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In vitro Biodegradation of Wheat straw and Wheat straw based Total Mixed Ration using Zoospores of different Elite Ruminal Fungi

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ABSTRACT

The elite anaerobic fungal cultures Neocallimastix sp GR-1, Piromyces sp WNG-12 andOrpinomyces sp C-14 were isolated from wild blue bull and cattle respectively of grazing and browsing goat. Attempts were made to grow thesecultures in normal media or using stress conditions by keeping them at lower or highertemperature than39°c or using a complex media; Normal media produced maximum number of fungal zoospores after 120hrs of incubation. The in-vitrodry matter, acid detergent fibre, neutral detergent fibre digestibilitywere studied using fresh zoospores; or those being kept at normal; lower orhigher temperature than 39°C. Based on in vitro digestibility of DM, NDF and ADF of wheat straw and wheat straw based totalmixed rations.The Neocallimastix sp GR-1was found better as compared to Piromyces sp WNG-12 andOrpinomyces sp C-14as this anaerobic fungiproduced maximum numbers of zoospores and also proved to be best performing for its abilityto degrade lignified feed material.With the addition of zoospores of Neocallimastix GR1 kept at different temperature for 45 days in treatments T2, T3, T4 and T5, NDF digestibility increased significantly ($P \leq 0.05$) and values were recorded as 42.54±0.54, 41.52±0.58, 41.00±0.56 and 42.27±0.55.respectively.With addition of zoospores of Piromyces sp.WNG-12, the NDF digestibility also increased significantly ($P \leq 0.01$) as compared to control both for WS and WS based rations.Addition of zoospores of Neocallimastix GR-1 to different treatments increased total number of zoospores significantly in all treatments compared to that of control.

Keywords: Elite, Anaerobic, Fungal zoospores, In-vitro, NDF

INTRODUCTION

Many attempts have been made to enhance the availability of digestible energy of straws, using physical, chemical and biological treatments. In case of physical treatments only chopping and socking were feasible. Chemical treatment of wheat straw using sodium hydroxide increased the voluntary feed intake of straw by cattle (Ng'ambi and Campling, 1991). But, NaOH is very costly and a hazardous chemical to be handle by dairy farmers under farm condition. Its cost has also increased because of its use in soap industry.

Urea is relatively a safe chemical to handle and its use to upgrade straw protein has been extensively studied (Owen and Jayasuriya, 1989), but the improvements in digestibility of urea-NH_3 treated wheat straw depends on temperature and moisture conditions and cannot be used when there is shortage of water. However, in vitro degradation of urea-NH_3 treated wheat straw using anaerobic ruminal fungi was successful to enhance the nutritive value of straw than urea-NH_3 treatment only. The unusual nature of anaerobic fungi or fungal zoospores and the potential importance of fibre-degrading fungi or fungal zoospores to herbivore nutrition have made these a subject of many studies over recent years. Rumen Anaerobic fungi, an emerging group of animal probiotics, account for up to 8-12% of the microbial biomass in the rumen and actively colonize plant cell-walls to degrade their lignin.

To alleviate the problem of delignification emphasis has been given to manipulate the rumen fermentation by isolating elite rumen anaerobic fungi and their fungal zoospores (having higher hydrolytic activities) from domesticated and wild ruminants. Among the rumen microbes, most of the anaerobic fungi have active and positive role to play in fibre degradation as evidenced by the plant cell wall degrading enzymes such as trans-feruloyl and trans-P.coumaroyl esterases, microcrystalline cellulase, hemicellulase, pectinase, protease etc. (Kopecny, 1995) and they have the unique ability to penetrate into the fibrous feed particles through rhizoids, which help in access of other rumen microbes to the secondary cell wall of feed particles to make their digestible energy available to the animal. These rumen anaerobic fungi play an important role in the digestion of poor quality straws and their proliferation in anaerobic media to enhance their activity to break down the bonds between the lignin and hemicellulose to improve the nutritive value of cereal straw based diets. In this direction many efforts have been made in recent past (Dey *et al.*, 2004) to enhance the digestibility of poor quality lignocellulosic roughages by the addition of various microbial or chemical feed additives. Among microbial additives, there are evidences of definite positive relationships between anaerobic fungi in the rumen and the increased voluntary intake of low digestible fibrous

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feeds (Kumar *et al.*, 2015, Ivarson *et al.*, 2016, Elghandour *et al.*, 2015).Manikumar *et al.* (2004) showed the ability of different elite fungal species on fiber digestibility in vitro and reported that Orpinomyces sp. (C-14) isolated from Cattle was most promising isolate for degradation of wheat and paddy straws.

Nagpal (2008) reported that zoospores developed from elite anaerobic fungus can be freeze dried and when subjected to in vitro degradation of wheat straw showed higher digestibility of nutrients. This paper reports on in vitro degradation of wheat straw and wheat straw based total mixed ration using zoospores of elite fungi.

MATERIALS AND METHODS

Revival, purification and maintenance of selected rumen fungal zoospores

Rumen anaerobic fungal isolates of Orpinomyces sp C-14, Neocallimastix sp GR-1 and Piromyces sp WNG-12, preserved in the anaerobic groth medium (Hungate, 1969) were revived by subculturing and tested morphologically by microscopy with cotton blue dye. These were cultured and purified in anaerobic broth (Joblin, 1981) to make them free from any contamination. These isolates of Neocallimastix sp. GR-1 were isolated from grazing and browsing goat (Thareja *et al.*, 2006), Piromyces sp. WNG-12 from wild nilgai (Tripathi *et al.*, 2007, Puniya *et al.*, 2015) and Orpinomyces sp. C-14 from cattle, were checked for purity, and chosen because of their maximum hydrolytic activity over the others normal fungi available in rumen.

These were also tested for type of colony: Monocentric or polycentric and Morphological characteristics with respect to zoospores feature and their enzymatic activities viz cellulase, cellobioses and xylanase to match them as per that of their parent culture.

Culture maintenance

Fungal cultures were maintained at refrigerator temp at 4^0C by sub-culturing every 7to 9 days in Orpin's medium containing ball-milled wheat straw (1% w/v) and agar (0.80), depending on the rate of growth by removing small sections of the fungal colony, which developed on the agar and transferring it to fresh soft agar medium. Rumen in vitro digestibility of wheat straw and wheat straw based total mixed ration (TMR)

Sampling of feed ingredients and Preparation of total mixed ration

Total mixed ration was prepared by mixing wheat straw, concentrate mixture in the ratio of 50:50 having app. CP 12% and TDN 52%. The samples of the wheat straw and concentrate mixture were collected for their proximate principle and Van soest analysis.

Rumen In- vitro digestibility experiments

Rumen In vitro digestibility of Wheat Straw and Wheat Straw based total mixed ration (TMR) was carried out by Tilley and Terry (1963) 1st stage technique.

a) Rumen In vitro digestibility of Wheat Straw

1gWheat Straw was taken in incubation flasks of 100 ml capacity along with 40 ml McDougall's buffer, 10 ml strained rumen liquor(SRL) and 1ml of selected anaerobic fungal zoospores (1 x 10^6 zoospores/ ml). The following treatments were made.

- T1(C) : Wheat Straw + buffer + SRL + 1ml broth without fungal zoospores
- T2 : Wheat Straw + buffer + SRL + 1ml broth containing fresh zoospores of Neocallimastix sp. GR1 or Piromyces sp. WNG-12 or Orpinomyces sp. C-14
- T3 : Wheat Straw + buffer + SRL + 1ml broth containing zoospores of Neocallimastix sp. GR-1 or Piromyces sp. WNG 12 or Orpinomyces sp. C-14 kept at normal room temperature for 45 days.
- T4 : Wheat Straw + buffer + SRL + 1 ml broth containing zoospores of Orponomyces sp. C-14 or Piromyces sp. WNG-12 or Neocallimastix sp. GR-1 kept at refrigerator temperature (4^0C for 45 days).
- T5 : Wheat straw + buffer+ SRL + 1ml broth containing zoospores of Neocallimastix sp. GR-1 or Piromyces sp. WNG-12 or Orpinomyces sp. C-14 kept at 45^0C for 45 days.

b) Rumen In vitro digestibility of Wheat Straw based total mixed ration (TMR)

Total mixed ration (1 gm) was taken in incubation flasks (100 ml capacity) along with 40 ml McDougall's buffer, 10 ml SRL and 1ml of selected anaerobic fungal zoospores (≈ 10^6 zoospores/ ml). The following treatments were made.

- T6(C) : TMR + buffer + SRL + 1 ml broth without fungal zoospores
- T7 : TMR + buffer + SRL + 1 ml broth containing fresh zoospores of Neocallimastix sp. GR1 or Piromyces sp. WNG-12 or Orpinomyces sp. C-14
- T8 : TMR + buffer + SRL + 1ml broth containing zoospores of Piromyces sp. WNG 12 or Orpinomyces sp. C-14 or Neocallimastix sp. GR-1 kept at normal temperature for 45 days.
- T9 : TMR + buffer + SRL + 1 ml broth containing zoospores of Orpinomyces sp. C-14 or Piromyces sp. WNG-12 or Neocallimastix sp. GR-1 kept at refrigerator temperature (4^0C) for 45 days.
- T10 : TMR + buffer + SRL + 1 ml broth containing zoospores of Orpinomyces sp. C-14 or Piromyces sp. WNG-12 or Neocallimastix sp. GR-1 kept at 45^0C for 45 days.

In vitro studies were repeated after 45 days to check survival of zoospores as per the above procedure adopted for wheat straw and wheat straw based TMR, respectively by keeping the zoosporic culture media at room temperature for 45 days, in incubator at 45^0C for 45 days and in a refrigerated temperature (4^0C) for 45 days.

Incubation period

In the above experiments, three replicates were taken. Before incubation, all flasks were flushed thoroughly with CO_2 gas and flasks were sealed immediately with Bunsen valve. The flasks were then placed in an incubator maintained at 39 ^{0}C. All the flasks were incubated for 48 h.

Enumeration of Zoospores

Zoospores were enumerated from the samples of fungal colonies in the roll-tubes and inoculated broth media using phase-contrast microscope, stained with lactophenol cotton blue.

Estimation of in- vitro dry matter digestibility (IVDMD)

After the incubation of substrates for 48 h, samples from each trial were centrifuged at 5000 rpm for 10 minutes; the pellets were dried at 100^{0}C for 24 h (Tilley and Terry, 1963).

Estimation of in- vitro neutral detergent fiber digestibility (IVNDFD)

The sample obtained after IVDMD was taken into a 1000 ml spout-less beaker. To this, 100 ml neutral detergent solution, 2 ml decalin and 0.5 g sodium sulphate were added and the contents of spout-less beaker were refluxed on hot plate for one hour. After refluxing, the sample was filtered through pre-weighed 50 ml capacity sintered glass crucible grade-I using vacuum pump. Residue was washed with hot boiling water and then acetone to remove all salts and moisture. The sintered crucible containing residue was dried in hot air oven at 100 °C and weighed.

The NDF was determined as follows:

$$\text{NDF \%} = \frac{\text{(Wt. of crucible + cell wall constituents) – Wt of empty crucible}}{\text{Wt. of sample taken}} \times 100$$

In- vitro acid detergent fiber digestibility (IVADFD)

The sample obtained after IVDMD treatment was also taken in a spout-less beaker of 1 L capacity. To this, 100 ml acid detergent solution and 2 ml decalin were added and the contents were refluxed on hot plate for 1 hour. After refluxing the residue was filtered through pre-weighed sintered glass crucible grade-I using vacuum pump, washed with hot water and then with acetone to remove all the salts and moisture. Then the residue was dried in hot air oven at 100°C and weighed.

The ADF was determined as follows:

$$\text{ADF \%} = \frac{\text{(Wt. of crucible + fibre) – Empty wt. of crucible}}{\text{(Wt. of crucible + fibre) – Empty wt. of crucible}} \times 100$$

Statistical analysis

All the data was subjected to the statistical analysis as per (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

In vitro dry matter digestibility (IVDMD) using fungal zoospores

The in vitro digestibility of WS alone was 36.75±0.71 percent at 48h, which with the addition of zoospores of Neocallimastix sp.GR-1, increased to42.87±0.72 percent, (Table 1) and the difference between the two was found to be statistically significant (P≤0.01). Likewise, digestibility of WS increased to

Table 1: Effect of elite fungal zoospores on Rumen in vitro dry matter digestibility of wheat straw and wheat straw based TMR

Treatment	Percent IVDMD (Incubation Time 48 h)		
	Orpinomyces C-14	Piromyces WNG -12	Neocallimastix GR -1
WS + Buffer + SRL + Broth without fungal zoospores (T1)	35.41± 0.26	35.32 ±0.46	36.75± 0.71
WS + Buffer + SRL + Broth containing fungal zoospores (T2)	37.17± 0.25	42.13** ±0.46	42.87** ±0.72
WS+ Buffer+SRL+Broth containing fungal zoospores (NRT) (T3)	36.86±0.28	41.34**±0.46	41.86** ±0.72
WS+ Buffer+SRL+Broth containing fungal zoospores (RF T) (T4)	36.49±0.28	41.87**±0.46	42.41** ±0.71
WS+ Buffer+SRL+Broth containing fungal zoospores (45 ^{0}) (T5)	36.07±0.28	41.44**±0.46	42.00** ±0.69
TMR + Buffer + SRL + Broth without fungal zoospores (T6)	48.31±1.14	48.20 ±0.26	47.91 ±0.40
TMR + Buffer + SRL + Broth containing fungal zoospores (T7)	49.55±1.13	52.15 **±0.25	52.92** ±0.41
TMR + Buffer + SRL + Broth containing fungal zoospores(NRT) (T8)	48.90±1.15	53.11 **±0.28	53.01** ±0.43
TMR + Buffer + SRL + Broth containing fungal zoospores(RF T) (T9)	49.44±1.14	51.91 ** ±0.27	52.63** ±0.39
TMR+ Buffer+SRL+Broth containing fungal zoospores (45^0) (T10)	49.52±1.14	51.70 ** ±0.26	52.32** ±0.38

**means within a row with no common superscript differ significantly (P≤0.01)

41.87±0.72 (T3, NRT), 42.41±0.71 (T4, RFT) and to 42.00±0.69 (T5, 45^{0}C) percent after 45 days. The results of DM digestibility indicate that zoospores produced by Neocallimastix sp. GR-1 can survive for longer period at room temperature, refrigerator temperature and at higher temperature (45^{0}C). Digestibility of TMR was observed to be 47.91±0.40 percent in treatment T6.The IVDMD of TMR increased significantly (P≤ 0.01) for Neocallimastix GR-1 to 52.92±0.41, 53.01±0.43, 52.63±0.39 and 52.32±0.38 with T7, T8, T9 and T10 respectively. The findings are in agreement with in-vitroor in-vivo studies conducted earlier with elite fungi (Paul *et al.,* 2004; Thareja *et al.,* 2006 and Shelke *et al.,* 2009).

Also with the addition of zoospores of Piromyces WNG-12 in T2, T3, T4 and T5 the IVDMD increased significantly (P≤0.01) to 42.13 ±0.41, 41.34±0.0.49, 41.87±0.48 and 41.44±0.46 percent respectively from 35.32±0.42 in control. The differences among IVDMD for different treatments were statistically significant (P≤ 0.01). The IVDMD of TMR without fungal zoospores (T6) was 48.20 ±0.26 percent which with the addition of zoospores of Piromyces sp WNG-12 kept at various temperatures(room temperature, refrigerator temperature and at 45^{0}C in incubator for 45days), increased to 52.15±0.25 (T7), 53.11±0.28 (T8), 51.91±0.27 (T9) and 51.70±0.26 (T10) respectively and the differences were highly significant (P≤ 0.01) compared to control (T6) indicating that these zoospores were able to resist lower or higher temperature for many days and can grew well when substrate was available under rumen environment.

The incorporation of fungal zoospores of Orpinomyces sp C-14 to wheat straw or wheat straw based total mixed ration did not have any effect on in-vitro DM digestibility as the zoospores production was very scanty. However, no significant differencesin in-vitro digestibility was observed between zoospores of Neocallimastixsp GR-1 and Piromyces sp.WNG-12 as the digestibility was more or less similar in both the cases. The efficiency of degradation of DM was quite comparable with the earlier observations (Manikumar *et al.,* 2004) where the fungal cultures were administered. Results were also comparable with finding of Shelke *et al.,* 2009 in which in-vitro DM digestibility were observed to be similar for normal zoospores of Neocallimastix sp.GR-1 and Piromyces sp.WNG-12. The results are also quite comparable with Paul *et al.,* 2004 and Thareja *et al.,* 2006 observed with fungal cultures.

In-vitroNeutral detergent fiber (NDF) digestibility

NDF represents the fibre content of cell wall .In the treatment T1(C), where only SRL was taken as the source of rumen microorganisms, percent NDF digestibility obtained after 48 hours of incubation was 30.60 ±0.58. But this increased with the addition of zoospores of Neocallimastix GR1 kept at different temperature for 45 days in treatments T2, T3, T4 and T5, significantly (P≤ 0.05) and values were recorded as 42.54±0.54, 41.52±0.58, 41.00±0.56 and 42.27±0.55.respectively

Table 2: Percent Rumen in vitro digestibility of neutral detergent fiber (NDF) of wheat straw and wheat straw based TMR using zoospores of elite fungi

Treatment	Percent IVNDFD (Incubation Time 48 h)		
	Orpinomyces C-14	Piromyces WNG-12	Neocallimastix GR -1
WS + Buffer + SRL + Broth without fungal zoospores (T1)	30.65± 0.69	29.91 ±2.34	30.60± 0.58
WS + Buffer + SRL + Broth containing fungal zoospores (T2)	33.64± 0.68	40.82** ±2.33	42.54** ±0.54
WS+ Buffer+SRL+Broth containing fungal zoospores (NRT) (T3)	34.18±0.67	41.12** ±2.35	41.52 **±0.58
WS+ Buffer+SRL+Broth containing fungal zoospores (RF T) (T4)	33.12±0.67	40.99** ±2.35	41.00 **±0.56
WS+ Buffer+SRL+Broth containing fungal zoospores (45^0) (T5)	32.80±0.70	39.92** ±2.36	42.27 **±0.55
TMR + Buffer + SRL + Broth without fungal zoospores (T6)	32.69±1.01	33.00 ±0.91	33.71 ±0.61
TMR + Buffer + SRL + Broth containing fungal zoospores (T7)	35.32±1.01	39.70** ±0.92	39.31** ±0.51
TMR + Buffer + SRL + Broth containing fungal zoospores(NRT) (T8)	35.30±1.11	41.45** ±0.99	40.59** ±0.61
TMR + Buffer + SRL + Broth containing fungal zoospores(RF T) (T9)	35.46.44±1.14	40.44** ±0.93	40.62 **±0.62
TMR+ Buffer+SRL+Broth containing fungal zoospores (45^0) (T10)	35.46±1.10	40.61 **±0.91	39.56 **±0.64

** means within a row with no common superscript differ significantly(P≤0.01)

(Table 2). In treatment T6 (C), the percent NDF digestibility was 33.71±0.61 for TMR. After addition of zoospores of Neocallimastix sp. GR-1 kept at different temperatures the values obtained for T7, T8, T9 and T10, were 39.31±0.51, 40.59±0.61, 40.62±0.62 and 39.56±0.64 and increased significantly (P≤0.01) than control.

With addition of zoospores of Piromyces sp.WNG-12, the NDF digestibility also increased significantly (P≤ 0.01) as compared to control both for WS and WS based rations. However the results were contrary with that of Orpinomyces sp.C-14 both for WS and WS based rations (Table 2).

Earlier studies showed that administration of elite fungal cultures led to a significant increase in the percent disappearance of NDF contained in WS and TMR based on WS (Thareja *et al.*, 2006; and Shelke *et al.*, 2009). The results indicated that fungal zoospores were quite efficient in reducing the NDF content in WS and WS based TMR under in-vitro system.

Acid detergent fiber (ADF)

ADF represents the cell wall components of plants except hemicelluloses. In Control, after 48 hours of incubation, the percent ADF digestibility obtained was 33.68±0.68. With addition of zoospores of Neocallimastix sp. GR-1, the percent digestibility increased significantly (P≤ 0.01) in all the treatments and values obtained were 41.87±0.69, 40.21±0.67, 40.45±0.66 and 40.15±0.70 for T2, T3, T4 and T5 respectively (Table 3). In TMR, the control (T6) value was 33.47± 0.60 and, the digestibility increased significantly for all the treatments and the values were 43.34± 0.51, 41.20±0.61, 41.18± 0.62 and 41.41± 0.65 for T7, T8, T9 and T10 respectively. Also with addition of zoospores of Piromyces sp.WNG-12, ADF digestibility of WS increased significantly (P≤0.01) compared to control (32.21±1.04) and the values were 39.52±1.04, 40.65±1.11, 39.69±1.12 and 39.90±1.05 for T2, T3, T4 and T5 respectively. Zoospores of Piromyces sp.WNG-12, resulted in significant increase of ADF digestibility of WS based TMR (P≤ 0.01) in comparison to control (33.62±0.79) and the values obtained were 40.55±0.80, 40.80±0.99, 40.40±0.98 and 40.71±0.79 for T7, T8, T9 and T10 respectively (Table 3).

Table 3: Percent Rumen in vitro digestibility of acid detergent fiber (ADF) ofwheat straw and wheat straw based TMR using zoospores of elite fungi

Treatments	Percent IVADFD (Incubation Time 48 h)		
	Orpinomyces C-14	Piromyces WNG-12	Neocallimastix GR-1
WS + Buffer + SRL + Broth without fungal zoospores (T1)	32.65± 0.92	33.21 ±1.04	33.68± 0.68
WS + Buffer + SRL + Broth containing fungal zoospores (T2)	35.05± 0.91	39.52 ** ±1.04	41.87** ±0.69
WS+ Buffer+SRL+Broth containing fungal zoospores (NRT) (T3)	35.35±0.93	40.65 **±1.11	40.21**±0.67
WS+ Buffer+SRL+Broth containing fungal zoospores (RF T) (T4)	36.02±0.95	39.69 **±1.12	40.45**±0.66
WS+ Buffer+SRL+Broth containing fungal zoospores (45^0) (T5)	34.95±0.86	39.90 **±1.05	40.15**±0.70
TMR + Buffer + SRL + Broth without fungal zoospores (T6)	32.66±1.02	33.62 ±0.79	33.47 ±0.60
TMR + Buffer + SRL + Broth containing fungal zoospores (T7)	36.21±1.11	40.58 ** ±0.80	43.34** ±0.51
TMR + Buffer + SRL + Broth fungal zoospores(NRT) (T8)	34.32±1.11	40.88 **±0.99	41.20**±0.61
TMR + Buffer + SRL + Broth containing fungal zoospores(RF T) (T9)	33.58±1.16	40.40 **±0.98	41.18**±0.62
TMR+ Buffer+SRL+Broth containing fungal zoospores (45^0) (T10)	34.34±1.03	40.71 **±0.79	41.41**±0.60

**means within a row with no common superscript differ significantly(P≤0.01)

Neocallimastix sp. GR-1 and Piromyces sp.WNG-12 yielded similar results in both WS and WS based TMR ration which is supported by the results obtained by Shelke *et al.*, 2009. Orpinomyces sp.C-14 did not give significant increase in digestibility for both WS and WS based TMR. The disappearance of ADF correlates positively to the degradation and utilization of highly complex polysaccharides present in dry roughages. Thus the present study showed that with the incorporation of fungal zoospores, complex polysaccharides present in WS could be utilized to release energy which can be further used by the animals for increasing their productive performance (Thareja *et al.*, 2006 and Jha, 2009). The ADL degradation were in concurrence with Lee *et al,* (2001), Manikumar *et al.*, 2002; 2004 and Shelke *et al.*, 2009.

Average number of zoospores

Addition of zoospores of Neocallimastix GR-1 to different treatments increased total number of zoospores significantly

in all treatments compared to that of control. Also in case of TMR, the addition of zoospores of NeocallimastixGR1 in different treatments (zoospores/ml) increased their number significantly ($P \leq 0.05$) compared to that of control. Similarly after the addition of zoospores of Piromyces WNG-12 in different treatments, number of zoospores also increased significantly (2.83×10^3, 2.83×10^3, 3.33×10^3 and 3.33×10^3, zoospores/ ml) in all treatments as compare to that of control (1.33×10^3 zoospores/ ml). In case of TMR similar trend was obtained, and with the addition of zoospores of PiromycesWNG-12, number of zoospores increased significantly compared to that of control (1.50×10^3 zoospores/ ml) in all treatments. In case of Orpinomyces sp.C-14, the numbers of zoospores obtained were very less and comparable to control for both WS and TMR (Table 4). It has also been reported in literature that polycentric fungi like Orpinomyces produce less zoospores compared to monocentric fungi (Fliegerova *et al.*, 2004).Thus, the results of the in-vitro trial clearly indicated that the zoospores of elite rumen fungi, Neocallimastix sp. GR-1 and Piromyces sp. WNG-12 were quite promising to be used as probiotics for ruminants to improve the nutritive value of WS and WS based TMR. Moreover, the zoospores of Neocallimastix GR1 showed better performance than zoospores of Piromyces WNG12.

Table 4: Average number of zoospores/ ml of fluid after 48 h of incubation

Treatments	Average number of zoospores/ ml of fluid (Incubation Time 48 h)		
	Orpinomyces C -14	Piromyces WNG -12	Neocallimastix GR -1
WS + Buffer + SRL + Broth without fungal zoospores (T1)	$1.66 \times 10^3 \pm 0.22$	$1.33 \times 10^3 \pm 0.33$	$1.66 \times 10^3 \pm 0.28$
WS + Buffer + SRL + Broth containing fungal zoospores (T2)	$1.83 \times 10^3 \pm 0.23$	$2.83 \times 10^3 \pm 0.38$ *	$3.50 \times 10^3 \pm 0.20$ *
WS+ Buffer+SRL+Broth containing fungal zoospores (NRT) (T3)	$2.00 \times 10^3 \pm 0.20$	$2.83 \times 10^3 \pm 0.34$ *	$3.33 \times 10^3 \pm 0.22$ *
WS+ Buffer+SRL+Broth containing fungal zoospores (RF T) (T4)	$1.66 \times 10^3 \pm 0.24$	$3.33 \times 10^3 \pm 0.28$ *	$3.50 \times 10^3 \pm 0.23$ *
WS+ Buffer+SRL+Broth containing fungal zoospores (45^0) (T5)	$1.66 \times 10^3 \pm 0.26$	$3.33 \times 10^3 \pm 0.30$ *	$3.00 \times 10^3 \pm 0.28$ *
TMR + Buffer + SRL + Broth without fungal zoospores (T6)	$1.16 \times 10^3 \pm 0.26$	$1.50 \times 10^3 \pm 0.38$	$1.50 \times 10^3 \pm 0.25$
TMR + Buffer + SRL + Broth containing fungal zoospores (T7)	$2.00 \times 10^3 \pm 0.23$	$3.83 \times 10^3 \pm 0.36$ *	$3.50 \times 10^3 \pm 0.26$ *
TMR + Buffer + SRL + Broth containing fungal zoospores(NRT) (T8)	$1.83 \times 10^3 \pm 0.24$	$3.16 \times 10^3 \pm 0.35$ *	$3.33 \times 10^3 \pm 0.30$ *
TMR + Buffer + SRL + Broth containing fungal zoospores(RF T) (T9)	$1.50 \times 10^3 \pm 0.20$	$3.66 \times 10^3 \pm 0.32$ *	$3.33 \times 10^3 \pm 0.29$ *
TMR+ Buffer+SRL+Broth containing fungal zoospores (45^0) (T10)	$2.16 \times 10^3 \pm 0.23$	$3.00 \times 10^3 \pm 0.34$ *	$3.16 \times 10^3 \pm 0.23$ *

* means within a row with no common superscript differ significantly($P \leq 0.05$)

CONCLUSION

The results of the in-vitro trial clearly indicated that the zoospores of elite rumen fungi, Neocallimastix sp. GR-1 and Piromyces sp. WNG-12 were quite promising to be used as probiotics for ruminants to improve the nutritive value of WS and WS based TMR. Capacity of fibre digestibility of Neocallimastrix sp. GR-1 was better than other anaerobic fungi.

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