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The Use of Colour Cue for Investigating the Ability of Laying Hens to Regulate Methionine Intake from Drinking Water*

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Abstract
The appetite of laying hens for methionine and the hens' ability to regulate methionine intake in drinking water were investigated. Birds were subjected to the combinations of diet adequate or deficient in methionine and methionine-treated or normal water. In regimen A, birds were fed diet adequate in methionine and normal water was provided from bottles. In order to enable laying hens to differentiate between the water-supply bottles containing normal and methionine-treated water, colour cues and training of the birds were introduced in Regimens B, C, and D. The birds were allowed to become accustomed to the colour cue of their water supply bottles, and to the physiological effects of their diet and drinking water, then, followed a "free choice" part of the experiment. The concentration of methionine in treated water was 0.15% (w/v) in Regiment E, and 0.30% (w/v) in Regiment F. Feeding a diet deficient in methionine resulted in a substantial reduction in the intake of both feed and water. When the drinking water was then supplemented with methionine, both feed and water intake was restored to the normal level, moreover, methionine consumption equalled or exceeded that attained when methionine was supplied in the feed alone. Finally, the hens were fed a methionine-deficient diet and were offered a choice of both normal and methionine-treated water. The hens fed methionine-deficient diet were able to select for water supplemented with methionine in preference to pure water by using colour cues. However, birds were not able to regulate methionine consumption for their optimum requirements from drinking water.

Key words: Laying hen, methionine, regulate, drinking water, colour cue

Renk Belirteci Kullanılarak Yumurta Tavuklarının İçme Suyundan Metiyonin İhtiyacını Düzenleyebilme Kabiliyetlerinin İncelenmesi

Özet
Yumurta tavuklarının metiyonine istek ve içme suyundan metiyonin alımlarını düzenleyebilme kabiliyetleri incelenmiştir. Tavuklar metiyonince yeterli veya yetersiz yem ile normal veya metiyonin karışımı su kombinasyonlarına tabi tutulmuştur. Tavuklara A beslenme döneminde metiyonince yeterli yem ve şişelerden normal su verilmiştir. Tavukların B, C ve D beslenme dönemlerinde normal ve metiyonin karışımı suyu ayırt edebilmeleri için renk belirteci kullanılmıştır. Tavuklarda yem ve içme suyunun fizyolojik etkileri farklı renkteki su şişeleri kullanılarak ilişkilendirilmiştir. Daha sonra denemenin serbest seçim dönemine geçilmiştir. Metiyoninle muamele edilmiş su konsantrasyonu E döneminde % 0.15 ve F döneminde % 0.30'dir. Metiyonince yetersiz besleme yem ve su tüketiminde önemli azalmayla sonuçlanmıştır. İçme sularına metiyonin eklendiğinde yem ve su tüketimi normal seviyesine gelmiştir. Diğer taraftan metiyonin tüketimi sadece yem ile verildiği zamandaki miktara eşit ya da yem ile verildiği seviyeyi geçmiştir. Sonunda tavuklara metiyonince yetersiz yem verilerek normal ve metiyonin karışımı su sunulmuştur. Metiyonince yetersiz yemle beslenen tavuklar renk belirteci vasıtası ile metiyoninli suyu normal suya tercih etmişlerdir. Ancak, tavukların içme suyundan metiyonini ihtiyaçları doğrultusunda alabilme yeteneği gözlenmemiştir.

Anahtar sözcükler: Yumurta tavugu, metiyonin, düzenlemek, içme suyu, renk belirteci

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Introduction

Methionine is an essential amino acid 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. Once the compound feed is mixed, both its amino acid content and also the extent to which it will meet the requirements of all the hens in any given flock are fixed. Cadirci et al., (2009) showed that layers fed methionine deficient diet are able to select for water supplemented with methionine in preference to pure water by using of two colour cues. However, 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 of this study. 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 to determine if hens can correct a feed deficient in methionine by choosing the methionine-treated water, while maintaining normal feed intake and to determine if the hens can regulate consumption of methionine-treated water in order to satisfy their optimum methionine requirement.

Material and Method

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 (2143.90 ± 92.23 g and 2087.80 ± 79.82 g) were not significantly different (P>0.05). Plastic water bottles (2000 ml) fitted with nipples at the base was used to supply water. According to the plan of the feeding regimens, bottles in three different colours (yellow, red and blue) 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 sides of the cages were closed with 3-ply wood. Two feed formulations (Feed 1, Feed 2) were used, the ingredients and estimated nutrient contents of which are shown in Table 1.

Table 1. The ingredient-and calculated 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	140.0	140.0
Calcium	37.5	37.5
Total Phosphorus	5.5	5.5
Sodium	1.8	1.8
Lysine	7.2	7.2
Methionine	3.7	2.1
Methionine + cystine	6.4	4.8
ME [kcal/kg]	2900	2900

[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

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 0.30% (regimen F). The regimens of the experiment are summarised in Table 2. Food and water intakes were recorded daily. Eggs laid each day were weighed. Body weights were measured at the beginning and end of the experiment. All data were obtained on an individual hen basis. 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.

Table 2. Training regimen of birds

	Regimens	Diet		Bottle colour	
		Feed	Water	Group 1	Group 2
	A (7 days)	F1	plain	yellow	red
4-day cycle repeated three times	B (1 day)	F2	plain	blue	blue
	C (1 day)	F2	treated*	red	yellow
	D (1 day)	F1	plain	yellow	red
	C (1 day)	F2	treated*	red	yellow
	B (1 day)	F2	plain	blue	blue
	E (10 days)	F2	plain and treated*	yellow red	red yellow
	F (10 days)	F2	plain and treated**	yellow red	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 0.30% methionine.

Results and Discussion

The body weights (mean and SEM) at the beginning and end of the experiment were 2113.90 ± 58.74 g and 1989.20 ± 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 63.90 g and 62.80 g, respectively.

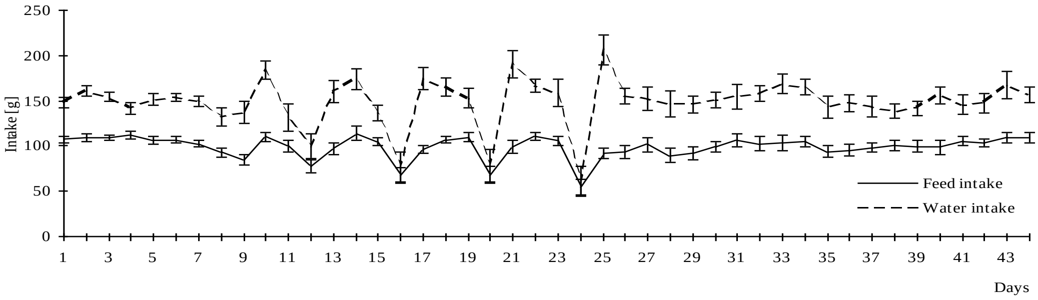

Figure 1. Daily changes in feed intake and water consumption in association with the six feeding regimens.

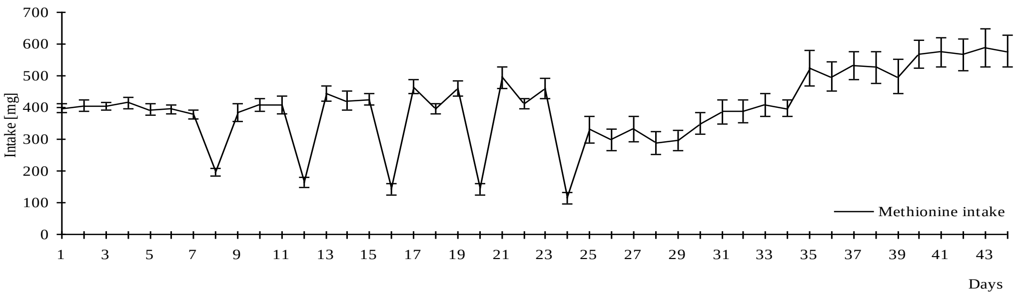

Figure 2. Daily changes in methionine intake in association with the six feeding regimens.

The daily feed and water intakes during each of the 44 days are presented in Figure 1, and the estimated methionine intake in the same period is shown in Figure 2. Each point is the mean ± SEM of the results from eighteen birds. Common in both figures those 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 2) follows the pattern of food and water intake (Figure 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.

Table 3. 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)
Feed intake [g/day]	107.7^{b}	72.3^{a}	99.8^{b}	110.8^{b}	98.6^{b}	101.2^{b}
Water intake [ml/day]	151.4^{b}	89.7^{a}	155.3^{bc}	172.6^{c}	160.7^{bc}	148.6^{b}
Methionine intake [mg/day]	398.5^{bc}	151.8^{a}	442.4^{c}	409.8^{bc}	348.3^{b}	544.2^{d}

^{abcd} Values within a row with no common superscript differ significantly (P<0.001).
Values are mean of n=18.

Feed-, total water-, and methionine intakes during the regimens of the experiment are shown in Table 3. 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 0.30% 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).

Water intake proportions during the choice period are presented in Table 4. 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 4. 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 =18, and ^{B}n=36.

Cadirci et al., (2009) demonstrated that more than 90% of the choices were made in favour of methionine-treated water. In contrast, in this study, 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 57.10% in the first choice phase (regimen E). When progressing to the next choice period (F), this figure increased to 73.50%. 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 0.30% 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., 1976). Methionine intake seems to be governed by the same mechanisms since the results of this study 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). 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. 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 73.50%. 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.

Conclusion

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. However, the birds were unable to regulate their methionine consumption from treated water.

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