case of water intake, values on day 7 do not differ considerably from those on the other days (Figure 8.5). In addition, between hours 4 and 13, large standard errors can be observed, indicating large individual variation in water intakes of the birds.

In group 2, where the birds were exposed to altering diets two-hourly, feed intake pattern was similar to that of group 1, i.e. no marked differences between the values recorded on the four days could be observed (Figure 8.6). Water intakes of these birds on all days were very similar up to the second exposure to treated water (i.e. normal diet, hour 8th), and values thereafter still followed closely those on day seven (Figure 8.7).

Whereas feed intakes in group 3 were similar at the first three diet-changes (i.e. at hours 4, 8 and 12) in all four days, the last values on days eight, nine and ten were considerably lower than that on day 7 (Figure 8.8). In the water intakes, no marked differences between the four days were found (Figure 8.9).

When the birds received the two different diets 8-hourly, their feed intake on the last training day (day 10) was lower than that on the other days, by the time of the first measurements had been taken (hour 8th). However, by the next measurement (hour 16th), feed intake on this day exceeded the others (Figure 8.10). In the case of water intake, the 10th-day value was again the lowest at the first measurement, but by the end of the day, birds drank as much on this day as on day 7 (Figure 8.11).

Figure 8.4. Hourly changes of feed intake of group 1, on days 7, 8, 9 and 10.

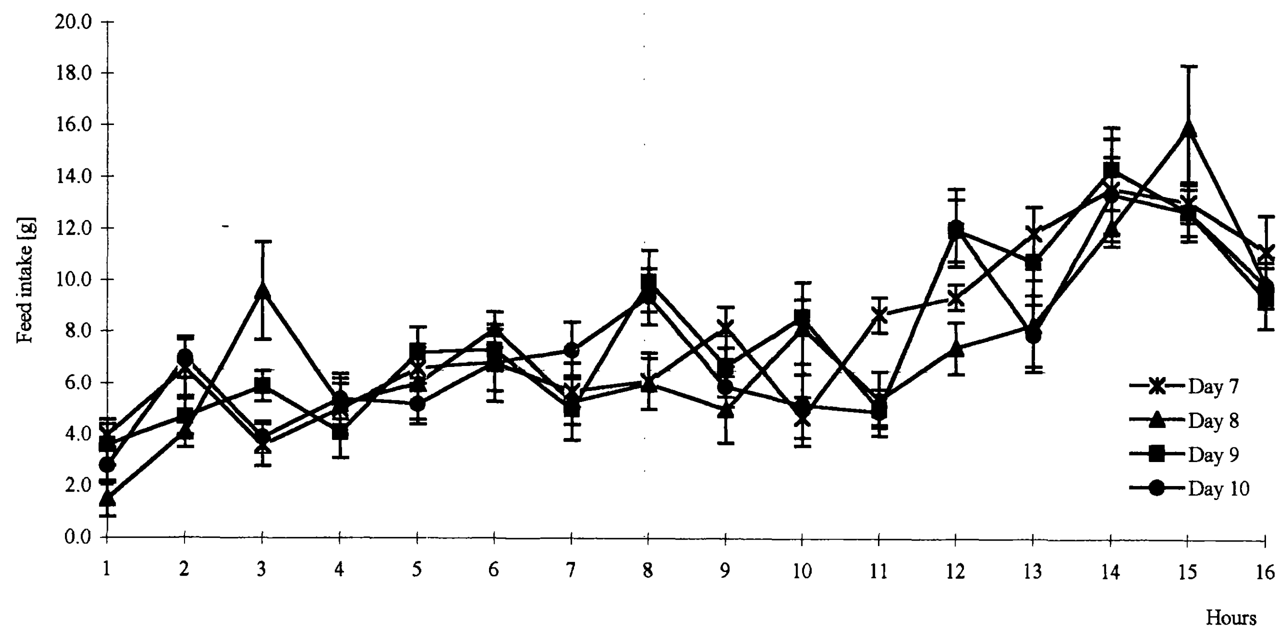


Figure 8.5. Hourly changes of water intake of group 1, on days 7, 8, 9 and 10.

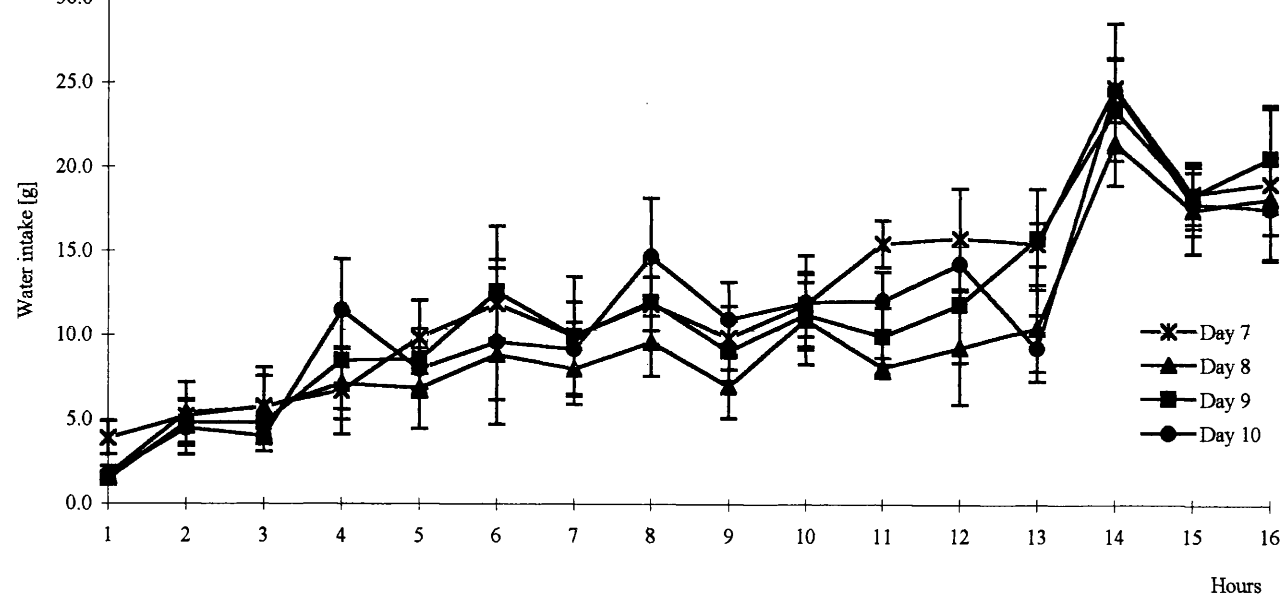


Figure 8.6. Hourly changes of feed intake of group 2, on days 7, 8, 9 and 10.

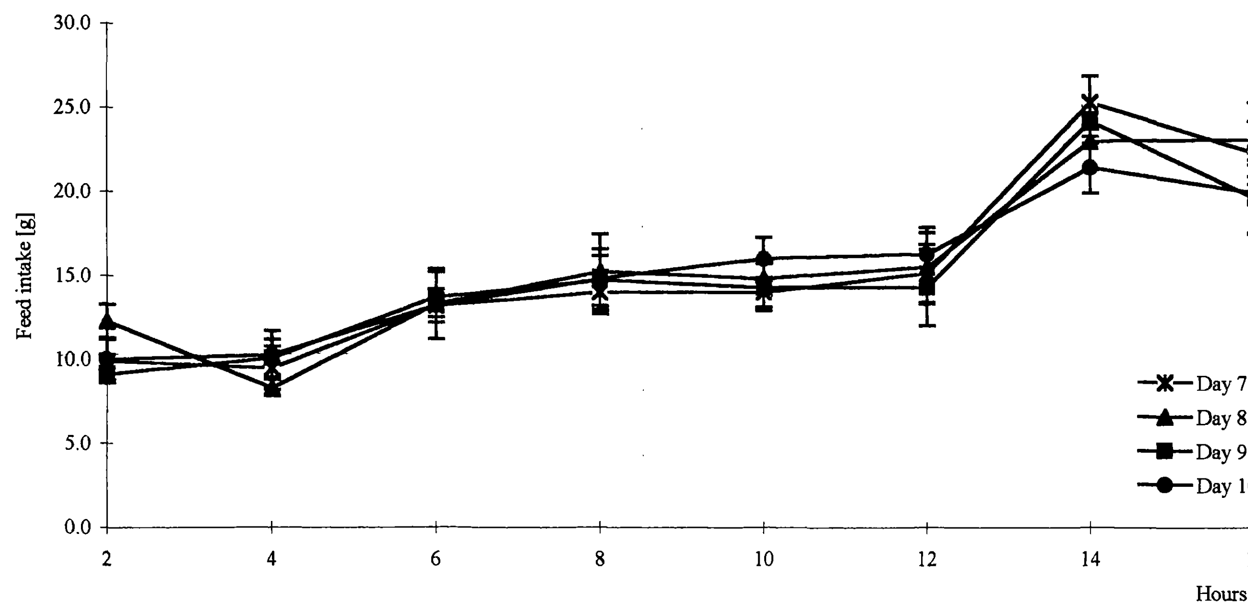


Figure 8.7. Hourly changes of water intake of group 2, on days 7, 8, 9 and 10.

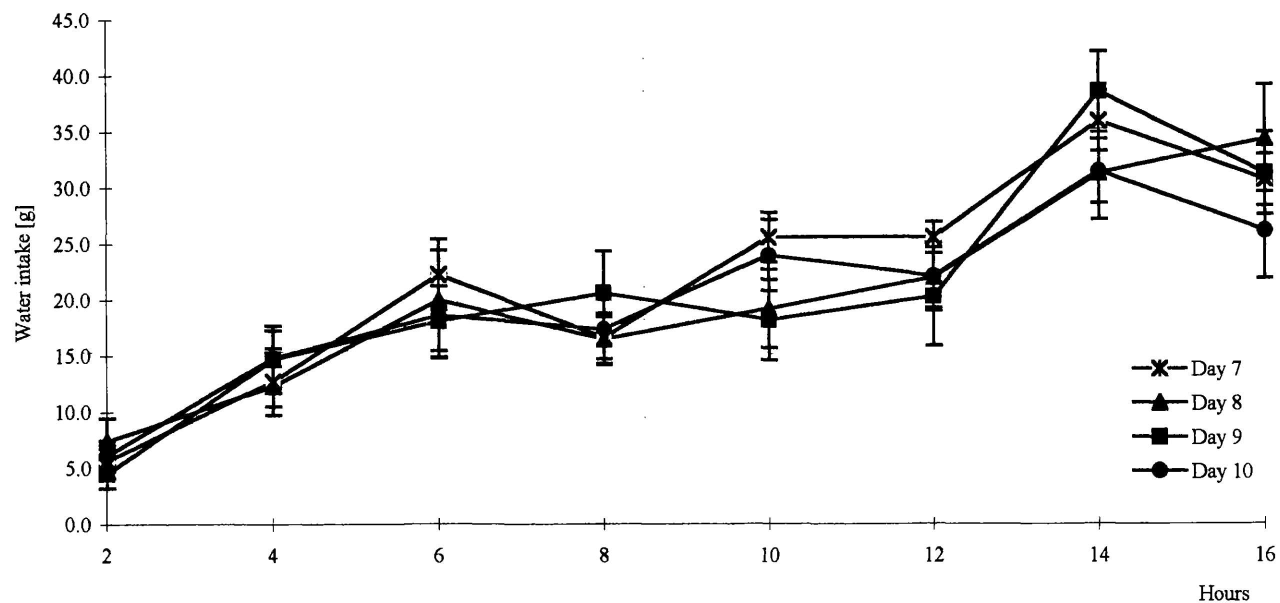


Figure 8.8. Hourly changes of feed intake of group 3, on days 7, 8, 9 and 10.

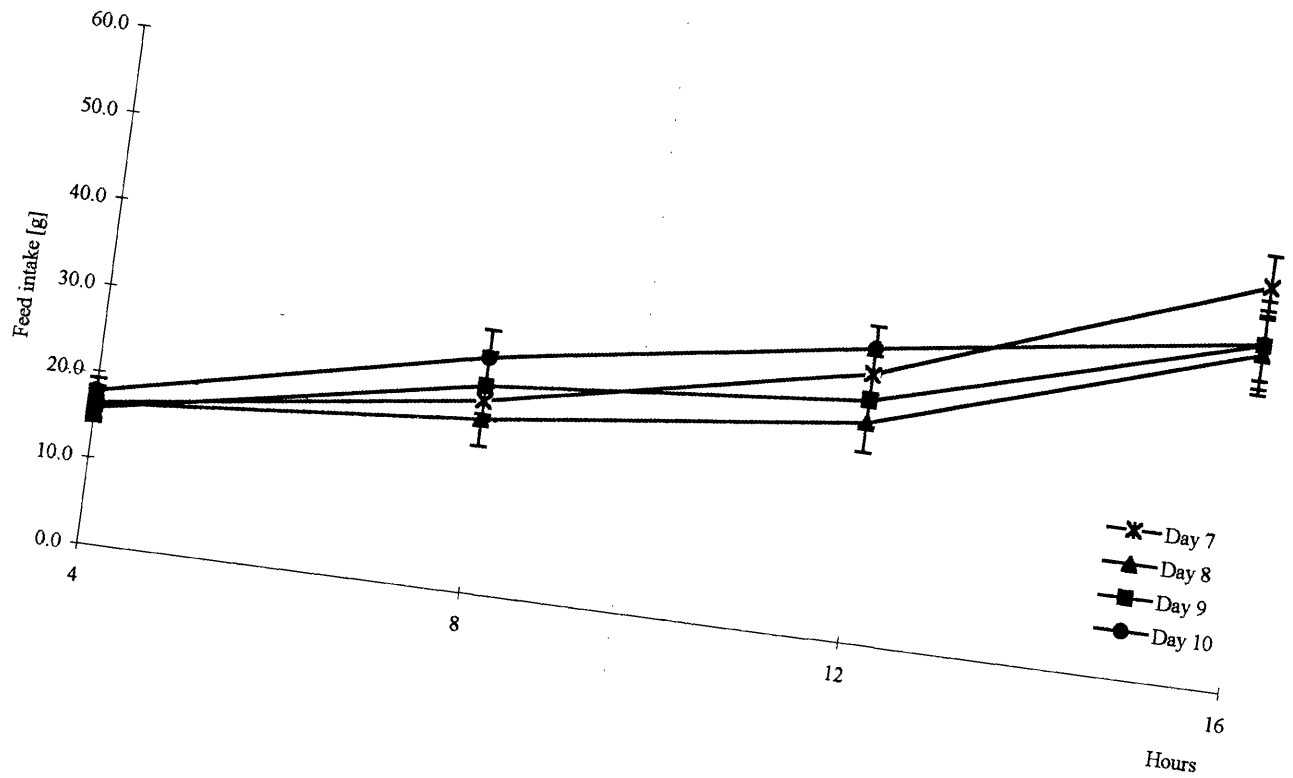


Figure 8.9. Hourly changes of water intake of group 3, on days 7, 8, 9 and 10.

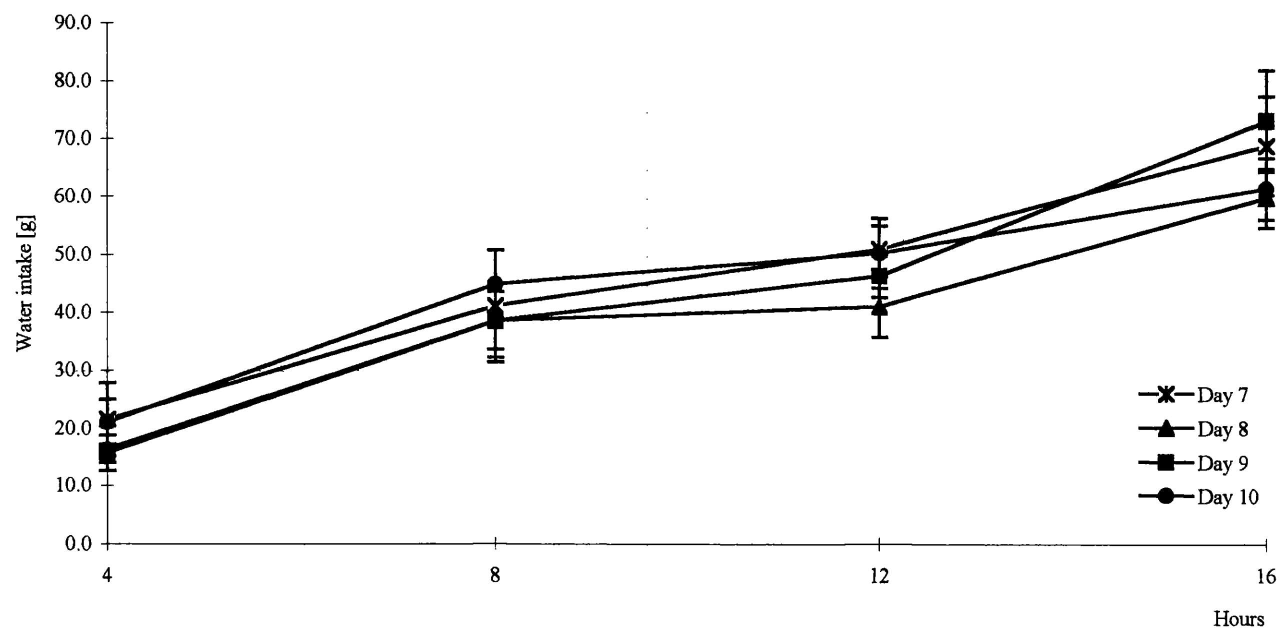


Figure 8.10. Hourly changes of feed intake of group 4, on days 7, 8, 9 and 10.

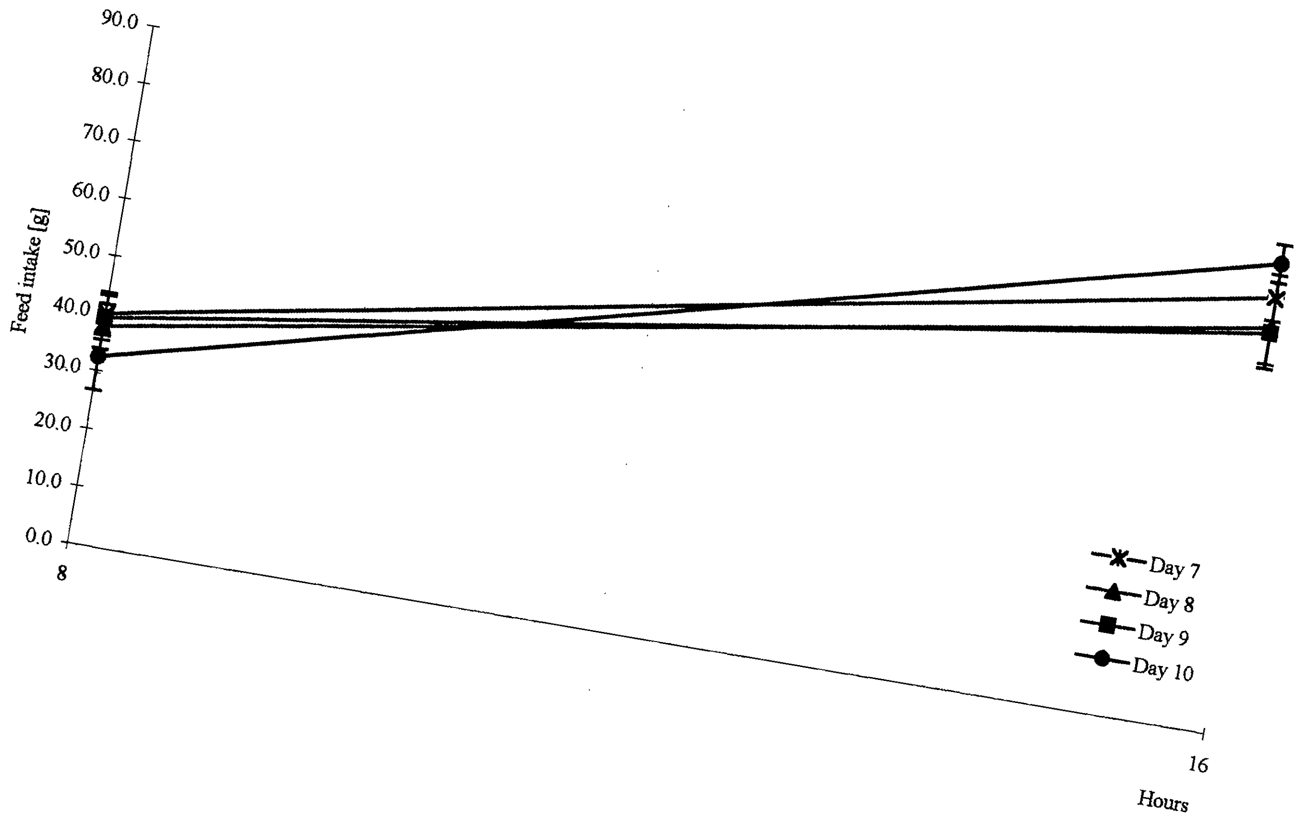
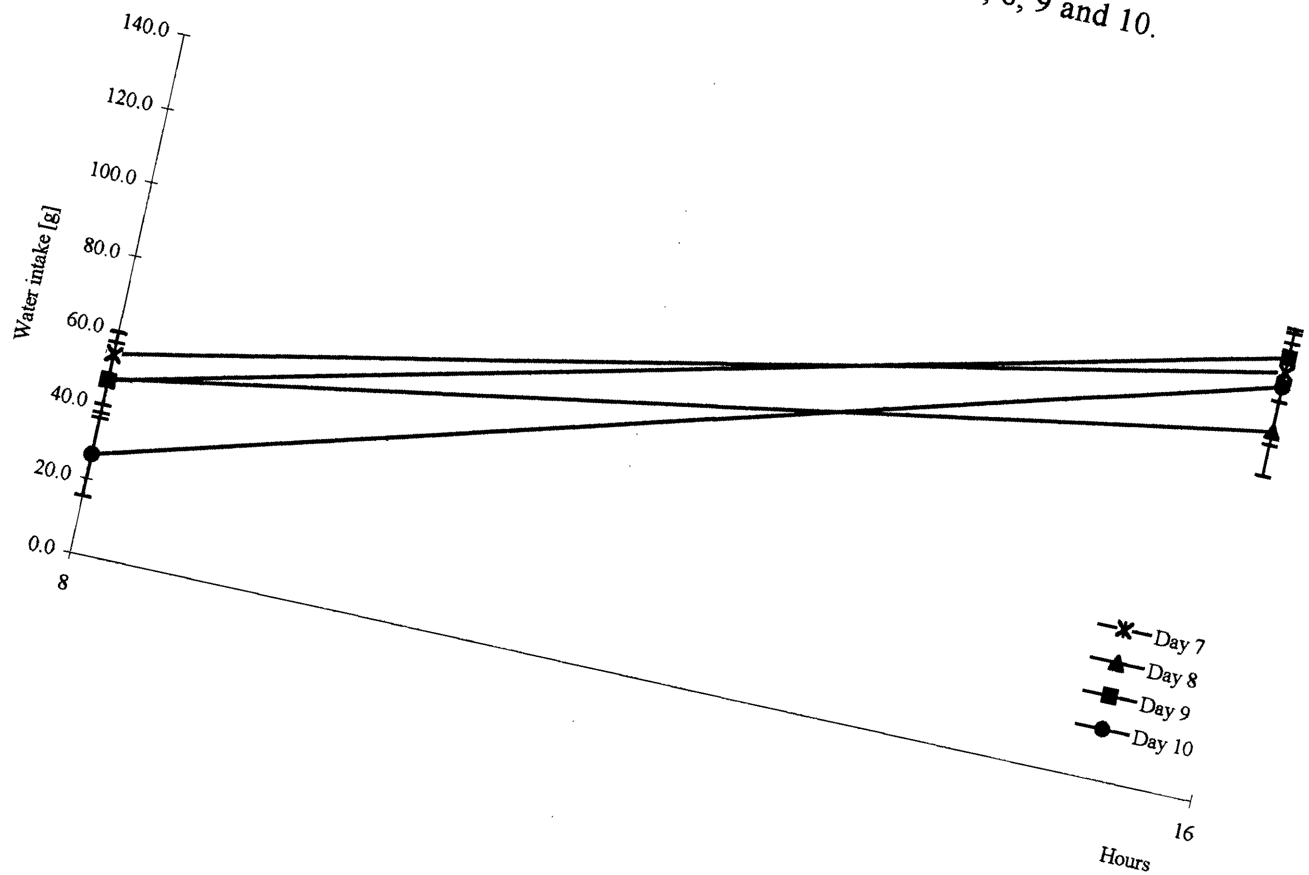


Figure 8.11. Hourly changes of water intake of group 4, on days 7, 8, 9 and 10.



8.1.4 Discussion

The present experiment demonstrated that applying 2-hour exposures to amino acid deficiency is already enough to enable the majority (approximately 73.5%) of the birds to make the association between the cue and the physiological effects of the diet. The 4-hour exposures gave similar results. However, a markedly higher proportion, i.e. almost all of the birds (over 90%) showed a preference for treated water during the choice period, when the 8-hour exposures were used. Comparisons between the 1, 2, 4, and 8 hourly feed and water intakes support these findings. By day 10, birds on 8 hours exposure had learned to make an association between the treatment and the colours, thus when experiencing methionine deficiency (first 8 hours) they reduced their feed and water intakes below the control values (day 7, i.e. normal diet), and when receiving treated water (second 8 hours) both intakes were similar to the control levels. In contrast, the remaining groups did not show such changes in feed and water intakes when compared to the control day. An explanation could be that the shorter exposures to deficiency were not long enough to derange the plasma amino acid pattern in all of the birds in these groups, therefore there was not a well pronounced physiological effect which, however, is a prerequisite for the association with a cue. The findings of the present experiment are also well supported by the earlier observations (Kirchegessner and Paulicks, 1994), that animals do not start to select nutrient before deficiency symptoms occur. At 1, 2, and 4 hour exposures, there was not enough time for symptoms to appear. Previously, the results in Experiment 6a indicated that birds reduce significantly ($p<0.05$) their feed intake after 8 hours of receiving the deficient diet.

After the training period, in regimen D, the highest methionine intake was observed in the group on 8-hour exposures, and it was near to the control value (regimen A). However, the other three groups were considerably below the control values. In addition, standard errors in these groups were higher than in group 4. This would indicate that while in group 4 the majority of the birds behaved similarly, in the other groups, individuals showed a great variation in behaviour when in a choice situation. As a consequence of inadequate methionine intake by some birds in groups 1, 2, and 3 in regimen D, average feed intakes in these groups were below the control values. A similar decrease was not observed in group 4.

When testing the birds' ability to recall the colour-treatment associations, group 4 was found to choose treated water in the highest percentage. An additional observation was that when returning to the experimental conditions, all groups showed a reduced feed intake from values in regimen A. This decrease was slight when returning after 15 days (regimen E), and more pronounced when the gap was 45 days (regimen F). The reason for this behaviour could be that, during the days out of the experiment, the birds were on commercial diet containing 155 g/kg CP, and having bigger particle size than the experimental feed. These features of the feed may have been more appealing to the birds than those of the experimental feed. Nevertheless, the results arising from this part of the experiment suggest that the physiological discomfort caused by the deficiency, and the colour cue associated relief of the discomfort, moreover, the associated set up of the two coloured bottles, are events which are committed to memory which lasts at least 45 days.

A reduction of average body weight, egg production and egg weight were also observed in all groups in this experiment, similarly to experiment 5. It is likely that in groups 1, 2 and 3, there were birds which were always on deficient diet because they were not consuming enough methionine. A reduction was also observed in group 4 where birds choose treated water at 90% frequency. Consequently, these birds received almost as much methionine as in regimen A. Therefore, the reductions are probably because of the total length methionine depletion (8 hours a day, in all groups). Possibly, in these birds a net proportion of body proteins were used to supply the amino acids for egg formation as it has been detailed in section 5.4.

The main conclusions of the experiments were:

1. applying two hour exposures to an amino acid deficiency is already enough for the majority of hens to detect a methionine deficiency in the diet, however, the exposures should be at least 8 hours to effectively train the whole of a given flock (above 90%) to associate the cue with methionine deficiency or adequacy;

2. the birds' memory of the associations between the colour cues and physiological needs can last at least 45 days.

9.0 DETERMINATION OF THE MINIMUM LEVEL OF METHIONINE DEFICIENCY FOR WHICH BIRDS CAN SHOW APPETITE

9.1 Experiment 8

9.1.1 Introduction

The results of Experiment 5 showed that after a training period, the birds exhibit an appetite for methionine-treated water even when the methionine content is very low (0.025%). However, it has not been examined if the laying hens would express appetite for methionine-treated water when the feed is NOT deficient in this amino acid (i.e. contains 3.7 g/kg methionine). In Experiment 8, different levels of methionine in the feed, ranging from adequate to very deficient, were used in combination with methionine-treated water to assess the extent of the metabolic discomfort that a hen must experience in order for the appetite to be expressed.

The aim was:
to determine the level of methionine deficiency in feed which enables birds to express an appetite for methionine treated water.

9.1.2 Materials and methods

9.1.2.1 Stock

A total of 32 Lohmann layers (43 weeks old), reared under the conditions of the current commercial practice, were used for the experiment. They were chosen at random from a flock of 1000 hens in the same house, and which had not been previously used in an experiment. The hens were distributed into four groups of eight birds. Each group was further divided into subgroups (A and B) of four birds in order to eliminate the effect of colour preference. The average body weights (mean ± SEM) of the four groups were 2044.5 ± 51.14 g for group 1, 2124.3 ± 56.92 g for group 2, 1962.0 ± 64.35 g for group 3, and 1932.3 ± 22.83 g for group 4; the differences between them were not significant ($p>0.05$). The birds were placed singly in cages. According to the feeding regimens, two different coloured water suppliers, two waste-water collector cups (yellow, red), and one trough was allocated to each cage. The sides of the wire cages were made solid with 3-ply wood.

91.2.2 Diets

Four feed formulations were used in this experiment, as shown in Table 9.1. Feed 1 contained 3.7 g/kg methionine (feed to group 1), Feed 2 contained 3.1 g/kg methionine (feed to group 2), Feed 3 contained 2.6 g/kg methionine (feed to group 3), and Feed 4 contained 2.1 g/kg methionine (feed to group 4). Feeds 2 and 3 were obtained from the appropriate mixture of Feed 1 and Feed 4. Water was given in bottles coloured accordingly to the treatment and the birds were trained to recognise which bottle had methionine supplemented water or plain water. The treated water contained three concentrations of methionine: 0.025% for group 1; 0.025% for group 2; 0.050% for group 3; 0.075% for group 4.

Table 9.1. The ingredients and estimated nutrient composition of Feeds 1, 2, 3, and 4.

Ingredient composition	**Feed 1 [g/kg]**	**Feed 2 [g/kg]**	**Feed 3 [g/kg]**	**Feed 4 [g/kg]**
Wheat (10.4 % CP)	714.0	713.6	713.2	712.8
H.P. Soya (46.2 % CP)	137.3	138.2	139.0	139.8
Limestone	90.3	90.3	90.3	90.3
Maize Oil	36.7	36.9	37.0	37.1
Dicalcium phosphate	11.4	11.4	11.4	11.4
NaCl	3.7	3.7	3.7	3.7
Vit/Min. Premix[1]	2.5	2.5	2.5	2.5
Yolk Colour A[2]	1.0	1.0	1.0	1.0
DL-Methionine	1.6	1.0	0.5	-
L-Lysine HCl	1.5	1.5	1.4	1.4
Calculated nutrient composition				
Crude protein	140	140	140	140
Calcium	37.5	37.5	37.5	37.5
Total Phosphorus	5.5	5.5	5.5	5.5
Sodium	1.8	1.8	1.8	1.8
Arginine	8.3	8.3	8.3	8.3
Isoleucine	5.3	5.3	5.3	5.3
Leucine	9.9	9.9	10.0	10.0
Lysine	7.2	7.2	7.2	7.2
Methionine	3.7	3.1	2.6	2.1
Methionine + cystine	6.4	5.8	5.3	4.8
Threonine	4.7	4.7	4.7	4.7
Tryptophan	1.7	1.7	1.7	1.7
AME [MJ/kg]	12.14	12.14	12.14	12.14

[1] The composition of vitamins and minerals in the premix provided the following amounts per kilogram of diet: vitamin A, 2400000 IU; vitamin D_3, 1200000 ICU; vitamin E (α-tocopherol acetate), 1600 IU; nicotinic acid, 4000 mg; pantothenic acid, 1600 mg; vitamin B_2 1000 mg; hetrazeen, 800 mg; iron ($FeSO_4$), 0.40%; cobalt ($CoSO_4$), 100 mg; manganese (MnO), 3.20%; copper ($CuSO_4$), 0.20 %; zinc (ZnO), 2.00%; iodine (CaI_2), 400 mg; selenium (Na_2SeO_3), 60 mg.
[2] Contains: canthoxanthin, ethyl ester of β-apo-8-carotenoic acid, citronaxanthin.
H.P. = high protein.

To determine normal feed intake, methionine intake and water consumption, the four groups of hens received Feed 1 and plain water for seven days (regimen A). During this time, each hen received plain water supplied in red (subgroup A) or yellow (subgroup B) plastic bottles. Subsequently, the birds were transferred to the four feed formulations (Feed 1 for group 1, Feed 2 for group 2, Feed 3 for group 3, and Feed 4 for group 4) and given plain water for two days to induce methionine deficiency (regimen B). The water was supplied in yellow (subgroup A) or red (subgroup B) plastic bottles. In this way the hens were exposed to the metabolic effects of a methionine deficiency and allowed to associate it with a colour cue. Over the following two days (regimen C), the four groups of birds were offered the same feed (Feed 1 for group 1, Feed 2 for group 2, Feed 3 for group 3, and Feed 4 for group 4), and water containing methionine (0.025% for group 1; 0.025% for group 2; 0.050% for group 3; 0.075% for group 4). The feed-water combinations for the four groups are summarised in Table 9.2. The water was given from red (subgroup A) or yellow (subgroup B) bottles. The above four-day cycle (regimens B and C) was repeated once more. Thereby the hens became accustomed to the effects of plain water and the three types of methionine-treated drinking water while consuming the deficient feed.

Table 9.2. Methionine content of feed and water formulations in Experiment 8.

Group		Methionine content in feed [g/kg]	in water [%]
1	Feed 1	3.7 (adequate)	0.025
2	Feed 2	3.1	0.025
3	Feed 3	2.6	0.050
4	Feed 4	2.1	0.075

Next, the birds received feed without methionine supplementation (Feed 1 for group 1, Feed 2 for group 2, Feed 3 for group 3, and Feed 4 for group 4) and plain water was given from yellow (subgroup A) or red (subgroup B) bottles for an additional two days. During this training period (a total of 10 days), the four types of feed were given to the hens alternately every two days in order to allow them to become accustomed to the colour cue and physiological effect of the diets adequate or deficient in methionine. Over the following five days, the birds, while still on same feeds, were offered a choice of both plain water and one of the three types of methionine-treated water, from red and yellow bottles, respectively (regimen D). Thereafter, the position of the bottles was changed and the choice offered for another five days (regimen E). The regimens of the experiment are summarised in Table 9.3.

Each day, the hens were allocated enough feed to just exceed their expected daily food intake.

Table 9.3. Training regimen of birds in Experiment 8.

	Regimens	Diet		Bottle colour	
		Feed	**Water**	**Subgroups A**	**Subgroups B**
	A (7 days)	F1	plain	red	yellow
4-day cycle repeated twice	B (2 days)	F1-4*	plain	yellow	red
	C (2 days)	F1-4	treated**	red	yellow
	B (2 days)	F1-4	plain	yellow	red
	D (5 days)	F1-4	plain and treated**	yellow red	red yellow
	E (5 days)	F1-4	plain and treated**	yellow red	red yellow

*F1 (3.7 g/kg methionine) for group 1;
F2 (3.1 g/kg methionine) for group 2;
F3 (2.6 g/kg methionine) for group 3;
F4 (2.1 g/kg methionine) for group 4

**methionine content of the treated water was 0.025% for groups 4 and 3; 0.05% for group 2; 0.075% for group 1.

9.1.2.3 Measurements

Daily feed intake and water consumption was measured gravimetrically every 24 hours. The plain water and four levels of water-methionine mixture remaining in the bottles was discarded daily and replaced with fresh. Body weights were recorded at the beginning and end of the experiment. Eggs were collected daily and weighed individually during regimens A and E. Methionine intake was calculated from the amounts consumed in the feed and water. All data were obtained on an individual hen basis.

9.1.3 Results

9.1.3.1 Egg production, egg weight and body weight

The body weights (mean ± SEM) at the beginning and end of the experiment were 2044.5 ± 51.14 g and 1988.8 ± 68.82 g for group 1, 2124.3 ± 56.92 g and 2065.9 ± 74.74 g for group 2, 1962.0 ± 64.35 g and 1893.5 ± 70.32 g for group 3, 1932.3 ± 22.83 g and 1856.1 ± 42.26 g for group 4, respectively. In the groups which had a reduced methionine level in the feed, body weights decreased by the end of the experiment, although not significantly ($p>0.05$). The average rate of egg production decreased during the experiment in all groups. The values (mean ± SEM) during regimens A and E were 87.5 ± 5.60 %HD and 85.0 ± 5.00 %HD for group 1; 96.4 ± 2.30 %HD and 87.5 ± 5.20 %HD for group 2; 94.6 ± 2.60 %HD and 87.5 ± 6.40 %HD for group 3; 94.6 ± 3.70 %HD and 90.0 ± 3.70 %HD for group 4, respectively. Egg weights decreased in group 4, while increased in the other three groups, however the differences were not significant ($p>0.05$) in neither cases. The values (mean ± SEM) during regimen A and E were 65.5 ± 1.40 g and 66.8 ± 1.40 g for group 1; 63.2 ± 1.40 g and 63.5 ± 1.00 g for group 2; 63.7 ± 1.10 g and 64.0 ± 1.60 g for group 3; 62.8 ± 1.50 g and 61.9 ± 1.60 g for group 4, respectively.

9.1.3.2 Daily feed-, water-, and estimated methionine intake

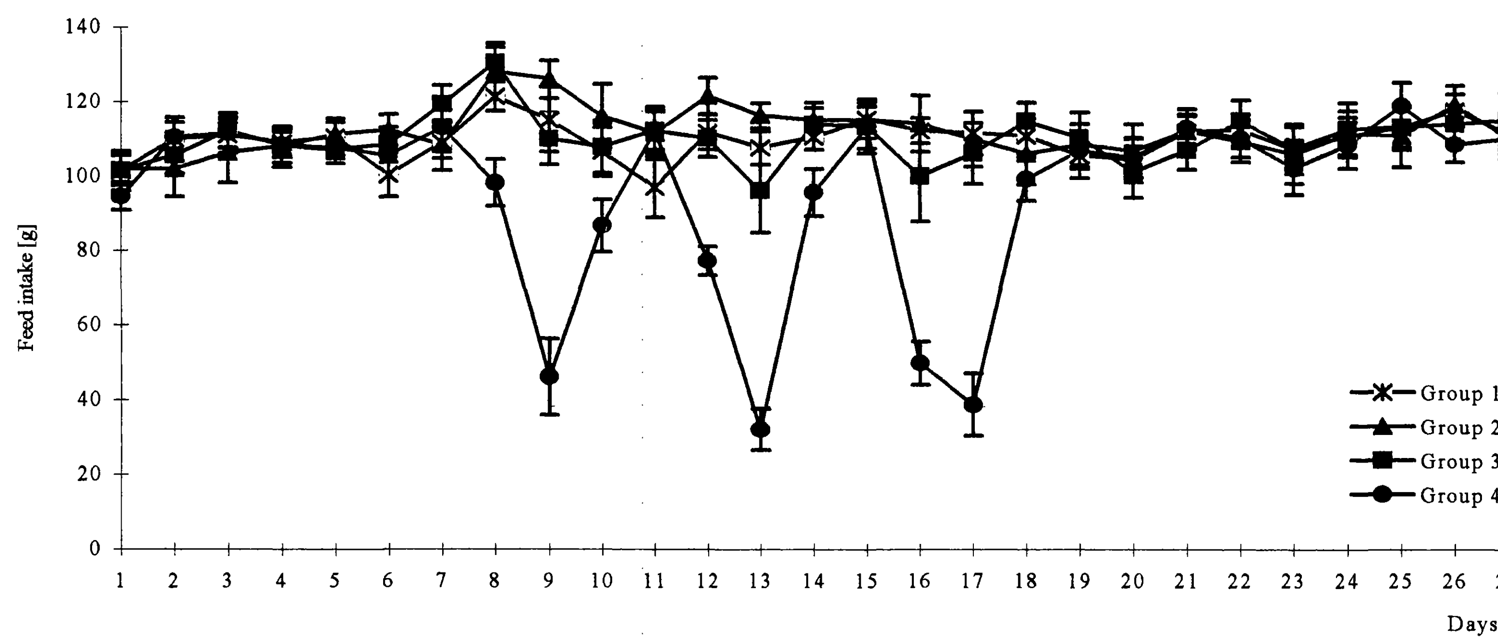
The daily feed and water intake, and the estimated methionine intake during each of the 27 days is presented in Figures 9.1, 9.2 and 9.3, respectively. Each point represents the mean ± SEM of the results from eight birds. In each graph, five distinct phases can be observed, corresponding to the regimens of the feeding. In general, during the first seven days of the experiment, i.e. when the birds received an adequate amount of methionine in the feed, the standard errors were small in all groups and the birds' appetite for feed, water and methionine was without dramatic changes. The most marked responses to the treatment were always observed in group 4 which experienced the greatest methionine deficiency. It is also worth mentioning that group 3 (i.e. birds with the second highest deficiency in their diet) often responded to the treatment similarly to group 4 but on a lower scale.

At days 8 and 9, when the birds received plain water and the four types of feed, a loss of appetite for feed was observed in group 4, and the decrease was more apparent by the second day of this regimen. In contrast, the other groups (with less or no deficiency) all showed some degree of increase in their feed intake. The pattern of water intake during this regimen was similar, in as much as there was an apparent drop observed in group 4 while values increased slightly in groups 1, 2, and 3. The increase of water intake was the greatest in group 2.

When the birds received methionine-treated water, their appetite for feed and water increased up to the level of that in regimen A in group 4 while the other groups stayed on the same level. When repeating the treatment, groups 3 and 4 lost appetite for food and water; the decrease was more apparent in group 4 than in group 3. In the case of water intake, when group 4 received treated water, appetite for water increased sharply on the second day after the deficiency. Groups 1 and 2 showed no difference. When repeating the treatment, the same responses were observed. During those days when the hens were on regimen B, the standard error of means in feed intake were high for groups 3 and 4, in water intake for groups 2, 3, and 4. This indicates that feed and water intake had a great variability within the groups. Variability diminished when the groups proceeded to regimen C. This was more apparent in regimens D and E. By day 18, it appeared that all groups of birds had become trained to the colour cues and it was decided to test the birds for their ability to choose water supplemented with different levels of methionine and feed contained different levels of methionine. In regimen D, the choices made in favour of treated water averaged 38.7% in group 1, 57.2% in group 2, 56.2% in group 3, 98.5% in group 4, while in regimen E the figure was 61.7% for group 1, 49.9% for group 2, 54.0% for group 3, 96.7% for group 4 (in addition see Table 9.6 in section 9.1.3.4).

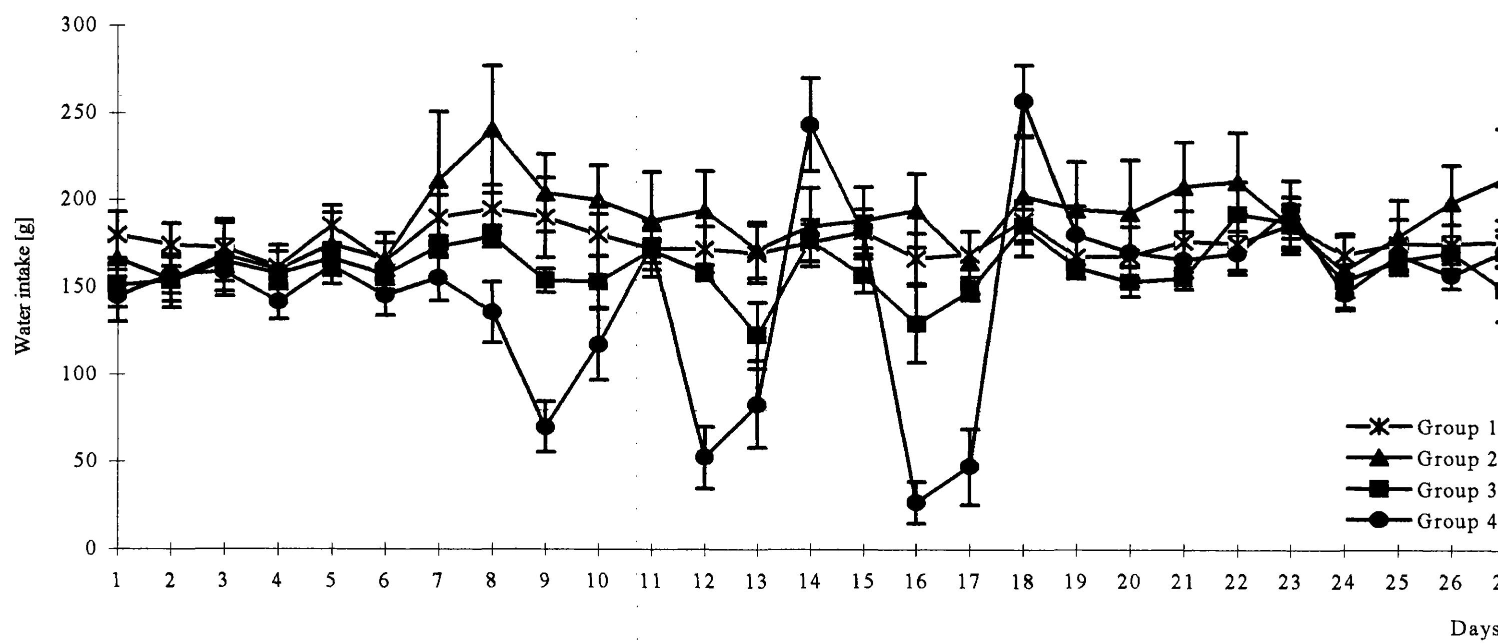
The general pattern of methionine intake was similar to the pattern of feed and water intake, with group 4 exhibiting the most dramatic changes. Moreover, it was noticeable that, after regimen A, group 1 had always the highest intakes of methionine.

Figure 9.1. Comparison of daily feed intakes in relation to the amount of methionine in the diet during the five regimens of the experiment.



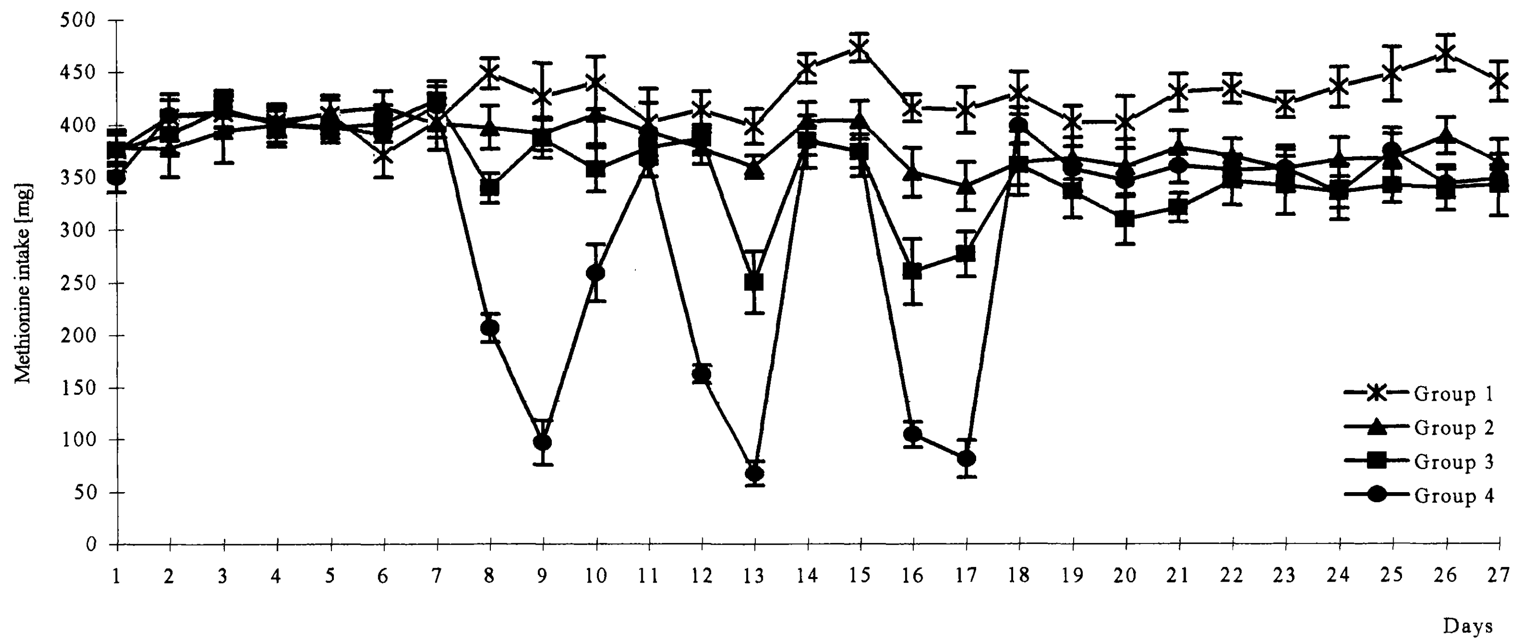
Regimens	A	B	C	B	C	B	D	E
Days	7	2	2	2	2	2	5	5

Figure 9.2. Comparison of daily water intakes in relation to the amount of methionine in the diet during the five regimens of the experiment.



Regimens	A	B	C	B	C	B	D	E
Days	7	2	2	2	2	2	5	5

Figure 9.3. Comparison of estimated daily methionine intakes in relation to the amount of methionine in the diet during the five regimens of the experiment.



Regimens	A	B	C	B	C	B	D	E
Days	7	2	2	2	2	2	5	5

9.1.3.3 Feed intake

Feed intakes of the birds during the experiment are presented in Table 9.4. When the effect of different methionine levels in the feed was examined, the mean feed intake of group 4 (smallest amount of methionine in feed) was found to be significantly lower than the other groups' ($p<0.05$). Comparing the effect of the regimens, the mean feed intake during regimen B was significantly lower than in the other regimens ($p<0.001$). Moreover, mean feed intake during regimen E was significantly higher than in regimen A ($p<0.001$). Differences between the regimens in response to methionine level treatment were confirmed by a significant interaction between the regimens and the effects of methionine level in the feed ($p<0.001$).

When the birds received adequate diet (regimen A), there was no significant difference ($p>0.05$) between the four groups in their feed intake. Progressing to regimen B, however, when the birds received the four levels of methionine in feed, the feed intake by group 4 decreased significantly ($p<0.001$), while groups 1, 2, and 3 showed no significant change ($p>0.05$) from values in regimen A. In addition, group 4's feed intake during this regimen (B) was also significantly lower ($p<0.01$) than in the rest of the regimens, while the other groups did not present such differences during the experiment. When they received only methionine-treated water in regimen C, the birds in group 4 recovered their feed intake so that there were no significant differences ($p>0.05$) compared to regimen A in any of the groups. Also, no significant differences ($p>0.05$) were observed between the groups within regimen C. After the training period, when the hens were allowed to choose between plain and treated water (regimen D), the feed intake of the four groups were not significantly different

($p>0.05$) from the values in regimen A. When the position of bottles was changed, similar observation was made in all groups. In addition, none of the feed intake values were significantly different ($p>0.05$) from one to another within regimens D and E.

Table 9.4. Feed intakes during the regimens of the experiment in relation to the level of methionine in the feed, and significance of effects of treatment, regimen, and their interaction.

Treatment	Regimens					
Methionine level in feed [%]	**A (7 days)**	**B (6 days)**	**C (4 days)**	**D (5 days)**	**E (5 days)**	**^{C}Mean feed intakes**
3.7 (group 1)^{A}	107.4^{b}	113.5^{b}	107.6^{b}	109.3^{b}	112.3^{b}	**110.0****
3.1 (group 2)^{A}	106.8^{b}	119.5^{b}	114.6^{b}	108.7^{b}	111.7^{b}	**112.3****
2.6 (group 3)^{A}	108.4^{b}	109.0^{b}	112.0^{b}	109.9^{b}	112.7^{b}	**110.4****
2.1 (group 4)^{A}	107.3^{b}	58.3^{a}	102.0^{b}	107.0^{b}	109.8^{b}	**96.9***
^{B}Mean feed intakes	**107.5^{2}**	**100.1^{1}**	**109.1^{2,3}**	**108.7^{2,3}**	**111.6^{3}**	

	Probability	**LSD**
Effect of treatment	0.046	11.8
Effect of regimen	0.001	4.1
Interaction between the effect of treatment and regimen	0.001	13.7

^{ab} Values within a column or row with different superscripts differ significantly ($p<0.05$).
Feed intakes are expressed as g/day.
Values are mean of $^{A}n=8$, $^{B}n=32$, and $^{C}n=40$.

9.1.3.4 Total water intake and water- (treated and untreated) intake proportions

The water intakes during the regimens of the experiment are shown in Table 9.5. It was observed that the adding of different amounts of methionine to the feed caused a significant reduction ($p<0.05$) of the mean water intake of group 4 (2.1% methionine) compared to group 2 (3.1% methionine). The examination of the effect of regimens showed that the mean water intake during regimen B is significantly lower ($p<0.001$) than the corresponding values of the other regimens. Moreover, the mean water intake values in regimens C and D are significantly higher ($p<0.001$) than in regimen A. The differences between the regimens in response to methionine level treatment were confirmed by a significant interaction between the regimens and the effects of treatment ($p<0.001$).

When the birds received adequate diet (regimen A), there were no significant differences ($p>0.05$) between water intakes by the four groups. When progressing to regimen B, water intake of group 4 reduced significantly ($p<0.001$), while values in other groups were not significantly different from regimen A. During regimen B, group 4 exhibited the smallest intakes, significantly different ($p<0.05$) from the other groups. The water intake of group 3 was also significantly ($p<0.05$) lower than group 2's but not was different from the intake of group 1. No significant difference ($p>0.05$) was observed between groups 1 and 2. In regimen C, when birds received only methionine-treated water, groups 3 and 4 increased their water intake. The intakes by all four groups during regimen C were not significantly different ($p>0.05$) from the intakes in regimen A. In addition, there were no significant differences ($p>0.05$) observed between the groups within regimen C. After the training period, when the hens were allowed to choose (regimen D), none of the groups showed significant difference ($p>0.05$) in water intake from the values in regimen A, and there were no significant differences ($p>0.05$) between the groups either. When the birds were transferred to regimen E, differences within regimen E and between regimen A and E were similar to regimen D in all four groups.

Table 9.5. Water- (treated and untreated) intakes during the regimens of the experiment in relation to the level of methionine in the feed, and significance of effects of treatment, regimen, and their interaction.

Treatment	**Regimens**					
Methionine level in feed [%]	**A (7 days)**	**B (6 days)**	**C (4 days)**	**D (5 days)**	**E (5 days)**	**^{C}Mean water intakes**
3.7 (group 1)^{A}	175.5^{bcde}	177.2^{bcde}	177.6^{bcde}	175.5^{bcde}	176.6^{bcde}	**176.5*, ** **
3.1 (group 2)^{A}	171.6^{bcde}	195.0^{de}	190.5^{cde}	202.1^{e}	187.6^{bcde}	**189.4** **
2.6 (group 3)^{A}	160.6^{bcd}	148.7^{b}	164.5^{bcde}	169.8^{bcde}	165.5^{bcde}	**161.8*, ** **
2.1 (group 4)^{A}	152.2^{bc}	69.4^{a}	182.3^{bcde}	188.9^{bcde}	167.9^{bcde}	**152.2***
^{B}Mean water intakes	**165.0^{2}**	**147.6^{1}**	**178.7^{3}**	**184.1^{3}**	**174.4^{2,3}**	

	Probability	**LSD**
Effect of treatment	0.001	35.3
Effect of regimen	0.001	11.3
Interaction between the effect of treatment and regimen	0.001	40.3

^{abcde} Values within a column or row with no common superscripts differ significantly ($p<0.05$).
Water intakes are expressed as ml/day.
Values are mean of ^{A}n=8, ^{B}n=32, and ^{C}n=40.

The birds' overall preference for treated and untreated water during the experiment was 64.1% and 35.9%, respectively, a significant difference from 50% ($p<0.001$).

The birds' proportional intakes of treated water during the choice period are shown in Table 9.6.

There was a significant ($p<0.001$) effect of treatment but not of the regimens on the preference for treated water indicating that position of the bottles was insignificant in water selection. There was no significant interaction found between the effect of regimen and the effects of methionine level in the feed.

Apart from group 4 (receiving the most deficient diet), the birds' proportional consumption of treated water was random in all groups during both regimens. This is reflected in the overall values inasmuch as differences 50% were not significant in groups 1, 2, and 3 but it was significantly ($p<0.05$) above 50% in group 4. The overall proportions during the two regimens were consistently not significantly different from 50% ($p>0.05$).

Table 9.6. Intake proportions of treated water during the choice period of the experiment in relation to the level of methionine in the feed, and significance of effects of treatment, regimen, and their interaction.

Treatment	**Regimens**		
Methionine level in feed [%]	**D (5 days)**	**E (5 days)**	**[C]Mean water intake proportions**
3.7 (group 1)[A]	38.7 n.s.	61.7 n.s.	**50.2*** n.s.
3.1 (group 2)[A]	57.2 n.s.	49.9 n.s.	**53.6*** n.s.
2.6 (group 3)[A]	56.2 n.s.	54.0 n.s.	**55.1*** n.s.
2.1 (group 4)[A]	98.5 s.	96.7 s.	**97.6**** s.
[B]Mean water intake proportions	**62.7** n.s.	**65.6** n.s.	

	Probability	**LSD**
Effect of treatment	0.001	30.1
Effect of regimen	0.693	21.0
Interaction between the effect of treatment and regimen	0.488	41.9

Water intakes are expressed as percentage of total (treated + untreated) water intake.
s.; n.s. Difference from 50% is significant ($p<0.05$), or not significant ($p>0.05$), respectively.
Values are mean of [A]n=8, [B]n=32, and [C]n=40.

9.1.3.5 Methionine intake

Methionine intakes during the regimens of the experiment are shown in Table 9.7. The comparing of mean methionine intakes showed that in the two groups most deficient in methionine (groups 3 and 4) the difference in treatment does not have significantly different effect on the mean methionine intakes ($p>0.05$). In contrast, different methionine levels in the feed resulted in a significant difference ($p<0.001$) between the two groups more adequate in methionine; the mean methionine intake of group 1 (adequate methionine in feed) was significantly higher than that of group 2 (second highest amount of methionine in feed). Moreover, both of these groups have significantly higher ($p<0.001$) mean methionine intake than groups 3 and 4. When the effect of the regimens was examined, mean methionine intake of the birds during regimen B was found to be significantly lower than the corresponding values in the other regimens ($p<0.001$). In addition, the means during the choice period (regimens D and E) are also significantly different from those in the previous regimens: higher than in regimen B but lower than regimens A and C ($p<0.001$). The differences between the regimens in response to methionine level treatment were confirmed by a significant interaction between the regimens and the effects of methionine level in feed ($p<0.001$).

When the birds received adequate diet (in regimen A), there were no significant differences ($p>0.05$) of methionine intake between the four groups. When the birds received four levels of methionine in the feed, methionine intake decreased significantly in groups 3 and 4 ($p<0.001$). When the comparison was made within regimen B, there was significant difference ($p<0.05$) observed between all the groups. In regimen C, when birds received only methionine-treated water, methionine intake increased in all groups, however this change was significant only in groups 3 and 4 ($p<0.001$). Values in this regimen were not significantly different from values in regimen A. When the comparison was made between the groups, group 4 was found to be significantly different ($p<0.05$) from groups 1 and 2. In addition, group 1 was also different ($p<0.05$) from group 3. After the training period, when the hens were allowed to choose (regimen D), group 3 showed a significant difference ($p<0.001$) from regimen A in methionine intake. However, a significant difference ($p<0.05$) was observed only between group 1 and the other groups. When the birds were transferred to regimen E, the significant differences were the same as what were observed in regimen D.

Table 9.7. Methionine intakes during the regimens of the experiment in relation to the level of methionine in the feed, and significance of effects of treatment, regimen, and their interaction.

Treatment	**Regimens**					
Methionine level in feed [%]	**A (7 days)**	**B (6 days)**	**C (4 days)**	**D (5 days)**	**E (5 days)**	**[C]Mean methionine intakes**
3.7 (group 1)[A]	397.4^{def}	419.9^{f}	442.4^{f}	420.0^{f}	442.3^{f}	**424.4*** **
3.1 (group 2)[A]	395.2^{def}	370.3^{cde}	403.0^{ef}	367.9^{cde}	369.2^{cde}	**381.1** **
2.6 (group 3)[A]	401.2^{ef}	283.3^{b}	373.5^{cde}	335.0^{c}	340.3^{c}	**346.7***
2.1 (group 4)[A]	397.0^{def}	122.5^{a}	350.9^{cd}	364.3^{cde}	352.3^{cd}	**317.4***
[B]Mean methionine intakes	**397.7^{3}**	**299.0^{1}**	**392.4^{3}**	**371.8^{2}**	**376.0^{2}**	

	Probability	**LSD**
Effect of treatment	0.001	37.4
Effect of regimen	0.001	15.2
Interaction between the effect of treatment and regimen	0.001	45.6

[abcdef] Values within a column or row (for same heading) with no common superscripts differ significantly ($p<0.05$).
Methionine intakes are expressed as mg/day.
Values are mean of An=8, Bn=32, and Cn=40.

9.1.4 Discussion

The design of this experiment was based on the assumption that methionine concentrations of the drinking water has no effect on the preference for treated water. This assumption arose in Experiment 5 where the birds were choosing methionine-treated water in equal amounts regardless of its methionine concentration. Therefore, in the present experiment, methionine concentrations of the treated water were chosen so that birds receiving adequate or near adequate levels in their feed (groups 1 and 2) should not exceed very much their actual needs, and those on deficient feed (groups 3 and 4) should be able to match their requirements from the treated water.

The present experiment intended to demonstrate that, under the conditions of the trial, the level of methionine adequacy of the feed is reflected in the selection-behaviour of the birds during the choice situation. The water intake results indicate that those birds that received feed most deficient in methionine (2.1 g/kg in group 4) experienced a well being from the methionine added via the drinking water during the training period. Thus, these birds were able to associate the cue with the treatment by the time the choice was offered (regimen D), and they could make the right choice according to their physiological needs even when the position of the bottles was changed (regimen E). In this group, the percentage of choice made in favour of treated water was virtually the same in regimens D and E, that is the birds have used the colour cue. The difference in methionine adequacy for these birds was manifested in the proportion of their choices between the two types of water. That is, choices made in favour of methionine-treated water in group 4 was more than 95.0%, whereas this figure was markedly lower (less than 60%) in the group (3) receiving the second lowest level (2.6 g/kg) methionine, and even lower in the other groups.

When the feed contained adequate or nearly adequate amounts of methionine (groups 1 and 2), the birds did not feel the physiological effects of deficiency. Thus, these birds did not follow the colour when the position of the bottles was swapped. Moreover, the proportion of treated water consumed had also swapped when the bottles were swapped (from regimen D to E). Therefore, it appears that the birds made their choices according to the bottle position. This response to position change was the most pronounced in group 1, where the intake proportions from treated water in regimens D and E were 38.7% and 61.7%, respectively. Such behaviour (i.e. position preference) of the birds has been described in an earlier experiment in this work (Experiment 2).

Daily feed intakes show that feed intake of birds on the most deficient feed declines at the first instance of experiencing the deficiency, whereas the second most deficient group reduced feed intake only at the second and third time, and in a more moderate way. A similar phenomenon has been described by Boorman (1979): a mildly imbalanced diet is initially not sufficient to cause the homeostatic reduction of feed intake. The author also noted that feeding the imbalanced diet continuously will eventually derange the amino acid pattern of tissues which, in turn, will lead to a decrease of feed intake that is sufficient to detect. Feed intake of those groups that received methionine in amounts above NRC (1994) requirement, showed no fluctuation during the experiment.

Feed intake results averaged by regimens suggest that a 70% reduction in methionine intake results in a 46% reduction in feed intake (group 4). A milder deficiency (i.e. 30% reduction in methionine intake in group 3) did not cause a similar decrease of feed intake. However, the big standard error in this group indicates great individual variation. Morris and Fisher (1970) have shown that individual methionine requirements vary greatly amongst the birds. Therefore, some birds perhaps feel only a slight deficiency thus increase their feed intake in compensation, whereas others experience a greater deficiency thus their feed intake decreases. The resulting average feed intake does not change from regimen A to B. A slight (7%) decrease in methionine intake (group 2) resulted in a 10% increase in feed intake, because some birds in the group may have attempted to compensate for the deficiency by increasing feed intake. These observations are in agreement with the report by Boorman (1979).

In group 4, where the feed was most deficient, water intake of birds after the first period of deficiency rose back to the level of the control period. However, after deficiency was experienced the second (or third) time, water intakes rose well above the control level, indicating the learning process. That is, with the help of the colour cue, the birds have learned the beneficial physiological effects of treated water.

Daily methionine intake results show that after the control regimen, methionine intake of group 1 was always above the other groups' intake, and also above the control level. This group had already received adequate methionine in the feed, the increased intake was caused by the additional methionine in the drinking water. That birds continued to consume an increased amount of methionine indicates that the 0.025% (in water) excess methionine had no adverse effect. As an effect of the previously mentioned positional preference, methionine intakes in the second choice period (regimen E) grew even slightly above the consumption in regimen D.

In general, when hens received enough (at least 300 mg/day) methionine in the drinking water (Experiment 8 group 4, Experiment 5 groups 2, 3 and 4, and Experiment 3), their daily feed and water intake patterns were similar. This was reflected in their proportional preference for treated and untreated water.

It should be noted that the confounding effects deriving from the design of this present experiment involving changes of methionine concentration in the feed and water simultaneously might give rise to problems, if the basic assumption for this experiment is not valid. Therefore, perhaps it would desirable to perform an additional experiment where various methionine concentrations in the feed are used while drinking water contains constant concentrations of methionine.

The main conclusion of the experiment was:

a feed containing 2.1 g/kg methionine enables birds to express an appetite for methionine-treated water.

10.0 General Discussion

There is a substantial body of evidence in the literature that fowls possess effective self-selection. They have been shown to be able to select for several essential nutrients such as calcium (Wood-Gush and Kare, 1966; Hughes and Wood-Gush, 1971b), phosphorus (Holcombe *et al.*, 1976a), zinc (Hughes and Dewar, 1971), thiamine (Hughes and Wood-Gush, 1971a), vitamin B_6 (Steinruck *et al.*, 1991), vitamin C (Kutlu and Forbes, 1993), for protein (Holcombe *et al.*, 1976b; Kaufman *et al.*, 1978; Shariatmadari and Forbes, 1993), amino acids (Newman and Sands, 1983; Steinruck *et al.*, 1990a; Picard *et al.*, 1993; Uzu *et al.*, 1993), and also for energy (Booth, 1972; Musten *et al.*, 1974; Bray, 1982). In contrast, there are far fewer reports on the mechanism and control of self-selection.

Complete diets are used world wide, and the standard of knowledge regarding their formulation is very high. However, choice feeding was also a recognised and widely practised method in the past (Winter and Funk, 1951) still, little is known about whether or not its application in formulating diets for choice of feeding regimes has any advantages over the feeding of complete diets.

The present project has focused on layers' specific appetite for methionine and the supplementation of this amino acid in the drinking water rather than in the feed. The aim was to investigate the mechanism of self-selection, and to provide answers to the following questions:

1) Can the birds show an appetite for methionine delivered in drinking water while fed on a methionine deficient diet?

2) What is the minimum level of methionine in the feed for birds to show an appetite for it in drinking water?

3) Does the concentration of methionine in drinking water affect the birds' appetite for this amino acid?

4) What is the minimum training time necessary for the birds to associate their physiological need and the colour cue?

5) Can the birds regulate their methionine intake from the drinking water?

6) What are the practical implications of the findings of this research?

The main findings of the research were:

1) a 140 g/kg CP diet is sufficient to support a high rate of egg production and suitable to assess a specific appetite for methionine in drinking water (Experiment 1a);

2) the typical feed and water intake of layers were 116.7 g/day and 168.5 g/day, respectively, resulting a 1:1.44 feed:water ratio (Experiment 1b);

3) a cue (colour) and training is necessary for the expression of appetite for methionine in the drinking water (Experiment 3), without them hens are unable to express appetite for methionine treated water even if they receive deficient diet (Experiment 2);

4) the route of methionine supplementation does not influence normal appetite (Experiments 3 and 6a);

5) in practice, birds seem unable to regulate their methionine intake once the requirement is satisfied (Experiment 4);

6) the detection threshold for methionine (i.e. the level of methionine in drinking water for which birds can express their appetite) is at least 0.025 % (Experiment 5);

7) the feed intake of birds experiencing a methionine deficiency returns to normal following the addition of methionine, regardless to whether it is supplied in feed or water (Experiment 6a);

8) the introduction of a methionine deficient diet to the birds induces a reduction of feed intake 5 hours later, and this decrease becomes significant after 8 hours;

9) if the birds consume a diet deficient in methionine for a short period (i.e. 5 hours), the feed intake of even those birds which have been transferred to an adequate diet is adversely effected (Experiment 6b);

10) the choices for methionine treated water are above 90% when an 8-hours exposure time is used, whereas choices are considerable lower at other exposure times (Experiment 7);

11) when testing the memory of the birds, those which showed the highest rate of right choice received the 8-hour exposures during training (Experiment 7);

12) a deficiency of 0.43% was necessary for the birds to express an appetite for methionine in water, whereas those receiving adequate or nearly adequate amounts of methionine in their feed did not select methionine-treated water in a choice situation (Experiment 8).

10.1 Specific appetite for amino acids

The ability to select for amino acids was first demonstrated in broilers for L-lysine (Newman and Sands, 1983), and for methionine, (Steinruck *et al.*, 1990a).

Day-old broiler chicks were also shown to be able to make appropriate selections between amino acid deficient (in lysine or methionine or tryptophan) and balanced diets within the first day, and within hours when 8 days old (Picard *et al.*, 1993). Similarly to broilers, laying hens can also adjust their feed intake in response to methionine deficiencies, and make appropriate selections when given a choice between methionine-deficient and adequate diets, as demonstrated by Uzu *et al.* (1993).

A crucial pre-requisite of effective self-selection for a nutrient is the deficiency of that particular nutrient, i.e. the animal has to experience the adverse physiological effects of the deficient diet. In other words, deficiency symptoms have to first appear before the birds start to select for the nutrient (Kirchegessner and Paulicks, 1994). Steinruck *et al.* (1991) reported that in a choice experiment with vitamin B_6, broilers started to avoid the deficient diet only at the third week, when the first deficiency symptoms appeared.

In the case of dietary amino acid deficiency or imbalance, the typical symptom is the suppression of food intake (Harper *et al.*, 1970; Okumura and Mori, 1979; Li and Anderson, 1983), although the animal's nutritional status (fed, fasted, or depleted) (Sanahuja and Harper, 1962; Kaufman *et al.*, 1978) and the type and degree of amino acid deficiency (Ousterhout, 1960; Boorman, 1979; Okumura and Mori, 1979; Muramatsu, 1985) may affect its latency and magnitude. In agreement with these, daily food intake was suppressed significantly in methionine-deficient birds as compared with the control or choice regimens during the course of the present research (Experiments 3, 4, 5, 6a, and 8). Moreover, since all nutrient values were based not on measurements but on book values (NRC, 1994), the birds' responses confirmed that calculated values were close to the actual composition. In addition, in these experiments, the introduction of a deficient diet resulted in increased SEM values compared to those in the control period (as seen in figures 5.1, 6.1, 6.3, 7.1, 8.1 and 9.1). This indicates that within a flock offered a diet deficient in methionine, there is a wide variation in the depression of feed intake.

Experiment 6a has shown that the reduction of feed intake followed five hours after the introduction of the deficient diet, and this decrease became significant after eight hours. In addition, food intake of those birds experiencing deficiency increased promptly and significantly once they were returned to normal diet or received methionine supplemented water.

On the other hand, food intake is increased if the deficiency is only marginal (as seen in Experiment 5). This response is actually an attempt to overcome the deficiency. A similar phenomenon has been observed (Kare and Ficken, 1963) when chicks were fed on a low calorie diet. Having their energy intake restricted, the birds showed a marked preference for sucrose solution to which they are otherwise indifferent, and increased fluid intake to overcome the energy deficiency. However, an adequate diet does not result such behaviour. Thus, being fed on a diet adequate in energy, chickens did not select the sucrose solution (Kare and Maller, 1967). In the present research, hens receiving a diet adequate in methionine do not show preference for the methionine-treated water (Experiment 8).

An other pre-requisite of expressing appetite for a diet component is the ability to associate non-nutritive properties (cues) of the food with nutritive qualities (Pick and Kare 1962; Brindley 1965; Schuler 1983; Kutlu and Forbes 1993). This ability is a result of a genetic predisposition and a learning process (Hughes, 1979). It was clearly demonstrated in the present work that without training, and without the use of a cue (colour), hens are unable to select for methionine (Experiment 2). However, when colour cue assisted recognition of the physiological effects of the diet, after a training period the birds were able to express an appetite for methionine and chose the treated water in the choice situation (Experiments 3, 4, 5, 7, and 8).

It is also shown by the present study that the duration of training, i.e. the time during which birds are exposed to the deficiency, is very important. The minimum (threshold) period of exposure to methionine deficiency proved to be eight hours (Experiment 7), which is the same length of time that is needed for a significant decrease of food intake after methionine deficiency (Experiment 6a). This is the period that is necessary for the birds to learn to associate the colour cue with the physiological effects of the appropriate diet. When compared with the shorter exposures (1hr, 2hrs, and 4hrs), the choices for the methionine water at the eight hour exposures were by far the highest, above 90% (Experiment 7). This indicates that during eight hours, most of the birds were able to discriminate the physiological effect of amino acid deficiency and adequacy coding the two different colours. This suggested that the animals did not immediately detect the food quality in terms of amino acid deficiency. Therefore, it can be concluded that the suppression of food intake caused by an amino acid-deficient diet is a phase of the mechanism that leads to effective selection. The above findings agree with the observations by Murphy and King (1987). To estimate the maximum learning-time required by sparrows, they conducted choice experiment using low- and high-SAA diets, and examined the birds' response to a reversal of diet location. After the birds had established a clear preference for the high-SAA diet, diet location was switched during the night. The birds responded by altering their feeding location and thereby maintained their preferential intake of the high-SAA diet. The authors could not determine the exact latency of this response, but they reported that it was less than 16 hours.

It had been suggested (Hughes and Wood-Gush, 1972; McFarland, 1973) that the intake of a nutrient is kept above the lower limit by a phenomenon called "positive postingestional feedback". It means that if the animal is deficient of a nutrient and experiences harmful physiological effects, it will select the appropriate nutrient in order to improve well-being. This, in turn, reinforces the animal's behaviour. In the case of methionine, a supplement of 0.025% in water already improved the hens well-being, and 90% of the choices were made for it (Experiment 5). It should be noted, however, that the intake of the first limiting amino acid methionine influences the intakes of the other amino acids (Gous and Kleyn, 1988), thus birds would probably show an appetite for much lower levels as well.

In contrast to the lower limit of nutrient intakes, the upper limit seems to be set by palatability and adverse physiological effects (such as poisoning) rather than nutritional requirements (Hughes, 1979). During the course of this research, the birds did not reduce their intake from methionine-treated water once they have satisfied their appetite, even when receiving the highest concentration of methionine (3.0% in Experiment 4).

In addition to the above results of this research, it is important to note that supplying methionine in water as opposed to feed has no known harmful effect on the animals. There are reports (Damron and Goodson-Williams, 1987; Damron and Flunker, 1992) on giving methionine in drinking water of broilers, and compared with the practical diet, this practice was found to have no adverse effect on production. They also showed that supplying methionine to broiler chicks through drinking water did not reduce their feed and water intake. Supplementing drinking water with methionine for the first 21 days in order to reduce the number of diet changes is a now routine practice by some producers (Damron and Flunker, 1992). Similarly to these reports, the present research (Experiments 3, 4, 5, 6a, 7, and 8) found no evidence of adverse effects on laying hens when methionine was added to their drinking water rather than to their feed. There was no suppression of feed and water intake in consequence of this way of methionine delivery. Moreover, the way of delivering methionine (in feed or water) to the birds seemed indifferent from the point of view of satisfying their requirements (Experiments 3, 4, 5, 6a, 7, and 8).

It was a general observation throughout the present study (Experiments 3, 4, 5, and 8) that some 10% of the flock fed the methionine-deficient feed did not chose the treated water in the choice situation. This might be an indication of that these birds do not experience the deficiency symptoms, probably due to an overconsumption of the feed.

In summary, this research has shown that, if fed on a diet deficient enough in methionine, layers can show an appetite for this amino acid supplied in the drinking water. For this, the physiological effects of the diets had to be coded, i.e. colour cue was introduced, and the birds were trained to recognise the deficient and adequate diets. Hourly feed-, and water intake measurements showed that 8 hours are needed for deficiency symptoms to occur after introducing low-methionine diets. Based on this result, it is suggested that for effective selection, birds should be exposed to methionine deficiency for 8 hours during training. This enables almost all birds in a flock to learn the colour-physiological effect associations, thereby more than 90% of the choices are made for methionine-treated water. Once trained, the smallest amount of methionine supplemented in the water (0.025%) seems to be already beneficial to the birds, and they select it in a choice situation. Thus, their selection threshold for methionine is at least 0.025%. Under the conditions of the experiments of this work, the birds were unable to regulate their methionine consumption after satisfying their requirements, i.e. there seemed to be no upper limit of intake. Finally, the way of methionine delivery does not seem to influence normal appetite, or the recovery of appetite after a period of deficiency.

10.2 Industrial implications of supplying methionine in the drinking water for layers

The present study has demonstrated that suppressed feed intake of the birds caused by methionine deficiency is corrected regardless of the way of methionine supplementation (in feed or water). Also, it has been shown (Experiment 5) that if the birds are fed on a diet deficient in methionine, in an attempt to meet their methionine requirement they drink 30-40 g/day more from the treated water than when on a normal diet. In addition, it has also been demonstrated (Experiment 7) that, after a sufficient period of training, birds can remember the colour-diet associations. Therefore, it is suggested that it could be an industrial practice to supply methionine in the drinking water instead of adding it to the mash feed. Layers could be trained at an early age to recognise methionine adequacy and deficiency, and this ability can then be recalled at a later stage of their life. Thus, in contrast to the present practice of satisfying the methionine requirement of 95% of a flock, diets could be formulated so that the methionine from the feed and water would satisfy a lesser percentage only, e.g. 70% (which means that, in contrast to the 94% at the present, only 69% of the birds would be over-fed). This would give a flexibility for the rest of the flock to match their exact requirement (from the drinking water only). The determination of the proportion of "flexible" hens, and the optimum level of methionine in the feed and water for maximum output and maximum income will be the task of a future, industrial-scale study. The experiments in the present project used only a small amount of birds.

It also has to be noted that methionine in the water can degrade into an aldehyde (methional) causing cabbage-like odour (Baker, 1977; Damron and Goodson-Williams, 1987). Therefore, in the case of any adverse effects of this oxidative reduction, it is advisable to flush methionine weekly from the water system. The effects of methional at various methionine concentrations in water should, however, be investigated precisely in a future study.

Finding ways of eliminating or reducing potential pollutants is a very important factor in all areas of the agricultural industry. Due to environmental pollution (e.g. contamination of surface and groundwater) the expansion of animal agriculture has to be severely limited in some regions, and legislation is now being established in various countries to control environmental contamination from animal wastes. The management of animal waste is a particularly important aspect of sustainable farming. In the poultry industry, nitrogen (ammonia) in the faeces, deriving from the metabolic break-down of surplus amino acids, is the major pollutant. An obvious solution is the avoidance of the overfeeding with amino acids. Methionine supplementation of the diet has been shown (Schutte *et al.*, 1983) to significantly improve feed efficiency in the later stages of laying. It is also known (see section 2.1.2.3) that a more efficient protein utilisation can be achieved by the closer matching of the birds' amino acid requirement from the diet.

The liquid egg sector, or breaking industry, grows every year. There is unfortunately little information concerning the management practices on the yield and composition of liquid egg, nevertheless, a flexible way of methionine supplementation to the hens' diet might offer a way to increase profit. It has already been mentioned in the literature review (section 2.1.2.4) that egg weight (e.g. Martin *et al.*, 1969; Shafer *et al.*, 1996; Harms and Russel, 1998) and composition (e.g. Shafer *et al.*, 1996, 1998) can be manipulated by methionine supplementation, without adversely affecting functionality, or mortality rate.

Delivering amino acid in drinking water can provide a degree of 'nutrient security' or diet formulation flexibility in a poultry starter feed programme and during the laying period. It would also give the opportunity of the consistent supply of methionine to the birds, and consequently, improved feed efficiency. In addition, this practice would aid a better handling of stressful conditions.

Finally, it should be noted that most of the results in this research are generalisations about the behaviour of a population since they represent the mean values of a group, however, studying the individuals can also provide some important information.

11.0 CONCLUSIONS

Based on the results of the experiments and the discussions, the following general conclusions can be drawn:

1. Birds can not select methionine-treated water without a discernible cue to assist their appetite memory.

2. Hens can be effectively trained during a daily exposure of minimum eight hours to a methionine deficiency to become accustomed to the colour cues of the water supply creating methionine deficient and normal diets, thereby associating a particular colour with the deficiency.

3. After a sufficient length of training during which both plain and methionine-treated water are associated with colour cues, birds are able to choose between the two types of nutrient supply.

4. When receiving a methionine deficient diet, hens are able to show an appetite for water containing a methionine concentration as low as 0.025% (although the threshold level might be lower).

5. The methionine deficiency disturbs their whole body metabolism and behaviour. Within a day of introducing the methionine deficiency, a reduction in feed intake is observed in proportion to the degree of the deficiency.

6. Within a flock offered a diet deficient in methionine, there is a wide variation in the depression of feed intake.

7. The hourly pattern and amount of feed intake of laying hens is the same when methionine supplement is given in the feed or the drinking water.

8. When hens are trained to recognise methionine-treated water, in response to a methionine deficiency, they can remember training and respond to consume the colour cue water.

9. The birds' memory for the associations between the colour cues and physiological needs can last for at least 45 days.

10. Most birds can detect a methionine deficiency in feed if subjected to it for at least two hour.

11. When subjected to a methionine-deficient diet, within the ranges of deficiency tested, hens with feed consumption levels in the upper 10% of a flock do not appear to experience a deficiency.

12. Deficiency symptoms (i.e. reduced feed intake) occur 8 hours after consuming a methionine-deficient diet.

13. When birds consume a methionine-deficient diet and are returned to the normal diet before the appearance of deficiency symptoms, their subsequent feed intake, and therefore their performance, might be adversely affected.

14. When the birds are fed a methionine-deficient feed, and methionine is supplied in the drinking water, in an attempt to meet their methionine requirement, hens, apart from polydipsic ones, can drink 30-40 g/day more than when on normal diet (i.e. adequate feed and plain water).

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Publications

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Cadirci, S. and Smith, W.K. (1999). Use of colour cue to determine the appetite of laying hens for methionine in drinking water. British Poultry Science, **40**:S7-8.