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Research Article

Evaluation of Se, Cr and Zn-enriched Yeast Culture in Improving *in vitro* Fermentation Characteristics of Cereal Straws

Liang CHEN [1,2] Bin LI [3] Ao REN [1,2] Zhiwei KONG [1] Zhiliang TAN [1,4] Mahmoud ALAGAWANY [5,a]
Mohamed EZZAT ABD EL-HACK [5,b] Chuanshe ZHOU [1,6]

[1] Key Laboratory for Agro-Ecological Processes in Subtropical Region, and Hunan Research center of Livestock & Poultry Sciences, and South-Central Experimental Station of Animal Nutrition and Feed Science in Ministry of Agriculture, Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Changsha, Hunan 410125, P.R. CHINA
[2] College of Animal Science and Technology, Hunan Agricultural University, Changsha, Hunan 410125, P.R. CHINA
[3] Institute of Animal Science of Tibet Academy of Agricultural and Animal Husbandry Sciences, Lhasa 850000, CHINA
[4] Hunan Co-Innovation Center of Animal Production Safety, CICAPS, Changsha, Hunan 410125, P.R. CHINA
[5] Department of Poultry, Faculty of Agriculture, Zagazig University, Zagazig 44511, EGYPT
[6] Postdoctoral Science Research Workstation, New Hope Group, Chengdu, Sichuan 610041, P.R. CHINA
[a] ORCID: 0971-8020-0002-0000; [b] ORCID: 0000-0002-2831-8534

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Abstract

The object of this study was to evaluate the effect of different sources and supplementation levels of yeast culture on *in vitro* fermentation characteristics of goats. The present study was performed in a 3×4 factorial design to examine the impacts of inclusion of three kinds of yeast culture (Se-enriched, Cr-enriched, Zn-enriched yeast culture) at four dose (0, 0.1%, 0.25%, 0.40% and 0.55%) on the *in vitro* fermentation characteristics of cereal straw. For maize stover, the results shown that the average values of *in vitro* gas production, DM and NDF disappearance, pH and the ratio of acetate to propionate were increased ($P<0.05$), while the concentration of ammonia nitrogen (NH_3-N) and butyrate were decreased ($P<0.05$) by the supplementation of Se-enriched yeast culture (YC-Se) compared with that of Cr-enriched yeast culture (YC-Cr) and Zn-enriched yeast culture (YC-Zn). For rice straw, the in vitro gas production, DM disappearance and pH were increased ($P<0.05$), and the concentration of ammonia nitrogen was decreased by the supplementation of YC-Se, while the concentration of acetate, propionate, butyrate and total VFA (TVFA) were increased ($P<0.05$) by the supplementation of YC-Zn. The current results indicate that YC-Se is more preferred as yeast culture supplements, and its optimal dose should be 0.25% substrates for maize stover and 0.10% substrates for rice straw *in vitro*. The present positive *in vitro* results should be tested using *in vivo* experiments in future.

Keywords: *Yeast culture, in vitro fermentation, Maize stover, Rice straw*

Tahıl Samanının *in vitro* Fermantasyon Özelliklerini Geliştirmede Se, Cr ve Zn İle Zenginleştirilmiş Maya Kültürünün Değerlendirilmesi

Öz

Bu çalışmanın amacı, farklı kaynak ve katkı düzeylerindeki maya kültürünün *in vitro* fermantasyon özelliklerine etkilerini değerlendirmektir. Çalışma üç çeşit maya kültürünün (Se ile zenginleştirilmiş, Cr ile zenginleştirilmiş ve Zn ile zenginleştirilmiş) dört farklı dozda (%0.01, %0.25, %0.40 ve %0.55) dahil edilmesinin mısır samanının *in vitro* fermantasyon özellikleri üzerine etkilerini incelemek için 3 × 4 faktöriyel tasarımda gerçekleştirilmiştir. Mısır samanı için, Cr ile zenginleştirilmiş maya kültürü ve Zn ile zenginleştirilmiş maya kültürü ile karşılaştırıldığında Se ile zenginleştirilmiş maya kültüründe in vitro gaz üretimi, DM ve NDF kaybolması, pH ortalama değerleri ile asetat propiyonat oranı artarken ($P<0.05$), amonyum nitrojen (NH_3-N) ve bütrat konsantrasyonlarında düşme ($P<0.05$) şekillendi. Pirinç samanı için, Se ile zenginleştirilmiş maya kültüründe *in vitro* gaz üretimi, DM kaybolması ve pH düşerken ($P<0.05$), amonyum nitrojen konsantrasyonu azaldı. Zn ile zenginleştirilmiş maya kültüründe asetat, propiyonat, bütrat ve total VFA (TVFA) arttı ($P<0.05$). Elde edilen sonuçlar, maya kültürü katkı maddesi olarak Se ile zenginleştirmenin daha ziyadesi ile tercih edilmesi gerektiğini ve *in vitro* optimal dozun mısır samanı için %0.25 ve pirinç samanı için %0.10 olması gerektiğini göstermiştir. Elde edilen *in vitro* sonuçlar *in vivo* çalışmalar ile test edilmelidir.

Anahtar sözcükler: *Maya kültürü, in vitro fermantasyon, Mısır posası, Pirinç posası*

İletişim (Correspondence)
+86 731 84615236; Fax: +86 731 84612685
zcs@isa.ac.cn

INTRODUCTION

During recent years, yeast culture has been used to enhance the utilization efficiency and nutritive value of low-quality roughages [1]. The supplementation of yeast culture to ruminant diets can increase dry matter intake (DMI), nutrient digestibility, cellulose degradation and production performance [1-4]. Many *in vitro* studies showed that yeast culture significantly altered the fermentation of mixed ruminal microorganism and activated digestion of cellulose through pure cultures of predominant ruminal bacteria [1,5,6].

Again, yeast has been a favorite cultivation medium which allows the incorporation of Zn, Cr and Se and other metals, such as Fe, Mn and Cu into biomass, in addition to other unicellular organisms such as Spirulina *(Arthrospira platensis)* and *Lactobacilli plantarum* [7]. Cr-, Zn-, and Se-enriched yeast cultures were produced by growing specific strains of yeast in Cr-, Zn-, and Se-enriched media. Ortman and Pehrson [8] reported that organic Se from Se-enriched yeast is an ideal supplement because animals retain and absorb it more efficient than the inorganic form of Se. Zn-enriched yeast is naturally integrated by the growing yeast into its own structure to improve the bioavailability of Zn and reduce the sides effects of Zn [9]. In mammals, chromium (Cr) has been recognized to be a biologically essential trace element. Researchers have reported that Cr played an essential role in carbohydrates and fats metabolism because of increasing the action of insulin when given to people [10,11]. In recent years, most reports focused on the effects of Se-enriched, Cr-enriched and Zn-enriched yeast supplementation on growth performance, physiology and biochemistry in animals [12-16]. While there is little available information about the use of Se-enriched, Cr-enriched and Zn-enriched yeast in *in vitro* or *in vivo* digestibility in goats or other ruminants. Therefore, the objective of the present study was to evaluate the effect of different sources and supplementation levels of yeast culture on *in vitro* fermentation characteristics of goats.

MATERIAL and METHODS

The present experiment was approved by the Animal Care Committee, Institute of Subtropical Agriculture (ISA), Chinese Academy of Sciences, Changsha, China. The experiment was performed during 2016.

Crop Straws, Yeast Culture and Experimental Design

Two kinds of crop straws, i.e., rice straw from Xiang 125s (a popular local breed) maize and stover from Kexiangtian 1 (bred by ISA), were selected as *in vitro* fermentation substrates. Straws were dried at 65ºC for 24 h, ground through a 1 mm sieve and stored in plastic bag until assay. Maize stover and rice straw contained (DM basis): 5.3% and 6.2% crude protein (CP), 63.6% and 63.2% neutral detergent fiber (NDF), and 38.6% and 43.4% acid detergent fiber (ADF), respectively.

Yeast cultures were purchased from Angel Yeast Co., Ltd (Yichang city, Hubei Province, China). The indexes of the three kinds of yeast cultures were as following: Zn-enriched yeast (YC-Zn): 2000 ppm, content of Zn element was 2479.52 mg/kg and water content was 3.81%. Se-enriched yeast (YC-Se): 2000 ppm, content of Se element was 0.2% and water content was less than 6.0%. Cr-enriched yeast (YC-Cr): 2000 ppm, content of Cr element was 2091.1 mg/kg and water content was less than 4.0%.

The experiment followed a blocked experimental design; each species of yeast culture was supplemented at five doses: 0%, 0.1%, 0.25%, 0.40% and 0.55% of fermentation substrates respectively, no addition of yeast culture (0%) was taken as control group.

In Vitro Gas Production and Sampling

Culture solutions, i.e., macro-element, buffered and reducing solutions used for *in vitro* fermentation, were prepared to form artificial saliva according to the procedures modified by Tang et al.[17]. The artificial saliva was maintained in an anaerobic environment by continuously pumping CO_2 around it for 2 h. Rumen fluids were collected before the morning feeding, from three rumen-cannulated *Xiangdong* black goats (a popular local goat, fed a rice straw based total mixed ration, the ingredients and chemical composition of diets were presented in *Table 1)*, and immediately transported to the laboratory. Rumen contents were strained through four layers of cheesecloth under a continuous flow of CO_2. Rumen fluids (5 mL) and artificial saliva (45 mL) were placed in pre-warmed (39°C) 145 mL fermentation bottles.

A sample of 500±5 mg of each straw type was placed in the 145 mL fermentation bottles. Each sample was measured three times at each incubation time point. Every species of yeast culture was added to the straw substrates based on experimental design when the *in vitro* fermentation was started.

All fermentation bottles were connected to pressure sensors and incubated at 39°C. Fermentation bottle pressure was recorded at 0, 1, 2, 4, 6, 12, 24, 36 and 48 h during the *in vitro* fermentation process. After 12, 24 or 48 h of incubation, fermentation was interrupted. Undegraded residues were filtered through 2 layers of nylon cloth (40-um pore size). The incubation solution from each treatment was sampled to determine NH_3-N and VFA concentrations at 12, 24 and 48 h, respectively.

Chemical Analysis

The DM (method 930.15) was analyzed using procedures from the Association of Official Analytical Chemists [18]. The NDF content was determined using a Fibretherm Fiber Analyzer (Gerhardt, Bonn, Germany) following Van Soest et al.[19] with the addition of sodium sulfite and alpha-amylase in the NDF analysis. The filtered residue was dried

Table 1. *Ingredients and chemical composition of the basal diets offered to goats (g/kg DM)*

Items	Diet
Dietary ingredient (g/kg DM)	
Forage	
Rice straw	300
Concentrate	
Soybeans	60
Maize	298
Wheat bran	280
$CaCO_3$	10
Fat	8
NaCl	10
Urea	14
Premix	20
Chemical composition (g/kg DM)	
DM	965
Organic matter	918
Ash	86.3
Crude protein	161
Starch	252
Neutral detergent fiber	332
Acid detergent fiber	118
Gross energy (MJ/kg)	17.2

Premix was formulated to provide the following (per kg of premix): 400 g of $NaHCO_3$, 2 g of Fe, 1 g of Cu, 0·01 g of Co, 0·05 g of I, 6·6 g of Mn, 4·4 g of Zn, 0·003 g of Se, 333 mg of retinol, 5 mg of cholecalciferol, 838 mg of α-tocopherol

at 105°C for 12 h and weighed to determine *in vitro* dry matter disappearance (IVDMD). The NDF content in the dried residues was determined to calculate *in vitro* NDF disappearance (IVNDFD).

VFA was measured as Wang et al.[20] described. Total molar concentration was calculated by taking the sum of individual VFA as 1. NH_3-N concentration was measured as Wang et al.[21].

Calculation and Statistical Analysis

The correlation between fermentation bottle pressure and gas volume was measured at 39°C, 20 bottles were used to determine the content in the equation**,** and the following regression equation was established:

$y=1.506x$ (n=20, $R^2=0.999$, $P<0.0001$) (1)

Where *y* represents gas volume (mL), *x* is bottle pressure (kPa), and 1.506 is a constant. Pressure measurements were then converted to gas production (mL). The following Logistic-Exponential equation [22] was fitted to *in vitro* gas production at 0, 1, 2, 4, 6, 12, 24, and 48 h:

$$GP = Vf * \frac{1-\exp(d-t*k)}{1+\exp(b-k*t)} \quad (2)$$

Where GP represents gas production at *t* time, *Vf* is the maximum gas production (ml), *k* is the gas production fraction (/h), *b* and *d* is the shape of the gas production curve. The following equation was used to calculate the elapsed time ($T_{0.5}$, h) until half of the maximum gas production was achieved [22].

$$T_{0.5} = \frac{In\left(\exp(b)+2\exp(d)\right)}{k} \quad (3)$$

FRD_0 was used to calculate the initial fractional rate of degradation (/h) as follows [22]:

$$FRD_0 = \frac{k}{1+\exp(b)} \quad (4)$$

Gas production (GP), IVDMD and IVNDFD were corrected by subtracting values obtained for the blanks.

Statistical Analysis

Data were analyzed by straw substrate using the PROC MIXED procedure in SAS software. For the statistical analyses of gas production parameters, the model included species, supplementation level and their interaction as fixed effects. For the analyses of pH, NH_3-N, VFAs, IVDMD and IVNDFD, the model included species, supplementation level and their interaction as fixed effects with incubation time as a repeated factor. Linear and quadratic effects of supplementation level were analyzed using orthogonal polynomial contrasts. Cubic effects of supplementation level were not examined due to inexplicability in a biological context. Least squares means are reported throughout the text, and significance was declared at $P<0.05$.

RESULTS

In Vitro Gas Production Parameters

Influences of different yeast culture supplementation on *in vitro* gas production parameters of maize stover and rice straw were listed in *Table 2a* and *Table 2b*, respectively. When selected maize stover as fermentation substrates, the average V_f value of YC-Se was significantly higher ($P<0.05$) compared to YC-Zn group. The largest V_f of YC-Cr was 82.41 mL, which was obtained at the supplementation dose of 0.25%, it is 13.23% higher than that of control group, and presented cubic effect with the dose increased ($P<0.01$). For YC-Zn group, the largest V_f was obtained when the supplemental dose was 0.10%, it is higher compared to that of control group, and there was cubic effect when the dose improved ($P<0.01$). The largest V_f was for the YC-Se group at the supplementation dose of 0.25%, and there was linear effect ($P<0.05$) with the dose improved. The average $T_{0.5}$ values of YC-Cr and YC-Zn group were significantly lower ($P<0.0001$) than that of the YC-Se group. When YC-Se supplementation dose improved,

Table 2a. *Effects of three kinds of yeast cultures supplementation on in vitro gas production parameters of maize stover*

Items[1]	Species[2]	Dose (%)						SEM[‡]	Significance (*P*<)[§]		
		Mean[†]	0	0.10	0.25	0.40	0.55		Species	Dose	Species × Dose
V_f (mL)	YC-Cr	76.58^{ef}	72.78^{bc}	69.35^{c}	82.41^{a}	80.25^{a}	78.12^{a}	1.54	<0.05	C(<0.01)	<0.01
	YC-Se	77.81^{e}	72.78^{b}	77.86^{ab}	80.43^{a}	79.20^{a}	78.83^{b}			L(<0.05)	
	YC-Zn	74.94^{f}	72.78^{b}	76.77^{a}	74.16^{ab}	75.33^{ab}	75.68^{ab}			C(<0.01)	
	SEM[¶]	0.69									
$T_{0.5}$ (h)	YC-Cr	14.58^{f}	14.21	15.57	12.52	13.99	16.59	0.70	<0.0001	NS	<0.05
	YC-Se	16.67^{e}	14.21^{b}	16.60^{a}	16.78^{a}	18.09^{a}	17.65^{a}			L(<0.0001)	
	YC-Zn	14.46^{f}	14.21	14.37	14.39	14.67	14.65			NS	
	SEM[¶]	0.31									
FRD_0 ($\times 10^{-2}$) mL/h	YC-Cr	3.82^{e}	2.83^{b}	3.69^{b}	4.91^{a}	4.33^{a}	3.32^{b}	0.19	<0.0001	Q(<0.05)	<0.0001
	YC-Se	2.83^{f}	2.83	2.77	2.69	2.71	3.13			NS	
	YC-Zn	2.88^{f}	2.83	2.86	2.79	2.92	3.00			NS	
	SEM[¶]	0.09									

[1] Vf, maximum gas production (mL); FDR_0, initial fractional rate of degradation at t-value=0; $T_{0.5}$, the elapsed time until half of the maximum gas production was achieved; [2] YC-Cr=Zn-enriched yeast culture; YC-Se=Se-enriched yeast culture; YC-Zn=Zn-enriched yeast culture; [ac] Means within a row for doses that do not have a common superscript differ (P<0.05); [e,f] Means within a column for species that do not have a common superscript differ (P<0.05); [†] Mean=mean for individual species across doses including the dose of 0; [‡] SEM for yeast culture dose; [§] NS, not significant (P>0.05); L, linear effect of dose, Q, quadratic effect of dose, C, cubic effect of dose; [¶] SEM for pooled mean of species including the dose of 0

Table 2b. *Effects of three kinds of yeast cultures supplementation on in vitro gas production parameters of rice straw*

Items[1]	Species[2]	Dose (%)						SEM[‡]	Significance (*P*<)[§]		
		Mean[†]	0	0.10	0.25	0.40	0.55		Species	Dose	Species × Dose
V_f (mL)	YC-Cr	71.77^{e}	72.68	69.76	72.36	72.47	72.60	2.35	<0.01	NS	NS
	YC-Se	72.58^{e}	72.78^{ab}	75.66^{a}	74.91^{ab}	70.86^{ab}	68.72^{b}			Q(<0.05)	
	YC-Zn	67.78^{f}	72.68	70.16	62.30	65.68	68.06			NS	
	SEM[¶]	1.05									
$T_{0.5}$ (h)	YC-Cr	17.97	22.08^{a}	16.78^{b}	16.45^{b}	16.07^{b}	18.46^{ab}	1.13	<0.0001	L(<0.001)	NS
	YC-Se	21.93	22.08	23.03	22.56	21.64	20.34			NS	
	YC-Zn	19.01	22.08^{a}	21.06^{ab}	17.25^{b}	17.15^{b}	17.50^{b}			L(<0.05)	
	SEM[¶]	0.51									
FRD_0 ($\times 10^{-2}$) mL/h	YC-Cr	2.35^{e}	1.79^{b}	1.93^{b}	2.79^{a}	2.84^{a}	2.41^{b}	0.21	<0.001	L(<0.001)	NS
	YC-Se	1.74^{f}	1.79	1.70	1.86	1.69	1.65			NS	
	YC-Zn	1.94^{f}	1.79	1.59	2.43	1.94	1.93			NS	
	SEM[¶]	0.09									

[1] Vf, maximum gas production (mL); FDR_0, initial fractional rate of degradation at t-value=0; $T_{0.5}$, the elapsed time until half of the maximum gas production was achieved; [2] YC-Cr=Zn-enriched yeast culture; YC-Se=Se-enriched yeast culture; YC-Zn=Zn-enriched yeast culture; [ab] Means within a row for doses that do not have a common superscript differ (P<0.05); [e,f] Means within a column for species that do not have a common superscript differ (P<0.05); [†] Mean = mean for individual species across doses including the dose of 0; [‡] SEM for yeast culture dose; [§] NS, not significant (P>0.05); L, linear effect of dose, Q, quadratic effect of dose; [¶] SEM for pooled mean of species including the dose of 0

the $T_{0.5}$ presented linear increasing effect (P<0.0001). For YC-Cr group, the greater value was obtained at the levels of 0.55%, and higher (P<0.05) than that of 0.25%. No significant difference (P>*0.05*) on the value of $T_{0.5}$ when YC-Zn supplementation dose increased. For FRD_0 of *in vitro* gas production, the FRD_0 value of YC-Cr group was 34.98% and 32.64% higher than that of YC-Se and YC-Zn, respectively (P<0.0001). With the supplemental level of YC-Cr, the FRD_0 presented quadratic effect (P<0.05). There were no significant difference in the value of FRD_0 when YC-Se and YC-Zn supplementation dose increased (P>0.05). Besides, there were interactive effects on V_f $T_{0.5}$ and FRD_0 for the three yeast culture (P<0.05).

For rice straw, the average V_f of YC-Cr and YC-Se was 5.89% and 7.08% higher than that of YC-Zn group, respectively (P<0.01). The V_f of YC-Se increased when the dose of YC-Se increased (quadratic, P<0.05), and the largest value was 75.66 mL, which was obtained at the supplementation level of 0.10%. While there were no significant difference on V_f when YC-Cr and YC-Zn supplementation level increased (P>0.05). There was no significant difference in $T_{0.5}$ when supplemented with three sources of yeast culture (P>0.05). $T_{0.5}$ increased when the dose of YC-Cr supplementation level increased (linear, P<0.01), while $T_{0.5}$ decreased when the dose of YC-Zn supplementation level increased (linear, P<0.01). Additionally, no significant

Table 3. *Effects of three kinds of yeast cultures on in vitro IVNDFD and IVDMD of maize stover and rice straw*

Items[1]	Species[2]	Dose (%)						SEM[‡]	Significance (*P*)[§]		
		Mean[†]	0	0.10	0.25	0.40	0.55		Species	Dose	Species × Dose
Maize stover											
IVNDFD (%)	YC-Cr	36.44^{f}	37.55	36.35	37.15	36.48	34.64	1.02	<0.01	NS	NS
	YC-Se	38.86^{e}	38.66	39.34	38.70	39.11	38.46			NS	
	YC-Zn	37.80^{ef}	38.66	36.88	38.66	37.63	37.17			NS	
	SEM[¶]	0.46									
IVDMD (%)	YC-Cr	51.79^{f}	51.60	52.27	52.80	48.70	53.59	1.25	<0.05	NS	NS
	YC-Se	53.86^{e}	51.62^{b}	54.64^{a}	54.75^{a}	54.46^{a}	53.86^{ab}			L(*<0.05*)	
	YC-Zn	52.27^{ef}	51.58	51.62	51.84	53.31	52.96			NS	
	SEM[¶]	0.56									
Rice straw											
IVNDFD (%)	YC-Cr	35.42	35.16	35.02	36.36	34.95	35.59	1.02	NS	NS	<0.05
	YC-Se	34.22	35.18^{ab}	36.22^{a}	33.69^{ab}	32.66^{b}	33.39^{ab}			L(*<0.05*)	
	YC-Zn	35.31	35.16^{ab}	33.81^{b}	34.46^{ab}	37.73^{a}	35.39^{ab}			Q(*<0.01*)	
	SEM[¶]	0.59									
IVDMD (%)	YC-Cr	45.79^{f}	47.40	45.25	45.68	45.33	45.30	0.81	<0.01	NS	NS
	YC-Se	47.47^{e}	47.41	48.31	48.01	46.98	46.68			NS	
	YC-Zn	46.40^{ef}	47.39^{a}	47.03^{a}	46.25^{ab}	46.43^{ab}	44.88^{b}			*<0.05*	
	SEM[¶]	0.36									

[1] IVDMD, in vitro dry matter disappearance; IVNDFD, in vitro neutral detergent fiber disappearance; [2] YC-Cr=Zn-enriched yeast culture; YC-Se=Se-enriched yeast culture; YC-Zn=Zn-enriched yeast culture; [ab] Means within a row for doses that do not have a common superscript differ (P<0.05); [e,f] Means within a column for species that do not have a common superscript differ (P<0.05); [†] Mean=mean for individual species across doses including the dose of 0; [‡] SEM for yeast culture dose; [§] NS, not significant (P>0.05); L, linear effect of dose; Q, quadratic effect of dose; [¶] SEM for pooled mean of species including the dose of 0

difference was observed when YC-Se supplementation dose increased (P>0.05). For *in vitro* FRD_0 of rice straw, the YC-Cr group was 35.06% and 21.13% higher than that of the YC-Se and YC- Zn (P<0.001). FRD_0 increased when the dose of YC-Cr supplementation level increased (linear, P<0.001). There was no significant difference in FRD_0 when YC-Se and YC-Zn dose increased (P>0.05). Besides, there was no interactive effects on FRD_0 for the three sources of yeast culture (P>0.05).

In Vitro NDFD and DMD

The influence of three sources of yeast culture on IVNDFD and INDMD of maize stover and rice straw was shown in *Table 3*. IVDMD and IVNDFD were affected by three yeast culture supplementation for maize stover (P<0.05), IVNDFD and IVDMD for YC-Se were higher by 2.42% and 2.07% compared with YC-Cr treatment, while there was no significant difference between YC-Se and the other two yeast culture supplemented treatments (P>0.05). IVNDFD were not affected (P>0.05) by the supplementation levels of three yeast culture, while the IVDMD of the YC-Se group increased when the dose of YC-Se supplementation level increased (linear, P<0.05).There were no interactive effects (P>0.05) on IVDMD or IVNDFD for maize stover.

For rice straw, the average IVNDFD was not affected by the supplementation of three sources of yeast culture (P>0.05), while IVDMD of YC-Se was significantly higher than that of YC-Cr treatment (P<0.05). IVNDFD of rice straw decreased linearly with the increased YC-Se supplementation levels (P<0.05) as shown in *Table 2*. The largest IVNDFD of rice straw was observed when YC-Zn supplemented at 0.40%, and it increased quadratically with the increased supplementation levels (P<0.01). Compared to YC-Cr treatment, IVDMD of rice straw increased by 1.68% when added YC-Se, but the lower IVDMD for the YC-Zn group was observed at the levels of 0.55%. No significant difference was observed in IVDMD by the supplementation levels of YC-Se and YC-Cr (P>0.05). There were interactive effects (P<0.05) on IVNDFD for rice straw.

In Vitro NH_3-N Concentration and pH

Effects of different yeast culture supplementation levels on *in vitro* NH_3-N concentration and pH were shown in *Table 4*. For maize stover, *in vitro* NH_3-N concentration was affected significantly by the supplementation of three yeast culture (P<0.05), the NH_3-N concentration of YC-Cr and YC-Zn were 35.14% and 32.07% higher than that of YC-Se (P<0.0001). As the *Table 3* described, the highest NH_3-N concentration of maize stover which obtained at the YC-Cr supplementation level of 0.10%, and it was 24.85% higher than the lowest concentration which obtained at the supplementation level of 0.55% (P<0.05). The NH_3-N concentration of control group was significantly higher than that of the other four groups after adding YC-Se, and presented cubic effect with the supplementation levels increased (P<0.01). The lowest (P<0.05) NH_3-N concentration for YC-Zn group was obtained at the levels

Table 4. *Effects of three sources of yeast cultures on in vitro pH and NH_3-N concentration of maize stover and rice straw*

Items[1]	Species[2]	Dose (%)						SEM[‡]	Significance (*P*)[§]		
		Mean[†]	0	0.10	0.25	0.40	0.55		Species	Dose	Species × Dose
Maize stover											
NH_3-N (mg/dL)	YC-Cr	7.46^{e}	7.78^{ab}	8.14^{a}	7.58^{ab}	7.26^{ab}	6.52^{b}	0.36	<0.0001	*<0.05*	<0.0001
	YC-Se	5.52^{f}	7.78^{a}	4.12^{b}	4.96^{b}	5.49^{b}	5.24^{b}			C(*<0.01*)	
	YC-Zn	7.29^{e}	7.78^{a}	7.30^{ab}	7.21^{ab}	7.64^{a}	6.50^{b}			*<0.05*	
	SEM[¶]	0.16									
pH	YC-Cr	6.38^{f}	6.38	6.38	6.39	6.38	6.38	0.007	<0.0001	NS	<0.0001
	YC-Se	6.43^{e}	6.38^{b}	6.44^{a}	6.43^{a}	6.45^{a}	6.45^{a}			C(*<0.05*)	
	YC-Zn	6.38^{f}	6.38^{ab}	6.39^{a}	6.38^{ab}	6.37^{ab}	6.37^{b}			L(*<0.05*)	
	SEM[¶]	0.003									
Rice straw											
NH_3-N (mg/dL)	YC-Cr	5.06^{ef}	5.64^{a}	5.21^{ab}	4.78^{ab}	5.17^{ab}	4.53^{b}	0.32	<0.0001	*<0.05*	<0.05
	YC-Se	4.70^{f}	5.64^{a}	5.22^{ac}	4.62^{abc}	3.84^{b}	4.18^{bc}			L(*<0.0001*)	
	YC-Zn	5.57^{e}	5.64	5.25	5.67	5.29	6.01			NS	
	SEM[¶]	0.14									
pH	YC-Cr	6.36^{f}	6.35^{b}	6.36^{ab}	6.36^{ab}	6.37^{a}	6.37^{a}	0.004	<0.0001	L(*<0.0001*)	<0.0001
	YC-Se	6.48^{e}	6.35^{b}	6.52^{a}	6.52^{a}	6.52^{a}	6.52^{a}			C(*<0.0001*)	
	YC-Zn	6.36^{f}	6.35^{b}	6.35^{b}	6.37^{a}	6.36^{ab}	6.38^{a}			C(*<0.01*)	
	SEM[¶]	0.002									

[1] NH_3-N=ammonia nitrogen; [2] YC-Cr=Zn-enriched yeast culture; YC-Se=Se-enriched yeast culture; YC-Zn=Zn-enriched yeast culture; [ac] Means within a row for doses that do not have a common superscript differ (P<0.05); [e,f] Means within a column for species that do not have a common superscript differ (P<0.05); [†] Mean = mean for individual species across doses including the dose of 0; [‡] SEM for yeast culture dose; [§] NS, not significant (P>0.05); L, linear effect of dose; Q, quadratic effect of dose; C, cubic effect of dose; [¶] SEM for pooled mean of species including the dose of 0

of 0.55%. The pH value of *in vitro* fermentation fluid of maize stover was also affected by adding yeast culture. It was significantly higher for YC-Se than that of the other two kinds of yeast culture (P<0.0001), and the pH value of the control group was significantly lower than that of the another four groups for YC-Se supplemented treatments (P<0.05), the pH of YC-Zn group at the supplementation level of 0.25% was higher (P<0.01) than that of 0.55%, besides, there were cubic effect (P<0.05) and linear effect (P<0.05) with the supplementation dose increased, respectively. There were interactive effects (P<0.0001) on NH_3-N concentration and pH value with the increased addition doses of yeast culture for maize stover.

For rice straw, NH_3-N concentration in *in vitro* fermentation fluid was not affected when supplemented with yeast culture. However, The NH_3-N concentration of rice straw for YC-Se was affected when the supplementation dose changed, the highest NH_3-N concentration was observed at the control group and presented linear decreasing effects with the increased supplementation level (P<0.0001), the lowest (P<0.05) NH_3-N concentration was obtained at the supplementation level of 0.55%. pH in *in vitro* fermentation fluid of rice straw was affected when supplemented with yeast culture. The pH value for YC-Se was significant higher than that of another two sources of yeast culture (P<0.0001). Besides, different supplementation levels also significantly influenced the pH value in *in vitro* fermentation of rice straw. From the *Table 3*, we known that YC-Cr and YC-Se presented linear and cubic increased effects, respectively (P<0.0001). For YC-Zn, it also presented cubic increased effect with increased levels (P<0.01). Additionally, there was interactive effect on NH_3-N concentration and pH with supplementation levels changed for rice straw (P<0.05).

In Vitro VFA Concentration

Effects of YC-Cr, YC-Se and YC-Zn supplementation on *in vitro* VFA concentration in fermentation fluid of maize stover and rice straw were shown in *Table 5a* and *5b*, respectively. For maize stover, the average acetate, propionate and TVFA concentration were not affected by three sources of yeast culture supplementation (P>0.05). It presented linear increasing effects on acetate, propionate, butyrate and TVFA concentration with increased supplementation dose for YC-Cr (P<0.01), while it presented a cubic effect on A: P (P<0.01). For YC-Se group, the largest acetate, propionate, TVFA concentration and A: P were observed at the supplementation dose of 0.55%, and presented linear increasing effects on acetate and TVFA concentration (P<0.05); For YC-Zn treatment. The largest acetate, TVFA concentration and were observed at the supplementation dose of 0.1%.While the largest propionate and butyrate concentration were observed at the supplementation dose of 0.55%. All of them presented cubic effect (P<0.01) with dose increased and without interactive effects (P<0.01) except A: P which presented quadratic effect (P<0.001).

For rice straw, effects of YC-Cr, YC-Se and YC-Zn on VFA

Table 5a. *Effects of three sources of yeast cultures on VFA concentration of in vitro incubation fluid of maize stover*

Items[1]	Species[2]	Dose (%)						SEM[‡]	Significance (*P*)[§]		
		Mean[†]	0	0.10	0.25	0.40	0.55		Species	Dose	Species × Dose
Acetate (mmol/L)	YC-Cr	21.05	17.66^{b}	21.87^{ab}	21.68^{ab}	22.83^{a}	21.18^{ab}	1.34	NS	L(<0.01)	NS
	YC-Se	21.73	17.67^{b}	21.53^{b}	23.47^{ab}	22.67^{ab}	23.70^{a}			L(<0.05)	
	YC-Zn	20.65	17.66^{b}	23.41^{a}	18.44^{b}	21.44^{ab}	22.27^{a}			C(<0.01)	
	SEM[¶]	0.59									
Propionate (mmol/L)	YC-Cr	6.81	5.37^{b}	7.15^{a}	7.05^{a}	7.49^{a}	6.97^{a}	0.43	NS	L(<0.01)	NS
	YC-Se	6.38	5.37^{b}	6.34^{ab}	6.86^{a}	6.47^{ab}	6.85^{a}			<0.05	
	YC-Zn	6.48	5.37^{b}	7.08^{ab}	5.58^{ab}	6.99^{ab}	7.36^{a}			C(<0.01)	
	SEM[¶]	0.19									
Butyrate (mmol/L)	YC-Cr	2.02^{e}	1.56^{b}	2.09^{a}	2.10^{a}	2.20^{a}	2.13^{a}	0.13	<0.05	L(<0.01)	NS
	YC-Se	1.79^{f}	1.56	1.53	2.08	1.81	1.98			NS	
	YC-Zn	1.93^{ef}	1.56^{b}	2.13^{a}	1.74^{ab}	2.02^{ab}	2.19^{a}			C(<0.01)	
	SEM[¶]	0.06									
TVFA (mmol/L)	YC-Cr	30.37	24.98^{b}	31.65^{ab}	31.35^{ab}	33.06^{a}	30.80^{ab}	1.93	NS	L(<0.01)	NS
	YC-Se	30.35	24.98^{b}	29.76^{ab}	32.81^{a}	30.93^{ab}	33.27^{a}			L(<0.05)	
	YC-Zn	29.53	24.98^{c}	33.14^{a}	26.17^{bc}	30.99^{ab}	32.34^{a}			C(<0.01)	
	SEM[¶]	0.86									
A:P	YC-Cr	3.14^{g}	3.33^{a}	3.09^{b}	3.09^{b}	3.09^{b}	3.10^{b}	0.05	<0.0001	C(<0.01)	<0.0001
	YC-Se	3.46^{e}	3.33	3.47	3.45	3.49	3.53			NS	
	YC-Zn	3.24^{f}	3.33^{a}	3.35^{a}	3.34^{a}	3.14^{b}	3.05^{b}			Q(<0.001)	
	SEM[¶]	0.02									

[1] TVFA, total volatile fatty acids; A:P, ratio of acetate to propionate; [2] YC-Cr=Zn-enriched yeast culture; YC-Se=Se-enriched yeast culture; YC-Zn=Zn-enriched yeast culture; [ab] Means within a row for doses that do not have a common superscript differ (P<0.05); [e,f] Means within a column for species that do not have a common superscript differ (P<0.05); [†] Mean=mean for individual species across doses including the dose of 0; [‡] SEM for yeast culture dose; [§] NS, not significant (P>0.05); L, linear effect of dose; Q, quadratic effect of dose; C, cubic effect of dose; [¶] SEM for pooled mean of species including the dose of 0

Table 5b. *Effects of three sources of yeast culture on VFA concentration of in vitro incubation fluid of rice straw*

Items[1]	Species[2]	Dose (%)						SEM[‡]	Significance (*P*)[§]		
		Mean[†]	0	0.10	0.25	0.40	0.55		Species	Dose	Species × Dose
Acetate, (mmol/L)	YC-Cr	19.72^{ef}	21.15^{a}	18.14^{b}	19.56^{ab}	18.48^{b}	21.29^{a}	0.98	<0.001	<0.05	NS
	YC-Se	18.31^{f}	21.14^{a}	20.47^{a}	16.45^{b}	16.31^{b}	17.17^{b}			C(<0.05)	
	YC-Zn	20.97^{e}	21.15	21.30	22.25	21.09	19.08			NS	
	SEM[¶]	0.44									
Propionate (mmol/L)	YC-Cr	5.80^{f}	6.87^{a}	5.26^{b}	5.58^{b}	5.18^{b}	6.11^{ab}	0.30	<0.001	C(<0.05)	<0.05
	YC-Se	5.38^{g}	6.87^{a}	5.78^{b}	4.79^{c}	4.64^{c}	4.85^{c}			Q(<0.05)	
	YC-Zn	6.19^{e}	6.87	6.34	6.33	5.95	5.43			NS	
	SEM[¶]	0.14									
Butyrate (mmol/L)	YC-Cr	1.69^{ef}	1.72	1.64	1.67	1.58	1.84	0.08	<0.05	NS	<0.001
	YC-Se	1.29^{f}	1.72^{a}	1.41^{b}	1.12^{c}	1.13^{b}	1.10^{c}			L(<0.01)	
	YC-Zn	1.77^{e}	1.72	1.79	1.85	1.75	1.73			NS	
	SEM[¶]	0.04									
TVFA (mmol/L)	YC-Cr	27.91^{e}	30.17^{a}	26.82^{ab}	27.22^{ab}	25.63^{b}	29.69^{ab}	1.33	<0.0001	L(<0.01)	<0.01
	YC-Se	25.30^{f}	30.17^{a}	27.96^{a}	22.63^{b}	22.36^{b}	23.41^{b}			L(<0.01)	
	YC-Zn	29.35^{e}	30.17	29.84	30.87	29.22	26.65			NS	
	SEM[¶]	0.60									
A:P	YC-Cr	3.59	3.16^{b}	4.03^{a}	3.58^{ab}	3.65^{ab}	3.58^{ab}	0.14	NS	<0.05	NS
	YC-Se	3.54	3.14^{b}	3.71^{a}	3.55^{a}	3.64^{a}	3.64^{a}			Q(<0.01)	
	YC-Zn	3.49	3.14^{c}	3.39^{b}	3.60^{a}	3.65^{a}	3.61^{a}			Q(<0.01)	
	SEM[¶]	0.06									

[1] TVFA, total volatile fatty acids; A:P, ratio of acetate to propionate; [2] YC-Cr=Zn-enriched yeast culture; YC-Se=Se-enriched yeast culture; YC-Zn=Zn-enriched yeast culture; [ac] Means within a row for doses that do not have a common superscript differ (P<0.05); [e,f] Means within a column for species that do not have a common superscript differ (P<0.05); [†] Mean=mean for individual species across doses including the dose of 0; [‡] SEM for yeast culture dose; [§] NS, not significant (P>0.05); L, linear effect of dose; Q, quadratic effect of dose; C, cubic effect of dose; [¶] SEM for pooled mean of species including the dose

concentration in *in vitro* fermentation fluid were shown in *Table 4*. The YC-Zn treatment obtained the higher acetate, propionate, butyrate, and TVFA concentration and lower A: P compared to another two treatments. For the YC-Cr treatment, the control group obtained the larger propionate (cubic, P<0.05), TVFA concentration (linear, P<0.01) and lower A: P values (P<0.05). For the YC-Se treatment, the larger acetate (cubic, P<0.05), propionate (quadratic, P<0.05), butyrate (linear, P<0.01), TVFA concentration (linear, P<0.0001) and lower A: P (quadratic, P<0.01) value were observed at the supplementation dose of 0% with dose increased. No significant difference in acetate, propionate, butyrate and TVFA concentration were observed when YC-Zn supplementation dose improved except A: P which the lower value was observed at the control group and presented quadratic effect (P<0.01). Besides, there were interactive effects on propionate, butyrate and TVFA for three sources of yeast culture.

DISCUSSION

Unlike the yeast cultures in which the influence on the rumen fermentation have been relatively intensively studied, available reports on the effects of Cr-, Se- and Zn-enriched yeast cultures on gas production parameters of *in vitro* ruminal fermentation are rather insufficient. In the current study, supplementation of three kinds of yeast cultures elevated the theoretical maximum of gas production (V_f) for maize stover, meanwhile, supplementation of YC-Se increased the V_f of rice straw. This finding was in accordance with Tang et al.[1], which reported that supplementation of yeast culture increased the theoretical maximum of gas production, rate of gas production and IVDMD and decreased the lag time for each type of straw (rice straw, wheat straw, maize stover and maize stover silage). However, Tang and Wang [23] reported that there was no significant influence of yeast culture supplementation on *in vitro* gas production of alfalfa, leymuschinensis, this was not in agreement with the results in the current study, the disparity was probably ascribed to the difference in yeast culture and fermentation substrates. Additionally, among the three sources of yeast culture, supplementation of YC-Se was more efficient in improving *in vitro* V_f of maize stover and rice straw compared to the other two yeast cultures. This findings possibly ascribed to that YC-Se element increased the activity of rumen microorganism in *in vitro* fermentation fluid. Wang et al.[24] reported that Se-yeast had a positive influence on rumen fermentation through improving the activity of rumen microorganism. Indexes of FRD_0 and $T_{0.5}$ usually reflect the rate of degradation at early incubation stages of '<12 h' and the incubation time of reaching half of the maximum gas production, respectively. Generally speaking, the faster FRD_0 is, the shorter $T_{0.5}$ becomes [25]. In *in vitro* fermentation of maize stover and rice straw, the addition of YC-Cr increased FRD_0 but decreased $T_{0.5}$ at the supplementation dose of 0.25% and 0.40%, suggesting that the rate of degradation would be accelerated at the early stage of *in vitro* fermentation. Besides, the addition of YC-Cr increased FRD_0 and decreased $T_{0.5}$ more efficiently than that of another two sources of yeast cultures. Wang et al.[20] reported that supplementation of two active yeast (Angel yeast & Lesaffre yeast) did not exert a significant influence on IVNDFD, parameters of LE model which including V_f, FRD_0 and $T_{0.5}$, but it increased the IVDMD of maize stover and rice straw, the difference in results between Wang et al.[20] and the current study may ascribe to the difference in yeast culture supplementation. Previous research indicated that *S. cerevisiae* culture filtrate stimulated the initial rate of cellulose degradation [26]. This may explain the increase in the rate of gas production by the addition of YC-Cr in the current study, the addition of YC-Cr might increase the number of total and cellulolytic bacteria in fermentation liquid.

In vitro DMD and NDFD disappearance were important indexes in the using of forage during the process of rumen fermentation. The findings in the current study indicated that supplemented with YC-Se got better effects on IVDMD and IVNDFD for maize stover and IVDMD for rice straw than that of YC-Cr and YC-Zn. Wang et al.[24] reported selenium yeast supplementation effectively increased the number of cellulose decomposition microbes or promoted the vitality of the cellulose decomposition microbes so that the degradation rate increased. These could be explained by that supplemented with YC-Se increased the number of total cellulolytic bacteria in *in vitro* fermentation fluid compared to that of the addition of the other two yeast cultures. However, except for YC-Zn, there was no effect on *in vitro* DMD and NDFD compared to control group with supplementation dose changed. Many similar results have been published; Tripathi and Karim [27] have reported that yeast culture supplementation did not influence intake and digestibility of organic matter, neutrtal detergent fiber (NDF) and acid detergent fiber (ADF). Fokkink et al.[28] have found that there was no effect on calf performance when Se was supplied in the form of YC-Se. Titi et al.[29] also found that addition of yeast culture had no effect on apparent digestibility of DM, CP and NDF. However, opposite finding to the results of the current results was also reported. Miranda et al.[30] had reported that *Saccharomyces cereuisiae* cultures increased *in situ* alfalfa NDF digestion at 48 h. This difference might be resulted from the different experiment method.

Since pH value is an important index reflecting the internal homeostasis of rumen environment, therefore maintaining a relatively stable ruminal pH is vital to assuring efficient rumen fermentation. Ruminants usually possess highly developed systems to maintain ruminal pH within a physiological range of about 5.5-7.0 [31]. In the current study, despite the varying drops in response to the addition of YC-Se, YC-Cr and YC-Zn, the pH value across all treatments ranged from 6.35 to 6.52, providing a suitable

circumstance for fermentation, growth of microorganisms and fiber degradation in the rumen [32].

Hristov et al.[33] suggested that deficiency of NH_3-N restricts the microbial protein synthesis, while the over-high NH_3-N concentration also inhibits the microbial utilization of NH_3-N. Satter and Slyter [34] reported that lowest NH_3-N concentration of rumen fluid should not be less than 5 mg/dL to maintain the higher growth rate of bacteria. In this study, concentration of NH_3-N in response to the addition of YC-Se was lower than that of YC-Cr and YC-Zn and the lowest concentration of NH_3-N was observed at the dose of 0.10% for maize stover and 0.40% for rice straw by adding YC-Se respectively, both of the lowest concentration were lower than 5 mg/dL. The reasons for these results may ascribe to the improvement in synthesis of microbial protein. Lu et al.[35] suggested that supplemented with Se-enriched probiotics could increase synthesis of microbe protein by rumen bacteria through using NH_3-N in fermentation fluid. Tripathi and Karim [27] have reported yeast culture supplementation improved microbial CP synthesis. Kamalamma et al.[36] also found that Yea-sacc^{1026} appeared to enhance incorporation of ammonia N into microbial cells. But many published results shown that lack of effect on ammonia concentration in the rumen using *Saccaromyces cerevisiae* yeast culture [37-39]. Different kinds of yeast culture and experiment method might result in this difference.

Ruminal volatile fatty acids (VFAs) are major energy sources for ruminants, rumen VFAs could provide 50-80% of the energy needed by ruminants [40,41], their content and composition are important physiological indexes that reflect rumen digestion and metabolism. In the current study, adding three kinds of yeast cultures to *in vitro* fermentation systems made changes to varying degrees in VFA concentration for maize stover and rice straw respectively, especially VFA concentration increased for maize stover with the enhancement of supplementation dose, while there was a decreased trend in that of rice straw. The changes in complex microflora were major reason for the difference in VFA concentration, *in vitro* fermentation was changed in response to the changes in microflora when yeast culture added. Wang et al.[13] reported that the VFA concentration was significantly related to the numbers of *Selenomonas ruminantium* and *Megasphaera elsdenii* organisms in the rumen. Pinloche et al.[42] found that diet supplementation of probiotic yeast changed the main fibrolytic group *(Fibrobacter* and *Ruminococcus)* and lactate utilizing bacteria *(Megasphaera* and *Selenomonas)*. The findings in TVFA concentration of maize stover increased due to three kinds of yeast cultures supplementation, which was similar to Guedes et al.[37]. Many published results shown that TVFA concentration was not affected in response to SC (*Saccaromyces cerevisiae*) yeast supplementation [38,43].

In this study, besides the stimulating effect of three kinds of yeast cultures on TVFA concentration, supplementation with them also changed the molar proportion of VFA in *in vitro* fermentation fluid. Different acetate: propionate ratio was obtained in response to different yeast culture and supplementation dose. Mwenya et al.[44] found an increase in acetate: propionate ratio when fistulated non-lactating cows were supplemented with *Trichosporum sericeum* yeast culture (forage to concentrate ratio, 70:30), while other researchers found a lower ratio due to SC (*Saccaromyces cerevisiae*) yeast supplementation [4,45].

In summary, YC-Se is preferred compared to YC-Cr and YC-Zn and its optimal dose should be 0.25% and 0.1% for maize stover and rice straw, respectively, as YC-Se improved the *in vitro* fermentation characteristics of maize stover and rice straw, *in vitro* DM and NDF disappearance, and enhanced most of VFA concentration.

FUTURE RECOMMENDATION

The present *in vitro* results should be tested further using *in vivo* experiments to explore the effects of different yeast culture on milk production in dairy cows in future.

Conflict of Interest

There was no Conflict of interest.

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