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Short Communication

Effect of Essential Amino Acids on Performance of Buffaloes

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ABSTRACT

The present study was designed to examine the influence of varying levels of limiting amino acids (lysine, methionine and threonine) supply to lactating Nili-Ravi buffaloes on their nutrient's intake and utilization, milk yield and composition, nitrogen balance and hematological parameters. Fifteen lactating Nili-Ravi buffaloes in mid lactation with almost similar milk production were randomly divided into three different groups of five females within each according to completely randomized design. Three iso-caloric and iso-nitrogenous diets with high, medium and low levels of lysine, methionine and threonine concentrations (% of crude protein) were formulated and represented as high essential amino acids (HEAA), medium essential amino acids (MEAA) and low essential amino acids (LEAA) concentrations, respectively. The study lasted for 100 days; first ten days were given for the adaptation to new diets while every six days after every month of the remaining period served as collection periods. The intake (% body weight) of dry matter (DM), crude protein (CP), metabolizable energy (ME), neutral detergent fiber (NDF) and acid detergent fiber (ADF) did not show any treatment effect. However, the intake of rumen un-degradable protein, lysine, methionine, and threonine reduced linearly ($P<0.01$) with decreasing concentrations of these nutrients in the experimental diets. A linear increase ($P<0.01$) in DM, NDF and ADF degradabilites was recorded with gradually decreased dietary RUP and EAA concentrations while CP digestibility remained unchanged. Milk yield and daily weight gain decreased linearly ($P<0.01$) with reducing dietary levels of EAA concentrations. A positive nitrogen balance was also observed in buffaloes fed all the experimental diets but it was similar statistically. Hematological analysis indicated decreased ($P<0.01$) mean corpuscular volume and hemoglobin concentrations in buffaloes fed gradual decreased dietary EAA concentrations. It is concluded that the varying levels of limiting amino acids have effect on nutrient intake, utilization, milk yield and hematological parameters. These results could be used for further studies of this field.

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Authors' Contribution
NAT designed research, finalized write up and data analysis also got the financial support from the BRI, Pattoki. MA and AR contributed in laboratory analyses. AF and AW helped in carrying out research work and writeup. IH wrote the draft manuscript initially. GM helped in write up, MP reviewed the article.

Key words
Nili-Ravi buffaloes, Milk yield, Essential aminoacids, Nutrient utilization, Nitrogen balance

The Nili-Ravi buffalo is a breed of domestic buffalo which is similar to the Murrah buffalo. It is distributed in Bangladesh, China, India, Pakistan, Philippines and Sri Lanka. But it is principally distributed in Pakistan and India, and is concentrated in the Punjab. It is mainly a dairy buffalo breed and used principally for milk production. The Nili-ravi buffalo breed date back to the Indus river valley civilizations when they were two different buffalo, nili and ravi. However, due to coincidental standards, both buffalo breeds looked very similar and it become hard to distinguish the two so the two breeds became one, Nili-Ravi in 1950. Large animals, massive body, docile temperament, the males generally mature within 30 months and females within 36 months, average first calving age is around 45 months, the breed is very good for milk production, they produce around 2000 kg of milk per year, the longevity of production is good, milk is of very good quality containing over 6% fat. Average body height is around 135 cm for males and around 125 cm for females. Average live body weight of the mature Nili-Ravi buffalo is around 700 kg for males and around 600 kg for females (Khan and Akhtar, 1999).

Sustainable milk production requires high intakes of protein especially essential amino acids (EAA; lysine methionine and threonine) for lactating animal to meet milk protein synthesis. Methionine and lysine have been suggested to be the most limiting AA for milk production when corn-based diets are fed to animals (NRC, 2001). Being cheaper high energy source, corn is extensively used as major feed ingredient in dairy rations worldwide. Since dietary proteins are largely degraded by microbes in the rumen, so formulating a ration to provide high EAA concentrations can be difficult using locally available feed resources. The difficulty is further confounded when

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rumen degradation of feed ingredients is considered during feed formulation.

Supplying rumen un-degradable protein (RUP) to meet the requirements of the host may balance the required nitrogen and energy ratio in the rumen but still may result in an inadequate supply of post ruminal EAA. In rapid growing and high producing dairy animals' rumen micro-organisms also cannot synthesize enough protein to support high level of milk production rather the microbial protein is deficient in EAA. Therefore, high producing animal diet demands escape of some dietary protein from rumen fermentation and ample concentration of EAA for milk production.

Additional RUP and EAA supply to dairy animals has been reported to optimize productivity (Ørskov, 1982). The NRC (2001) have suggested that in a typical diet, approximately 40% of the protein eaten must be true protein that escapes rumen degradation, whereas 60% of the protein value can be a mixture of protein and non-protein nitrogen that is degraded and incorporated into the rumen microbes. The present study has been designed to examine the influence of varying levels of EAA supply to lactating buffaloes on their nutrient's intake and utilization, milk yield and composition, nitrogen balance and hematology.

Materials and methods

Fifteen lactating Nili-Ravi buffaloes having similar milk production (Group I- 9.88±1.59; Group II- 9.11±2.10; Group III- 9.52±1.07) were divided in to three groups (five buffaloes in each group) according to CRD for this study. Three iso-caloric and iso-nitrogenous diets with 3 levels of EAA (lysine, methionine and threonine) were formulated using NRC (2001) standards for energy, protein and EAA (Supplementary Table I).

The buffaloes fed individually at *ad libitum* intakes and were housed on concrete floor in separate pens (40 square feet covered area + double open space). The study lasted for 100 days (10 adaptation + 90 experimental), first ten days were adaptation period while every first week of each month of the remaining period served as collection periods. The buffaloes were weighed at start of the study and fortnightly thereafter.

Milk samples were taken for the analysis of milk fat, crude protein (CP), total solid contents (AOAC 1990). Feed offered, refusals and fecal samples were analyzed for dry matter (DM), CP (AOAC 1990), neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest *et al*., 1991) while metabolizable energy (ME) was estimated according to Conrad *et al*. (1984).

Nutrients digestibilities and nitrogen balance were determined by the complete collection of urine and feces by the methods of Williams *et al*. (1984). Blood samples were collected from the jugular vein for hematological analysis by using standard kits method in hematology analyzer (AOAC 1990).

The data were analyzed according to Completely Randomized Design (SAS, 1988) Statistical model used for all parameters was; $Y_{ij} = \mu + \tau_j + \varepsilon_{ij.}$ Where, μ was overall mean, τ_j was the effect of treatment (3 treatments) and ε_{ij} was difference within treatments (error term). In case of significance differences, the means were separated by DMR test (Steel *et al*., 1997).

Results and discussion

The DM, CP, NDF, ADF and ME intake and nitrogen balance in buffaloes fed different EAA concentrations remained unaltered. However, there was a linear decrease in EAA and RUP intakes with reducing levels of these nutrients in the diets (Table I, II). The linear decrease in intake of EAA and RUP in buffaloes fed HEAA, MEAA and LEAA diets was because of gradual decreased dietary concentration of these nutrients in the experimental diets.

Table I. Nutrients intake and their digestion

Parameters	HEAA	MEAA	LEAA	SE	Linear	Quadratic
Nutrients Intake % body weight						
Dry matter	2.85	2.92	2.78	0.09	0.78	0.65
Crude protein	0.40	0.41	0.40	0.01	0.84	0.73
Rumen un-degradable protein	0.20^{a}	0.17^{b}	0.12^{c}	0.01	0.001	0.70
Lysine	0.49^{a}	0.37^{b}	0.28^{c}	0.03	0.00	0.16
Methyonine	0.29^{a}	0.19^{b}	0.15^{c}	0.02	0.00	0.00
Treonine	0.51^{a}	0.43^{b}	0.29^{c}	0.03	0.00	0.09
ME	0.25	0.25	0.25	0.02	0.94	0.93
NDF	6.14	6.40	6.33	0.56	0.90	0.91
ADF	3.51	3.65	3.61	0.32	0.91	0.91
Nutrient digestion						
DM	63.95^{c}	65.83^{b}	68.8^{a}	0.63	0.001	0.16
CP	75.00	76.50	76.05	0.98	0.69	0.67
NDF	53.82^{c}	55.66^{b}	57.7^{a}	0.56	0.001	0.88
DWG	0.81^{a}	0.56^{b}	0.51^{b}	0.04	0.001	0.06

HEAA, MEAA and LEAA diets contained high, medium and low essential amino acids concentrations, respectively, means within row bearing different superscripts differ significantly ($p<0.05$), SE standard error of the means; ME, metabolizable energy; NDF, neutral detergent fiber; ADF, acid detergent fiber; DM, dry matter; CP, crude protein; DWG, daily weight gain.

Dry matter and NDF digestibility increased linearly with gradually decreased of dietary concentration of EAA and RUP in the diets but CP digestibility was similar statistically. The diets were iso-nitrogenous which did not influence CP digestibility, but improved DM and NDF digestibility in buffaloes fed decreased EAA and RUP concentrations might be attributed to gradual dietary increased RDP concentration which might have increased supply and availability of N in diverse forms (ammonia

N, peptides and amino acids). This might have accelerated rumen microbial fermentation activities leading to improved feed consumption (DelCurto *et al*., 1990).

Table II. Nitrogen balance.

Parameters	HEAA	MEAA	LEAA	SEM	Linear	Quadratic
Nitrogen intake g/d)	353.37	339.39	340.30	4.59	0.28	0.47
Fecal nitrogen	77.60 ^{c}	106.4 ^{b}	128.0^{a}	6.91	0.00	0.62
Urine nitrogen	152.06^{a}	134.8^{ab}	131.7^{c}	3.76	0.02	0.28
Milk nitrogen	22.73	22.19	22.65	0.24	0.91	0.38
Nitrogen balance	89.93	91.46	89.04	2.94	0.91	0.78
BUN	21.07 ^{a}	18.00 ^{b}	16.16 ^{c}	0.67	0.00	0.25

BUN, blood urea nitrogen. For other abbreviations, see Table I.

A positive correlation between increased rumen fermentation and dry matter digestibility has been reported previously by Nisa *et al*. (2004). Availability of N in diverse forms has also been documented to facilitate the synchronization of carbon and nitrogen units in rumen which has direct positive influence on rumen microbial proliferation and enzyme production which might have favored increased DM digestibility in buffaloes fed decreased EAA and RUP diets (Javaid *et al*., 2008; Nisa *et al*., 2008). Increased nutrient digestibility due to increased ruminal dynamics by availability of nitrogen in diverse form has also been documented by Haddad and Goussous (2005). Therefore, improvement in nutrient digestion in the current investigation might be due to increased ruminal dynamics by availability of nitrogen in diverse form to the host.

A linear (P<0.05) decrease in daily weight gain and milk yield was observed in buffaloes fed reducing EAA and RUP concentrations, however, milk fat content didn't differ (Table III). This increased milk production and weight gain with increasing EAA concentrations might be attributed to better availability of dietary nitrogen in diverse forms which might have enhanced rumen microbial multiplication, per unit enzyme production and overall fermentation activities in the rumen leading to more volatile fatty acid production (Javaid *et al*., 2008). This might have enhanced nutrients supply for more milk synthesis. Furthermore, increasing dietary RDP concentration has been reported to increase rumen ammonia content (Nisa *et al*., 2008).

In the current study all the buffaloes were in positive N balance which led to spare more nutrients for milk production and daily gain. Ammonia, being alkaline in nature might have increased rumen pH which might have favored shifting the fermentation pattern in favour of acetate and butyrate production and ultimately increased milk production. The weight gain was also found to be affected by high input feeding systems in Nili-Ravi buffalo calves (Faraz *et al*., 2019). In addition to this, weight gain was also found affected by different feeding systems in dromedary camel calves (Faraz *et al*., 2017, 2018).

Table III. Milk production and weight gain.

Parameters	HEAA	MEAA	LEAA	SEM	Linear	Quadratic
Milk production, Kg/day	14.86^{a}	12.2 ^{b}	10.84 ^{c}	0.52	0.00	0.09
Fat%	6.08	6.18	6.33	0.11	0.39	0.92
4%FCM, Kg/day	15.06	14.16	13.82	0.24	0.03	0.52
Daily weight gain	0.81^{a}	0.56^{b}	0.51^{b}	0.04	0.001	0.06

FCM, fat corrected milk. For other abbreviations, see Table I.

Table IV. Hematology.

Parameters	HEAA	MEAA	LEAA	SEM	Linear	Quadratic
WBC Nx10^3/uL	10.91	12.80	11.19	0.96	0.91	0.25
RBC Lx10^6/uL	5.45	4.88	5.58	0.28	0.98	0.25
Hb g /dL	10.54	8.91	9.00	0.47	0.10	0.16
MCV H fL	66.60^{a}	59.39^{ab}	57.08^{b}	1.91	0.04	0.45
Hct L %	32.84	30.13	30.95	1.22	0.35	0.22
Plt *L x10^3/uL	188.01	248.44	218.31	22.0	0.68	0.37
MCH pg	23.90^{a}	17.45^{ab}	16.38^{b}	1.55	0.04	0.38
MCHC L g/dL	33.30	29.10	28.70	0.99	0.05	0.41

WBC, white blood cell count; RBC, red blood cell count; Hb, hemoglobin; MCV, mean corpuscular volume; Hct, hematocrit; Plt, platelets; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration. For other abbreviations, see Table I.

Observations of WBC, RBC, Hct, and MCHC remained unaltered across all diets, however, MCV and MCH concentrations decreased with gradual reduction of dietary RUP concentration (Table IV). Gradual reduction in MCV and MCH concentration with decreased dietary EAA and RUP concentration might have decreased the sustainable supply of EAA required for the normal biosynthetic activities aimed to synthesize these blood constituents. Gradual reduction in dietary RUP concentration might have limited the supply of these amino acids leading to their respective decreased contents in the blood, despite the fact that diets were iso-nitrogenous (Bergman and Heitmann, 1980).

Another plausible explanation of decreased MCV and MCH concentration might be that gradual increase in RDP fraction of dietary CP couldn't ensure similar amino acid supply at post ruminal level to avoid any variation in blood amino acid profile which might have altered availability and sequence of amino acid supply for the synthesis of these blood constituents (Bergman and Heitmann, 1980). While Faraz *et al*. (2019) studied feedlot performance and serum profile of Nili-Ravi buffalo (*Bubalus bubalis*) calves under high input feeding systems and reported that hematological and biochemical profile was found to be altered in response to different dietary regimens.

A few reports available about hematology in Pakistan, however, in comparison to this, some studies also verified the effects of diet and feeding system on hematological parameters in dromedary camels (Faraz *et al*., 2017) and these measures could be used as indirect parameters for the evaluation of dietary management (Faraz *et al*., 2018) while these are the mirrors of general health status and physiological condition which may serve as a guide for these parameters (Faraz *et al*., 2020).

Conclusion

The results of present study testified the fact that varying levels of limiting amino acids have definite impact on the nutrient intake, utilization and milk yield. In this, milk production and daily weight gain decreased linearly with reducing dietary levels of EAA concentrations. While a positive nitrogen balance was also observed in buffaloes fed all the experimental diets. However, hematological analysis indicated decreased MCV and Hb concentrations in buffaloes fed gradual decreased dietary EAA concentrations. These results could be used for future studies in this field.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20200419160408

Statement of conflict of interest

The authors have declared no conflict of interest.

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Supplementary Material

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Supplementary Table I. Ingredients and chemical composition of experimental diets, Gram/ Kg.

Ingredients	HEAA	MEAA	LEAA
Maize Broken	30.00	200.00	150.00
Wheat Straw	450.00	250.00	120.00
Wheat bran	150.00	200.00	300.00
Rice Polishing	10.00	0.00	50.00
Cotton seed cake	60.00	15.00	20.00
Maize oil cake	0.00	0.00	95.00
Maize gluten Meal 30%	83.50	50.00	10.00
Canola meal	10.00	10.00	10.00
Soy bean Meal	48.00	55.00	20.00
Corn steep liquor	60.00	60.00	60.00
Cane Molasses	80.00	142.00	148.00
Mineral Mix	15.00	15.00	15.00
Urea	3.50	3.00	2.00
Chemical Composition, %			
Dry matter	87.62	85.83	85.37
Crude protein	14.02	14.18	14.12
Rumen un-degradable protein	50.31	41.31	32.17
Lysine %CP	3.10	2.43	1.85
Methyionine %CP	1.86	1.20	0.95
Treonine %CP	3.20	2.84	1.86
Lysine%of RUP	1.56	1.01	0.60
Methionine %RUP	0.94	0.50	0.31
Threonine %RUP	1.61	1.17	0.60
Neutral detergent fiber	46.87	32.35	30.88
Acid detergent fiber	26.98	17.56	13.86
Acid detergent lignin	5.44	3.35	2.85
Total Ash%	8.89	9.30	9.19
Metabolizable energy Mcal/ kg	2.09	2.22	2.25

HEAA, MEAA and LEAA diets contained high, medium and low essential amino acids concentrations, respectively.

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0030-9923/2021/0001-0001 $ 9.00/0