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Bioavailability and Effects of Rumen Protected Methionine on Milk Yield and Amino Acid Metabolism in Lactating Crossbred Frisan Cows

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Abstract

The objective of this study was to evaluate ruminal degradation and productive performance of lactating cows fed rations supplemented with one of two different sources of methionine products [methionine hydroxy analogue (HMB) or DL-methionine (DL-Met)]. The effect of the two sources of methionine on ruminal degradations, milk yield and plasma essential amino acids concentrations were measured in two experiments. In experiment 1, the ruminal degradation of methionine was assessed using *in situ* bag techniques with three Barki female sheep (with an average of 44kg) fitted with permanent ruminal fistula. In the second experiment nine lactating Crossbred Friesian multiparous cows in similar condition were randomly assigned to three experimental treatments to determine milk yield and milk composition. Methionine concentration was assayed in blood plasma. The obtained results indicated that the rate of disappearance of HMB was significantly (P>0.05) lower than DL-Met. (52.12. vs. 86.73). While the highest

(P>0.05) rumen escapes were recorded for HMB. Methionine hydroxy analogue had more resistance to ruminal degradation than DL-Met. The results also revealed that daily milk production and FCM of cows fed 25g day HMB was significantly (P>0.05) higher than those fed the control or ration supplemented with DL-Met. Treatment had no effect on percentage of lactose. However, total solids, fat and milk protein significantly (P>0.05) increased with 25g day HMB supplementation. The results also showed that methionine concentration in blood plasma increased significantly (P>0.05) with cows fed ration supplemented with HMB compared with those fed control ration or those fed ration supplemented with DL-Met. Results of the present study also showed that supplementation of 25g day HMB improved, bioavailability of methionine and promoted amino acid utilization in lactating cows which increase milk yield

Introduction

As milk production of dairy cows continues to increase,meeting nutrient requirements especially for amino acids (AA),becomes more difficult. Protein available for absorption in the ruminant in intestine is derived from ruminal microbes and dietary protein that escapes degradation during passage through the rumen ***(Dhiman and Satter,1997)***. Protein is one of the major limiting nutrients in the diets of lactating dairy cows ***(Koenig and Rode,2001).*** Feeding diet containing more protein may lead to more waste and environmental pollution. Excess rumen degradable protein in the form of urea will be expost for degradation by ruminal microbes. It is important to increase milk yield, especially milk protein yield and the efficiency of protein utilization and to avoid protein deficiency in early lactation ***(Xu et al., 1998 and Blum et al., 1999).***

Many studies have been focused on increasing amounts of limiting Amino Acids (AA) especially methionine (Met.) in the diet of high yielding dairy cows to increase milk and milk protein yield ***(Rulquin et al., 2006)*** and the efficiency of protein utilization. **(Broderick *et al*., 2008)**, reducing nitrogen excretion ***(Leonardi et al., 2003)*** and reducing protein intake ***(Broderick et al., 2008).*** Methionine has been identified as one of the most limiting AA for the synthesis of milk and milk protein by dairy cows fed diets based on corn ***(Leonardi et al., 2003)***. Unfortunately, crystalline methionine (Met) may be degraded by ruminal bacteria before it passes to the small intestine. Because free Met. would be mostly degraded in the rumen, it needs to be supplemented in the rumen- protected form to be available in sufficient amounts for absorption in the duodenum and for metabolic purposes. Methionine has been successfully protected by a number of coating techniques. Methionine hydroxy analogue (HMB) are generated from the substation of the a- amino group of methionine with a non-nitrogenous group such as hydroxyl ***(Storm and Ørskov,1983, Samuelson et al., 2001 and Leonardi et al., 2003)***.Many studies indicate that HMB is more resistant to ruminal degradation than free Met.,which it can be absorbed from the rumen and omasum through passive diffusion, and that ruminants have enzymes for the conversion of HMB to methionine. ***(Charles et al., 2003)*** reported that HMB has the potential to increase milk production, but would only be expected to have such an effect when its resistance to ruminal degradation and its intestinal digestibility are high. Addition of HMB to diets of dairy cows frequently resulted in increased protein content of milk ***(Lundquist et al., 1983)*** milk and production ***(Casper et al., 1987).*** The objective of this study was to evaluate the effect of supplementing lactating rations with DL-

methionine or methionine hydroxy analogue on milk yield and composition and plasma AA concentration of dairy cows.

Materials and Methods

Experimental diets:

The ingredients and chemical composition of the experimental diets are presented in Table (1). Animals were fed total mixed rations (TMR) consisting of concentrate, corn silage and rice straw with approximate ratio of 60:30:10, respectively on dry matter (DM) basis. The tested animals were fed TMR supplemented with two different sources of methionine. The first ration was supplemented with methionine hidroxy analogues (HMB) 25g / head / day, the second ration was supplemented with DL- methionine 25 g / head / day while, the third ration (control) was without methionine supplement. The methionine products were mixed well into a portion of the concentrates then mixed with the TMR. Animals were fed individually twice daily (08:00 and16:00). and had free access to water throughout the experiment.

First experiment:

In situ trials:

Three Barki female sheep fitted with permanent rumen fistula (with an average of 44 kg ± 1.50 live body weight) were used in ruminal degradation. The animals were fed TMR for two weeks as preliminary period before they were fed the methionine sources. An average of 2.5±0.04 g of each HMB or DL-Met was weighed into dacron polyester bags (100 % dacron polyester) with a mean pore size of 45 µm and the bags dimension 7 X 10 cm. Samples were incubated in the rumen for 2, 4, 8, 12, 24, and 48 h. Each sample

was incubated in duplicate bags for each incubation time. A pair of bags was also incubated in distilled water at 38˚C for 10 min to estimate time zero. After removal from the rumen, bags were hand-washed with cold tap water for at least 10 min until no color was visible and dried for 24 h in a 60˚C forced-air oven. After drying, bag contents were weighed and duplicates of bags were analyzed for Met contents. Methionine was determined by performic acid to oxidize methionine to methionine sulfone. Sodium metabisulfite ($Na_2S_2O_5$) is added to decompose performic acid and analyzed for Met. content using ion-exchange chromatography according to ***AOAC (2000)*** method 994.12.

The linear model described by ***Mathers and Miller (1981)*** was used to estimate the rate of degradation from the Dacron bags. ruminal degradation was calculated as follows:
Ruminal degradation = soluble fraction + degradable fraction {kd / (Kd+Kp)}.

Where: soluble fraction: is the percentage of disappearing at time zero, degradable fraction: is potentially degradable fraction = {100- fraction completely resistant to ruminal degradation at time (t)}. Kd: the rate of ruminal degradation (h^{-1}), and kp: the rate of passage (assumed to be 0.06 h^{-1}). Rumen escape = {kd / (Kd+Kp)}.

Second experiment:

Lactation trials:

Nine lactating crossbred Friesian multiparous cows were chosen based on their live body weight (450 kg±6.5 in average). The cows were allocated to 3 dietary treatments (3 animals each). The experiment lasted 45 days of which the first 15 days was considered

as preliminary period followed by 30 days trial period. During the adaptation period all cows received the control ration (no Met.). On day 16, cows were assigned randomly to one of the three treatments. The cows were fed to cover their maintenance and production requirements. Maintenance requirement was calculated according to ***NRC (1990)*** and requirement for the production was calculated according to ***Barney Harris (1992).*** Cows were housed individually tie stalls and had free access to water throughout the experiment.

Sampling, measurement and analyses:

Cows were milked twice a day 8.00 and 16.00 and milk yields of individual cows were recorded at each milking. Milk was sampled weekly from two consecutive milking, and preserved with 2-bromo-2 nitropropan-1, 2diol and composited according to milk yield and kept at 4 °C for analysis. Fat corrected milk (4 %) was calculated according to ***Gaines (1923)*** using the following equation: FCM = M (0.4+0.15 F %) Where M= milk yield, F = fat percentage. Milk fat percentage was determined according to Gerber's method as described by ***Ling (1963).*** Total solids percent (TS), total protein and ash were determined according to the standard methods of ***AOAC (1995).***

Blood samples:

Blood samples were obtained from the jugular vein with evacuated tubes containing Li-heparin (LH) as anticoagulants during the last day of the experiment at 3h post feeding. Blood samples tubes were centrifuged at 1500 x g at 4^{o}C for 10 min. Plasma was separated and directly stored at frozen -20^{o}C until analysis. Plasma AA was analyzed using a Beckman system 6300 high performance amino acid analyzer cation-ion exchange column. AA was

determined according to method reported by ***Fontaine and Eudaimon (2000) and (Fontaine et al., 1998).*** Samples were deproteinized using sulfosalicylic acid. Plasma samples were composited prior to being sent for analysis.

Data were statistically analyzed using the method of least squares analysis of variance using General Linear Models (GLM) procedure ***(SAS, 2000).*** Duncan's Multiple Range Test ***(Duncan, 1955)*** was used to compare among means of each trait.

Results and Discussion

There are various techniques for measuring the ruminal rate of degradation of feedstuffs. The *in-situ* technique is one of the approaches that are most commonly used for assessing ruminal resistance and availability of rumen protected methionine (RPM) products. This technique is also the most currently used to study amino acid degradation. ***(Jarrige et al., 1987).*** But it is not in-agreement with those obtained by ***(Abdi- Benemar et al., 2016)*** who suggested that the *in-situ* method may not adequately characterize the availability of rumen protected amino acids. However the protected methionine technique may underestimate ruminal degradation as well as bio-availability of certain ruminally protected methionine ***Koenig and Rode (2001)*** and ***(Berthiaume et al., 2000).***

Ruminal degradation patterns of both source of methionine whether DL- Met or HMB are showed in table (2) and the rumen escape of methionine at different times of ruminal incubation are shown in Fig.(1). The results indicated that the solubility value (Soluble fraction) of DL- Met (38.19) was significantly ($p<0.05$) higher

than that of HMB (34.05) with a significant differences. This result was in-agreement with those of ***Koenig and Rode (2001)*** they found that DL-Met had higher solubility fraction than ruminal protected methionine (RPM), such as methionine hydroxyl analog. While, ***Patterson and Kung (1988)***. Found that both of DL-Met and hydroxyl protected methionine (HMB) had the same results of higher solubility fraction.

Ruminal disappearance of methionine from DL- Met or HMB increased with residence time. Disappearance of DL-Met was greater than HMB at the different times of incubation. The results indicated higher ($p<0.05$) resistance of HMB to ruminal degradation than DL-Met. This results were in-agreement with those obtained by ***(Overton et al., 1996); (Blum et al., 1999); (Berthiaume et al., 2006), Belasco (1980), (Jones et al., 1988)*** and ***(Oh et al., 2008)***. They reported that HMB was more resistant to degradation in the rumen than DL- methionine. Previous studies have reported that feeding ruminally protected Methionine (RPM) had an increased passage of Met to the small intestine than unprotected methionine and increased the amount of bioavailability Met.

Rumen escape of Met from HMB was greater than dl-Met. This results in-agreement with those of ***(Charles et al., 2003), Loerch and Oke, (1989)*** and ***(Schwab et al., 2001)*** who showed that HMB enhance ruminal escape, at least in part because of their apparent ability to be absorbed across the rumen wall through passive diffusion and there is evidence that extent of ruminal escape is greater with amino acid analogue are generated from the substitution of the -amino group of the AA with a non-nitrogenous

group such as a hydroxyl group. Studies indicate that HMB is more resistant to ruminal degradation than free Met.

Results for the daily milk, FCM (4%) production and milk composition are presented in Table (3). The present study indicated that daily milk production and daily milk yield 4% FCM of cows fed on ration supplemented with HMB had higher (P< 0.05) yield, than those fed on ration supplemented with DL-Met or no supplemented (control). These results were in-agreement with ***Čermakova et al., (2012)*** and ***(Noftsger et al., 2005).*** They noted that dietary supplementation with ruminally protected methionine (RPMet) has the potential to increase milk production, but it is not in-agreement with the results of ***(Berthiauml et al., 2006)*** who found that there was no effect of RPMet on milk yield. In the experiment of ***Casper and Schigoethe (1988)*** on cows fed barley and corn silage- based total mixed diet, found that supplementation with RPMet did not increase milk production but increased milk protein percentage. They concluded that Met increased in mammary synthesis, but it was not first factor limiting milk production.

Milk compositions (%) of cows fed ration supplemented with 25 g day- of HMB significantly (p<0.05) higherpercentage of milk protein had fat compared with cows fed ration supplemented with 25g DL-Met and control group. Percentage of milk protein may be more sensitive index to estimate the effect of RPMet on cows ***(Samuelson et al., 2001).*** Rumen-protected Met tended to increase protein percentage in milk, which agreed with the data from other experiments ***(Wu et al., 1997*** and ***Misciattelli et al., 2003).*** Indeed several studies have indicated that content of protein in milk is sensitive to adequacy of Met in diet and that milk protein percentage

increases when the content of Met in diets from protected methionine source such as HMB ***(Guinard and Rulquin, 1995)*** and ***(Pisulewski et al., 1996).*** While ***Blum et al., (1999)*** reported that the non-significant effect of RPMet on protein percentage in milk may be due to low bioavailability of methionine from (Mepron M85) for protein synthesis.

Milk fat percentage increased (P< 0.05) by 5% and 3% for cows received 25g/ day HMB compared with control group and those supplemented with 25g/ day DL- Met. These results were in-agreement with those of previous studies they documented an increase of milk fat synthesis following feeding supplementation with RPMet. ***Overton et al. (1996), Schwab (1995)*** and ***(Robinson et al., 1998)*** and ***(Clark and Davis 1983).*** In this respect ***Oldham, (1984)*** suggested that supplementary dietary HMB was associated with an increase in milk fat production. The specific reason for the increased percentage of milk fat was unknown; however, several possibilities had been suggested in the literature. ***(McCarthy et al., 1968)*** reported that Met might be important for synthesis of phospholipids, suggesting that a post–absorptive effect of Met on lipid metabolism is possible. ***Sharma and Erdman (1988)*** speculated that choline synthesized from Met was likely to have been at least partially responsible. In this study, percentage of lactose and ash in milk were not significantly affected when HMB was fed, which was inagreament with ***Overton et al. (1996).*** In general previous research reported that protected Met products fed to lactating cows had no positive effect on milk yield and milk protein content ***(Schingoelhe et al., 1988, Casper et al., 1987, Chalupa, 1975*** and ***Schwab et al., 2003)*** increased both milk yield and milk protein content from these experiments might be caused by differences in status of methionine

or other amino acid of cows the and amount of methionine supplied in the protected form and the efficacy of the protection scheme in delivering Met to the small intestine.

The effect of supplemented DL-Met or HMB in lactating cows ration on blood plasma essential AA (umol/dl) and methionine concentration showed in table (4). The results indicated that methionine concentrations in blood plasma increased significantly (P< 0.05) with cows fed ration supplemented with HMB compared with those fed control ration or fed ration supplemented with DL-Met. These results are in-agreement with ***Abdi-Benemar et al., (2016)*** who reported that concentrations of Met in plasma were higher (P < 0.05) for cows fed rations supplemented with RP Met. ***(Alex Bach et al., 2000)*** who compared the results obtained from the in situ methods and those derived from plasma concentrations, estimated that the greatest (P < 0.05) measured plasma methionine concentration occurred with cows fed diets supplemented with RPMet also, they found that based on *in situ* and *in vitro* intestinal digestion the amount of methionine available for absorption increased with the same group of cows which fed diets supplemented with RPMet. On the other hand, ***(Sylvester et al., 2003)*** reported increased plasma Met concentration on diets supplemented with HMB and had no effect on free plasma amino acids. However, ***(Overton et al., 1996)*** and ***(Blum et al., 1999)*** illustrated that feeding Ruminally Protected Met (RPM) to dairy cows has resulted in increased passage of Met to the small intestine and increased the amount of Met in the plasma. On the other hand there were no significant (P < 0.05) differences in concentrations of other EAA between supplemented with DL-Met or HMB compared to control theresult may be related to the three groups of animals feed the same ration which contained the same

ratio of EAA. This is agreement with ***Noftsger et al., (2005)*** who found that dietary treatments with different three sources of Met had no effect on free plasma amino acids. Nevertheless**, *(Yang et al., 2010)*** indicated that addition of RPMet improved the balance of plasma amino acids and increased the utilization of all amino acids. In theory, when the supply of the most limiting amino acids is increased postruminally, other essential amino acids in plasma while, ***(Overton et al., 1996)*** reported concentration of Met in plasma increased, in contrast, concentrations of Gly, Ile, Leu, Thr, Tyr and Val in plasma tended to decrease.

Conclusion

In conclusion, milk yield had significantly (P< 0.05) increased and had tended to promote the synthesis of milk protein and milk fat. The ultimate goal of supplying protected methionine, or other AA, is to improve performance of dairy cattle. Selection of the ruminally protected AA products by dairy producers should be based on the cabability of the product at escaping the rumen intact and releasing absorbable AA to the small intestine. This study demonstrated that addition of 25g methionine hydroxy analog in the diet could improve dairy performance and utilization of amino acids. Further work is needed to determine the suitable level to be used with dairy cows during the different stages of lactation.

Table (1): Ingredients and chemical composition of basal ration (DM basis).

Ingredients	Basal diet (% of DM)
Corn silage	30
Rice straw	10
Yellow corn grain	24.3
Soybean meal, solvent, 48% CP	12
Wheat bran	12
Distiller Dried Grain with solubls (DDGS)	5
Undecorticated cotton seed meal	5
Calcium carbonate	0.4
Salt	0.5
Dicalcium phosphate	0.3
Min. and vit. mix[1]	0.5
Items	Chemical composition%
Organic matter	91.3
Crude protein	16.4
Crude fiber	18.1
Fat	3.1
NFE	53.7
Ash	8.7

[1]Contains: 5.0% Mg, 7.5% K, 10.0% S, 3.0% Zn, 3.0% Mn, 2.0% Fe, .5% Cu, 0.025% I, 0.015% Se, 0.004% Co, 2200 IU of vitamin A/g, 660 IU of vitamin D_3/g, and 8 IU of vitamin E/g.

Table (2): Degradation of soluble, degradablefractions for sheep supplemented HMB or DL-Met (mean ± SE).

Item	HMB	DL- Met
Soluble fraction	34.05 ± 0.22[a]	38.19 ± 0.54[b]
Degradable fraction	53.31 ± 0.23[a]	91.41 ± 0.12[b]
Kd	0.031 ± 0.003[a]	0.068 ± 0.001 [b]
Ruminal degradation	52.17± 0.85[a]	86.73 ± 0.79[b]

[a&b] Means within column with different superscript are significantly differ (P<0.05).

Table (3): Milk yield and milk composition of lactating cows fed ration supplemented with different sources of methionine (mean ± SE).

Item	Control	DL- Met	HMB
Milk yield kg/d	15.78 ± 0.87[b]	16.01±0.95[b]	16.94±0.85[a]
4 % FCM	14.88 ± 0.82[c]	15.24±0.74[b]	16.41±0.71[a]
Fat, kg/d	0.57 ± 0.03[b]	0.59± 0.02[b]	0.64±0.03[a]
Milk composition (%):			
Total solids	12.37± 0.32[c]	12.75±0.33[b]	13.16±0.58[a]
Fat	3.62 ± 0.18[b]	3.68± 0.16[b]	3.79 ± 0.13[a]
Protein	3.29 ± 0.29[c]	3.35 ± 0.44[b]	3.47 ± 0.38[a]
Lactose	4.69 ± 0.22	4.73 ± 0.19	4.72± 0.17
Ash	0.92 ± 0.01	0.91 ± 0.02	0.93± 0.01

A&bc Means within rows with different superscripts are significantly differ (P<0.05).

Table (4): Blood plasma essential amino acids concentrations in lactating cows fed ration supplemented with different sources of methionine.

Item / Essential AA (umol/dl)	Control	DL- Met	HMB
Histidine	5.10±0.42	4.96±0.53	4.92±0.49
Isoleucine	14.58±1.22	14.70±1.14	14.61±1.18
Leucine	12.22±085	11.95±0.83	12.01±0.91
Lysine	9.74±0.66	9.81±0.69	9.79±0.62
Methionine	1.91± 0.37[b]	1.98± 0.41[b]	2.24± 0.33[a]
Phenylalanine	4.93±0.43	4.96±0.41	5.10±0.47
Threonine	10.51±1.01	10.55±0.83	10.58±0.89
Tryptophan	3.80±0.34	3.78±0.38	3.81±0.41
Valine	19.95±1.52	20.10±1.47	19.99±1.36

A&b Means within rows with different superscript are significantly differ (P<0.05).

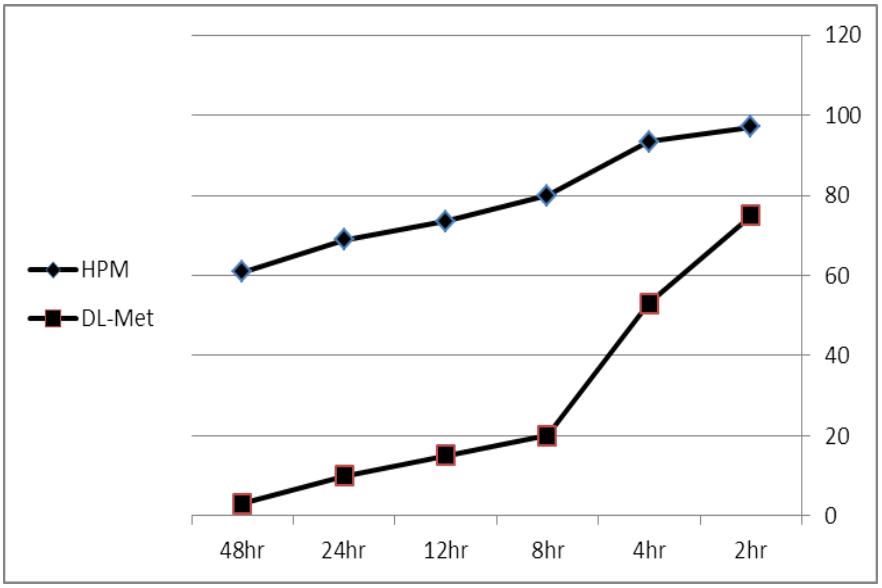

Figure (1): Rumen escapes of methionine for sheep supplemented with DL-Met or HMB.

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الإستفادة الحيوية للمثيونين المحمي في الكرش ومقارنتة بـ د.ل- مثيونين المضاف إلي علائق الأبقار الحلابه وتأثير ذلك على إنتاج اللبن وتمثيل الأحماض الأمينيه

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***المركز الأقليمي للأغذية والأعلاف – مركز البحوث الزراعية.**
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الملخص العربى

أجريت هذه الدراسة لتقييم التحلل في الكرش والمظاهر الانتاجية للابقار الحلابة المغذاة علي علائق مدعمه وتقييم تأثير مصدرين مختلفين من منتجات المثيونين (مثيونين هيدروكسي أنالوج او د-ل مثيونين) المصدرين علي التحلل في الكرش و إنتاج اللبن و تركيز الأحماض الأمينية الضرورية في بلازما الدم من خلال تجربتين.

التجربة الأولي تم تقدير تحلل المثيونين داخل الكرش (*in-situ*) بتقنية إستخدام أكياس البولي أستر بواسطة ثلاث من النعاج المزودة بفستيولات مستديمة في الكرش.
التجربة الثانية تمت علي تسع أبقار خليط فرزيان حلابة متماثلة تم تقسيمها عشوائياً إلي ثلاث مجاميع لتقدير إنتاج اللبن ومكوناته – كما تم تقدير المثيونين في الدم.

وقد أظهرت النتائج المتحصل عليها أن سرعة إختفاء المثيونين هيدروكسي أنالوج كان منخفض معنوياً عن ال- د-ل مثيونين بينما كان معدل هروب مثيونين هيدروكسي أنالوج مرتفعا معنويا مما يدل علي انة أكثر مقاومة للتحلل في الكرش عن ال- د-ل مثيونين. واوضحت النتائج أن إنتاج اللبن أو اللبن المعدل الدهن للأبقار المغذاة علي عليقة مضاف اليها 25جم/يوم من المثيونين هيدروكسي أنالوج كان مرتفعاً معنوياً عن تلك المغذاة علي عليقة المقارنة أو التي غذيت علي عليقة مضاف اليها 25جم/يوم من د-ل مثيونين. لم يكن للمعاملة تأثير علي النسبة المئوية للاكتوز بينما

أرتفعت نسب الجوامد الكلية و الدهن والبروتين للمجاميع التي غذيت علي 25جم/يوم من المثيونين هيدروكسي أنالوج مقارنة بالمجاميع الأخري.

كما اظهرت نتائج تركيز المثيونين في بلازما الدم أن تركيز المثيونين كان مرتفعاً معنوياً مع الأبقار المدعمة بالمثيونين هيدروكسي أنالوج مقارنة بمجوعة المقارنة أو المدعمة ب د-ل مثيونين.

وتظهر الدراسة أن التدعيم بالمثيونين هيدروكسي أنالوج بمعدل 25جم /يوم قد ادي إلي تحسين الأستفادة من المثيونين وعززت من تمثيل الاحماض الامينية للأبقار الحلابة مما ادي إلي زيادة إنتاج اللبن.