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# Research Article
# Impact of Dietary Methionine Levels and Sources on Performance and Health of Foot Pad in Broilers

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## Abstract

**Background and Objective:** Foot Pad Dermatitis (FPD) is a widespread problem in poultry production and constitutes a welfare issue. The objective of this study was to test the surplus dietary levels of methionine and its sources (DL-Met/MHA) on performance and health of foot pad in broilers. **Methodology:** One-week-old ♀ broilers were divided randomly into 5 groups for 28 days. All diets were basically composed of wheat (64%) and soybean meal (30%). One group was fed a basic level of Met in the diet (3.5 g $kg^{-1}$ diet) without any supplementation. The other groups were fed with different Met levels (+1/+2 g $kg^{-1}$ diet) as DL-Met or as methionine hydroxy analogue. External assessment of foot pads was done weekly. Individual body weight was recorded weekly on the day of scoring. **Results:** Met supplementation improved the body weight gain, so that the means of Body Weights (BW) differed among the groups after 4 weeks of trial. Feeding unsupplemeted Met diet resulted in the significantly lowest BW (1627 g). Furthermore, feeding higher levels of Met in form of DL-Met (+1 g $kg^{-1}$ diet) led to the highest BW (1916 g) numerically. The highest significantly FPD scores (5.2±0.6) was found in the control group in comparison to other experimental groups. However, using dietary DL-Met in higher levels (+2 g $kg^{-1}$ diet) led to numerically lower FPD scores (3.2±0.8) than using only+1 g $kg^{-1}$ diet (4.7±0.4). **Conclusion:** It means that there is a specific function of Met regarding performance and foot pads.

Key words: Dietary methionine, performance, foot pad dermatitis, broilers

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Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Methionine (Met) is an essential amino acid and is considered a first limiting amino acid because being limited in plant protein sources for poultry. Methionine primarily serves as a methyl donor for transmethylation reactions and also as a sulphur donor especially in the biosynthesis of lipids and other compounds and is involved in lipid transport in the blood[1,2]. Within these two roles Met is a major component in protein synthesis. Several analogues of Met exist, but Methionine Hydroxy Analogue (MHA) is the most common analogue used in animal diets. Its chemical structure is similar to that of Met with a hydroxy group substituted in the amino component. Historically analogues have been known to be inhibitors of enzymes specific to the nutrient it mimics. This is true for MHA as well. In some poultry studies MHA exhibited lower biological activity in comparison to DL-Met[3]. However, in some animal trials DL-Met and MHA exhibited similar results when they were directly compared for weight gains and feed conversions over the whole range of suboptimal to optimal supply for both Met sources.

Methionine is normally supplemented only when crude protein cannot provide the required levels of Met. Nevertheless, there are many factors that could impact nutritional requirements of Met as immunological functions, health, environmental temperature, sex, age and maintenance[4]. As previously mentioned, Met is a primary limiting amino acid and is often supplemented to balance the ratio of amino acids. Methionine can however, be supplemented in excess, which can lead to deleterious consequences. A deficiency of the nutrient has been a historical problem of nutritional interest. Examples of deficiency have been described more extensively but examples of excess that can cause problems are documented as well. Chi and Speers[5] and Waldroup and Hellwig[6] found that excess Met supplemented to a diet containing 14% corn depressed growth. However, greater body weight from increased dietary protein and added methionine were additive in all cases except when Met was added to the 30% protein diet or when protein was added from 27-30% in the presence of 0.1% added Met[7]. Increased Met has also been shown to increase egg size and numbers by moulted hens[8].

The incidence and severity of Foot Pad Dermatitis (FPD) is of great concern to the poultry industry and recently it has attracted additional attention in terms of animal welfare, food safety and also consumer protection[9]. At the end of the fattening period this disease can reach a prevalence of 91-100% in turkeys[10]. The aetiology of FPD is a complex interaction of different factors[9]. However, the most important factor causing the onset of FPD is the moisture content of litter. Abd El-Wahab *et al.*[11] noted a significant increase in FPD lesions in young turkeys exposed for only 4 h day$^{-1}$ to a litter with a 'critical moisture content' of 35% is accompanied.

The proposed legislative changes in the European Union may force producers to reduce the prevalence and severity of FPD. Therefore, there is a high need to develop preventive measures against FPD. Against this background, the main objective of this study was to test potential effects of different levels of dietary Met and its sources (DL-Met/MHA) on the performance and health of foot pads in broilers.

## MATERIALS AND METHODS

**Housing and experimental design:** One-day-old ♀ broilers (Cobb) were obtained from a local hatchery. The birds were housed with wood shavings, kept dry and clean before the experiment. All birds were fed *ad libitum* with a commercial pelleted diet (containing lasalocid-A-sodium, 110 mg kg$^{-1}$ diet) for the first 7 days, afterwards all 150 birds were divided randomly into 5 groups for 28 days. Each treatment was replicated three times with ten chicks (n = 10). The experimental pens (1.40×0.85 m) were bedded with approximately 1 cm (1 kg m$^{-2}$) of wood shavings. All diets were basically composed of wheat (64%) and soybean meal (30%). One group was fed a basic level of Met in the diet (3.5 g kg$^{-1}$ diet) without any supplementation. The other groups were fed with different Met levels (+1/+2 g kg$^{-1}$ diet) as DL-Met or as MHA (containing 84% methionine). The birds were initially kept at 34-36°C using one heating lamp in each pen and the temperature was lowered by 1°C/2 days. The photoperiod from d 4 onwards was 16 h of light and 8 h of darkness. A typical ventilation system in this study was not used (no need for ventilation) due to the small rearing groups. No growth promoting substances were used in any group and no birds were treated otherwise throughout the whole experimental period. In each group, the individual BW was recorded weekly. Feed and water intakes were measured daily at group level.

**Litter measurements:** Litter samples for measuring the Dry Matter (DM) content were collected weekly from 3 sites (2 peripheral samples and 1 central one) in each pen. At each area, a sample (~50 g) over the whole bedding height was punched out using a tin with a diameter of 5 cm from the full depth of the litter. Samples were oven-dried at 103°C for the time needed to reach constant weight. Ammonia in the air in

each pen was measured weekly by using a hand-held Dräger meter tube (sample tube: Ammonia 2-30 ppm) attached to a Dräger pump (Dräger Accuro, Dräger Interservices GmbH, Lübeck, Germany). The glass Dräger tube was broken at both ends and inserted into the Dräger pump. The pump was then held about 10 cm over the litter in the middle of the pen. The extent of the discolouration within the Dräger tube was then read off the tube and recorded. The litter pH values was measured by making a suspension (1 part of material: 9 parts of water) then by using a pH meter (WTW, Weilheim, Germany).

**Excreta measurements:** Pure/fresh excreta of the birds were collected from each pen once a week by putting a plastic sheet in each pen for approximately 1 h until ~80 g pure excreta per pen had been obtained. The collected excreta were then removed from each pen, thoroughly mixed and dried at 103°C to determine the DM content. The pH value of excreta was measured by using pH meter.

**FPD scoring criteria:** External assessment of foot pads was done weekly. Only the central plantar area was scored, signs of foot pad lesions were recorded on an 8-point scale (0 = normal skin, 3 = small black necrotic areas and 7 = over half of the foot pad is covered with necrotic scales) according to Mayne *et al.*[12].

**Statistical analysis:** The foot pad scores were evaluated by using the mean of both feet. The data from the foot pad scoring and body weight were analyzed separately for each sampling point using the GLM procedure of the[13] software. For body weight and FPD scores the Tukey test for pair-wise multiple means comparison of the GLM procedure of SAS Institute Inc.[13] software was used. All statements of statistical significance are based upon $p<0.05$.

## RESULTS

As planned the nutrient contents of experimental diets differed mostly in level and source of methionine (Table 1 and 2). The DL-Met was added to the diets at a concentration of 1.2 g $kg^{-1}$ diet. The MHA was also added to the other experimental diets (1.2, 2.4 g $kg^{-1}$ diet) to raise the Met contents in the diets equally (+1/+2 g $kg^{-1}$ diet). The analyzed levels of Met in all experimental diets were as planned. The vitamin and mineral mixtures (commercially produced) were added to all diets at the same amount (10 g $kg^{-1}$).

The feed intake for the unsupplemented group (2492 g) was markedly lower than for the other treatments (~2846 g±25.0) during the experimental period (data not shown).

As shown in Table 3, feeding unsupplemented Met diet resulted in the significantly lowest BW (1627 g) at day 35 in comparison to those fed +1 g Met/kg diet either in form of DL-Met or MHA. Furthermore, feeding higher levels of Met in form of DL-Met (+1 g $kg^{-1}$ diet) led to the highest BW (1916 g) numerically in comparison to other groups. Moreover, using dietary Met (+1 g $kg^{-1}$ diet) in form of MHA resulted in higher BW (1892 g) vs. (1855 g) for group fed dietary Met (+2 g $kg^{-1}$ diet) as MHA.

It has to be stressed that the data of excreta/litter quality not shown. Regarding excreta DM content, no marked differences were found among the experimental groups (~16.3%±0.56). The pH values in the excreta for the experimental were about 6.27±0.07. At the end of the trial (day 35) the highest DM content of litter (48.7%) was found in group fed +2 g Met/kg diet as DL-Met than other groups. The lowest litter DM content (39.3%) was noted only for the control group. Increasing level of Met (+2 g $kg^{-1}$ diet) as MHA had negative effect on litter DM content (42.2%) vs. (45.5%) for (+1 g $kg^{-1}$ diet as MHA). No marked differences in

Table 1: Feed composition (g $kg^{-1}$ diet as fed) of the experimental diets (days 7-35)

| | Control 3.5 g Met/kg | +1 g Met/kg as DL-Met | +1 g Met/kg as MHA | +2 g Met/kg as DL-Met | +2 g Met/kg as MHA |
|---|---|---|---|---|---|
| Wheat | 640.0 | 640.0 | 640.0 | 640.0 | 640.0 |
| Soybean meal | 300.0 | 300.0 | 300.0 | 300.0 | 300.0 |
| Oil | 22.1 | 22.1 | 22.1 | 22.1 | 22.1 |
| Mineral and vitamins mixture[1] | 4.2 | 4.2 | 4.2 | 4.2 | 4.2 |
| $CaCO_3$ | 7.7 | 7.7 | 7.7 | 7.7 | 7.7 |
| Dicalcium phosphate | 23.0 | 23.0 | 23.0 | 23.0 | 23.0 |
| NaCl | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| DL-Met | - | 0.1 | - | 0.2 | - |
| MHA[3] | - | - | 0.12 | - | 0.24 |

[1]Vitamin and mineral mixture supplies the following per kilogram of diet: 13000 IU vitamin A, 4000 IU vitamin D3, 0.0250 mg 25-hydroxycholecalciferol, 100 mg vitamin E, 12 mg copper, 75 mg iron, 75 mg zinc, 90 mg manganese, 1.8 mg iodine, 0.3 mg selenium, 0.04 mg cobalt, [2]99% methionine and [3]Methionine hydroxy analogue = 84% methionine

Table 2: Chemical analysis of the experimental diets (analysed values on DM basis)

| Parameter | Control 3.5 g Met/kg | +1 g Met/kg as DL-Met$^{-1}$ | +1 g Met/kg as MHA$^{-1}$ | +2 g Met/kg as DL-Met$^{-1}$ | +2 g Met/kg$^{-1}$ as MHA |
|---|---|---|---|---|---|
| DM (g kg$^{-1}$) | 897.00 | 897.00 | 898.00 | 894.00 | 896.00 |
| CP (g kg$^{-1}$) | 237.00 | 238.00 | 234.00 | 234.00 | 223.00 |
| EE (g kg$^{-1}$) | 49.10 | 49.40 | 46.90 | 47.20 | 48.40 |
| CF (g kg$^{-1}$) | 38.30 | 39.00 | 39.40 | 38.50 | 38.80 |
| NfE (g kg$^{-1}$) | 508.00 | 505.00 | 513.00 | 509.00 | 521.00 |
| Starch (g kg$^{-1}$) | 433.00 | 427.00 | 434.00 | 434.00 | 434.00 |
| ME (MJ kg$^{-1}$) | 13.20 | 13.20 | 13.10 | 13.20 | 13.20 |
| Ca (g kg$^{-1}$) | 12.60 | 12.50 | 12.70 | 12.60 | 12.50 |
| P (g kg$^{-1}$) | 7.57 | 7.75 | 8.15 | 8.34 | 8.19 |
| Na (g kg$^{-1}$) | 1.54 | 1.56 | 1.49 | 1.61 | 1.43 |
| K (g kg$^{-1}$) | 9.36 | 9.28 | 9.20 | 8.90 | 8.86 |
| Cl (g kg$^{-1}$) | 3.10 | 3.13 | 3.03 | 3.14 | 3.00 |
| Cu (mg kg$^{-1}$) | 54.60 | 54.80 | 45.40 | 48.40 | 51.10 |
| Zn (mg kg$^{-1}$) | 138.00 | 141.00 | 137.00 | 142.00 | 140.00 |
| Cys (g kg$^{-1}$) | 4.61 | 4.36 | 4.64 | 4.32 | 5.28 |
| Met (g kg$^{-1}$) | 3.58 | 4.59 | 3.58 | 5.45 | 3.55 |
| Lysin (g kg$^{-1}$) | 12.30 | 12.30 | 11.70 | 11.80 | 11.50 |

[1]ME calculated by using the official formula for complete diets in poultry: $ME_n$ (MJ kg$^{-1}$) = 0.01551 crude protein+0.03431 crude fat+0.01669 starch+0.01301 sugar (nutrients in g kg$^{-1}$ diet, FMVO, 2007) (Source: FMVO[23])

Table 3: Body weight (g) of broilers fed different exprimental diets

| Days of life | Control3.5 g Met/kg | +1 g Met/kg as DL-Met | +1 g Met/kg as MHA | +2 g Met/kg as DL-Met | +2 g Met/kg as MHA |
|---|---|---|---|---|---|
| 7 | 171.0±17.9 | 172.0±16.5 | 170.0±15.6 | 172.0±16.5 | 167.0±14.7 |
| 14 | 386.0±50.4$^{b}$ | 432.0±57.4$^{a}$ | 438.0±63.1$^{a}$ | 435.0±58.3$^{a}$ | 431.0±49.3$^{a}$ |
| 21 | 656.0±106$^{b}$ | 774.0±138$^{a}$ | 777.0±130$^{a}$ | 788.0±134$^{a}$ | 768.0±114$^{a}$ |
| 28 | 1138.0±146$^{b}$ | 1336.0±271$^{a}$ | 1347.0±215$^{a}$ | 1339.0±257$^{a}$ | 1361.0±127$^{a}$ |
| 35 | 1627.0±242$^{b}$ | 1916.0±390$^{a}$ | 1892.0±336$^{a}$ | 1870.0±418$^{ab}$ | 1855.0±193$^{ab}$ |

[a,b]Means in the same row with different superscripts are significantly different ($p<0.05$)

Table 4: Development of external foot pad scores during the experimental period (Mean±SD) days of life

| Days of life | Control 3.5 g Met/kg | +1g Met/kg as DL-Met | +1 g Met/kg as MHA$^{-1}$ | +2 g Met/kg as DL-Met | +2 g Met/kg as MHA |
|---|---|---|---|---|---|
| 14 | 1.9±0.8$^{a}$ | 1.1±0.5$^{b}$ | 0.8±0.4$^{b}$ | 0.7±0.4$^{bc}$ | 0.6±0.6$^{bc}$ |
| 21 | 3.9±0.9$^{a}$ | 3.5±0.5$^{a}$ | 1.7±0.7$^{bc}$ | 2.2±0.9$^{b}$ | 3.8±0.5$^{a}$ |
| 28 | 4.2±0.6$^{a}$ | 4.1±0.4$^{a}$ | 2.3±0.9$^{b}$ | 2.6±1.0$^{b}$ | 4.2±0.5$^{a}$ |
| 35 | 5.2±0.6$^{a}$ | 4.7±0.4$^{b}$ | 3.5±0.5$^{bc}$ | 3.2±0.8$^{b}$ | 4.4±0.4$^{b}$ |

[a,b]Means in the same row with different superscripts are significantly different ($p<0.05$)

pH values of the litter were observed among the groups (~5.92±0.39). No marked differences in ammonia levels in the air above litter surface were noted among the experimental groups (2.50 ppm±0.22).

Birds in the control group (without any Met supplementation) had significantly the highest FPD scores (5.2±0.6) in comparison to other experimental groups (Table 4). However, using dietary DL-Met in higher levels (+2 g kg$^{-1}$ diet) led to numerically lower FPD scores (3.2±0.8) than using only +1 g kg$^{-1}$ diet (4.7±0.4).

Interestingly, feeding only +1 g MHA/kg diet was associated with numerically lower FPD scores than feeding diets with +2 g MHA/kg diet (3.5±0.5 vs. 4.4±0.4).

## DISCUSSION

Met supplementation affected markedly the body weight, which could be due to the requirements of Met for protein synthesis. Bunchasak[14] stated that decrease in dietary Met level led to growth inhibition, the induction of metabolic disorder and the reduction of disease defensive potential. Thus, in previous studies Met has been added to overcome growth depression caused by dietary tannic acid and mild arginine toxicity[1]. The addition of Met to the poultry diet has been correlated with the tendency to have less total body fat[15], to improve growth performance and to reduce odor-related compounds in excreta[16].

Nevertheless, Chi and Speers[5] and Waldroup and Hellwig[6] found that excess methionine supplemented to a diet containing 14% corn depressed growth. Moreover, feeding surplus levels of dietary methionine has been reported to impair body weight gain[17]. Although, Han and Baker[18] demonstrated that an excess of 0.5% of methionine is not harmful for young broiler chicks fed corn-soybean meal diets. The results in this study are in agreement with the previous study of Abd El-Wahab *et al.*[19] who noted that feeding diets of

young turkeys with a supplementation of +1 g Met/kg diet either as DL-Met or as MHA led to significantly higher BW (1300 and 1338 g, respectively) in comparison to the control group (884 g).

Foot pad dermatitis is a widespread challenge in poultry production. Previous study[11] has shown that the first significant increase in FPD lesion was observed after exposure of young turkeys for only 4 h days to a "Critical moisture content" (35%) of litter and the severity of FPD increased with increasing litter moisture. This could be explained by the fact that standing on wet litter brings the feet in constant contact with moisture and has been suggested to cause the foot pad to soften and become more prone to damage, this being a predisposing factor for the bird to develop FPD[20]. In this study there is a positive association between the mean of DM contents (measured weekly) in the litter and FPD scores. However, in further investigations it has to be tested whether surplus dietary levels of Met could decrease the severity of FPD even with wet litter. Abd El-Wahab *et al.*[19] concluded that at almost identical litter DM contents (measured weekly) of groups fed +2 and +3 g $kg^{-1}$ diet in form of DL-Met, the FPD scores for birds fed +3 g $kg^{-1}$ diet had markedly lower FPD scores than those fed +2 g $kg^{-1}$ diet. It means that level of dietary Met plays an important role for health of skin rather than moisture content in the litter.

Generally, supplementing the diet with any concentration of Met, regardless of the source, reduced the development and severity of FPD significantly. Surplus levels of dietary methionine will increase the growth and reduce the severity of FPD significantly. These results indicate that a marginal Met deficiency could a role in the severity of FPD in broilers. However, it is questionable whether a high dietary supplementation of Met can reduce the severity of FPD even on wet litter (with critical moisture content). Chavez and Kratzer[21,22] reported that the source-and not only the dosage-of Met might play an important role for the foot pad health of turkeys. They indeed recorded an improved status of foot pad health with increased amounts of Met in the diets (0.3% DL-Met vs. a basal diet without supplementation), where a decrease of prevalence and severity of FPD was found, when 1, 2 or 3 g DL-Met/kg was added to the diets. Furthermore, they observed a reduced FPD score when a diet with DL-Met was fed, compared with a diet in which the same amount of Met as MHA was included. Abd El-Wahab *et al.*[19] found that supplementation of Met in diets of young turkeys resulted in a significant reduction in FPD scores compared to the control group, independent of level and/or source of added Met. Also, the previous authors noted that using DL-Met in higher levels (+2 or +3 g $kg^{-1}$ diet) led to significantly lower FPD scores (4.54-4.12, respectively) than using only +1 g $kg^{-1}$ diet (5.33), whereas this effect could not be observed for MHA.

## CONCLUSION

There seems to be a specific function of Met regarding foot pad health (as known for skin and feathers), but in further investigations it has to be tested whether at the end of the fattening period (with reduced levels of protein and also of Met) the lower levels of Met could impair foot pad health even more distinct. May be Met levels required for high growth rates do not cover the needs for optimizing foot pad health (or the quality of feather coat), especially at the end of fattening period. Further more, MHA can be used in the diet with a positive effects regarding performance and foot pads.

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