



## Effect of supplementation of rumen protected methionine and lysine on some physiological aspects of fattening calves

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### Abstract

This study was carried out during the period from February-April 2019 at a private breeding field. A total of 82 imported calves for fattening 9-12 month of age. These calves were divided randomly into 3 groups and treated for 90 days as follows: G1 (27 calves) supplied with basal diet only and considered as control group, Calves of G2 (27 calves) of were supplied with basal diet complemented with RPM in complete feed using 15 g/animal/day, while G3 (28 calves) was supplied with basal diet complemented with RPL with 10g/animal/day. The results showed a significant increase in growth hormone value in the G2 in comparison with G1 and G3 at 90 day post treatment. While the mean values of body weight were (310.8±12.97) and (334.3±15.41) in G2 at 60 and 90 days respectively with significance deference in compare with G1and G3 ( $p \leq 0.05$ ). The significant increase in red blood cells count (RBCs) showed in G2, while the White Blood Cells (WBCs) increased in G1 when compare between groups. In addition, the results showed a significant increase ( $p < 0.05$ ) in cholesterol and triglycerides values in G2 in compare with G1 and G3. While no significant changes in total protein values revealed between groups. The results of this study confirmed that supplementation of RPM has the potential to improve body weight in fattening calves with enhancement of immune status of animals and an enhancement of oxygen flow to the tissues by increasing of RBCs counts especially with RPM, but lasser effects with RPL.

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### Introduction

Fattening calves plays a crucial role in Iraqi market and most of these calves are kept by smallholder farmers, in latest years, demand for beef is growing. Livestock and poultry products are presently of two main kinds of meat resources and the major kinds of livestock in world are beef cattle, chicken, swine, dairy cows, and buffalo (1). Amino acids are the building parts of polypeptides and proteins that are the main elements of animals' muscles and tissues, these amino acids also form an essential unit in some fluids such as milk and play a significant role in many principle biochemical and metabolic events within the animal cells (2). The protein-producing ability of an animal is restricted by the quantities of amino acids within its body. Later some proteins require specific amino acids, if

the body cannot create sufficient amount of a single amino acid or it is not supplemented in sufficient amount in the food (3). One vital Amino acid that could possibly be restraining for reproduction in lactating dairy cows is methionine (Met) (4). Preceding studies that assessed the effects of consuming rumen-protected methionine (RPM) on milk production recognized a reliable improve in milk protein profit and in general milk protein yield in high producing dairy cows (5,6) as well as improving the reproductive performance in animals (7) and has a similar effect to other many essential amino acids like arginine supplementation for ruminants (8). Supplementation of rumen protected methionine is helpful for developing of the Performance of calves weight gain and efficiency of feed utilization (9).

Lysine (Lys) is one of fundamental amino acid required for the synthesis of protein in growth, milk production, tissue healing and gestation (10). Lys is cited as the most-limiting amino acid in feed thus Supplementation of rumen-protected Lys (RPL) could be helpful if Lys is limiting in diets (11). Met and Lys Are the 1<sup>st</sup> limiting amino acids in microbial protein. If specific amino acids are limiting, complementing cattle with bypass protein has been shown to increase gains and feed efficacy especially for young and fast growing calves (12). Met/lys supplementation lead to elevate in protein scores, water keeping capacity and lesser shear force marks (13) Thus, protecting the amino acids from ruminal degradation has made it achievable to supplement diets with particular amino acids that will become obtainable directly for absorption in intestine (14). Therefore, due to the few studies that evaluate of Met and Lys supplementation as a ruminal protected amino acid in fattening animals, the present study aimed to evaluate the influence of daily providing coated Met and Lys in mixture protein sources based diets on growth performance, blood parameters, serum biochemical indices of fattening calves.

**Material and methods**

**Animals and experimental design**

This study was carried out during the period from February-April 2019 at private breeding field located at Bartella district, Mosul, Iraq. A total of 82 imported calves for fattening 9-12 month of age. Calves were housed in separated pens (18x20 meter). Clean drinking water *ad libitum* throughout the fattening period (3 month). The constituent and nutrient levels of the basal diet recommendation National Research Council (14). All calves were kept under standard hygienic circumstances. Both (MetiPEARL)<sup>®</sup>: Rumen Protected Methionine Consist of protected or encapsulated D-L methionine (3c301) 550 g/kg and LysiGEM)<sup>®</sup>: Rumen Protected Lysine Consist of protected or encapsulated L-lysine monohydrochloride (3.2.3.) 680 g/kg (Packing 25 kg bags separately) were homogenates with the feed prior to its providing for each group. Calves were divided randomly into 3 groups and treated for 90 days as follows: G1 (27 calves) supplied with basal diet only and considered as control group. Calves of G2 (27 calves) of were supplied with basal diet complemented with (MetiPEARL)<sup>®</sup> Mix carefully in complete feed using 15 g/animal/day, while G3 (28 calves) was supplied with basal diet complemented with (LysiGEM)<sup>®</sup> with 10 g/animal/day (Table 1).

**Data collection**

The primary and final body weight (kg) was recorded monthly. Blood samples were collected from jugular vein at 90 days post supplementation and each samples were divided into two parts one for measuring the complete blood picture and another for serum collection. The sera were separated at 3000 rpm by centrifugation for 15

minutes. Sera were stored at -20 °C until further processing. The parameters analyzed in sera include: total protein, cholesterol and triglyceride using commercial kits (Biolabo, France) and growth hormone measured using bovine GH ELISA kit. Data were expressed as mea ±SE and analyzed by using of one - way analysis of variance (ANOVA). The significance of differences between means was tested by Duncan multiple range test at P<0.05 (15).

Table 1: Ingredient and chemical composition of basal diets

Item	Groups (DM%)		
	First	Second	Third
Corn 7.5%	10	10	10
Barley 10% CP	20	20	20
Wheat bran 13.5%	17.3	17.3	17.3
Wheat hard 12%	23.0	23.0	23.0
Soya bean meal 47%	13.8	13.8	13.8
Wheat flour 12%	10	10	10
Soya oil	1.0	1.0	1.0
Premix	2.5	2.5	2.5
Lime stone	0.25	0.25	0.25
Salt	0.65	0.65	0.65
dicalcium Phosphate	1.25	1.25	1.25
Sodium bicarbonate	0.25	0.25	0.25
<b>Nutrient composition</b>			
Dry matter DM	86.92	86.92	86.92
Crude protein CP	15.5	15.5	15.5
Metabolic energy	2726	2726	2726
Crude fat CF	3.06	3.06	3.06
Fiber	4.9	4.9	4.9
Ash	5.2	5.2	5.2
Acid detergent fiber	6.47	6.47	6.47
Neutral detergent fiber	13.83	13.83	13.83
Starch	39.6	39.6	39.6
Hemicellulose	2.49	2.49	2.49
Total digestible nutrient	71.8	71.8	71.8
RPM (g/animal)	-	15	-
RPL (g/ animal)	-	-	10

**Results**

The results in table 2 revealed a significant increase of growth hormone value in the G2 in comparison with G1 at 90 day post treatment. The mean values of body weight showed no significant differences between groups at 30 day post treatment, While, a significant increase in the mean values of body weight were reported in G2 received RPM at 60 and 90 days of the treatment as compared to the control group. The mean values were 310.8±12.97 and 334.3±15.41 in G2 at 60 and 90 days respectively (Table 2).

The significant increase in red blood cells count (RBCs) showed in G2, while the White Blood Cells (WBCs) reveled significant decrease in G2 when we compare with G1. There were no significant differences detected in lymphocytes, Hemoglobin (HGB), Hematocrit (HCT),

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), Platelets (PLT) and Red cell distribution width RDW between the groups (Table 3).

The results of biochemical parameters showed significant increase in cholesterol and triglycerides values in G2 in compare with G1. While no significant changes in total protein values revealed between groups (Table 4).

Table 2: Effect of RPM and RPL on growth hormone and body weight

Groups	GH ng/ml after 90 days post treatment	Mean of body weight (kg) ± St. error			
		0 day	30 day	60 day	90 day
G1 (control)	7.8±0.33 b	247.2±18.13a	260.4±8.7a	279.9±8.2 b	301±10.8 b
G2 (MetiPEARL)	19.3±0.46 a	259±20.27a	279.8±10.6a	310.8±12.97 a	334.3±15.41 a
G3 (LysiGEM)	7.4±1.06 b	246.8±7.7a	262.2±8.7a	284±7.2 ab	312.4±12.03 ab

The different superscript in each column means statistically different significantly at P< 0.05.

Table 3: Effect of RPM and RPL on some blood parameters

Parameters	Groups		
	G1 (control)	G2 (MetiPEARL®)	G3 (LysiGEM)
WBCs	15.06± 0.7 a	11±1.2 b	13.06±1.24 ab
Lymphocytes	8.12±0.4 a	7.6±0.8 a	8.6±1.19 a
RBCs	6.09±0.39 c	8.61±0.45 a	7.48±0.17 b
HGB	90.4±8.51 a	107.6±3.6 a	94.8±2.39 a
HCT	40.9±3.99 a	44.7±1.2 a	40.1±1.34 a
MCV	52.8±1.6 a	52.5±2.65 a	53.6±1.21 a
MCH	12.8±0.25 a	12.2±0.67 a	12.6±0.12 a
MCHC	235.4±2.1 a	234±2.25 a	236.4±3.2 a
PLT	196.6±35 a	296.8±94 a	422±880.9 a
RDW	17.4±0.6 a	19.4±0.21 a	19.1±1.1 a

The different superscript in each column means statistically different significantly at P< 0.05.

Table 4: Effect of RPM and RPL on some biochemical parameters

Groups	Biochemical values after 90 day post treatment		
	total protein	cholesterol	triglycerides
G1 (control)	7.08±0.1 a	66±3.14 b	20.8±1.01 b
G2 (MetiPEARL)®	7.16±0.2 a	94.2±5.8 a	36.8±2.76 a
G3 (LysiGEM)	7.14±0.13 a	66.4±3.07 b	21.6±0.74 b

The different superscript in each column means statistically different significantly at P< 0.05.

## Discussion

Free methionine and lysine undergo degradation in the rumen; coating of these amino acids is one way to protect it from this degradation in rumen and comes obtainable in the small intestine for absorption. Innovate unique coating procedure is based on advanced technology. This technique ensures that the maximum amount of covered nutrients is able to bypass rumen fermentation and stay available at the small intestine (16).

The results of significant increasing of body weight of G2 in compare with G1 and G3 agreed with Singh *et al.* (17) when they studied the effect the same products in heifers, but not agreed with Lopes *et al.* (18) suggesting that the response of increasing of body weight was due to lysine, not methionine (treated group with a mixture of Met+Lys), although non significance increasing of body weight in G1 to G2 occur as a result of dietary protein supplementation that are respected to be beneficial resources of "bypass" or rumen non degradable protein have been used in expansion of animal performance (19), and Dietary supplementation of rumen protected protein and amino acids are suggested to keep the physiological and productive requirements of livestock for amino acids (11), while this results agreed with one study that evaluated the effect of supplementing crossbred steers with rumen protected methionine, rumen protected lysine or a mixture of both rumen protected amino acid through the entire feeding interval 134day (20).

The varied results of growth hormone (GH) is due to the restrictions in Methionine reduce the capability of GH to promote insulin like growth factor one (IGF1) production (21). The decrease in receptor expression of GH in the liver of peri-partal cows is a single cause that accounts for decrease of messenger RNA expression and blood quantity of IGF1, mainly after calving (22), or the Diverse in results of growth hormone because the various in dry matter intake and this remarks is matched with Trinacty *et al.* (23) when they declared that entire dry matter intake (DMI) was lesser in control than Rumen protected lysine group and Rumen protected methionine group. Changes in DMI resulted in notably superior intake of other nutrients (crud protein, fat) in which may led to significant increase of body weight with (MetiPEARL)®, or there is established an elevation the level of plasma GH in early lactating ruminants triggers metabolic alterations in liver, skeletal muscle and adipose tissue (24) thus this metabolic alteration may perform a role in the increasing the level of this hormone in G2.

Normal physiology and pathology of the bovine blood picture were examined. The results of a complete blood count (CBC) calculation are usually beneficial in the monitoring, diagnosis and prediction of an illness. If computerized cell counts are utilized to achieve a CBC,

results should be understood with carefully to avert false +ve or false -ve results (25). An increasing of WBCs counts in G1 in comparison with G2 and G3 agreed with study that well proven that metabolic outcomes of Met metabolism perform a crucial function as a preservative and supportive ingredients for immunity, and this effect of RPM may lead to decrease the infections with different microbes (26,6). The immune-modulator effects of these metabolites are featured by the decline in lymphocyte count and phagocytic process during taurine metabolite deficiency as well as an increase in Polymorph nuclear cell attachment when homocysteine metabolite concentration mounted (27).

The counting of RBCs in this study disagreed with Blum *et al.* (28) that mentioned no significant change in RBCs counts when it measured at 68 day post treatment with protected methionine, while other the values of other parameters including MCV, RDW, hemoglobin, platelets and hematocrit agreed with Roland *et al.* (25).

The total protein values in this study was not significantly affected (28,29). While increased total cholesterol in G2 is due to glucagon increase and this will lead glucose yield from the liver and drops insulin-glucagon fraction, increase plasma glucose (30,28) and ultimately to rise the concentration of HDL2-cholesteryl ester and LDL-free cholesterol in plasma (31). The rumen protected methionine might have an effect on the endocrine system, leading to possible expand in growth hormone and glucagon, thus the increase in entire cholesterol in our experts might have been produced by GH (28).

Triglycerides value was elevated significantly in G2 and this results agreed with (30) that mentioned to increasing the levels of plasma triglycerides and very low density lipoproteins were higher in treated group with methionine and lysine at ( $P < 0.01$ ) in compare with control group, this results is due to decrease in plasma NEFA (non-esterified fatty acid) levels suggested that there was esterification of NEFA into triglycerides and VLDL which might have been used as energy sources in the body (32).

## Conclusion

It can have concluded that the dietary protected methionine improved the body weight and RBCs count, furthermore fattening calves fed on RPM had higher cholesterol and triglycerides. Methionine is required in the diet in the rumen protected form to enhance the immune status in fattening calves.

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## Conflict of Interest

The author declares that there is no conflict of interest.

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## تأثير إضافة الميثيونين واللايسين المحمي على بعض المعايير الفسلجية في عجول التسمين

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### الخلاصة

أجريت هذه الدراسة خلال الفترة من شباط إلى نيسان ٢٠١٩ في مزرعة خاصة لعجول التسمين. تضمنت الدراسة استخدام ٨٢ من العجول المستوردة للتسمين بعمر ٩-١٢ شهر. تم تقسيم هذه العجول عشوائياً إلى ٣ مجاميع: المجموعة الأولى (٢٧ عجل) تم تغذيتها على علف قياسي فقط (مجموعة ضابطة)، غذيت عجول المجموعة الثانية (٢٧ عجل) على علف قياسي معاملة بـRPM بعد مزجه مع العلف بجرعة ١٥ جم / حيوان / يوم، بينما تمت تغذية عجول المجموعة الثالثة (٢٨ عجل) على علف قياسي معاملة بـRPL وجرعة ١٠ غم / حيوان / يوم. أظهرت النتائج زيادة معنوية في مستوى هرمون النمو في المجموعة الثانية G2 مقارنة مع المجموعتين الأولى G1 والثالثة G3 بعد مرور ٩٠ يوماً من المعاملة، بينما كان متوسط وزن الجسم ٣١٠,٨±٩٧,١٢ و ٣٣٤,٣±٤١,١٥ في المجموعة الثانية G2 عند ٦٠ و ٩٠ يوم بعد المعاملة على التوالي مقارنة مع المجموعة الأولى G1 والثالثة G3 حدوث زيادة معنوية في عدد خلايا الدم الحمراء (RBCs) في المجموعة الثانية G2، في حين زادت خلايا الدم البيضاء (WBCs) في المجموعة الأولى G1 عند المقارنة بين المجاميع، وفي الاختبارات الكيموحيوية كانت هناك زيادة معنوية (P<0.05) في قيم الكوليسترول والدهون الثلاثية في المجموعة الثانية G2 مقارنة مع المجموعتين الأولى والثالثة G1 و G3، بينما لم تظهر هناك تغييرات معنوية في قيم البروتين الكلي بين المجاميع الثلاثة، وبذلك تؤكد نتائج هذه الدراسة أن RPM كان لديه القدرة على تحسين وزن الجسم وتعزيز الحالة المناعية للحيوانات، بينما كان RPL أقل تأثيراً في بعض معايير هذه الدراسة.