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Influence of 2-hydroxy-4-(Methylthio)butanoic Acid on Early Egg and Chick Weights of Broiler Breeders

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Abstract: This trial was conducted to determine if early egg and chick weights of broiler breeders are increased by dietary 2-hydroxy-4-(methylthio)butanoic acid (HMB) supplementation. Hens of two strains were provided feed with HMB levels equivalent to 0, 0.5, 1 and 1.5 kg methionine/tonne from 21 to 35 wk. Chick yield (chick weight/egg weight) increased significantly as dietary HMB level was increased from 0 to 1 kg/tonne; however, egg and chick weights were not individually affected by increasing HMB level. Egg production was increased from 29 to 35 wk by the 0.5 kg/tonne treatment; however, there was neither improvement prior to this period nor further increases due to greater HMB levels. Mortality, primarily due to fatty-liver hemorrhagic syndrome, was markedly elevated at low HMB levels in one strain. Although increasing HMB levels had some affect on chick yield, results were not as evident as what has been measured in commercial layers.

Key words: Early egg weight, diet, chick weight, 2-hydroxy-4-(methylthio)butanoic acid

Introduction

Broiler growth rate (McNaughton *et al.*, 1978; Wilson 1991) and livability (Wyatt *et al.*, 1985) are affected by chick weight at hatch, which is principally dependent on egg weight (McNaughton *et al.*, 1978; Wilson, 1991). Based on previous reports, a 1 g increase in broiler chick weight results in a 10 to 23 gram increase in live weight and a 0.2 to 0.6% improvement in livability at 6 to 8 wk of age (Wyatt *et al.*, 1985). Egg weights of broiler breeders are low early in the production cycle (Yuan *et al.*, 1994; Lien and Yuan, 1994). Although the onset of lay usually occurs at 23 to 24 wk, eggs are generally not saved until 25 to 26 wk since few eggs produced before this age are settable (egg weight > 50 g). Egg weights remain low up to 30 wk and a sizable percentage of eggs produced during this period are undesirably small (Lien and Yuan, 1994).

Dietary supplementation with either DL-methionine or HMB (Reid *et al.*, 1982; Van Weerden and Schutte, 1984; Harms and Russell, 1994; Manning and McGinnis, 1986) increases egg weight in commercial layers. Only a single report has documented the influence of either methionine or HMB supplementation on egg weights of breeders (Sutton and McDaniel, 1985). In that study, dietary supplementation with either methionine or HMB increased egg weights of breeders provided sorghum but not corn-based rations; however, specific effects on early egg or chick weights were not reported.

Based on the above observations, dietary HMB or methionine supplementation may increase early egg and chick weights of broiler breeders. This effect could

increase the percentage of settable eggs produced and improve the performance of broilers produced by young breeders. This trial was conducted to determine if dietary HMB supplementation will increase early egg and chick weights of breeders.

Materials and Methods

A total of 480 Arbor Acres (Strain A) and 480 Cobb (Strain C) broiler breeder pullets were randomly placed by strain in 12 rooms (11.2 m², 80 birds/room) of an insulated building at 1 day of age. The rooms were heated and ventilated according to standard industry practices. Incandescent lights provided an 8h L:16h D cycle and intensity of 2 foot candles from 1 day to 20 wk. Common controlled feeding programs provided a total of 7.85 kg of feed per pullet (1200 g CP, 22,600 kcal ME and 28 g methionine) through 20 wk.

At 20 wk, 240 pullets from each strain were randomly selected and distributed by strain among 32 floor pens (4.1 m², 15 birds/pen). Four pens per strain were randomly assigned to each of 4 HMB treatments. Strains and treatments made up a 2 by 4 factorial arrangement. Incandescent lights provided a 15h L:9h D cycle and intensity of 4 foot candles from 20 to 35 wk. Ventilation and evaporative cooling were provided according to standard industry practices. High and low temperatures in each room were recorded daily (Table 1).

Breeder feeds (Table 2) were supplemented with HMB levels equivalent to added methionine levels of 0, 0.5, 1 and 1.5 kg per tonne of feed (0, 0.057, 0.115 or 0.172% HMB; treatments 0, 0.5, 1 and 1.5, respectively).

Samples of the basal feed (treatment 0) were subjected to proximate and amino acid analyses and HMB inclusion (Table 2).

Equal daily allotments (Table 1) of a single prebreeder feed (15.5% CP, 1325 kcal ME/lb or 2915 kcal ME/kg, 1.3% Ca) were provided to all birds during wk 21. Equal daily allotments (Table 1) of the 4 treatment feeds were provided from 21 to 35 wk. The total body weight of each pen was determined weekly. Mean body weights and egg production levels were determined weekly to adjust feed allotments for the following week.

Hens were artificially inseminated at 23, 24, 26 and 28 wk with 50 μ l aliquots of pooled undiluted semen collected from Ross strain breeder males. Eggs laid between 24 and 30 wk were collected 3 times daily, stored in an egg cooler and set in an incubator weekly beginning at 26 wk. Eggs were incubated under standard conditions in a single incubator for 18 days, then segregated by pen in hatching baskets and transferred to a single hatcher for 3 days and 6 hours.

Individual egg weights were determined for all eggs laid between 24 and 28 wk and eggs laid on 2 days per wk between 28 and 35 wk. Mean egg weights were calculated by pen weekly and on a production-weighted basis for the duration of the trial. Double yolked, soft shelled, broken, misshapen and very small eggs (egg weight < 35 g) were not incubated or included in determinations of egg weight, composition or production. There were no differences in the numbers of these defective eggs produced between treatments or strains. Individual chick weights were determined after 21 days and 6 hours of incubation. Mean chick weights and yields (chick wt/egg wt) were calculated by pen for the period from 24 to 30 wk. Yolk and albumen percentages of eggs and dry matter percentages of yolk and albumen were determined for eggs laid on 2 days per wk during wk 31, 33 and 35. Egg yolk and albumen percentages and dry matter percentages were calculated by pen for the period from 31 to 35 wk. Egg production by each pen was recorded daily. Ages at 20 and 50% hen-day production (HDP), and total and settable (egg weight > 50 g) egg production per hen to 35 wk were determined for each pen.

Mortalities were recorded daily. Weekly and cumulative percent mortality was calculated by pen. Mortality in 7 of 11 cases diagnosed by the Alabama State Veterinary Diagnostic Laboratory between 30 and 34 wk was attributed to fatty liver hemorrhagic syndrome. Subsequently, 2 birds were randomly selected from each pen and killed at 35 wk to obtain liver samples. Livers were weighed and scored based on color and macroscopic condition. A sample (1.5 cm³) was taken from each liver, fixed in formalin, and later microscopically examined for histological lesions. All data were subjected to statistical analysis. Data were analyzed as a completely randomized design using the

General Linear Models procedure of SAS according to the following statistical model; $Y_{ijk} = u + H_i + S_j + (HS)_{ij} + e_{ijk}$, where Y_{ijk} = observed dependent variables, u = mean of population, H_i = effect of i^{th} HMB treatment, S_j = effect of j^{th} strain, $(HS)_{ij}$ = HMB treatment by strain interaction, and e_{ijk} = experimental error. Since pens were experimental units, $n = 8$ for each HMB treatment, $n = 16$ for each strain, and $n = 4$ for each HMB treatment and strain. Duncan's multiple range test was used to separate differences between means. Statistical significance was set at $P < 0.05$.

Results and Discussion

Mean egg weights for the entire trial were greatest in HMB treatments 0.5 and 1.5, lowest in treatment 1, and intermediate in treatment 0 (Table 3). Egg weights increased with dam age; however, there were no interactions between dam age and HMB treatment or strain (Fig. 1). Therefore, there was not a consistent positive effect of increasing dietary HMB on early egg weights of either strain.

When basal rations containing from 0.213 to 0.24% methionine were supplemented with from 0.025 to 0.20% methionine, egg weights of laying hens were increased (Harms and Russell, 1994; Manning and McGinnis, 1986). Early egg weights were increased in methionine-supplemented layers from 33 to 39 (Harms and Russell, 1994), and 24 to 40 wk of age (Manning and McGinnis, 1986). Jackson *et al.* (1987) observed a greater responsiveness of young layers to methionine and concluded that this effect "may be due to the fact that younger birds have a greater physiological potential to achieve higher egg sizes through dietary means due to the smaller eggs produced". Similarly, supplementation with from 0.028 to 0.20% HMB from 25 to 37 wk (Van Weerden and Schutte, 1984), and 24 to 40 wk of age (Manning and McGinnis, 1986) increased early egg weights in layers.

Differences in, and absolute levels of, methionine and HMB reported to increase egg weights of layers are in the same range as HMB levels that had no affect on breeder egg weights in the present study. In a previous report, both HMB and methionine supplementation increased mean egg weights of breeders provided sorghum but not corn-based rations from 22 to 52 wk (Sutton and McDaniel, 1985). Observations that HMB and methionine supplementation of corn-based rations did not increase egg weights imply that methionine intake may not limit early egg weights in breeders provided typical corn-based rations. However, since sorghum is lower in available methionine than corn, methionine levels in sorghum-based rations can apparently limit egg weights in breeders. The substantial increase in body weight that breeders must attain during the onset of production (Table 1) (Lilburn and Myers-Miller, 1990a) may result in the diversion of

Table 1: Body weights, daily feed allotments and environmental temperatures to which broiler breeder hens were exposed

Age (Week)	Body weight (kg)		Feed provided (g hen/day)	Mean temperatures (F)	
	Strain A	Strain C		High	Low
20	1.91 ^b	2.03 ^a
21	2.25 ^b	2.36 ^a	104.9	84.5	69.7
22	2.43 ^b	2.54 ^a	115.8	87.3	71.2
23	2.58 ^b	2.68 ^a	125.8	92.0	75.6
24	2.75 ^b	2.86 ^a	135.3	89.0	74.2
25	2.95 ^b	3.04 ^a	143.0	88.8	76.7
26	3.03 ^b	3.15 ^a	149.8	91.5	76.1
27	3.12 ^b	3.20 ^a	154.8	87.1	74.5
28	3.18 ^b	3.30 ^a	157.1	83.5	75.9
29	3.25 ^b	3.32 ^a	158.9	84.1	76.6
30	3.32 ^b	3.41 ^a	158.9	83.8	74.7
31	3.41 ^b	3.49 ^a	160.7	81.7	73.9
32	3.49 ^b	3.57 ^a	160.7	83.0	75.0
33	3.55 ^b	3.64 ^a	158.0	83.3	76.0
34	3.59 ^b	3.66 ^a	155.3	83.0	75.1
35	3.64 ^b	3.72 ^a	155.3	82.2	75.0

^{a,b}Body weight means within rows with different superscripts are significantly different ($P < 0.05$).

supplemental dietary methionine from egg formation to muscle growth. Whereas in laying hens, which experience relatively slight increases in body weight during the onset of production (Roland, 1980), supplemental methionine remains available for egg formation and results in increased egg weight.

Few studies have directly addressed dietary methods for increasing early egg weights in breeders. Mean breeder egg weights for entire production cycles were increased by increasing dietary protein (Robey *et al.*, 1988), fat (Brake *et al.*, 1989), and protein or energy (Pearson and Herron, 1981). Increasing dietary protein and lysine during the period from 40 to 48 wk also increased egg weights (Harms and Ivey, 1992). In addition, breeder egg weights were increased by increasing dietary protein level from 12.7 to 16.7% or energy level from 2165 to 2996 kcal ME/kg during the period from 19 to 41 wk (Spratt and Leeson, 1987). However, breeder egg weights were not affected by increasing dietary protein level from 14 to 18% during the period from 18 to 24 wk (Lilburn and Myers-Miller, 1990a) or increasing daily protein intake from 18.6 to 23.0 g/hen and the daily sulfur amino acid intake from 682 to 850 mg/hen throughout an entire production cycle (Wilson and Harms, 1984). Additionally, egg weights were numerically decreased ($P < 0.067$) from 28 to 32 wk by providing breeders a diet high in energy and protein from 18 to 25 wk (Lilburn and Myers-Miller, 1990b). Care must be taken to ensure that supplementation of breeder diets does not result in excessive body weights and/or fat levels which result in declines in production (Yuan *et al.*, 1994), fertility and hatchability (Pearson and Herron, 1981; Brake *et al.*, 1989) later in the laying cycle. There is potential for use of protein and/or amino acid supplementation to

increase early egg weights of breeders since these nutrients would not be expected to result in later declines in performance associated with increased fat deposition.

Although common controlled-feed-allotment programs provided equal amounts of feed and nutrients during rearing and laying, strain C was heavier than strain A throughout the trial (Table 1). However, egg weights did not differ between strains (Table 3 and Fig. 1). This was probably due to the equal feed allotments provided during lay and the fact that the two strains initiated lay at similar ages (Table 3). Although increased egg weights have been associated with increased body weights (Lilburn and Myers-Miller, 1990b), they often do not differ in breeder hens of the same age that have widely different body weights, if they are provided similar feed allotments during lay (Lien and Yuan, 1994). Body weights of the different HMB treatments did not differ at any time during the trial (data not shown).

Mean chick weight for the period from 24 to 30 wk was greatest in treatment 0.5, least in treatment 1, and intermediate in treatments 0 and 1.5 (Table 3). Chick weight increased with dam age but was not influenced by strain. There were no interactions between dam age and HMB treatment or strain (Fig. 2). However, chick yields increased as dietary HMB supplementation was increased from 0 to 1 kg/tonne (Table 3). No additional increase in chick weight occurred when HMB level was increased to 1.5 kg/tonne. These data indicate that chick yields may increase in response to HMB supplementation; however, due to inconsistent effects on egg weights, chick weights may not show a similar response.

Table 2: Composition and analysis of feeds provided to broiler breeder hens

Ingredients (%)	Dietary treatment			
	0	0.5	1	1.5
Corn	69.03	68.97	68.92	68.86
Soybean meal (48% CP)	20.80	20.80	20.80	20.80
Limestone	7.40	7.40	7.40	7.40
Dicalcium phosphate	1.60	1.60	1.60	1.60
Salt	0.38	0.38	0.38	0.38
Poultry Oil	0.29	0.29	0.29	0.29
Vitamin-mineral premix	0.50	0.50	0.50	0.50
2-hydroxy-4-(methylthio)butanoic acid	0.00	0.057	0.115	0.172
Calculated analysis				
Crude protein (%)	15.5	15.5	15.5	15.5
ME (kcal/lb)	1303	1303	1303	1303
Methionine (%)	0.28	0.28	0.28	0.28
TSAA (%)	0.52	0.52	0.52	0.52
Lysine (%)	0.79	0.79	0.79	0.79
Calcium (%)	3.20	3.20	3.20	3.20
Chemical analysis ^A				
Crude protein (%)	15.64	14.59	15.16	14.07
Methionine (%)	0.25	----	----	----
TSAA (%)	0.53	----	----	----
2-hydroxy-4-(methylthio)butanoic acid	ND	0.052	0.118	0.172
Lysine (%)	0.80	----	----	----

^AThe basal diet (Treatment 0) was subjected to amino acid analysis. All diets were subjected to protein and 2-hydroxy-4-(methylthio)butanoic acid analyses.

Table 3: Influence of dietary 2-hydroxy-4-(methylthio)butanoic acid (HMB) and strain on egg production by broilers breeders

VARIABLE	Dietary treatment ^A				Strain	
	0 HMB	0.5 HMB	1 HMB	1.5 HMB	A	C
Egg weight ^B (g)	54.5±0.3 ^{ab}	55.9±0.9 ^a	53.0±0.3 ^b	54.9±0.8 ^a	54.3±0.3	54.9±0.6
Chick weight ^C (g)	36.7±0.2 ^{ab}	37.1±0.2 ^a	36.1±0.4 ^b	36.8±0.3 ^{ab}	36.5±0.2	36.9±0.2
Chick yield ^{CD} (%)	69.6±0.2 ^b	70.1±0.4 ^{ab}	71.3±0.5 ^a	70.2±0.6 ^{ab}	70.2±0.3	70.4±0.4
Total production (eggs/hen)	31.0±6 ^b	42.9±1.0 ^a	38.0±2.0 ^{ab}	37.6±3.9 ^{ab}	39.9±1.7	34.6±2.6
Settable production ^E (eggs/hen)	25.2±3.0	35.4±2.2	28.5±1.8	30.5±4.1	31.6±1.7	27.9±2.8
Age at 20% HDP ^F (day)	173.1±1.0	173.4±1.2	173.0±0.9	173.4±1.0	173.9±0.8	172.5±0.6
Age at 50% HDP ^F (day)	185.9±0.9	182.7±1.3	182.7±1.1	184.0±1.6	184.7±1.0	182.9±0.8
Albumen ^G (% of EW ^H)	59.2±0.3	59.4±0.2	59.3±0.3	58.7±0.5	58.4±0.2 ^b	59.9±0.1 ^a
Yolk ^G (% of EW ^H)	28.7±0.2	28.5±0.2	28.6±0.3	29.1±0.5	29.4±0.2 ^a	28.1±0.1 ^b
Dry albumen ^G (% wet weight)	13.3±0.2	13.8±0.2	13.9±0.3	13.8±0.3	13.5±0.2	13.9±0.2
Dry yolk ^G (% wet weight)	48.8±0.1	49.1±0.2	48.8±0.4	49.5±0.5	49.0±0.2	49.1±0.3

^{a,b}Means±EM within rows, dietary treatments, and strains with different superscripts are significantly different ($P < 0.05$).

^ATreatments 0, 0.5, 1 and 1.5 provided HMB levels equivalent to added methionine levels of 0, 0.5, 1 and 1.5 kg/tonne, respectively. ^BFrom 24 to 35 wk of age. ^CFrom 24 to 30 wk of age. ^DChick yield = chick weight/egg weight. ^EEgg weight >50 g or 21.1 oz/dozen. ^FPercent hen-day egg production. ^GFrom 31 to 35 wk of age. ^HEgg weight.

Breeder egg weight is the primary factor influencing broiler chick weight which typically averages about 62 to 74% of egg weight (Wilson, 1991; Wyatt *et al.*, 1985). However, reports that methionine supplementation of layer diets increased dry matter percentages of both yolk and albumen (Carey, 1990; Carey *et al.*, 1991) imply that it may be possible to increase chick yield (chick wt/egg wt), and hence chick

weight independent of egg weight, by increasing the concentration of the egg contents. Calculations of chick yield made in this study support this hypothesis (Table 3). In addition, chicks hatching from eggs laid by hens provided dietary HMB supplementation may have greater protein and/or methionine stores which may result in improved early livability and/or growth rates. This hypothesis was not tested in this study.

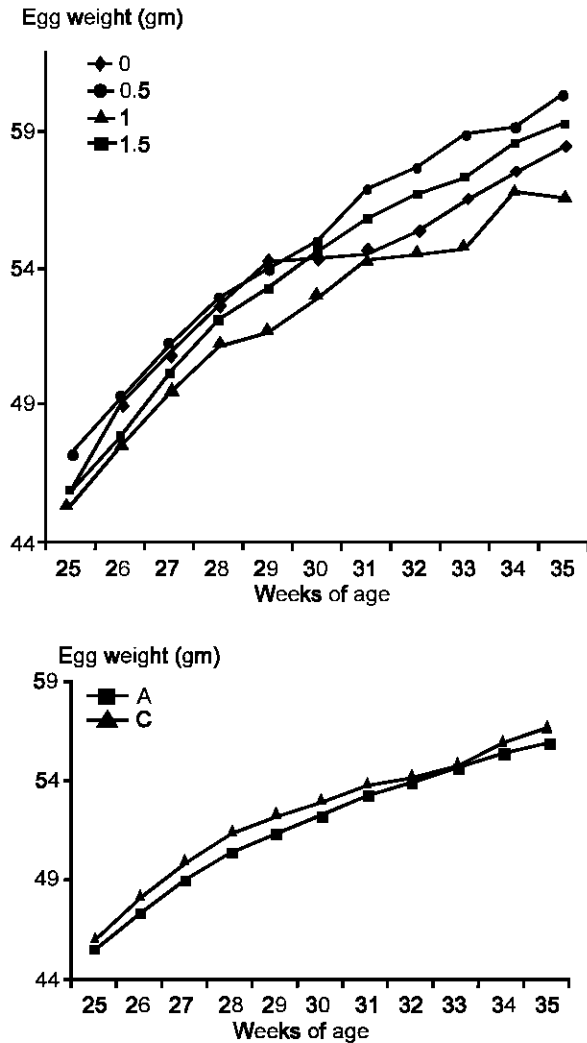


Fig. 1: Effects of dietary 2-hydroxy-4-(methylthio)butanoic acid (HMB) and strain on egg weights of broiler breeder pullets. Treatments provided HMB levels equivalent to 0, 0.5, 1 and 1.5 kg of added methionine per tonne of feed (HMB Trt 0, 0.5, 1 and 1.5, respectively). Pullet strains were Arbor Acres (A) and Cobb (C)

Although egg yolk and albumen percentages were not significantly affected by HMB treatments, when considered together, it appears that dietary HMB supplementation above 1 kg/tonne had a positive effect on yolk percentage at the expense of albumen percentage. Reports on layers (Carey, 1990; Carey *et al.*, 1991) indicated that methionine supplementation which increased dietary methionine from 0.28 to 0.43% increased egg weights as well as absolute albumen and yolk weights. However, neither yolk nor albumen percentages were affected. In addition, albumen and yolk dry matter percentages were increased by 0.3 and 0.5%. In the present study, an equivalent degree of HMB

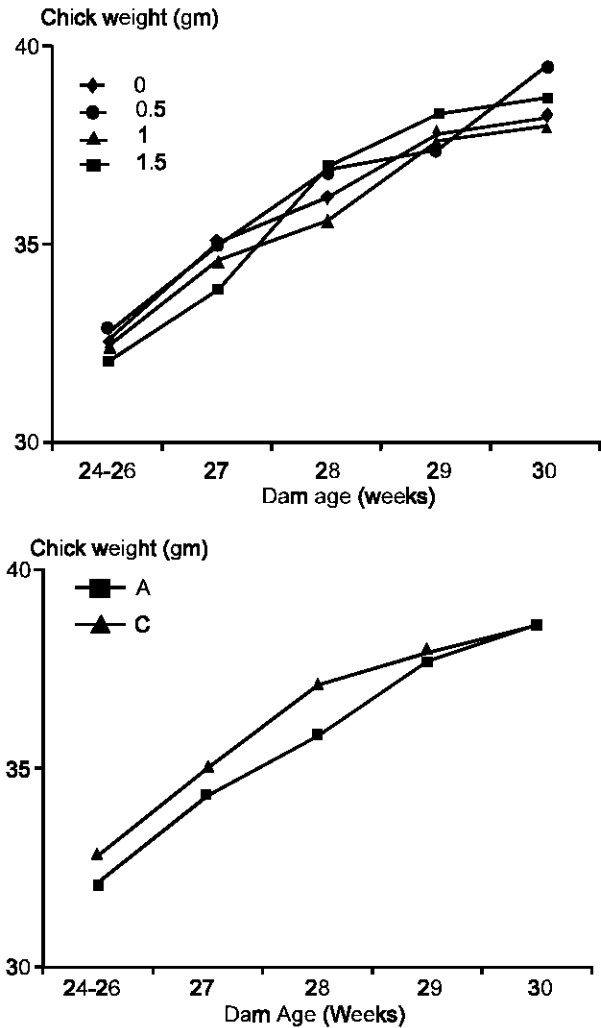


Fig. 2: Effects of dietary 2-hydroxy-4-(methylthio)butanoic acid (HMB) and strain on chick weights of broiler breeder pullets. Treatments provided HMB levels equivalent to 0, 0.5, 1 and 1.5 lb of added methionine per ton of feed (HMB Trt 0, 0.5, 1 and 0.5, respectively). Pullet strains were Arbor Acres (A) and Cobb (C)

supplementation increased dry matter percentages of both albumen and yolk to a similar extent. However, differences were not statistically significant, although they may have contributed to differences in chick yield (Table 3). Strain A had greater yolk and lesser albumen percentages than strain C (Table 3); however, dry matter percentages of albumen and yolk did not differ between strains. Differences in the composition of eggs from different turkey strains were hypothesized to be related to differences in poult livability (Reidy *et al.*, 1994). Total and total settable egg production were greatest in HMB treatment 0.5, intermediate in treatments 1 and 1.5,

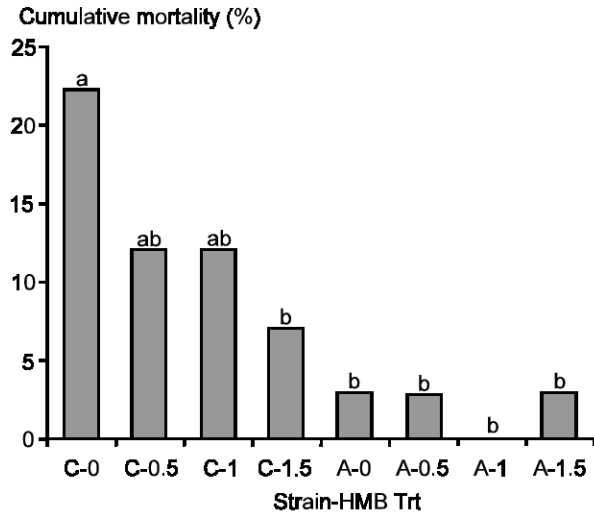


Fig. 3: Effects of dietary 2-hydroxy-4-(methylthio)butanoic acid (HMB) and strain on cumulative mortality of broiler breeder pullets from 21 to 35 wk of age. Treatments provided HMB levels equivalent to 0, 0.5, 1 and 1.5 kg of added methionine per tonne of feed (HMB Trt 0, 0.5, 1 and 1.5, respectively). Pullet strains were Arbor Acres (A) and Cobb (C)

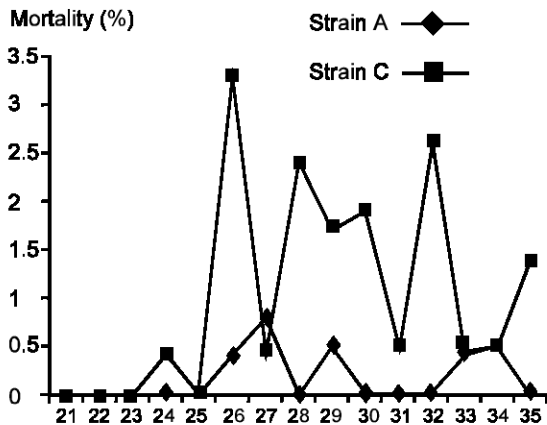


Fig. 4: Effect of strain on weekly mortality of broiler breeder pullets from 21 to 35 wk of age. Pullet strains were Arbor Acres (A) and Cobb (C)

and least in treatment 0 (Table 3). Ages at 20 and 50% HDP were not influenced by HMB treatment (Table 3). Weekly egg production levels of the 4 HMB treatments were similar through near-peak production (28 wk, data not shown); however, weekly production levels differed in a manner similar to total production from 29 to 35 wk. Therefore, although dietary HMB supplementation equivalent to 0.5 kg of methionine per tonne of feed increased egg production after near-peak production levels were attained, production was not increased by

further increases in HMB supplementation levels.

Based on previous reports, egg production by breeders was increased when dietary protein intake was increased during the prelay period (Lilburn and Myers-Miller, 1990a; Lilburn and Myers-Miller, 1990b) and, daily dietary protein and lysine intakes per hen were increased up to 18.55 g and 824 mg, respectively, during the period from 40 to 48 wk (Harms and Ivey, 1992). However, breeder egg production throughout entire production cycles was not increased by increasing daily dietary protein and sulfur amino acid intakes per hen from 18.6 to 23.0 g and from 682 to 850 mg, respectively, (Wilson and Harms, 1984) or increasing dietary protein from 12 to 18% (Robey *et al.*, 1988). Based on these results and those of this study, it appears that breeders can attain high egg production levels when provided rations with a relatively broad range of methionine levels. Although, adequate protein and amino acid intakes must be provided to support maximal production levels, greater levels of these nutrients do not appear to consistently result in increased production.

There was an interaction between HMB treatment and strain on mortality (Fig. 3). Mortality was minimal and unaffected by HMB treatment in strain A. A similar mortality level occurred in strain C at the highest level of HMB supplementation; however, mortality increased markedly as dietary HMB level was decreased in this strain and was most severe at the 0 kg/tonne level. Between 30 and 35 wk, the cause of death was diagnosed as fatty-liver hemorrhagic syndrome (FLHS) in 6 of 9 mortalities from strain C and 1 of 2 from strain A.

The occurrence and etiology of FLHS in laying chickens has been reviewed by several authors (Meijering, 1979; Squires and Leeson, 1988; Hansen and Walzem, 1993). Several factors previously associated with the development of FLHS were probably involved in its occurrence in the present study. Hens in this study were likely in a positive energy balance since environmental temperatures were quite high (Table 2), and standard feed (and hence, energy) allotment increases were made during the onset of lay (Table 2). Factors such as birds being in a positive energy balance (Polin and Wolford, 1976; Polin and Wolford, 1977) exposure to high temperatures or other stressors (Meijering, 1979) and elevated plasma estrogen levels associated with egg production (Polin and Wolford, 1977) have been implicated in the development of FLHS. The use of corn-soy diets without added fat, as were used in the present study, has also been indicated as a causative factor for FLHS since they apparently encourage excessive hepatic lipogenesis (Squires and Leeson, 1988). In addition, supplementary methionine was hypothesized to decrease the incidence of FLHS due a lipotropic affect on fat transport from liver cells (Meiring, 1979). Although all of these factors may have contributed to the

occurrence of FLHS in this study, the interaction between strain and treatment on the incidence of mortality, which was apparently due primarily to this ailment, indicates that strain C was particularly sensitive to decreased dietary methionine levels. Apparently, this strain has a greater tendency to accumulate lipid in the liver when provided lower dietary methionine (HMB) levels. Different strains of chickens have been observed to have different propensities for developing FLHS (Couch, 1956), and these differences have been loosely attributed to selection for increased egg production (Couch, 1956) and body weight (Squires and Leeson, 1988). The most obvious differences between the two strains tested in the present study, based on information provided by primary breeders and producers, are that strain C had been selected for high breast meat yield and is a heavier type bird than strain A.

When livers were examined at 35 wk, there were no differences between strains or treatments for liver weight, color, macroscopic condition or microscopic lesions (data not shown). This may have been because those birds that experienced FLHS had already died and been removed from the population and/or that the disease had run its course and birds in which it was not fatal had recovered. These possibilities are supported by the decreasing trend in mortality from 26 to 35 wk (Fig. 4).

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