THE USAGE OF THE 2-HYDROXY-4-(METHYLTHİO) BUTANOİC ACİD'S ISOPROPYL ESTER (HMBİ) IN DAİRY CATTLE RATİONS

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ABSTRACT

Methionine and lysine have been reported to be limiting AA for milk yield and protein production. The main objective of this trial was to test the efficacy of 2-hydroxy-4-(methylthio)-butaonic acid's isopropyl ester on milk production and composition, rumen volatile fatty acids values, blood glucose, plasma urea nitrogen, plasma triglyceride and creatinine values compared with a positive control (dietary supplemented corn gluten meal instead of HMBi) and control. Feeding for 8 week of 44 g per day of 2-hydroxy-4-(methylthio) butanoic acid isopropyl ester (HMBi) was tested on 30 cows (10 controls, 10 gluten meal treatment, 10 HMBi treatment) in Baskent University herd. There was no significant difference in dry matter intake across treatment groups. Neither milk yield nor milk composition (protein yield, lactose yield, fat yield, solids-not-fat, total solids) was affected significantly by supplementation HMBi or corn gluten meal. The %4 fat-corrected milk for the gluten meal and HMBi supplemented groups was numerically (1.86 kg/day and 1.39 kg/day respectively) higher than for the control group. Milk tota solids for the gluten meal and HMBi supplemented groups was numerically (188.32 g/day and 70.66 g/gün respectively) higher than for the control group. Milk fat yield for the gluten meal and HMBi supplemented groups was numerically (111.56 g/day and 98.71 g/gün respectively) higher than for the control group. Treatments did not affect rumen volatile fatty acids values. Dietary supplementation of corn gluten meal significantly (p=0.026) reduced the mid-trial blood glucose values than for the control and HMBi groups. However end of the trial blood glucose values for the corn gluten meal were obtained significantly (p=0.004) higher than for the control and HMBi groups. Supplementation of HMBi significantly (p=0.032) increased plasma urea nitrogen value than for the control and corn gluten meal groups. Plasma triglyceride and creatinine value was not affected treatments.

Key words: *2-hydroxy-4-(methylthio)-butanoic acid's isopropyl ester, methionine hydroxy analog, HMBi, milk yield, milk composition.*

INTRODUCTION

Methionine have been identified first limiting AA in dairy cows (Polan et al., 1991; Wu et al., 1997). Several technologies have been developed to achieve efficient methionine against ruminal degradation by microorganisms. As an example, lipid-protected product, It is a matrix compound that contains %65 DL-Met embedded in a mixture of calcium salts of fatty acids. Surface-coated, carbohydrate-protected product consist of core of methionine and starch coated with several thin layers of ethyl cellulose and stearic acid. Lipid/pH sensitive polymer-protected product consist of a core of DL-methionin which is covered with a coat of stearic acid its resistant to ruminal degradation but in the abomasum's low pH allows for rapid release of the methionin. Amino acid analogs are generated from the substitution of the α-amino group of the AA with a non-nitrogenous grup such as a hydroxyl group and the final product called 2-hydroxy-4-methylthio butanoic acid (HMB). The isopropyl ester of HMB (HMBi) is considerably more effective than HMB (Schwab et al., 2011). The isopropyle ester of HMB was shown to have 40 to %58 bioavailability based on blood kinetics of a pulse ruminal dose (Robert et al., 2001,2002). During recent years several investigators have reported that methionine hydroxy analog in diets of lactating cows has increased milk production (Wang et al., 2010; Piepenbrink et al.,2004; Patton et al., 1969; Lundquist et al.,1983) and fat production (Wang et al., 2010; Holter et al., 1971; St-Pierre and Sylvester., 2005; Lundquist et al., 1983; Bhargava et al., 1977; Patton et al., 1969; Lundquist et al., 1985). While

others found no effect on milk production (Hindle et al., 2008; Huber et al.,1984; Holter et al., 1971; Bhargava et al.,1977; Lundquist et al., 1985; Juranz et al., 2006; Piepenbrink et al., 2004) and fat production (Noftsger et al., 2005; Rulquin et al., 2006; Hutjens and Schultz., 1971).

The objective of this trial was to compare the effects of HMBi and corn gluten meal on milk production and composition , rumen volatile fatty acids values, blood glucose, plasma urea nitrogen, plasma triglyceride and creatinine values.

MATERIALS AND METHODS

Feeding for 8 week of 44 g per day of 2-hydroxy-4-(methylthio) butanoic acid isopropyl ester (HMBi) was tested on 30 multiparous cows were assigned to 1 of 3 dietary treatments (10 controls, 10 gluten meal treatment, 10 HMBi treatment) in Başkent University's herd. Control diets contained, with 6 kg/day alfalfa hay,4 kg/day dry sugar beet pulp, 2 kg/day barley hay and 10 kg/day pelleted concentrate made of % 41.69 barley, % 22.87 corn, % 23.39 soybean meal, % 5 wheat bran, % 4 sugar beet molasses, % 1,65 limestone, and DCP,salt,vitamin mineral mix. Corn gluten meal group diets contained, with 6 kg/day alfalfa, hay,4 kg/day dry sugar beet pulp, 2 kg/day barley hay and 10 kg/day pelleted concentrate made of % 40 barley, % 24.69 corn, % 18.21 soybean meal, % 6.5 wheat bran, % 4 sugar beet molasses, % 3.3 corn gluten meal % 1,8 limestone, and DCP,salt,vitamin mineral mix HMBi treatment group consisted of the basal diet plus 44 g/day HMBi (Meta Smart Dry) per cow (Tale 1).

Table1. Ingredient of nutrient composition of treatment groups of concentrate

	control	**Gluten treatment**	**HMBi treatment**
Barley	41,69	40	41,69
Corn	22,87	24,69	22,87
Soybean meal	23,39	18,21	23,39
Wheat bran	5	6,5	5
Sugar beet molasses	4	4	4
Corn gluten meal	-	3,3	-
HMBi	-	-	*
Limestone	1,65	1,8	1,65
DCP	0,5	0,5	0,5
Salt	0,7	0,8	0,7
Vitamin mineral mix	0,2	0,2	0,2

*HMBi treatment consist of basal diet plus 44 gr/day HMBi per cows.

All treatment groups diets were formulated to isocaloric and iso nitrogenic. Milk yield were recorded daily, milk was sampled during eight evening and eight morning milkings weekly and analyzed for protein, fat and lactose with Milkana Multi Test Milk Analyzer. Blood samples were taken from the jugular vein in the middle and at the end of the experiment to determine glucose. Principles of procedure ; glucose is phosphorylated by hexokinase (HK) in the presence of adenosine triphosphate (ATP) and magnesium ions to produce glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G-6-PD) specifically oxidizes G-6-P to 6-phosphogluconate with the micromole of NADPH is produced for each micromole of glucose consumed. The NADPH produced absorbs light at 340 nm and can be detected spectrophotometrically as an increased absorbance Burtis et al., (1994). Triglycerides are enzimatically hydrolyzed by lipase to free fatty acids and glycerol. The glycerol is phosphrylated by ATP with glycerol kinase (GK) to produce glycerol-3-phosphate and adenosine diphosphate (ADP). Glycerol-3-phosphate is oxidized to dihyrdocyacetone phosphate (DAP) by glycerol phosphate

oxidase (GPO) producing hydrogen peroxide . In a color reaction catalyzed by peroxidase, the hydrogen peroxide reacts with 4-aminoantipyrine (4-AAP) and 4-chlorophenol (4-CP) to produce a red colored dye.The absorbace of this dye is proportional to the concentration of triglyceride present in the sample. This analytical methodology is based on the reaction sequence described by Fossati et al.,1982 and by McGowan et al.,(1983). Plasma urea nitrogen principles of procedure; the urea nitrogen assay is a modification of a totally enzymatic provedure first described by Talke and Schubert., (1965) the test is performed as a kinetic assay in which the initial rate of the reaction is linear for a limited period of time. Urine in the sample is hydrolyzed by urease to ammonia and carbon dioxide. The second reaction, catalyzed by glutamate dehydrogenase (GLD) converts ammonia and α-ketoglutarate to glutamate and water with the concurrent oxidation of reduced nicotinamide adenine dinucleotide (NADH) to nicotinamide adenine dinucleotide (NAD). Two moles of NADH are oxidized for each mole of urea present. The initial rate of decrease in absorbance at 340 nm is proportional to the urea concentration in the sample. and creatinine principles of procedure; At an alkaline pH, creatinine in the sample reacts with pictare to form a creatinine-picrate complex. The rate of increase in absorbance at 500 nm due to the formation of this complex is directly proportional to the concentration of craetinine in the sample Fabiny and Ertingshausen.,(1971) ; Soldin et al.,(1978) . Rumen fluid samples were collected by stomach tube at the end of the trial to determine volatile fatty acids. Analytical procedures; Ruminal short chain fatty acids (SCFA) samples were allowed to thaw completely at 4°C before analysis. Samples were then acidified (pH<3) with 90 µL of 12 N H2SO4 vortexed and centrifuged (Universal 32R, Hettich Zentrifugen, Germany) in Eppendorf tubes for 30 min at 13000 rpm. The supernatant was filitered through a 0.2 µm PTFE membrane (Millex-GN, Millipore). Concentrations of SCFA in the supernatant were then determined by HPLC (Dionex Summit P680, ASI100) equipped with an UV absorbance detector (Dionex UVD170) operated at 210 nm. Separation of acids was conducted using an organic acid analysis column (300x7.8mm; Rezex ROA-Orgnic Acid column) with 0.005 M H2SO4 as eluent at flow rate of 0.6 mL min and with the column temperature of 60°C. A Rezex ROA Organic Acid precolumn (50x7.8 mm) was used to protect the column from any particles than could have been injected together with the samples Oeztuerk et al., (2010).

RESULTS AND DISCUSSION

The chemical analyses of alfalfa hay, sugar beet pulp, barley hay and concentrate feed are reported in table 2

Table2. Chemical composition of feeds

	DM	CP	Crude fat	Crude fiber	Ash	Ca	P
Control and HMBi treatment's concentrate feed	91.00	18.22	1.58	4.58	5.50	1.06	0.54
Gluten treatment concentrate feed	91.05	16.30	1.99	5.33	5.45	1.00	0.53
Corn gluten meal	94.70	63.17	0.47	1.90	1.45	0.32	0.46
Sugar beet pulp	90.40	8.75	0.56	22.25	3.35	0.70	0.06
Alfalfa hay	90.50	14.50	1.41	20.40	12.4	1.28	0.25
Barley hay	93.40	1.39	0.78	40.15	4.30	0.52	0.35

There was no significant difference in dry matter intake across treatment groups which averaged 22 kg/d across all 3 treatments.

Milk production was not affected by HMBi or corn gluten meal (p=0.93). Cows supplemented with HMBi milk produced nonsignificant 0.42 kg/d lower of milk compared with the control cows. However cows supplemented with corn gluten meal group produced an additional 0.26 kg/d of milk

compared with the control cows (table 3). These findings are similar to others (Stokes et al., 1981; Hutjens and Schultz., 1971). However some researchers found an increase in milk yield (Wang et al., 2010; Piepenbrink et al., 2004; Patton et al., 1969; Lundquist et al., 1983). 4% fat corrected milk production (FCM) was not affected treatment (p=0.23). The FCM for the supplemented HMBi and corn gluten meal diets was numerically higher for the control (respectively 1.38 kg/d, 1.86 kg/d). These observations agree with some reports (Huber et al., 1984; Holter et al., 1971), and disagree with those reporting significatly increase in FCM (Bhargava et al., 1977; St-Pierre and Sylvester., 2005; Piepenbrink et al., 2004; Lundquist et al., 1983).

Table 3. Influence of HMBi and corn gluten meal on milk production and composition, rumen volatile fatty acids values, blood glucose, plasma urea nitrogen, plasma trigliceride and creatinine values.

	control	Gluten treatment	HMBi treatment	sem	p
Milk yield kg/day	27.86	28.12	27.44	0.69	0.93
%4 Fat-corrected milk kg/day	21.86	23.68	23.24	1.11	0.23
Total solids gr/day	3323.32	3511.64	3393.98	87.13	0.68
Solids-not-fat g/day	2607.32	2680.96	2575.99	63.68	0.8
Fat yield g/day	714.73	826.29	813.44	34.85	0.38
protein yield g/day	979.55	1008.01	968.72	23.92	0.8
Lactose yield g/day	1432.73	1472.54	1420.26	35.11	0.82
Acetate mmol/l	45.03	46.57	45.04	2.11	0.95
Propionate mmol/l	21.53	22.8	19.12	1.1	0.38
Butyrate mmol/l	5.67	5.76	6.17	0.35	0.84
Glucose mid-trial mg/dl	51.13a	43.44b	53.25a	1.64	0.026
Glucose end of the trial mg/dl	41.25b	51.33a	39.13b	1.76	0.004
Glucose average trial mg/dl	46.19	47.39	46.19	1.14	0.89
Triglyceride average trial mg/dl	6.19	7.06	6.81	0.45	0.73
creatinine average trial mg/dl	0.73	0.75	0.79	0.02	0.48
Plasma ureaN end of the trial mg/dl	12.13b	11.89b	13.88a	0.35	0.032
Plasma ureaN average trial mg/dl	13.5	14.28	14.88	0.32	0.23

Milk total solids for the gluten meal and HMBi supplemented groups was numerically (188.32 g/day and 70.66 g/gün respectively) higher than for the control group. Similar resuts were obtained by Piepenbriks et al., (2004) and Lundquist et al., (1985).

Milk fat yield for the gluten meal and HMBi supplemented groups was numerically (111.56 g/day and 98.71 g/gün respectively) higher than for the control group. In this experiment is consistent with results from other studies (Huber et al., 1984; Wang et al., 2010; Holter et al., 1971; St-Pierre and Sylvester., 2005). However some researchers found an significant increase in milk fat yield (Lundquist et al., 1983; Bhargava et al., 1977; Patton et al., 1969; Lundquist et al., 1985).

Milk protein yield for the gluten meal was found numerically higher (28.46 g/d) than control and HMBi supplemented group was found numerically lower (10.83 g/d) than for the control group. Similar to lactos yield was found for gluten meal numerically higher (39.81 g/d) than control and HMBi supplemented group was found numerically lower (12.47 g/d) than for the control. These findings are similar to others (Wang et al., 2010; Piepenbrink et al., 2004). However some researchers found an significant increase in milk protein yield (St-Pierre and Sylvester., 2005; Rulquin et al., 2006; Noftsger et al., 2005; Phipps et al., 2008; Hindle et al., 2008; Juranz et al., 2006)

Treatments did not affect rumen volatile fatty acids values. In this experiment is consistent with results from other studies (Stokes et al., 1981; Lundquist et al., b1985).

Dietary supplementation of corn gluten meal significantly (p=0.026) reduced the mid-trial blood glucose values than for the control and HMBi groups. However end of the trial blood glucose values for the corn gluten meal were obtained significantly (p=0.004) higher than for the control and HMBi groups. Supplementation of HMBi significantly (p=0.032) increased plasma urea nitrogen value than for the control and corn gluten meal groups. Plasma triglyceride and creatinine value was not affected treatments.

CONCLUSION

As a result in the presented study, dietary supplementation of %0.15 HMBi did not shown a significant difference milk yield or milk composition however trial has shown that the milk fat content numerically increased by the HMBi supplementation. Treatments did not result in a significant increase in yield in terms of other parameters.

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REFERENCES

1. **BHARGAVA, P. K., OTTERBY, D. E., MURPHY, J. M., DONKER, J. D. (1977).** Methionine hydroxy analog in diets for lactating cows. *J.Dairy Sci*. 60: 1594 – 1604.

2. **BURTIS, C. A., ASHWOOD, E. R., eds.(1994)** Tietz Textbook of Clinical Chemistry, 2nd ed. Philadephia, PA: WB Saunders 959 – 960.

3. **FABINY, D. L. , ERTİNGSHAUSEN, G. (1971).** Automated reaction-rate method for determination of serum creatinine with the centrifichem. *Clin Chem* **17:** 696 – 700.

4. **FOSSATİ, P., PRENCİPE, L. (1982).** Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin Chem.* **28:** 2077 – 2080.

5. **HİNDLE, V. A., KAN, C. A., ROBERT, C. J., VAN VUUREN, A. M. (2008).** Effect of the isopropylester of the hydroxylated analogue of methionin (HMBi) on feed intake and performance of dairy cowa in early lactation. *J.Dairy Sci*. **89(Suppl. 1):** 401.

6. **HOLTER, J. B., KIM, C. W., COLOVOS, N. F. (1971).** Methionine hydroxy analog for lactating dairy cows. *J.Dairy Sci.* **55:** 460 – 465.

7. **HOLTER, J. B., KIM, C. W., COLOVOS, N. F. (1971).** Methionine hydroxy analog for lactating dairy cows. *J.Dairy Sci.* **55:** 460 – 465.

8. **HUBER, J. T., EMERY, R. S., BERGEN, W. G., LIESMAN, J. S., KUNG, L., KING, K. J. (1984).** Influences of methionine hydroxy analog on milk and milk fat protduction, blood serum lipids, and plasma amino acids. *J.Dairy Sci.* **67:** 2525 – 2531.

9. **HUTJENS, M. F., SCHULTZ, L. H. (1971).** Addition of soybeans or methionin analog to high-concentrate rations for dairy cows. *J.Dairy Sci.* **54:** 1637 – 1644.

10. **JURANZ, S., ROBER, J. C., LAURENT, F. (2006).** Effects of the isopropylester of the hydroxylated analogue of methionine (HMBi) on production performance of dairy cows in early lactation. *Jornal of Animal Scn*. **84:** 1.

11. **LUNDQUİST, R. G., LINN, J. G., OTTERBY, D. E. (1983).** İnfluence of dietary energy and protein on yield and composition of milk from cows fed methionine hydroxy analog. *J.Dairy Sci.* **66:** 475 – 491.

12. **LUNDQUİST, R. G., OTTERBY, D. E., LINN, J. G. (a1985).** Influence of three concentrations of DL-methionine or methionine hydroxy analog on milk yield and milk composition. J.Dairy Sci. **68:** 3350 – 3354.

13. **LUNDQUİST, R. G., STERN, M. D., OTTERBY, D. E., LINN, J. G. (b1985).** Influence of methionine hydroxy analog and DL-methionine on rumen protozoa and volatile fatty acids. *J.Dairy Sci*. **68:** 3055 – 3058.

14. **McGOWAN, MW., ARTİSS, D. J., STRANDBERG D. R. (1983).** A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin Chem.* **29:** 538 – 542.

15. **NOFTSGER, S., St-PİERRE, N. R., SYLVESTER, J. T. (2005).** Determination of Rumen Degradability and Ruminal Effects of Three Sources of Methionine in Lactating Cows. *J.Dairy Sci.* **88:** 223 – 237.

16. **OEZTUERK, H., EMRE, B., SAGMANLIGIL, V., PISKIN, I., FIDANCI, U. R., PEKCAN, M. (2010b).** Effects of nisin and propolis on ruminal fermentation in vitro. *Journal of Animal and Veterinary Advances*. **9(21):** 2752 – 2758.

17. **PATTON, R. A., McCARTHY, R. D., GRIEL, JR. (1969).** Observations on rumen fluid, blood serum and milk lipids of cows fed methionine hydroxy analog. *J.Dairy Sci.* **53:** 776 – 780.

18. **PHIPPS, R. H., REYNOLDS, C. K., GİVENS, D. I., JONES, A. K., GERAERT, P. A., DEVİLLARD, E., BENNETT, R. (2008).** Effects of 2-Hydroxy-4 (Methylthio) Butanoic Acid Isopropy Ester on Milk Production and Composition of Lactating Holstein Dairy Cows. *J.Dairy Sci.* 91: 4002 – 4005.

19. **PIEPENBRINK, M. S., MARR, A. L., WALDRON, M. R., BUTLER, W. R., OVERTON, T. R., VAQUEZ-ANON, M., HOLT, M. D. (2004).** Feeding 2-hydroxy-4-(methylthio)-butanoic acid to periparturient dairy cows improves milk production but not hepatic metabolism. *J.Dairy Sci.* 87: 1971 – 1084.

20. **POLAN, C. E., CUMMINS, K. A., MUSCATO, T. V., SNIFEN, C. J., VICINI, J. L., CROOKER, B. A., CLARK, J. H., GUILLAUME, B., OTTERBY, D. E., JOHNSON, D. G., MULLER, L. D., VARGA, G. A., MURRAY, R. A., PEIRCE-SANDNER, S. B. (1991).** Responses of dairy cows to supplemental rumen-protected forms of methionine and lysine. *J.Dairy Sci.* 74: 2997 – 3013.

21. **ROBERT, J. C., d'ALFONSO, T., ETAVE, G., DEPRES, E., BOUZA, B. (2002).** Quantifying the metabolisable methionine contribution of a liquid or powder presentation of 2-hyrdroxy-4(methyl thio) butanoic acid isopropyl ester (HMBi). *J.Dairy Sci*. **85 (Suppl.1):** 71.

22. **ROBERT, J. C., RICHARD, C., BOUZA, B. (2001).** Influence of monomer or dimer forms of isopropyl ester of HMB, on the suppl of metabolisable methionine to the blood of ruminants. *J.Dairy Sci*. **84(Suppl.1):** 281.

23. **RULQUİN, H., GRAULET, B., DELABY, L., ROBERT, J. C. (2006).** Effect of Different Forms of Methionine on Lactational Performance of Dairy Cows. *J.Dairy Sci.* **89:** 4387 – 4394.

24. **SCHWAB, C. G., ORDWAY, R.S. (2011).** Methionine supplementation options. Department of animal and nutritional sciences., University of New Hampshire Durham NH., Access: [http://www.formulate2.com/schwab_methionine.pdf.] Erişim tarihi 06/03/2011.

25. **SOLDIN, S., HENDERSON, L., HILL, G. (1978).** The effect of bilirubin and ketones on reaction rate methods for the measurementt of creatinine. *Clin Biochem*. 82 – 86.

26. **STOKES, M. R., CHLARK, J. H., (1981).** Performance of lactating dairy cows fed methionine or methionine analog at two concentrations of dietary crude protein. *J.Dairy. Sci*. **64:** 1686 – 1694.

27. **ST-PİERRE, N. R., SYLVESTER, J. T. (2005).** Effects of 2-Hydroxy-4-(Methylthio) Butanoic Acid(HMB) and Its Isopropyl Ester on Milk Production and Composition by Holstein Cows. *J.Dairy Sci*. **88:** 2487 – 2497.

28. **TALKE, H., SCHUBERT, G. E. (1965).** *Klinische Wochenschrift* 43 – 174.

29. **WANG, C., LİU, H. Y., WANG, Y. M., YANG, Z. Q., LİU, J. X., WU, Y. M., YAN, T., YE, H. W. (2010)** Effects of dietary supplementation of methionine and lysine on milk production and nitrogen utilization in dairy cows. *J.Dairy Sci.* **93:** 3661 – 3670.

30. **WU, Z., FISHER, R. J., POLAN, C. E., SCHWAB, (1997).** Lactational performance of cows fed low or high ruminally undegradable protein prepartum and supplemental methionine and lysine postpartum. *J.Dairy Sci*. **80:** 722 – 729.