Journal of Poultry Science, 41 : 213–222, 2004

Use of Alimet® Feed Supplement (2-Hydroxy-4-(Methylthio) Butanoic Acid, HMTBA) for Broiler Production

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Alimet and MHA for Poultry Production

Controversy about the efficacy of Alimet as a source of methionine arises from the fact that when it is ingested, it is fundamentally different from methionine. Thus, broiler performance studies comparing efficacy of Alimet and DL-methionine (DL-met) continue to be of interest. This report will cover two studies using Alimet Feed Supplement (an 88% aqueous solution of 2-hydroxy-4-(methylthio)butanoic acid, HMTBA). The objective of the first was to compare Alimet with DL-met as sources of supplemental L-methionine (L-met) for broiler production. The sources were fed on an equal dry-matter basis at a level approximating 85% of that used in commercial practice in the UK, where the study was run. A basal diet was used to confirm sensitivity to methionine source addition. No antibiotics were fed. Results confirmed a significant response to supplemental methionine over the 0–42 day study for gain and feed conversion efficiency with no significant difference between Alimet and DL-met. The objective of the second study was to compare performance of Alimet with DL-met when birds were fed high inclusion levels. In this experiment, conducted at Novus International, performance in a basal diet was compared to five supplemental levels of Alimet or DL-met (0.2, 0.5, 1.0, 1.5 and 2.0%). The first level of supplementation was designed to represent an adequate diet with subsequent levels representing excessive addition. Results confirmed that the performance and feed intake of birds fed DL-met was more negatively affected than those fed Alimet, with differences becoming significant above the 0.5% level. Data presented here confirm full molar equivalency of HMTBA to DL-met in broilers fed commercial diets and illustrate the relatively greater safety of Alimet when fed at higher inclusion levels.

Key words : methionine, poultry, 2-hydroxy-4-(methylthio) butanoic acid, Alimet, MHA

Introduction

The active component in Alimet and MHA is 2-hydroxy-4-(methylthio) butanoic acid (HMTBA). Its chemical structure is depicted in Fig. 1. HMTBA differs from methionine by having a hydroxyl group on the alpha carbon rather than an amino group. That is the only difference between the two compounds. The chemical structure

Received : March 1, 2004, Accepted : April 21, 2004
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of HMTBA resembles commonly used organic acids such as lactic acid or fumaric acid and, like them, HMTBA is absorbed primarily by diffusion in the upper gut (Knight and Dibner, 1984). Once inside the tissue of the animal, however, HMTBA is rapidly converted to L-met (Fig. 1) and shows identical metabolism to any other source of L-met, including intact protein: HMTBA will have the same availability for methyl group transfer or cysteine synthesis as L-met from any other source. A recent review of the metabolism of HMTBA described the biochemistry of its conversion to L-met, and the activity of the polymer fraction of the liquid product, Alimet (Dibner, 2003).

Alimet and MHA are often used in the production of agricultural animals, including poultry, to satisfy a methionine deficiency. Despite many decades of use in the U.S. and Europe, the introduction of this methionine source to new world areas must be accompanied by demonstrations of its use in the feed mill as a liquid product and its activity as a methionine source in the animal. This report will cover HMTBA performance in broilers, one of the major types of poultry production in Japan. Comparison of HMTBA performance in laying hens has recently been described (Harms, 2004). In addition, advantages for Alimet fed birds following the feeding of excess levels of methionine supplementation will be described.

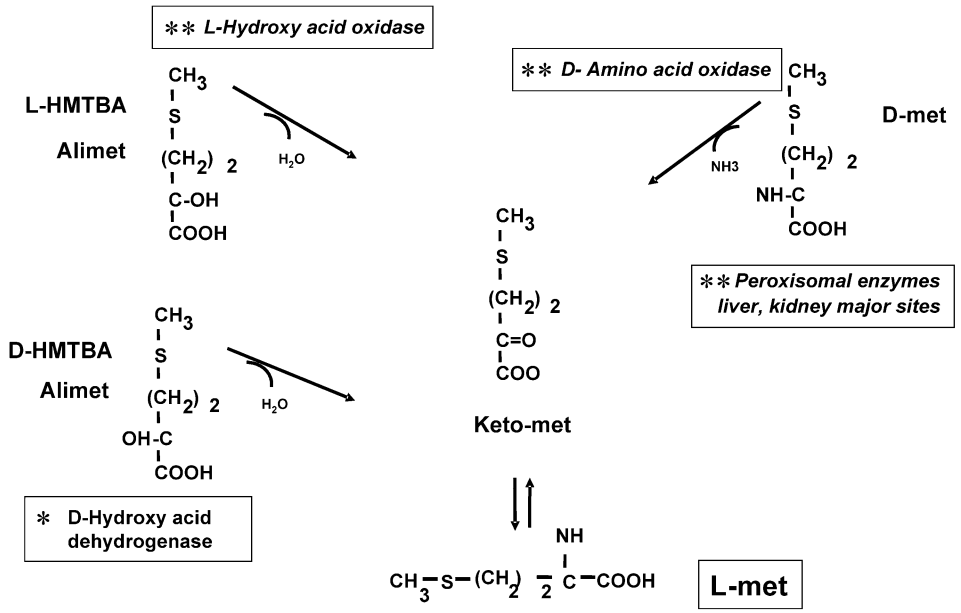

Fig. 1. Schematic of the conversion of D-HMTBA, L-HMTBA and D-met to L-met. All the isomers have the same general conversion pathway with an initial oxidation step to keto-methionine followed by transamination to L-met. The major difference among the sources is that D-met yields ammonia as a byproduct of oxidation while HMTBA yields water. HMTBA=2-hydroxy-4-methylthio-butanoic acid. D-met=D-methionine.

Materials and Methods

Broiler Trial

This broiler trial was conducted at the Roslin Institute in Scotland. In this trial, Alimet and DL-met were compared on an equal dry matter (equimolar) basis in a wheat soy diet supplemented to 85% of typical levels of methionine supplementation used in commercial practice. Final methionine levels in the diets were : 0.47, 0.44 and 0.41% in the starter, grower and finisher, respectively. Diet composition for this study can be seen in Table 1.

One-day-old Ross 308 broiler chicks (960 birds) were used in 8 replicate floor pens of 40 male broilers. The birds were at commercial stocking density of 13 birds/m^2. Light was provided for 23 h/day. Feed intake, bird weight and mortality were determined for periods 0–14, 14–28, 28–42 and 0–42 days. The experimental data were analysed as a randomised block design using source (HMTBA or DL-met) by analysis of variance, using the general linear models procedure of SAS (1999).

Performance at high inclusion levels

In this study, 792 Ross×Ross male chicks were raised in battery cages during the

Table 1. Composition of broiler diets (%)

Ingredients and nutrients	Starter	Grower	Finisher
Wheat	63.24	65.15	68.08
Soybean meal	23.78	22.02	19.08
Fish meal	2.50	1.00	0.93
Peas	3.00	3.00	3.00
Soybean oil	2.66	4.24	4.64
Limestone	1.00	0.86	0.71
Dicalcium phosphate (18.5%)	1.32	1.40	1.25
Sodium chloride	0.25	0.21	0.29
Choline chloride (50%)	0.03	0.03	0.03
DL-Methionine	0.08	0.09	0.08
L-Lysine Hydrochloride	0.47	0.35	0.32
Avizume 1300	0.10	0.10	0.10
Vitamin/mineral premix	0.50	0.50	0.50
Pellet binder	1.00	1.00	1.00
Elancoban G200	0.05	0.05	0.00
Nutrient content			
ME (MJ/kg)	12.51	12.95	13.12
CP (%)	21.64	19.80	18.49
Lysine (%)	1.40	1.20	1.10
Methionine (%)	0.372	0.347	0.331
TSAA (%)	0.712	0.697	0.661

Table 2. Composition of broiler basal diets : High inclusion Levels (%)

Ingredients and nutrients		Starter (1–17 days)	Grower (18–35 days)
Corn		60.55	64.00
Soybean meal		32.25	29.25
Animal fat		3.66	3.84
Vitamin/mineral Premix		3.81	3.61
Lysine HCl		0.10	0.09
Threonine		0.05	0.07
Nutrient Content			
ME (MJ/kg)		12.97	13.18
CP (%)		21.42	20.17
Fat (%)		6.61	6.85
Lysine	Total	1.21	1.13
	Digestible	1.09	1.01
Methionine	Total	0.33	0.31
	Digestible	0.30	0.29
TSAA	Total	0.71	0.67
	Digestible	0.61	0.58

first 36 d of age. Corn soy based mash diets were deficient in TSAA (Table 2) and included a starter (17 d) and grower (18 d) period. Five supplemental levels of the two L-met sources (HMTBA, DL-met) were added on an equimolar activity basis for each diet (0.2, 0.5, 1.0, 1.5 and 2.0%). The first level of supplementation was defined to supply L-met at nutritional requirements and the rest of the levels supplied L-met in excess. Ten chicks per pen were housed in 72 batteries and allowed to consume feed and water ad libitum. Feed consumption and BW were measured at d 35 of age. Blood, liver, kidney, and spleen were removed from one bird per pen, weighed and frozen for later determination of free methionine and HMTBA in plasma, and iron content in tissues. The effect of L-met source and level of supplementation was analyzed as a 2×5 factorial with a basal treatment. In addition, the experimental data were analysed by source, level, and their interaction, omitting the data for the basal diet. These studies were analyzed as a randomised block design by analysis of variance, using the general linear models procedure of SAS (1999).

Results

Broiler trial

Table 3 shows the weight gain and feed conversion results for this study. During the starter period birds on Alimet gained significantly more weight than those on the control treatment. The weight gained by the birds fed DL-met diet did not differ significantly from the amounts gained by birds on either the control or HMTBA groups

Table 3. Bird performance

Weight gains (g/bird) Treatment				
Age (days)	Control	DL-Met	Alimet	SEM[1]
0–14	299.9[b]	314.9[ab]	330.1[a]	6.76
14–28	682.5[b]	764.2[a]	804.1[a]	13.19
28–42	1151	1187	1212	26.2[NS]
0–42	2133[b]	2266[a]	2346[a]	33.2
Feed conversion efficiency (g feed/ g gain)				
Age (days)	Control	DL-Met	Alimet	SEM[1]
0–14	1.664	1.648	1.544	0.0440[NS]
14–28	1.982[b]	1.901[a]	1.877[a]	0.0268
28–42	2.294	2.277	2.222	0.0443[NS]
0–42	2.103[b]	2.060[ab]	2.008[a]	0.0237

Means with no common superscripts differ significantly ($P < 0.05$).

($P > 0.05$). During the grower phase of the experiment, both of the treatments on supplemented diets gained significantly more than birds on the control treatment. Birds fed DL-met did not differ from birds fed Alimet ($P > 0.05$). During the 28–42 day period, although birds fed Alimet gained the most weight, there was no significant difference between that treatment and the control or DL-met supplemented birds. Over the experiment as a whole, birds on DL-met and on Alimet gained significantly more than those on the control treatment (2133 g). There was no significant difference between gain of DL-met fed birds (2266 g) and Alimet fed birds (2346 g).

Feed conversion results are shown in Table 3. During the starter period birds on DL-met and Alimet were not significantly better than the basal treatment. During the grower phase of the experiment, both of the birds on both of the supplemented diets were significantly more efficient than birds fed the unsupplemented basal. For the finisher period, the control had an efficiency which was numerically poorer than birds supplemented with DL-met and Alimet which were not different from each other. For the overall study, the control birds had a feed conversion efficiency of 2.103, significantly poorer than either the DL-met fed birds (2.060) or the birds fed Alimet (2.008).

Mortality for the study (data not shown) was 2.2%, with 7 birds lost from each treatment. Two birds were lost in the starter, 8 during the grower and 11 during the finisher. No single cause of death was dominant, with post mortem findings including chronic polyserositis, heart failure, and necrotic enteritis. Two birds were culled for bad legs.

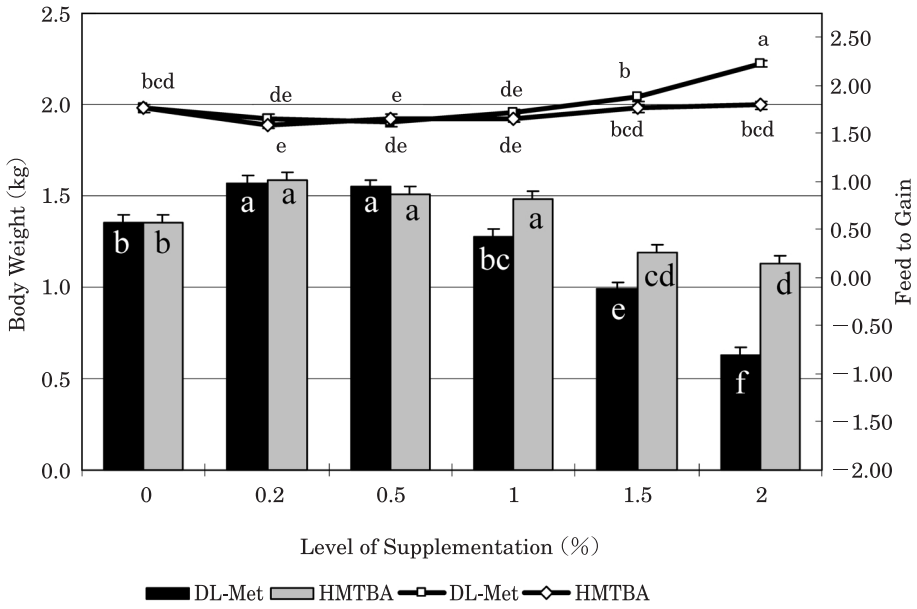

Fig. 2. Effect of methionine source and level of supplementation on day 35 body weight (bars, □=HMTBA, ■=DL-met) and dead bird corrected feed conversion (lines, ◇=HMTBA, □=DL-met) in birds fed methionine sources at high levels of inclusion. There was a significant source ($P<0.001$), level ($P<0.001$) and source by level ($P<0.001$) interaction for both body weight and feed conversion. Within a data set, means with no common superscripts differ significantly ($P<0.05$). HMTBA=2-hydroxy-4-methylthio-butanoic acid, DL-met=DL methionine.

Performance at high inclusion levels

Bird response to L-met supplementation followed a quadratic response (Fig. 2). Birds responded to the first level of L-met (0.2% ; $P<0.05$) by increasing BW, feed intake, and dead-bird corrected feed efficiency equally for both sources, indicating the basal diet was deficient in L-met. Performance reached a plateau at the next level of supplementation (0.5%) and further supplementation induced a gradual ($P<0.05$) reduction in BW, feed intake and feed conversion efficiency, indicating L-met excess. The response to supplementation differed among sources. The negative impact on BW ($P<0.0001$) and feed conversion efficiency ($P<0.001$) was more severe and occurred sooner (1.0 vs 1.5% ; $P<0.001$) in birds fed DL-met than birds fed HMTBA (Fig. 2). Changes in breast, leg, liver, kidney and spleen weights followed the same response (data not shown). All tissue weights increased during first and second level of supplementation and started to decrease when the level of DL-met was at and above 1%, and at and above 1.5% for HMTBA fed birds. The differences in the response to over-supplementation between the two sources might be due to the differences in how the two sources are metabolized at the tissue level. Plasma free methionine increased with level of supplementation but the magnitude of the increase differed among sources

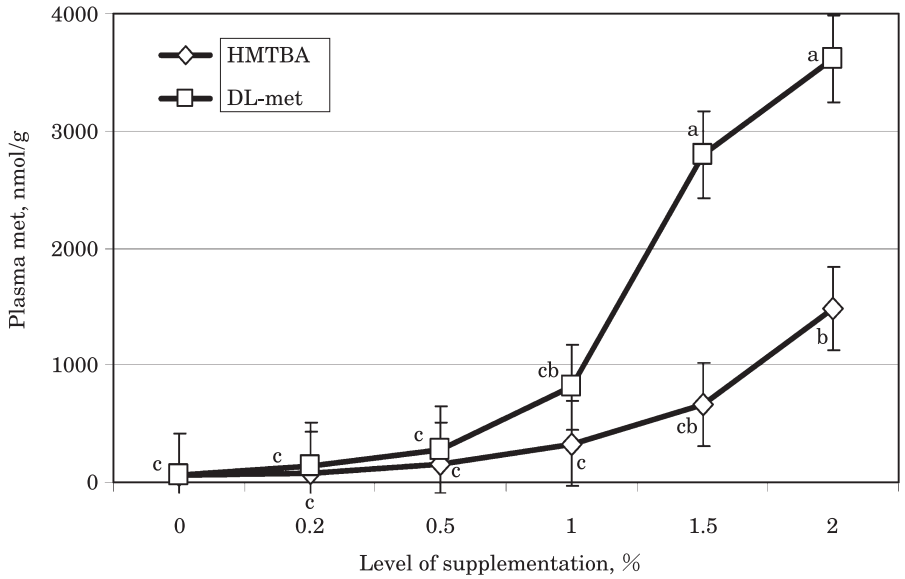

Fig. 3. Effect of methionine source and level of supplementation on day 35 plasma free methionine concentration in birds fed methionine sources at high levels of inclusion (◇=HMTBA, □=DL-met). There was a significant source ($P<0.001$), level ($P<0.001$) and source by level ($P<0.05$) interaction. Means with no common superscripts differ significantly ($P<0.05$). HMTBA=2-hydroxy-4-methylthio-butanoic acid, DL-met=DL methionine.

(Fig. 3). At and above 1% supplementation, plasma free methionine from birds fed DL-met increased exponentially but birds fed HMTBA only showed a gradual increase. A significant decrease in feed intake (Fig. 4) was observed for both sources, but was significant above the 0.5% level for DL-met while Alimet fed birds did not show the decline in feed intake until the 1.5% supplementation levels.

Discussion

Broiler trial

Over the years, many studies have been done comparing the bioavailability of Alimet and MHA to the other major source of supplemental methionine activity for poultry, DL-met. However, the genetics of broilers are constantly changing, and contemporary studies provide the most relevant data for the poultry producer. This report provides data from very recent studies comparing Alimet and DL-met.

This broiler trial was designed to measure the response of birds fed a methionine deficient diet under commercial conditions. The basal diet was wheat and soy based and had a methionine level of 0.37% in the starter phase, 0.35% in the grower and 0.33% in the finisher. The supplemented treatments received this control diet with 0.1, 0.09 and 0.08% of each methionine source on an equimolar basis (HMTBA added in the form of Alimet). We concluded that birds on HMTBA performed best, while those on

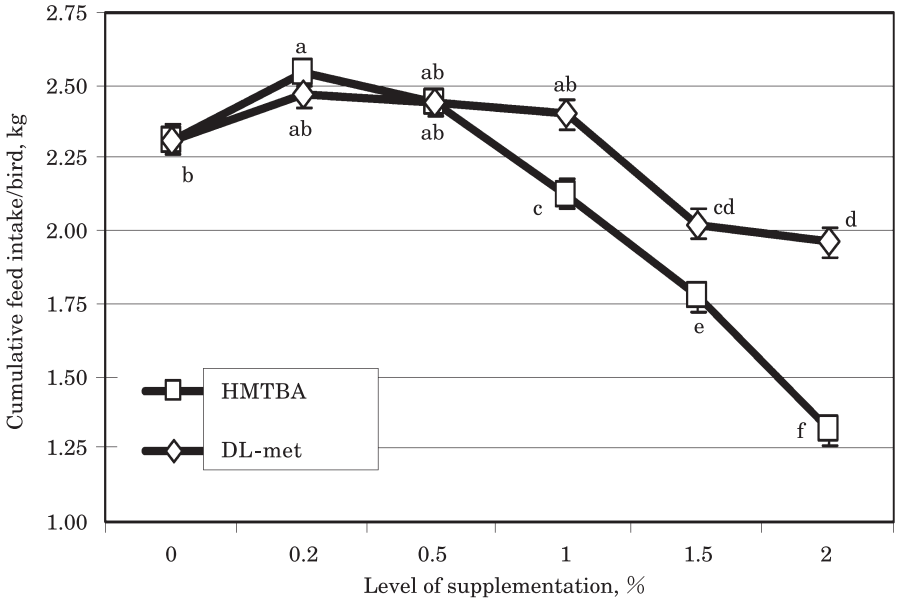

Fig. 4. Effect of methionine source and level of supplementation on cumulative feed intake per bird at day 35 (◇=HMTBA, □=DL-met). There was a significant source ($P<0.001$), level ($P<0.001$) and source by level ($P<0.001$) interaction. Means with no common superscripts differ significantly ($P<0.05$). HMTBA=2-hydroxy-4-methylthio-butanoic acid, DL-met=DL methionine.

DL-met diets performed better than the birds on the control treatment, particularly during the two earlier stages over which growth was monitored.

Performance at high inclusion levels

Methionine is one of the most toxic of the essential amino acids. The negative impact on growth and mechanisms of methionine toxicity at the tissue level are described extensively for rats (Mitchell and Benevenga, 1978) with additional reports describing toxicity for chicks and poults (Katz and Baker, 1975). The toxicity of HMTBA relative to DL-met has been reported to be lower in chicks fed crystalline amino acid diets (Katz and Baker, 1975). The impact of excess methionine activity from HMTBA and DL-met on performance and metabolism in chickens was evaluated in this study.

Differences in metabolic parameters between the two sources have been previously observed and reflect the fact that the two L-met sources are metabolized differently (Lobley *et al.,* 2001). HMTBA, once absorbed and taken up by the tissues (Knight and Dibner, 1984) is rapidly converted to L-met by the enzymes described by Dibner and Knight (1984). The L-met derived from HMTBA is further utilized by the cells and only secreted into the blood stream in conditions of excess supply of methionine (Lobley *et al.,* 2001). When over-supplemented, DL-met accumulates in plasma prior to its uptake and further metabolism by the tissues, and D-met is the isomer responsible for this accumulation (Vázquez-Añón *et al.,* 2000). In this study, approximately 25% of

the free met in plasma was in the form of D-met in birds fed DL-met. In contrast, HMTBA fed birds had lower plasma free-methionine, all of which was the L-met isomer. Elevation of plasma free methionine has been associated with reduction in feed consumption and this may be responsible for the significantly poorer performance seen in DL-met birds at the supplementation levels above 0.5% in this study The iron content increased in all tissues when levels of supplementation were at and above 1.5%. Hepatic iron content was lower for birds fed HMTBA than DL-met (634 vs 455 ppm ; $P < 0.02$), however no differences were observed in spleen and kidney iron content with L-met source (data not shown), suggesting that the effect is one of reduced feed intake associated with high circulating methionine causing a reduction in bird performance.

Benefits that go beyond methionine

Alimet is chemically an organic acid until it is taken up by host cells and converted to methionine. For this reason, there are several benefits HMTBA brings to the animal that occur in the feed and in the gastrointestinal system prior to its conversion by enterocytes. The first is that it is an antimicrobial organic acid both in the feed and in the digesta of the animal (Dibner and Buttin, 2002). HMTBA has activity against both acid intolerant species such as *Salmonella spp* and *E. coli,* and also against fungi that can contaminate feed and crop. The second benefit is that HMTBA is absorbed by diffusion (Knight and Dibner, 1984). This means that the absorption is not energy requiring, unlike that of DL-met. It also means that the presence of specific amino acid transporters is not required for uptake. This brings the benefit of absorption in the proximal gut, where transporters are rare, and indicates that HMTBA could be absorbed by immature enterocytes that are found in young animals and following stress situations such as heat and disease (Dibner *et al.,* 1992 ; Dibner and Atwell, 1993 ; Knight *et al.,* 1994).

In conclusion, this report presents data that confirm the full molar efficacy of HMTBA as a methionine source for broilers and the reduced performance problems at high inclusion levels. Thus HMTBA provides a methionine source in liquid (Alimet®) or solid (MHA®) form with equivalent performance to DL-met in broilers at equal dry matter inclusion levels and with the advantages of organic acid activity, superior performance in heat stress and no intake depression at inclusion levels up to 1%.

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