Araştırma Makalesi

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

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ABSTRACT

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 for laying hen. In addition, the memory of the birds was assessed using the colour cue associations with the physiological needs. Birds were subjected to the combinations of diet adequate or deficient in methionine and normal or methionine-treated water. The concentration of methionine in treated water was 0.1%. First information was obtained on the birds' normal intake of feed, water and methionine. For this, the hens were fed diet adequate in methionine and normal water. The colours were associated with the two types of water (normal or methionine-treated). Hens were exposed to different time of methionine deficiency, by being fed a diet deficient in methionine. Finally, the hens were fed a methionine-deficient diet and were offered a choice of both normal and methionine-treated water. The birds showed a clear preference for methionine-treated water even after changing the position of colour-cued drinking bottles. 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.

Key words: Appetite; Laying hens; Methionine; Drinking water; Colour cue; Exposure time

TAVUKLARIN İÇME SUYUNA KATILAN METİYONİNE İŞTAH GÖSTEREBİLDİĞİ MİNİMUM ALIŞTIRMA ZAMANININ BELİRLENMESİ

ÖZET

Bu çalışmayla, seçmeli yemleme öncesi alıştırma döneminde tavukların metiyonine iştah göstermelerini sağlayacak, metiyonince yeterli ve yetersiz rasyonlarla en az besleme süresini belirlemek amacıyla tasarlanmıştır. Buna ek olarak, tavukların fizyolojik ihtiyaçları ile renk ilişkilendirilip hatırlama yeteneği değerlendirilmiştir. Metiyonince yeterli ve eksik rasyonlar kullanılarak, tavuklar normal ve metiyonin karışımı su bileşimlerine tabi tutulmuştur. Suyun metiyoninle muamele edilmiş konsantrasyonu %1 olarak ayarlanmıştır. İlk olarak günlük normal yem, su ve metiyonin tüketimleri elde edilmiştir. Bunun için, tavuklar metiyonince yeterli yem ve normal su ile beslenmiştir. Metiyonince yeterli ve yetersiz yemler renklerle ilişkilendirilmiştir. Metiyonince yetersiz yem aynı gün içinde farklı sürelerde ardışık olarak verilmiştir. Tavukların içmiş oldukları su şişesi renkleriyle yem ve su kombinsyonlarının fizyolojik etkilerini tanımaları sağlanmıştır. Sonunda tavuklar metiyonince yetersiz yem ile beslenip, normal ve metiyonin muamele edilmiş su seçenek olarak farklı renklerde sunulmuştur. Metiyonince yetersiz yemle beslenen tavuklara metiyoninli ve normal su tercihi sunulduğunda, tavuklar metiyoninli suyu normal suya tercih etmiş olup, sulukların yerlerinin değiştirilmesi sonucu etkilememiştir. Tavuklar iki saatlik metiyonince yetersiz yeme maruz bırakıldığında, büyük çoğunluğun metiyonin yetersizliğini algılaması sağlanabilmiştir. Fakat, tavuklar 8 saat metiyonince yetersiz yeme maruz bırakıldığında, sürünün % 90'ı fizyolojik ihtiyaçları ile renk arasında ilişki kurup, eksikliği durumunda metiyoninli suyu seçebilmişlerdir.

Anahtar sözcükler: İştah; Yumurta tavugu; Metiyonin; İçme suyu; Renk uyarısı; Maruz kalma süresi

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INTRODUCTION

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. In order for animals to differentiate between foods to make up an appropriate diet, sensory cues are important. These can be, for instance, colour, and smell, the taste or texture of the food (Forbes, 1995). Birds rely primarily on their vision to identify foods. Research of the past years has made it clear that animals learn to associate the sensory properties of foods with the metabolic consequences of eating those foods (Kutlu and Forbes, 1993; Cadirci *et al*., 2009). Methionine is the first limiting amino acid in the conventional corn-soybean and wheat-soybean diets (Leong and Mc Ginnis, 1952; Harms and Damron, 1969; Fisher and Morris, 1970; Schutte and Van Weerden, 1978; Schutte *et al.,* 1983, 1984, 1994; Waldroup and Hellwig, 1995), and synthetic methionine has been used for over six decades as a supplement to these diets. In addition to its main role as a component of proteins it also acts in various metabolic pathways. With the use of sensory cues it is possible to envisage a learned appetite for an essential nutrient (e.g. methionine) (Cadirci and Smith, 1999). Birds quickly learn to associate the sensory properties of a food with the metabolic consequences of eating it. Cadirci *et al*. (2009) reported that layers fed methionine deficient diet were able to select for water supplemented with methionine in favour to pure water. 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. 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. However, Kirchgessner and Paulicks (1994) reported that animals do not start to select a nutrient before deficiency symptoms occur. This study 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 the previous study (Cadirci *et al*., 2009) that, after a training period, the birds exhibits an appetite for methionine-treated water. 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. In addition, the memory of the birds was assessed using the colour cue associations with the physiological needs.

MATERIALS AND METHODS

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.

Diets

Two feed formulations (Feed 1, Feed 2) were used in this experiment, as shown in Table 1. 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 pre-training 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.

Table 1. The ingredient- and calculated nutrient composition of Feeds 1 and 2.
Çizelge 1. Rasyon 1 ve rasyon 2 için kullanılan yem maddeleri ve hesaplanmış besin içerikleri.

Ingredient composition	Ration 1 [g/kg]	Ration 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

The exposure regimens for each group are shown in Table 2. 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 2. Exposure time and training regimen of birds.
Çizelge 2. Alıştırma ve uygulanma zaman rejimleri.

Group	Exposure time [hours]		Repetitions
	Diet 1	Diet 2	in a day (16 hrs)
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.

Measurements

Food 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.

The results of experiment was 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.

RESULTS

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.

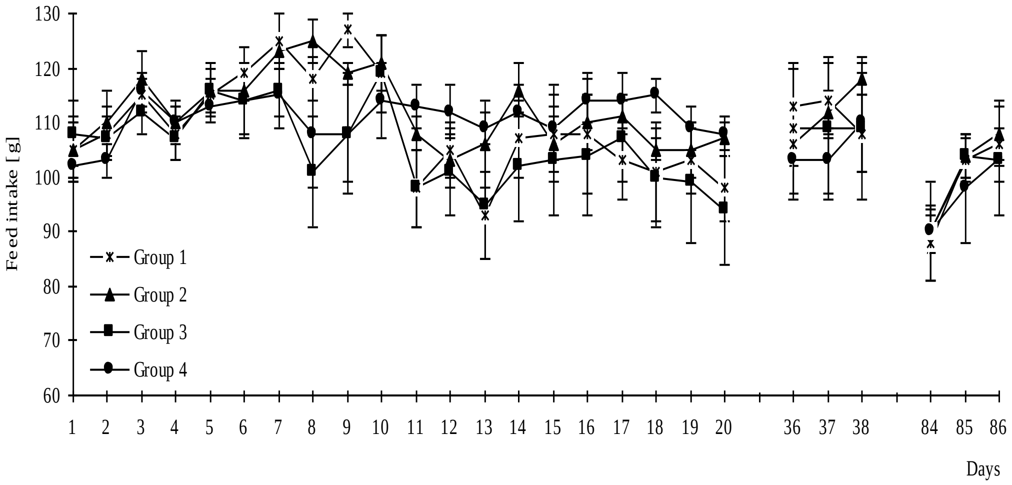
Daily feed-, water-, and estimated methionine intake

The daily feed and water intake, and the estimated methionine intake during each of the 26 days is presented in Figures 1, 2 and 3. 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,

Figure 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

Şekil 1. Suyla verilen metiyoninin uygulanma zaman uzunluğu ile günlük yem tüketimi arasındaki ilişki

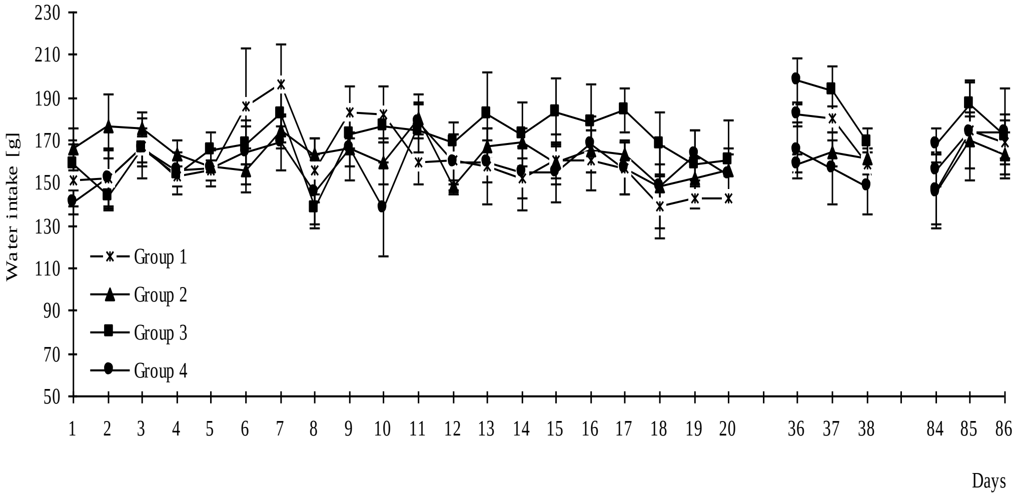


R	A	B	C	D	E	F
D	7	1 1 1	5	5	3	3

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.
R=Regimens
D=Days

Figure 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

Şekil 2. Suyla verilen metiyoninin uygulanma zaman uzunluğu ile günlük su tüketimi arasındaki ilişki

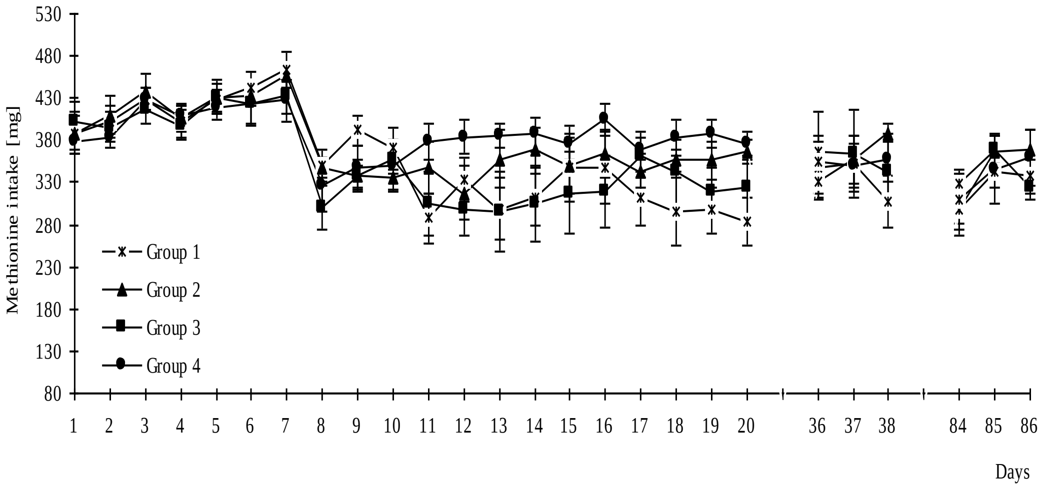


R	A	B	C	D	E	F
D	7	1 1 1	5	5	3	3

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.
R=Regimens
D=Days

Figure 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

Şekil 3. Suyla verilen metiyoninin uygulanma zaman uzunluğu ile günlük metiyonin tüketimi arasındaki ilişki



R	A	B	C	D		E		F
D	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.
R=Regimens
D=Days

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 (Table 3):
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.

Feed intake

Feed intakes during the regimens of the experiment are shown in Table 3. 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.

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

Daily water intakes (total) during the regimens of the experiment are shown in Table 4. 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 5. 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 ($p<0.05$).

Table 3. 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

Çizelge 3. Suyla verilen metiyoninin uygulanma zaman uzunluğu ile rejimlere ait ortalama günlük yem tüketimi arasındaki ilişki

Treatment Exposure Time [Hour]	Regimens A (7 days)	B (3 days)	C (5 days)	D (5 days)	E (3 days)	F (3 days)	^{C}MFI
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}MFI	$113.3^{3,4}$	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.
Mean feed intakes (MFI)
^{1,2,3,4} Values within a row with no common superscripts differ significantly ($p < 0.05$).

Table 4. 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

Çizelge 4. Suyla verilen metiyoninin uygulanma zaman uzunluğu ile rejimlere ait ortalama günlük su tüketimi arasındaki ilişki

Treatment Exposure Time [Hour]	Regimens A (7 days)	B (3 days)	C (5 days)	D (5 days)	E (3 days)	F (3 days)	^{C}MFI
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}MFI	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.
Mean feed intakes (MFI)

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.

Mean methionine intake

The mean methionine intakes during the regimens of the experiment are shown in Table 6. 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 5. 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

Çizelge 5. Suyla verilen metiyoninin uygulanma zaman uzunluğu ile rejimlere ait metiyoninli su tüketim oranları arasındaki ilişki

Treatment Exposure Time [Hour]	Regimens B (3 days)	C (5 days)	D (5 days)	E (3 days)	F (3 days)	^{C}MIP
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}MIP	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.
Mean intake proportions (MIP)
^{1,2} Values within a row with no common superscripts differ significantly ($p < 0.05$).

Table 6. 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

Çizelge 6. Suyla verilen metiyoninin uygulanma zaman uzunluğu ile rejimlere ait ortalama günlük metiyonin tüketimi arasındaki ilişki

Treatment Exposure Time [Hour]	Regimens A (7 days)	B (3 days)	C (5 days)	D (5 days)	E (3 days)	F (3 days)	^{C}MFI
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}MFI	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.
Mean feed intakes (MFI)
^{1,2}Values within a row with no common superscripts differ significantly ($p < 0.05$).

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. Cadirci. (2001) reported 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 study. 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.

CONCLUSION

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.

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