

INFLUENCE OF SUPPLEMENTING FREE METHIONINE AND LYSINE AT POST-WEANING ON PERFORMANCE, BLOOD PLASMA AMINO ACID PROFILE AND IMMUNITY IN BUFFALO CALVES

Allam, A.M.; A. M. Nour; M. E. A. Nasser; S. M. A. Sallam and Yosra A. Sultan

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ABSTRACT

Post-weaned buffalo calves ($n = 12$) were used in four treatments to examine the effect of different levels of DL-methionine and L-lysine HCl on the performance of calves receiving diets based on corn-soybean meal. Three different levels of methionine and lysine were used as 100%, 105% and 110% from the recommended requirements (11.5g lysine + 3.5g methionine). Administration of lysine and methionine increased ($P < 0.01$) average daily gain, feed intake and gain/feed ratio. Plasma concentration of methionine and lysine were increased; calves in treatment 4 (110%) had the greatest values.

No differences were detected in plasma concentrations of both branched chain amino acids and amino acids associated with methionine metabolism except serine which was decreased ($P < 0.05$) in all treated groups. Plasma concentrations of total amino acids associated with urea cycle and arginine were significantly ($P < 0.01$) increased. Calves in treatments 3 and 4 had the greatest ($P < 0.05$) values as compared to other treatments. No significance differences were detected for phenylalanine, histidine, aspartic, glutamine, proline and tyrosine due to supplementation.

Free methionine and lysine supplementation increased the lymphocytes proliferation (%); the fourth treatment had the highest values compared with other treatments ($P < 0.01$).

Key words: dietary lysine and methionine; buffalo calve performances.

INTRODUCTION

Post weaning period is the most stressful time in the life of the buffalo calf due to the weaning shock, resulting in depressing growth rate and calf performance (Soliman, 2000 and Nasser *et al.*, 2004), and increasing possibility of diseases and mortality (Sun *et al.*, 2006). Mortality rate of Egyptian buffalo calves range from 16.7 to 23% in post weaning period (Allam 1979). The most important causes of calves mortality are pneumonia, digestive trouble and naval infections (Mohamed *et al.*, 1994),

Several suggestions have been recommended to overcome these problems. Supplementing the most limiting amino acids to the calf starter in protected or encapsulated forms is one of these suggestions. Methionine and lysine were found to be the first and second limiting amino acids in starters based on corn-soybean meal proteins (Robinson *et al* 2005b and Socha *et al.*, 2005). Supplementing diets of weaned buffalo calves with protected methionine and lysine have resulted in an improvement in growth performance (Abd-El All, 1997 and Nasser *et al.*, 2004). In addition to production responses, protected methionine and lysine were important in thymus-derived T helper lymphocytes function in dairy cattle (Anabael *et al.*, 2001 and Kurz, 2004). However, little information is available about the effect of dietary limiting amino acids on immune function for buffalo calves. It is important to maintain herd health through proper nutrition and handling in order to achieve efficient growth and production of beef calves (Tayade *et al.*, 2006).

The lack of commercial availability of ruminal protected forms of methionine and lysine, in addition to the increase in costs of protection formulation of these protected amino acids (Noftsker *et al.*, 2005), also the difficulty of having these amino

acids especially in capsulated forms by large numbers of buffalo calves in big farms, or calves towards the small farmers prevent the use of protected amino acids in practical ways. If calf performance can be improved by supplementing unprotected methionine and lysine to diets, taking into account the greater quantities that would have to be supplemented to accommodate ruminal degradation, then these amino acids supplementation may be economically feasible, and using availability of these amino acids in easier way.

The aim of the present study is to test the effects of different levels of dietary free amino acids (L-lysine HCl and DL-methionine) supplementation on the performance of weaned buffalo calves.

MATERIAL AND METHODS

1. Animals and general management:

Twelve male nursing buffalo calves were purchased from neighboring farms around the Alexandria Governorate at average body weight of 53.5 kg during the period from February to March 2004. The calves were kept in individual stalls and bedded on wooden beds covered with rice straw that was changed daily.

Calves were weaned at an average body weight of 66 kg on buffalo milk replacer (1 kg of milk replacer powder resuspended in 8 liter of warm water). Calves were suckled twice daily at 8.30 a.m. and 4.30 p.m. Chemical composition of milk replacer is presented in Table (1). Calves were fed starter concentrate mixture based on corn-soybean (composition and the nutritive values are presented in Table 2). Berseem hay was introduced to calves as *ad lib* feeding. The chemical composition of the starter concentrate mixture and berseem hay are presented in

Table (3). Feed residues were collected, weighed and recorded daily. Fresh water was offered four times a day. The animal weights were recorded biweekly during the experimental period (85 days).

2. Treatments:

After weaning the calves were divided into four equal groups (3 calves each) according to their body weights. Group 1 (G1) was fed on the control diet containing starter concentrate mixture (SCM) and berseem hay. Group 2 (G2), 3 (G3) and 4 (G4) were fed on the same control diet as in G1, however the SCM was supplemented with 100% (11.5g + 3.5 g), 105% (12.08g + 3.6g) and 110% (12.65g + 3.85g) free amino acids* (lysine + methionine), respectively.

3. Sampling of blood:

Blood samples were taken from all animals (15 ml) among the different treatments every 3 weeks throughout the experimental period. Plasma samples were obtained by centrifugation of the heparinized blood at 4000 r.p.m for 20 minutes. The separated plasma were stored at -20°C till the determinations of free amino acids

4. Analytical methods:

Chemical composition (%) of SCM and berseem hay were determined according to the official

method of the A.O.A.C. (1990). Plasma free amino acid concentrations were determined according to the method described by Hamilton (1962) and Spackman *et al.* (1958), respectively. Lymphocyte proliferation assay was carried out at Faculty of Veterinary Medicine (Edfena), Alexandria University as indicator for the animals immunity according to El-Balhaa *et al.* (1985).

5. Statistical analyses:

The experimental results were analyzed using GLM procedure according to Statistical Analysis System, (SAS) User's Guide (2000). Using Duncan multiple tests, the statistical model describing each trait as follows, carried out separation among means

$$Y_{ijk} = \mu + T_i + G_j + TG_{ij} + e_{ijk}$$

Where:

- Y_{ijk} = an observation of trait on individual k,
- μ = Over all mean,
- T_i = Fixed effect of ith treatment,
- G_j = Fixed effect of jth period,
- TG_{ij} = Fixed effect of jth interaction,
- e_{ijk} = Random error assumed to be normally distributed with mean = 0 and Variance = σ^2 .

Table 1: Chemical composition (%on DM basis) of buffalo milk replacer*.

Item	% (on DM basis)
Crude protein (CP)	22.5
Ether extract (EE)	23.5
Crude fiber (CF)	0.5
Nitrogen free extract (NFE)	44
Ash	9.5

*The buffalo milk replacer was obtained from Egavet company-Cairo.

Table 2: Composition and the nutritive value of the experimental starter concentrate mixture (SCM) fed to the buffalo calves.

Ingredients	%
Yellow corn	60.0
Barley grains	13.0
Soybean meal	24.5
Calcium carbonate	1.5
Common salt	0.7
Mineral. mix*	0.2
Vit. AD ₃	0.1
<i>Nutritive value (calculated)% **</i>	
Total digestible nutrients (TDN%)	76.13
Digestible crude protein (DCP%)	17.74

*Each 1kg contains 15g magnesium sulphate, 1.380g ferrous sulphate, 0.0641g zinc oxide, 0.150g manganese sulphate, 0.310 copper sulphate, 0.12 sodium borate, 0.005g cobalt chloride, 0.0040g sodium fluoride, 0.0018g sodium selenate, 0.004g molybdenum oxide, 0.0831g calcium iodine 483.40g dicalcium phosphate, 479.47g sodium chloride, 20.008g magnesium sulphate.

**Calculated from feeding tables (NRC,1988)

* Amino acids = (L-lysine HCl 98% & DL-methionine 99%)

Table 3: Chemical composition (%on DM basis) and amino acids content (g AA/100 g sample) of the experimental starter concentrate mixture (SCM) and berseem hay.

Item	Starter conc. mix.	Berseem hay
<i>Chemical composition (% on DM basis)</i>		
Crude protein (CP)	18.84	16.05
Ether extract (EE)	3.42	2.83
Crude fiber (CF)	2.11	29.38
Nitrogen free extract (NFE)	69.53	38.84
Ash	6.10	12.90
<i>Amino acid (g AA/100 g sample)</i>		
Methionine	0.20	0.17
Lysine	0.77	0.50
Theronine	0.65	0.66
Glycine	0.75	0.63
Serine	0.80	0.67
Arginine	0.73	0.56
Proline	1.22	0.99
Isoleucine	0.69	0.64
Leucine	1.30	1.01
Valine	0.94	0.74
Phenylalanine	0.82	0.67
Histidine	0.41	0.26
Aspartic	2.06	1.87
Glutamic	4.04	2.03
Alanine	0.86	0.61
Tyrosine	0.53	0.36

RESULT AND DISCUSSION

1. Buffalo Calves Performance:

Table 4 shows the response of ADG, DMI and feed/gain to the graded amounts of supplemental lysine and methionine.

Values of ADG are within the range obtained by (Yossef, 1992; El-Basiony, 1994; Soliman, 2000 and Nasser *et al.*, 2004). The ADG tended to increase ($P < 0.01$) with increasing amounts of lysine and methionine and values were greatest with group 4. These results are in agreement with those reported by (Han *et al.*, 1995; Abe *et al.*, 2000; Nasser *et al.*, 2004; Chang and Weiwei, 2005 and Cheng *et al.*, 2006). They stated that the dietary supplementation of free or protected DL-methionine and L-lysine to corn-soybean meal based diets fed to buffalo or Holstein calves improved the daily gain due to their limitation in the diet in post-weaning period.

Free DL-methionine and L-lysine supplementation did not significantly affect the DMI, which agreed with those reported by (Figueroa *et al.*, 2002; Bernard *et al.*, 2004; Noftzger *et al.*, 2005 and Sun *et al.*, 2006). The present values are lower than those obtained by Soliman, (2000) who found that normal mean value was 5.25 kg DMI/ day for buffalo calves in post- weaning period and fed on corn-soybean meal based diet. This discrepancy can be

attributed to the variation in the length of nursing period and weaning weight.

The feed/gain value tended to decrease ($P < 0.01$) with increasing amounts of supplemental DL-methionine and L-lysine. The present results clearly showed that the addition of free DL-methionine and L-lysine supplementation to SCM improved significantly ($P < 0.01$) the values of feed utilization as compared with the control group (Table 4). Similar trend of these results was observed by Chang and Weiwei (2005) and Cheng *et al.* (2006). The present results suggest that DL-methionine and L-lysine supplemented to corn-soybean meal diets improved the buffalo calves performance during the post-weaning period of its life due to its limitation overcoming of such nutrient of these diets, and it clearly indicated that buffalo calves can benefit from DL-Met and L-Lys as free supplemental amino acids to its diets.

2. Blood plasma amino acid profile:

Table 5 shows plasma concentration of free amino acids in response to graded amounts of DL-Met and L-Lys. Plasma methionine and lysine concentrations tended to increase ($P < 0.05$ and $P < 0.01$) with graded amounts of additional DL-Met and L-Lys.

The greatest increase occurred in the fourth group. The present results suggest that free amino acids in the fourth group used in this trial may satisfy the requirements, because calves of this group had the highest values of daily gain. These results agree with those reported by Allam *et al.* (1995) and Abe *et al.* (1998) that the plasma amino acid concentrations did not increase unless their requirements were first satisfied.

No differences were detected in plasma concentration of threonine and glycine, only plasma concentration of serine tended to decrease ($P < 0.05$) with the incremental amounts of free DL-methionine and L-lysine HCL supplementation. This is the cause methionine often causes serine deficiency because serine is needed for methionine degradation through the transsulfuration pathways (Harter and Baker, 1978). However, serine is synthesized from threonine via glycine. There are no significant decrease in plasma concentration of glycine and threonine, probably because the levels of free methionine used in the study were not in excess. The same results were found in Holstein bull calves in post-weaning period that were treated with 0.111g methionine and 0.333 g lysine/kg body weight (Abe *et al.*, 1998, 1999b and 2001).

Plasma arginine tended to increase ($P < 0.01$), whereas plasma proline was not significantly affected. Abe *et al.* (1998 and 1999a) observed a linear increase in plasma concentration of arginine with continuous infusion of increasing quantity of DL-methionine into the abomasum of Holstein bull calves, and attributed the increase to reduced urinary excretion and the associated reduction of arginine used for urea cycle activity.

Plasma concentrations of branched-chain amino acids (BCAA; leucine, iso-leucine and valine) showed a trend towards a constant value. Abe *et al.* (1998) and Varcikko *et al.* (1999) observed a linear decrease in plasma concentration of branched-chain amino acids with cow calves, this discrepancy may be due to differences between the two species. No significant differences were noted in plasma concentrations of supplemental phenylalanine, histidine, aspartic, glutamate, alanine and tyrosine among the experimental groups.

Table 6 presents the lymphocyte proliferation (%) for different experimental groups. Values were 37.4, 40.9, 38.5 and 54.4% for G1, G2, G3 and G4, respectively. The present results indicated that incremental amounts of DL-Met and L-Lys added to corn-soybean meal based diets led to increase ($P < 0.01$) the lymphocytes proliferation. Such increase reflects an improvement in buffalo calves immunity, because lymphocytes are responsible for recognizing foreign antigens and mounting immune responses (Tizard, 2000). The greatest values ($P < 0.01$) of lymphocyte proliferation occurred with group 4. This may be due to the higher plasma methionine and lysine concentration which increased the activity of peripheral T lymphocytes, increase splenocyte (Konashi *et al.*, 2000) and increase IgG and Ig M concentrations (Sun *et al.*, 2006). Soder and Holden (1999) and Anabael *et al.* (2001) found that methionine and lysine increased proliferate responses to mitogen concavalin A (Con A) and phytohemagglutinin (PHA) because both (Con A) and (PHA) stimulate T cells division (Kurz, 2004).

It is clear from the present results (Table 5) that methionine and lysine supplementation resulted an increase in arginine concentration ($P < 0.01$) which can affect the immune system by increasing growth hormone with consequent effects on thymus weight. Moreover, arginine enhance thymic lymphocyte blastogenic response and resuced posttraumatic thymic involution (Suncher *et al.*, 2002; Tsai *et al.*, 2002 and Tayade *et al.*, 2006).

The present results agree with those reported by Nauss *et al.* (1982) Hall *et al.* (1986) Anabael *et al.* (2001) and Sun *et al.* (2006) that dietary methionine and lysine not only play an important nutritional role, but also have essential function of regulating the immune response in growing animals.

CONCLUSIONS

Supplementation of free amino acids (DL-Met. and L-Lys) in post-weaned buffalo calves may require particular care, because even a modest excess could cause an imbalance. For buffalo calves raised on starters based on corn-soybean meal, DL-methionine and L-lysine could be used in the form of powder taking into account the amount degraded in the rumen due to the lack of commercial availability of ruminal protected forms.

Table 4: ADG, DMI and feed/gain in response to graded amounts of DL- methionine and L-lysine supplementation

Item	Treatment				Mean ± SE
	G1	G2	G3	G4	
Initial weight (kg)	64.30	69	65.33	64.67	-
Final weight (kg)	107.33	133.33	126.33	131	-
Total gain (kg)	43.03	64.33	61	66.33	-
Treatment period (days)	85	85	85	85	-
Daily gain (kg/head/day)	0.51 ^b	0.76 ^a	0.72 ^a	0.78 ^a	0.69±0.03
Total intakes (Kg/ DMI/head/day)	2.72	3.07	2.75	2.82	2.84±0.08
(Kg/ TDMI/head/day)	1.89	2.18	1.93	1.99	1.99±0.06
Feed efficiency (kg DMI/kg gain)	5.33 ^a	4.04 ^b	3.82 ^b	3.62 ^b	4.20±0.21
(kg TDNI/kg gain)	3.71 ^a	2.87 ^b	2.68 ^b	2.56 ^b	2.96±0.15

a, b Means within row with different superscripts differ significantly at (p<0.01).

G1= control group,

G2= 100% of daily met and Lys requirements,

G3=105% of daily met and lys requirements, G4=110% of daily met and lys requirements.

Table 5: Plasma concentrations (mg/100 ml) of free amino acids (AA) in response to graded amounts of DL- methionine and L-lysine

Amino Acid	Treatment				SEM	P <
	G1	G1	G1	G1		
<i>AA associated with supplementation</i>						
Methionine	0.48b	0.49b	0.59ab	0.66a	0.04	0.05
Lysine	1.23b	1.34b	1.61a	1.71a	0.09	0.01
<i>AA associated with Methionine metabolism</i>						
Threonine	2.55	2.65	2.50	2.58	0.25	NS
Glycine	3.17	2.66	2.84	3.32	0.23	NS
Serine	1.87	1.55ab	1.32b	1.50b	0.10	0.05
<i>AA associated with Urea Cycle</i>						
Arginine	2.53b	2.47b	3.05a	3.09a	0.08	0.01
Proline	1.67	1.33	1.74	1.63	0.11	NS
<i>Branched-chain AA</i>						
Iso-Leucine	1.38	1.31	1.35	1.33	0.08	NS
Leucine	2.02	2.07	2.13	2.04	0.18	NS
Valine	2.62	2.31	2.64	2.62	0.40	NS
<i>Remaining essential and Non-essential AA</i>						
Phenylalanine	1.02	0.84	0.95	0.96	0.06	NS
Histidine	2.47	1.86	2.35	2.15	0.16	NS
Aspartic	1.28	1.31	1.46	1.49	0.11	NS
Glumatic	3.38	3.31	3.69	3.65	0.19	NS
Alanine	2.59	2.71	2.66	2.98	0.20	NS
Tyrosine	0.95	0.89	0.85	0.93	0.06	NS

a, b means within column with different superscripts differ significantly at (P < 0.05), (P < 0.01)** , respectively*

Table 6. Effect of different dietary levels of (DL-methionine and L-lysine HCL) on lymphocyte proliferation (%) of buffalo calves in post weaning period

Treatment	Lymphocyte proliferation (%)±SE
G1	37.41±3.70b
G2	40.92±2.23b
G3	38.50±2.59b
G4	54.44±0.55a

a, b Means in the same column with different superscripts differ significantly at (p<0.01).

G1= control group, G2 100% of daily met and lys requirements, G3= 105% of daily met and lys requirements, G4= 110% of daily met and lys requirements.

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المخلص العربي

تأثير إضافة الأحماض الامينية الحرة (الميثا يونين واليسين) على الأداء وتركيز الأحماض الامينية في بلازما الدم والمناعة في عجول الجاموس بعد الفطام

على علام، عبد العزيز نور، محمد عماد، صبحي سلام، يسرا سلطان
كلية الزراعة - جامعة الإسكندرية - قسم الإنتاج الحيواني

تهدف هذه الدراسة للتعرف علي تأثير إضافة مستويات مختلفة من الأحماض الامينية الحرة (اليسين و ميثايونين) لبداي العلف المركز و المستخدم في تغذية العجول الجاموسي حديثة الفطام و المتكون أساسا من الذرة و فول الصويا، و تأثير هذه الإضافة علي أداء العجول الجاموسي بطريقة عمالية مشابهة للنطاق التجاري.

استخدم في هذه الدراسة ١٢ عجل ذكر جاموسي رضيع تم شرائها من المزارع المجاورة لمدينة الإسكندرية بمتوسط وزن يتدائي ٥٥,٣ كجم ، و عند الفطام (بمتوسط وزن ٦٦ كجم) تم تقسيم الحيوانات إلي أربعة مجاميع متماثلة و هي مجموعة الكنترول و التي تغذت علي بادئ العلف المركز و دريس البرسيم دون أي إضافات من الأحماض الامينية، المجموعة الثانية و التي تغذت يوميا علي نفس العليقة الكنترول بالإضافة إلي الأحماض الأمينية الحرة بنسبة ١٠٠ % من الاحتياجات اليومية الموصي بها (١١,٥ جم ليسين + ٣,٥ جم ميثايونين) و المضافة للبداي المركز، المجموعة الثالثة و التي تغذت يوميا علي نفس العليقة الكنترول بالإضافة إلي الأحماض الامينية الحرة بنسبة ١٠٥ % من الاحتياجات اليومية الموصي بها (١٢,٠٨ جم ليسين + ٣,٦٧ جم ميثايونين) و المضافة للبداي المركز، المجموعة الرابعة و التي تغذت يوميا علي نفس العليقة الكنترول بالإضافة إلي الأحماض الامينية الحرة بنسبة ١١٠ % من الاحتياجات اليومية الموصي بها (١٢,٦٥ جم ليسين + ٣,٨٥ جم ميثايونين) مخلوطة بالبداي المركز .

و كانت النتائج المتحصل عليها هي :

- ١- إضافة الأحماض الامينية الحرة أدت لحدوث زيادة معنوية ($p < 0.01$) في معدل الوزن اليومي (كجم/عجل/يوم) بالأضافة لحدوث تحسن معنوي ($p < 0.01$) في معدل تحويل الغذاء مقارنة بمجموعة الكنترول دون حدوث أي تغير معنوي في كمية المأكول الجاف من العلف .
- ٢- ازداد مستوي الأحماض الامينية (اليسين و ميثايونين) في البلازما زيادة معنوية نتيجة للزيادة للتدرجية من الأحماض الامينية المضافة و كانت أعلى زيادة في المجموعة الرابعة مقارنة بباقي المجاميع ($p < 0.05$) .
- ٣- لم تؤثر المعاملات معنويا علي تركيزات الأحماض الامينية المتشعبة أو المرتبطة بميتابولزم الميثايونين في البلازما عدا الحامض الاميني سيرين و الذي إنخفض ($p < 0.05$) تركيزه بزيادة إضافة الأحماض الامينية .
- ٤- أدت المعاملات إلي حدوث زيادة معنوية في تركيز البلازما من المجموع الكلي للأحماض الامينية و المرتبطة بميتابولزم دورة اليوريا و الحامض الاميني أرجينين ($p < 0.01$) و احتلت كل من المجموعة الثالثة و الرابعة أعلى القيم.