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THE EFFECTS OF DIETARY EUBIOTICS OR INTRAVENOUS AMINO ACID INFUSIONS ON NUTRIENT DIGESTIBILITY, RUMEN FERMENTATION, PERFORMANCE AND BLOOD PARAMETERS OF BUFFALO CALVES UNDER SUBTROPICAL CLIMATIC CONDITIONS

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Abstract: This study was conducted to compare the effects of dietary eubiotics or intravenous amino acid infusions (IVAAI) as two different growth promoters on nutrient digestibility, rumen fermentation, performance, and blood biochemical parameters of buffalo calves in subtropical climatic conditions. Thirty male buffalo calves (284.40 ± 18.45 kg) were randomly distributed into three groups and fed a basal diet (BD) of concentrate feed mixture and roughages. The first group was fed BD and considered as the control, the second group was fed the BD supplemented with eubiotics at 1.0 kg/ton of concentrate, whereas the third group was intravenously infused with amino acid (IVAAI) injection at a dose of 2.0 ml/100 kg body weight. Results showed that the total gain and the average daily gain were improved ($P < 0.05$) with dietary eubiotics. The digestibility of some nutrients was increased ($P < 0.05$) with dietary addition of eubiotics. In addition, eubiotics stabilize ($P < 0.05$) the rumen pH, which reduce the risk of subacute ruminal acidosis but increased ($P < 0.05$) ruminal NH3-N and total volatile fatty acids. The rectal temperature was decreased ($P < 0.05$) with eubiotics supplementation. In conclusion, the use of eubiotics induced superior positive effects on the digestibility of nutrients, rumen fermentation, rumen enzymes, rumen protein concentration, growth performance, feed conversion, blood parameters and ameliorated the harmful effects of thermal stress of buffalo calves in comparison with intravenous infusion of amino acids.

Key words: eubiotics; amino acids; buffalo calves; nutrient digestibility; rumen fermentation; growth performance

Introduction

Recently, there has been increasing worldwide legislation dedicated to limiting or banning the use of chemically synthesized antibiotics, which have been used for decades as growth promoters in animal production at sub-therapeutic doses under normal conditions; in order to prevent the emergence of drug resistance in human pathogens (1). This has accelerated the efforts of nutritionists to present natural, new, and safe antibiotic alternatives that are adequate for the animal production industry. Under stress conditions, banning or limiting the use of antibiotic growth promoters in animal feed would lead to highly reduced profits due to deteriorated intestinal barrier immunity and increased risk of pathogenic invasions (2).

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Dysfunction of the intestinal barrier causes intestinal epithelium failure and reduces the absorption of nutrients in animals. Various alternatives have been proposed, including probiotics, prebiotics, organic acids, exogenous enzymes, essential oils, amino acids, and vitamins to improve gut health and intestinal barriers (3, 4). Eubiosis means the correct balance of microflora in the intestinal tract, which is necessary for healthy gut performance. Supporting good animal gut health leads to reducing the need for antibiotics, ensuring effective utilization of natural resources, reducing the environmental pollution of farms, and improving farmers' incomes. eubiotic feed additives include five categories: organic acids, probiotics, prebiotics, phytogenics or essential oils, and gut health enzymes. All of these products positively contribute to microbiome modulation, leading to improved gut health. The combination of these different eubiotics would have a synergistic effect in improving animal performance (5).

Feeding of exogenous enzymes in the diet boosts the ruminal microbial enzyme activity, improves microbial adhesion to feed particles, and increases breakdown of dietary fiber (6). It has been demonstrated that amino acid infusion or ingestion is required to promote whole-body protein synthesis, minimize protein breakdown, and so elicit a positive net protein balance under heat-stressed conditions (7). Little work has gone into evaluating the feasibility of eubiotic combinations and amino acid supplementation in buffalos under heat-stress conditions. The use of combinations from these additives is hypothesized to show more pronounced benefits utilizing their cumulative and synergistic effects. Hence, the objective of this study was to evaluate the effects of dietary multicomponent eubiotics, or intravenous amino acids infusions (IVAAI) as two different growth promoters on nutrient digestibility, rumen fermentation, performance, and blood biochemical parameters of buffalo calves in subtropical climatic conditions.

Materials and methods

Animals, diets, and management

Thirty male buffalo calves that are clinically healthy, aged eleven to twelve months and weighing 284.40 ± 18.45 kg on average were used in this study. The animals were divided into three groups, each with ten animals. The experimental period was divided into two parts: a 15-day adaption period and a 110-day experimental period. The animals of each group were housed separately in pens, throughout the experiment, and were fed individually. The average ambient temperature inside the bunkers ranged between 36.0 to 45.0˚C and the relative humidity ranged between 60.5% to 65%. The temperature humidity index ranged from 75.33 to 83.01 on average. at 02:00 pm. The basal diet was prepared to meet the nutritional needs of growing calves according to NRC (8) recommendations. All the calves were fed a basal diet of 60% concentrate feed mixture (CFM) and 40% roughage (wheat straw (20 %) and Egyptian clover (20%)). The control group (1) received only the basal diet and no supplements. Group 2 were dietary supplemented with eubiotics at a rate of 1.0 kg/ton of concentrate mixture. The calves in group 3 were infused intravenously with amino acids (IVAAI) at a dose of 2.0 ml/100 kg body weight, through using jugular vein catheters with an automated peristaltic pump, and this dose was repeated biweekly. The chemical compositions of the experimental diet are presented in Table 1.

The multicomponent eubiotics consisted of: *Saccharomyces cerevisiae* ($20x10^{10}$ CFU), *Lactobacillus acidophilus* ($2x10^{9}$ CFU), *Lactobacillus plantarum* ($1.6x10^{9}$ CFU), *Lactobacillus casei* ($0.4x10^{8}$ CFU), total live bacteria ($2x10^{10}$ CFU), *Entrococcus faeciun, Bacillus licheniformis* ($6 x10^{9}$ CFU), ($4.0x10^{9}$ CFU), *Bacillus subtilis* ($6 x10^{9}$ CFU), lipase (2400 U), xylanase (1200 U), phytase (2400 U), cellulase (2400 U), amylase (20000 U), pectinase 400 U, β-gluconase (1000 U), protease (40000 U), fructo oligosaccharides (10 g), mannan oligosaccharides (10 g), calcium propionate (24 g), copper penta sulphate (10 g). The second growth promoter is an intravenous amino acid supplement, which contains 13 amino acids and vitamins. Each 100 ml of IVAAI contains Arginine 144 mg, Cysteine 320mg, Glycine 320mg, Glutamine 320mg, Histidine 132mg, Isoleucine 360mg, Leucine 428mg, Lysine 544mg, Methionine 320mg, Threonine 320mg, Tryptophan 86mg, Phenylalanine 500mg, Valine 360mg, Vitamin B1 400mg, Vitamin B2 17mg, Vitamin B6 34mg, Nicotinamide 800 mg.

Table 1: Chemical composition of the experimental diet (% on dry matter basis)

Items	CFM*	Egyptian clover	Wheat straw
Dry matter (DM)	89.40	18.43	91.2
Organic matter (OM)	94.70	93.78	92.7
Crude protein (CP)	15.22	18.78	3.55
Crude fiber (CF)	12.57	21.45	36.40
Ether extract (EE)	2.47	4.03	1.76
Nitrogen free extract (NFE)	69.74	55.74	58.29
Ash	5.30	6.22	7.30

*CFM, concentrate feed mixture. The concentrate feed mixture (CFM) consists of: 50% corn, 20 % wheat bran, 20% undecorticated cotton seed meal, 8 % soybean meal, 1% limestone, 0.5 % salt and 0.5% mineral -vitamin premix

To determine the animals' daily feed intake, the feed was daily offered to the animals, and the refusals were collected and weighed. The animals were weighed at the beginning and end of the experiment and biweekly throughout the experiment. By dividing the feed intake by the weight growth, the feed conversion ratio was obtained. Fresh water was available *ad libitum*. The calves were dewormed with an anthelminthic injection before the start of the trial and clinically examined to be sure that they are healthy.

Blood sampling and analysis

Every month, blood samples were collected from each calf's jugular vein 6 hours after the morning feeding. Then the blood samples were centrifuged at 3000 rpm for 15 minutes to separate the serum. The serum was collected and kept at 20°C until chemical analysis. Serum glucose, total protein, albumin, globulin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, and total cholesterol were tested colorimetrically using commercial kits and a spectrophotometer (Hitachi 911 automated analyzer) (Spinreact, Spain), and the analyses were performed according to the manufacturer's company.

Thermoregulatory responses

The number of flank movements per minute (breath/min) was used to calculate the respiratory rate (RR). Pulse rate (PR) was recorded using pulse oximeter apparatus (CMS60D- VET Handheld Veterinary Pulse Oximeter). A clinical thermometer was gently inserted into the rectum for one minute to determine rectal temperature (RT, °C). A portable infrared thermometer intended for temperature measurements was used to measure skin temperature (ST, °C), hair temperature (HT, °C), and ear temperature (ET, °C). All measurements were done at 2.00 pm every two weeks during the experimental period. The pulse rate and respiration rate were counted before measuring the body temperature. Air temperature and humidity were recorded during the experimental period using temperature / humidity thermometer at two pm. Temperature Humidity Index (THI) was estimated according to Mader et al. (9).

Digestibility trials

The nutrient digestibility of the experimental diets was determined at the end of the feeding trial using chromic oxide as an external marker in three digestibility trials. Each digestibility trial for each diet lasted for 14 days, with the first 7 days serving as an adaptation period, followed by 7 days of data collection. Each calf got exactly 10 g of powdered Cr2O3 on the first day of the preparatory period, which was manually mixed with the concentrate mixture. For a chemical analysis, daily feed samples were collected, mixed, dried, and ground through a 1 mm sieve screen. In addition, from day 8 to day 14, about 200 g of fresh feces were gathered twice daily by fecal grabbing and kept in a refrigerator.

The fecal samples from each animal were collected at the end of digestibility trial, dried at 60°C, and ground through a 1 mm mesh screen for chemical analysis. Using AOAC (10) methodologies, chemical analysis of feeds and excrement was

carried out. Goering and Van Soest (11) methods were utilized (ADL) to identify neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin. The nutrient composition of feces and feed from chromium was estimated using atomic absorption spectrophotometry (12). The nutrient digestibility was calculated using Maynard and Loosli (13) equations:

Digestibility of nutrient (%) = 100–(100 x % marker in feed / % marker in feces x % nutrient in feces / % nutrient in feed).

Rumen liquor parameters

Using a stomach tube, samples of rumen contents were obtained from each calf at the end of the digestibility trails, on the two successive days next to the collection period. Samples were taken immediately prior to feeding time, then three and six hours later, samples were filtered through four layers of cheesecloth to determine the pH using a digital pH meter (Beckman, Model 45, USA) and ammonia N content using the method of Conway (14). Furthermore, 0.8 mL of ruminal liquor filtrate was combined with 0.2 mL of a solution containing 250 g of metaphosphoric acid/L to halt microbial activity before being stored at −20 °C for volatile fatty acid (VFAs) measurement. The total volatile fatty acids were determined by the steam distillation method according to methodology of Warner (15).

Rumen enzyme activities

A subsample of 5 mL from the whole samples, collected at 3 h after the morning feeding of each calf, was preserved by the addition of a few drops of saturated mercuric chloride solution to inhibit microbial activity, and then refrigerated at -20 °C for rumen enzyme activities measurement. The enzyme activity in rumen fluid was determined using spectrophotometry (Unico, USA). The activities were determined following previously published protocols: cellulose and alpha-amylase were estimated according to Miller (16), lipase was determined according to Peled and Krenz (17), protease was measured according to Folin and Ciocalteu (18), and urease was measured according to Weatherburn (19). According to Lowry et al.(20) the quantities of extracellular protein in the crude enzyme were measured

Statistical analyses

The general linear model (G.L.M) of the SAS (21) program was used for statistical analysis. One-way ANOVA was used to assess the effect of treatments on feed intake, growth rate, feed conversion ratio, blood parameters, nitrogen retention, nutritional digestibility, feeding value, and rumen enzyme activities. Data on rumen liquid parameters were analyzed using SAS's procedure, which used time as a repeated measure and each animal as

Table 2: Effect of dietary eubiotic supplementation or intravenous infusion of AA on growth performance of buffalo calves

Items	Treatment			P-value
	Control	Eubiotics	IVAAI	
Initial weight (kg(	284.20 ± 19.51	284.60 ± 18.60	284.40 ±17.24	0.999
Final weight (kg(	376.80 ± 17.95	394.00 ± 17.71	383.00 ± 14.41	0.768
BW gain (kg)	92.60^{b} ± 3.54	109.40^{a} ± 2.52	98.60^{b} ± 3.68	0.011
Average daily gain (g)	841.82^{b} ± 38.22	994.54^{a}± 22.93	896.36^{b} ± 33.48	0.011
Feed intake (kg/d)				
DMI of concentrate	7.01 ± 0.21	7.22 ± 0.24	7.21± 0.22	0.756
DMI of roughages	3.22 ± 0.07	3.18 ± 0.04	3.13 ± 0.04	0.447
Total DM intake	10.22 ± 0.27	10.40 ± 0.25	10.33 ±0.25	0.886
Feed conversion ratio g DM/g gain	12.15^{a} ± 0.32	10.46^{b} ± 0.25	11.53^{a} ± 0.28	0.001

$^{a, b}$ *Means within the same row carrying different superscripts are significantly different at ((P<0.05).* IVAAI, intra venous amino acid infusions.

the experimental unit. Treatment, time, and the treatment x time interaction were all included in the model. The Duncan multiple range test (22) was used to evaluate the impact of treatments on parameters that were investigated. The results are shown as means and standard error (SE). Significant P values were defined as those less than 0.05 (P < 0.05).

Results

Growth performance

Calves fed the diet containing eubiotics gained more total body weight and daily weight gain than those fed IVAAI and control diets (Table 2). The feed intake of concentrate and roughages as well as the total DM intake were not significantly (P > 0.05) differed between groups. The feed conversion ratio was considerably (*P* < 0.05) improved in eubiotics group in comparison to the IVAAI and control groups (10.46 vs. 11.53 and 12.15 g DM/g gain, respectively).

Blood constituents

The results of blood metabolite analysis are shown in Table 3. The IVAAI increased (P < 0.05) the serum cholesterol concentration and decreased (P < 0.05) the urea-N and glucose concentrations as compared with other groups. However, there were no significant (P > 0.05) variations in other blood constituents among the treatments.

Table 3: Effect of dietary eubiotic supplementation or intravenous infusion of AA on blood serum constituents of growing buffalo calves.

Items	Treatment			P-value
	Control	IVAA		
Total protein, g/dl	7.56 ± 0.17	7.31 ± 0.21	7.34 ±0.15	0.581
Albumin (g/dl)	4.07 ± 0.17	3.86 ± 0.12	4.22 ± 0.11	0.207
Globulin (g/ dl)	3.41 ± 0.15	3.13 ± 0.10	3.04 ± 0.19	0.224
A/G ratio	1.22 ± 0.09	1.25± 0.05	1.46 ± 0.15	0.260
AST (U/l)	65.44 ± 1.37	65.91± 1.57	65.68 ± 1.67	0.830
ALT (U/l)	14.11 ± 0.48	14.22 ± 0.36	14.67± 0.44	0.635
Cholesterol, mg/dl	179.78^{b} ± 12.37	181.89^{b} ± 16.38	252.33^{a} ± 13.11	0.001
Urea-N (mg/dl)	11.67^{a} ± 0.51	11.70^{a} ± 0.73	9.30^{b} ±0.94	0.001
Creatinine, mg/dl	1.37 ± 0.07	1.39 ± 0.05	1.28 ± 0.04	0.388
Glucose, mg/dl	101.20^{a} ± 6.49	97.07^{a} ± 10.90	78.27^{b} ± 7.40	0.001

[a, b] Means within the same row carrying different superscripts are significantly different at ((P<0.05). IVAAI, intra venous amino acid infusions.

Table 4: Effect of dietary eubiotic supplementation or intravenous infusion of AA on thermoregulatory responses

	Treatment			P-value
Parameter	Control	Eubiotics	IVAAI	
RT (ºC)	39.06^{b} ± 0.08	38.82^{c} ± 0.10	39.37^{a} ± 0.06	0.021
ST (ºC)	37.22 ± 0.81	37.13 ± 0.56	37.43 ± 0.45	0.621
ET (ºC)	35.44± 0.87	35.38 ± 0.65	35.29 ± 0.87	0.835
RR (breath/min)	37.27 ± 0.45	37.77 ± 0.40	36.88 ± 0.40	0.354
PR (beat/min.)	65.56^{a} ± 1.45	64.22^{b} ± 1.50	64.12^{b} ± 1.51	0.002

[a,b,c] Means within the same row carrying different superscripts are significantly different at (P < 0.05). RT, Rectal temperature ; ST, Skin temperature; ET , Ear temperature ; RR, Respiration rate; PR, Pulse rate IVAAI, intra venous amino acid infusions.

Table 5: Effect of dietary eubiotic supplementation or intravenous infusion of AA on nutrients digestibility and nutritive value of experimental rations (%)

Item, %	Treatment			P-Value
	Control	Eubiotics	IVAAI	
Nutrient digestibility				
Dry matter, DM	63.66^{c} ± 0.04	65.97^{a} ± 0.41	65.03^{b} ± 0.21	0.002
Organic matter, OM	63.62^{b} ± 0.14	64.00^{ab} ± 0.28	64.88^{a} ± 0.31	0.034
Crude protein, CP	76.55 ± 0.56	77.52 ± 0.12	77.52 ± 0.40	0.226
Crude fiber, CF	55.75^{b} ± 0.30	64.79^{a} ± 0.98	57.45^{b} ± 0.42	0.001
Ether extract, EE	73.67^{b} ± 1.76	81.86^{a} ± 1.87	77.52^{ab} ± 0.64	0.018
Nitrogen free extract, NFE	62.50^{b} ± 0.78	64.26^{a} ± 0.20	64.56^{a}± 0.50	0.049
NDF	58.19^{c} ± 0.58	66.67^{a} ± 0.35	62.41^{b} ± 0.46	0.001
ADF	42.52^{c}± 1.58	49.26^{a} ± 0.45	46.14^{b} ± 0.87	0.001
Cellulose	37.29^{b} ± 1.76	43.43^{a} ± 0.69	40.46^{ab} ± 1.14	0.039
Hemicellulose	15.67 ± 1.62	17.41 ± 0.70	16.28 ± 1.11	0.609
ADL	5.23 ± 3.67	5.83 ± 0.24	5.68 ± 0.34	0.597
Nutritive value				
TDN	65.41 ± 0.32	65.73 ± 0.23	66.06 ± 0.25	0.255
SV	63.57^{b} ± 0.26	64.10^{b} ± 0.16	64.76^{a} ± 0.17	0.001
DCP	11.65^{b} ± 0.06	11.80^{a} ± 0.04	11.80^{a} ± 0.04	0.050

^{a,b} Means within the same row carrying different superscripts are significantly different at (($p<0.05$). NDF, Neutral detergent fiber; ADF, Acid detergent fiber; ADL, Acid detergent lignin; TDN, total digestible nutrient; SV, Starch value; DCP, digestible crude protein; IVAAI, intra venous amino acid infusions

Table 6: Effect of dietary eubiotic supplementation or intravenous infusion of AA on rumen parameters

Item	Treatment	Hours after feeding			Means of treatment	P- value	
		zero	3 hours	6 hours		Treat. effect	Treat. X time
pH	Control	6.53± 0.05	6.36^{B} ± 0.07	6.14 ± 0.02	6.36B ± 0.07	0.001	0.001
	Eubiotics	6.54 ± 0.04	6.87± 0.01	6.74 ± 0.03	6.72^{A} ± 0.05		
	IVAAI	6.56 ± 0.07	6.19 ± 0.1	6.29 ± 0.05	6.35^{B} ± 0.06		
Means of time		6.54 ± 0.03	6.48 ± 0.11	6.39± 0.09			
P- value (time)		0.46					
NH3-N, mg/100ml	Control	14.88 ± 0.07	23.23 ± 0.18	20.11 ± 0.57	19.41C± 0.68	0.001	0.055
	Eubiotics	15.08 ± 0.14	25.37 ± 0.0.23	19.09 ± 0.27	19.84^{B}± 0.84		
	IVAAI	15.26 ± 0.10	25.21 ± 0.21	20.18 ± 0.15	20.21^{A}± 0.80		
Means of time		15.07^{c}± 0.07	24.60^{a}± 0.22	19.79^{b}± 0.15			
P- value (time)		0.001					
TVFAs, meq/100ml	Control	8.78 ± 0.05	8.77 ± 0.12	8.77 ± 0.12	9.23C ± 0.14	0.001	0.376
	Eubiotics	9.40 ± 0.08	10.42 ± 0.10	9.49 ± 0.07	9.77A ± 0.10		
	IVAAI	8.98 ± 0.13	10.23 ± 0.18	9.06 ± 0.10	9.42B ± 0.13		
Means of time		9.05C ± 0.29	9.10^{b} ± 0.08	9.10b ± 0.08			
P- value (time)		0.001					

^{a,b,c} Means within the same row carrying different superscripts are significantly different at P (($p<0.05$). ^{A,B,C} Means within the same column (within each parameter) different superscripts differ significantly ($p<0.05$). IVAAI, intra venous amino acid infusions.

Table 7: Effect of dietary eubiotic supplementation or intravenous infusion of AA on ruminal enzymes activity

Items	Treatments			P-value
	Control	Eubiotics	IVAAI	
α-amylase activity (µg glucose/min/ml)	$4.73^{b} \pm 0.13$	$5.71^{a} \pm 0.08$	$4.88^{b} \pm 0.05$	0.001
Cellulase activity (µg glucose/min/ml)	$3.03^{b} \pm 0.08$	$4.46^{a} \pm 0.01$	$3.12^{b} \pm 0.08$	0.001
Lipase activity (µg p- nitrophenol/min/ml)	$4.73^{b} \pm 0.14$	$7.29^{a} \pm 0.22$	$5.22^{b} \pm 0.20$	0.001
Urase activity (µg NH_3/min/ml)	$34.47^{c} \pm 0.58$	$53.20^{a} \pm 2.83$	$42.04^{b} \pm 0.71$	0.001
Protease activity (µmol of tyrosine/min/ml)	$3.84^{b} \pm 0.24$	$4.68^{a} \pm 0.21$	$4.64^{a} \pm 0.21$	0.020
Rumen protein concentration (mg/ml)	$2.54^{b} \pm 0.09$	$3.11^{a} \pm 0.14$	$2.80^{ab} \pm 0.11$	0.006

^{a,b,c} Means within the same row carrying different superscripts are significantly different at (($p<0.05$). IVAAI, intra venous amino acid infusions

Thermoregulatory responses:

The rectal thermoregulatory responses are presented in Table 4. It was found that the rectal temperature of growing calves was increased ($P < 0.05$) with the infusion of IVAAI, and decreased ($P < 0.05$) with supplementation of dietary eubiotics in comparison with the control group but they were still within the normal range of body temperatures. The pulse rate was lower ($P < 0.05$) in all treatment groups than in the control. However, the skin temperature, ear temperature, and respiration rate were not significantly affected among the groups.

Nutrient digestibility and nutritive value

Dietary supplementation of eubiotics as a source of a microbial feed additive to the diets of growing calves increased ($P < 0.05$) the DM, CF, NDF, and ADF digestibility rates in comparison with IVAAI and control groups (Table 5). In addition, the digestibility of EE, NFE, and cellulose was significantly ($P < 0.05$) higher in the eubiotic group than in the control one. However, no significant ($P > 0.05$) differences were found between the eubiotic group and IVAAI group. Moreover, the intravenous (I/V) infusions of AA to growing calves improved ($P < 0.05$) the OM digestibility when compared to the control one. In addition, the feeding values including the starch value (SV) and digestible crude protein (DCP) were increased ($p < 0.05$) in treatment groups, when compared to the control group.

Rumen fermentation activities

Supplementation of eubiotics significantly increased ($P < 0.05$) the pH values in comparison to the control and IVAAI treated groups (Table 6). However, the concentrations of ruminal NH3-N and total short chain fatty acids (VFAs) were higher ($P < 0.05$) with the inclusion of eubiotics or I/V infusion of IVAAI to growing calves than the control one. The concentrations of total short-chain volatile fatty acids of calves that received dietary eubiotics were higher ($p < 0.05$) than in other groups. In terms of how sampling time affected rumen fluid parameters, the mean pH values were higher ($p < 0.05$) before feeding time and, then decreased at 3 and 6 hours after feeding. However, the concentrations of NH3-N and total VFAs decreased before to feeding, increased following feeding to reach their peak at 3 hours post feeding, and then began to decline once more at 6 hours post feeding. The interactions between the treatments and the time effect for ruminal pH and NH3-N concentration were significant ($P < 0.05$). The interaction between treatment and the time effects on total VFAs is not statistically significant ($P > 0.05$).

Rumen enzymatic activities

Regarding the effect of treatments on ruminal enzymatic activity, we found that ruminal α-amylase, cellulose, lipase and urase activities and rumen protein concentrations were significantly ($P < 0.05$) increased in eubiotic group when compared

to IVAAI and control groups (Table 7). Moreover, the protease activity was improved ($p < 0.05$) in both treatment groups when compared with the control one.

Discussion

Growth performance

In comparison to the control and IVAAI, intra venous amino acid infusions. groups, eubiotic supplementation in calves' diet enhanced the body weight and average daily gain by 18.14 and 10.95 %, respectively. This improvement might be due to increased nutrient digestibility in the eubiotic diet. This impact was found to be beneficial in the current study when calves were fed a fibrous diet (40% wheat straw and Egyptian clover) that was predicted to ferment and create a few amounts of lactobacili in the rumen. Furthermore, the product comprises two Bacillus bacteria strains (*B. subtilis* and *B. licheniformis*), both of which can suppress or create antimicrobials in the gastrointestinal tract and maintain gut health and improve the animal's performance (3, 23). In this context, the inclusion of exogenous enzymes in the diet of lambs enhanced the daily weight gain because of higher N intake and retention, as well as fiber digestion (24). The average daily gain of IVAAI treated growing calves was slightly higher than that of the control group. The numerical increase in body weight in AA supplemented group might be due to the better availability of infusing amino acids into the blood for metabolism and absorption by 100% and utilization sites by the various tissues in the body, resulting in better performance (25). The results reported by Kassube et al. (26), who suggested that infusing essential amino acids into cows exposed to the heat stress environment improved whole-body protein synthesis, confirm our findings. Similarly, supplementation of metabolizable amino acids to the finishing calves improved the performance and feed efficiency (27, 28). The addition of dietary eubiotics or IVAAI to calves had no impact on their intakes from concentrate, roughage, or total DM. This result confirms the finding of previous studies who stated that infused methionine, lysine, and branched-chain over needs (i.e. 135% of requirements) decreased dry matter intake and milk

Blood constituents

Except for cholesterol, urea nitrogen, and glucose, treatments did not affect the blood parameters examined in this study. The higher cholesterol concentration with infused amino acids in growing calves could be ascribed to decreased lipid metabolism in this group in comparison with the control group (30). The decreased blood urea nitrogen in the IVAA group may be due to lower amino acid deamination and improved AA absorption and utilization efficiency for tissue growth (31). The reduced serum glucose levels in the IVAA group could imply glucose elimination in peripheral tissues (e.g. muscle or adipose) (32). Similarly, infusions of methionine, lysine, and branched-chain AA decreased (P 0.01) plasma glucose levels, glutamate concentrations, plasma alanine, and aspartate compared to the control group (26). This suggests that glucose was absorbed in the small intestine and had an impact on the calves' growth.

Thermoregulatory responses

One of the strategies of supplementing eubiotics or infusing amino acids to calves in our study is to ameliorate the effects of thermal stress. The calves in this study were subjected to heat stress, and the addition of dietary microbial additives had only small impacts on rectal temperature and pulse rate. Supplementing heat-stressed dairy cows with a mixture of exogenous enzymes and yeast cultures decreased rectal temperature, suggesting a role in thermoregulatory processes (33, 34). Exogenous enzymes in eubiotics improve dry matter intake, nutrient digestibility, energy-use efficiency, water absorption, and the intestinal permeability and consequently could reduce the metabolic heat load coming rumen fermentation and reduce heat stress as clarified in previous study (35). Animals receiving feed additives/ supplements had lower rectal temperature (RT), respiratory rate (RR), and pulse rate (PR), because of a decrease in cortisol, indicating increased thermotolerance and performance (33, 34(. In addition, animals in the control group were unable to dissipate heat efficiently due to the high-temperature humidity index, resulting in increasing in rectal temperature. The increase in rectal temperature with an infusion of amino

acids could be related to protein and amino acid metabolism, which produces more heat as compared to fat and carbohydrate metabolism (36).

Nutrient digestibility

The majority of nutrients' digestibility rates were increased by the dietary addition of eubiotic to growing calves, especially crude fiber and its fractions, which may be related to its role in the establishment a healthy ruminal microflora and maintaining a ruminal pH to be more appropriate for ruminal digestion (37). The eubiotic contain multi-enzymes like cellulases, xylanase, and pectinase that have been found to increase fiber digestibility compared to components containing a single enzyme (38). Such additions enhanced the bacterial adhesion, stimulated the rumen microbiota, and interacted with other ruminal microorganisms. Eubiotic decreased the digesta viscosity and creates anaerobic conditions for cellulolytic bacteria, as well as provides vital nutrients for microbial activity and growth in the rumen (3, 37). Our findings are consistent with those of Sallam et al. (24), who found that adding eubiotics to sheep diets enhanced NDF and ADF digestibility by roughly 10% and 7.9%, respectively. The improvement in OM, NDF and ADF in IVAAI supplemented calves in comparison with the control group coincided with the results of many previous studies who found that supplementing protected proteins and amino acids to ruminant animals fed poor quality forages enhanced feed intake and nutrient digestibility (39, 40).

Rumen fermentation activities

The purpose of eubiotic supplementation in the diets of calves is based mainly on its positive effects on rumen fermentation. The components of eubiotics are bacteria, yeast, and exogenous enzymes are thought to have the potential to maintain ruminal pH and VFA, particularly lactate (3, 37). The rumen pH was increased when the eubiotics were added to the diet. This could be because probiotics stabilize rumen-dominant bacteria that consume more ruminal lactate, and consequently stabilize rumen pH at the range of (6.6 – 6.8) (41). The main benefits of probiotics for ruminants were improved ruminal digestion by increasing rumen pH (42), fiber digestion (43), and the production of microbial proteins (44). Probiotics decrease the concentration of rumen organic acids and may decrease the risk of SARA (subacute ruminal acidosis) (45). The higher ammonia nitrogen concentration in the rumen fluid of growing calves fed a diet containing eubiotics or intravenously infused with IVAAI may be attributed to the conversion of peptides and amino acids to ammonia by microbial activity. The energy needed for microbial protein synthesis is insufficient, and not all ammonia is converted into protein lead to elevation of rumen ammonia nitrogen (46). On the other side, the intravenous infusions of AA to growing calves improved the ruminal fermentation and production of VFA. Intravenous supplementation with amino acids could alter N recycling and thus potentially affect rumen microbial fermentation especially when the diet was deficient in RDP (47). These findings were agreed with those of Russell et al. (48) who found that adding free amino acids to the rumen ecosystem could be an important source of nitrogen and improves ruminal fermentation.

Rumen enzyme activities

The efficiency of the enzyme system found in the gastrointestinal tract determines how well animals digest and utilize nutrients present in feeds (49). Heat stress could affect the rumen microbial composition and metabolism (50). However, it was shown that adding microbial feed additives to the heat-stressed calves improved enzyme activity. This improvement in the enzyme activity might be attributed to an increase in the rumen's hydrolytic capability, owing to enhanced bacterial attachment, rumen microbial population stimulation, and synergistic actions with ruminal microorganism hydrolases (24, 51), since these enzymes are secreted by microbes in the GI tract (48). Eubiotics contain enzymes such as cellulases, xylanase, and pectinase, has been shown to improve microbial activity and rumen enzyme activity. These results could indicate a change in the colonizing bacteria's species profile because of the pre-feeding enzyme treatment of the feeds (6). Also, increases cellulolytic activity with enzyme treatment *in vivo* (52).

Conclusion

From the current study's findings, it could be concluded that both dietary supplementation of

eubiotics and intravenous amino acid infusions (IVAAI) improved the growth of buffalo calves through improving the digestibility rate of nutrients (particularly of cell wall constituents), rumen fermentation, and rumen enzyme **activities.** However, the addition of dietary eubiotics induced superior positive effects than IVAAI on all studied parameters, and ameliorated the harmful effects of thermal stress in growing buffalo calves.

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