

## **EFFECT OF BIOLOGICAL TREATMENTS ON THE NUTRITIVE VALUE OF RICE STRAW**

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### **SUMMARY**

The objective of this study was to the effect of biological treatments (ZAD, fungus and ZAD with fungus) on feed intake, digestibility coefficients, nutritive value and nitrogen balance of rice straw and some rumen liquor and blood parameters.

Twelve adult Ossami rams were divided into four similar groups and used to carried out four metabolic trials using three animals for each group (all groups were fed the rice straw ad. Libitum) the first (T1) was the control (untreated rice straw), the second (T2) was rice straw treated with ZAD, the third (T3) was rice straw treated with fungus (*Pleurotus osteratus*) and the fourth (T4) was rice straw treated with ZAD and fungus. T2, T3 and T4 increased ( $P<0.05$ ) crude protein of rice straw and decreased ( $P<0.05$ ) dry matter, crude fiber, NFE, NDF, ADF, ADL and hemicellulose contents than the control group. Dry matter intake (DMI) increased ( $P<0.05$ ) the groups fed rice straw treated with ZAD, fungus and ZAD+ fungus than the control group. ZAD, fungus and ZAD + fungus treatments increased ( $P<0.05$ ) digestibility coefficients of CP, ADF, NDF, ADL, cellulose and hemicellulose than the untreated rice straw. Total digestible nutrients (TDN) and digestibility of crude protein (DCP) for T2, T3 and T4 were higher ( $P<0.05$ ) than untreated rice straw. The rumen liquor parameters (NH<sub>3</sub>-N) and TVFA's concentrations were highest value after 3 hours of feeding in all groups. All of treated rice straw groups had no significant differences ( $P<0.05$ ) for urea, total protein, albumin, globulin, GOT and GPT than untreated rice straw.

Twenty-four Ossimi lambs averages ( $20.0 \pm 0.3$  kg) were used in feeding trial lasted 120 days. Feeding trial results showed all biological treatments had higher dry matter intake (1037, 990, 896 and 786g/d) and average daily gain (169.2, 163.3, 134.3 and 92.5g) for T2, T3, T4 and T1, respectively. Biological treatments indicated that it they could be good method to improve the nutritive value of rice straw,

**Keywords:** *rice straw, fungus, biological treatments, feeding value, sheep.*

## INTRODUCTION

Large quantities of agricultural residues with low feed value exist through the world. At present most of these materials are not only a wasting natural resource but also an important sources of environmental pollution.

In Egypt, the agricultural crops generate plentiful and inexpensive by-products available around the year but are not efficiently used (22 million tons of plant by-products produced annually (Agriculture Economic and Statistics Institute, 1999).

Residues are burned or wasted, and hence lead to environmental pollution and consequently health hazards. The primary factors limiting the utilization of crop residues are low digestibility, low protein content and low palatability. Thus, to increase digestibility of crop residues, it is important to destroy the linkage between cellulose, hemicellulose and lignin or to destroy the compact

nature of the tissue, so that lignified tissue is separated from non-lignified one. There have been attempts to do that by mechanical, chemical or biological treatments (Baker *et al.*, 1975 and Jackson, 1978).

Biological treatments of some agricultural by-products become essential in order to degrade lignocelluloses into lignin, cellulose, hemicellulose and improve crude protein content. It is well known that some micro-organisms, including celluloses enzymes from anaerobic bacteria and white rot fungi (*Pleurotus ostreatus*) can degrade lignin in the cell walls and attempts have been made to improve digestibility of plant residues (Abdel-Aziz and Ismail, 2001).

This study aimed to investigate, the ability of biological treatments to improve nutritive value as total digestible nutrient (TDN) and digestibility crude protein (DCP) of

rice straw as crop residues and the effect of biological treatments of rice straw on chemical composition, nutrient digestibility, nitrogen metabolism, ruminal and blood parameters of sheep fed the experimental rations.

## **MATERIALS AND METHODS**

### **Microorganisms:**

#### **1-ZAD treatment:**

ZAD (Patent on : 22155) is bio-ech product made from natural sources to elevate the level of celluloses enzymes from anaerobic bacteria. It is produced in the Molecular Biology Lab., Animal Production Department, Ain Shams University according to the procedure of (Gado, 1997).

#### **2- Fungal treatment :**

*Pleurotus ostreatus* NRRL 3780 was obtained from the National Center of Agricultural Utilization Research, Agricultural Research Service, US, Department of Agriculture, Peoria, USA.

#### ***Media used***

This fungus was maintained on Czapek's medium (Difco, 1984) or

molasses medium and grown at 25-25° for 5 days.

### **Substrate preparation and cultivation**

Rice straw was chopped into 3-5 cm. It was boiled for 2 hours and strained until moisture level was to 65-70%. The wet rice straw (100kg) was put in plastic bags and inoculated with ZAD (1 litter/500kg rice straw) and/or fungus (1 litter/100kg rice straw), layer by layer in order. Then the plastic bags were plugged and a moderate temperature (28-30°C).

### **Analytical methods:**

Chemical composition of feed, feces and urine were determined according to A.O.A.C (1990) method. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL,,) were determined by the methods of Van Soest (1982).

### **In vitro rate of degradation of thy matter**

In vitro extent (48 hrs.) was used to determine the rate of degradation of dry matter per hour. The natural log method was used to convert data to linearity (Mertens, 1977). The apparent in vitro digestibility of the

rice straw was also determined through three periods (30, 45 and 60 days of ensiling) according to Tilley and Terry (1963).

Two tubes as a replicate each sample were used in incubation times (0, 2, 4, 6, 8, 24 and 48 hours). Two blank tubes were prepared for each incubating time. Rumen, liquor was collected by stomach tube from the rumen of three mature Ossami sheep, which were fed only berseem hay as a basal diet. Fluids from different sheep were mixed together to have one representative sample of rumen fluid. Fluid was squeezed through 4 layers of cheesecloths, placed in prewarmed thermos, and flushed with CO<sub>2</sub>.

### **Digestibility trial**

Twelve adult Ossinii rams were divided into four similar groups and used to carry out four metabolic trials using three animals for each group as follows:

**T<sub>1</sub>**: Untreated rice straw (control). **T<sub>2</sub>**: Rice straw with ZAD litter/500kg rice straw). **T<sub>3</sub>**: Rice straw with (*Pleurotus osteratus*) (1 litter/100kg rice straw), **T<sub>4</sub>**: Rice straw with ZAD + (*Pleurotus osteratus*)

Preliminary period lasted 21 days followed by 7 days for collection period, The nutrient intake and excretion were determined to calculate their digestibility and utilization by conventional balance trial method. The experimental animals were fed concentrate feed mixture (CFM) to cover their maintenance requirement (NRC, 1985). CFM consists of 40% undecorticated cotton seed meal, 17% yellow corn, 20% wheat bran, 5% molasses, 15% soybean meal, 2% limestone and 1% salt. All groups were fed the rice straw ad libitum. Feces were collected quantitatively every day during the collection period sprayed with 10% 1-12SO<sub>4</sub>. At the end of the collection period, fecal samples from each ram were ground mixed well and kept in the refrigerator for chemical analysis. Samples of feed, feces and urine were analyzed according to A.O.A.C. (1990).

Rumen liquor samples were taken from each animals at the end of collection period at 0, 3 and 6 hours after morning feeding by stomach tube. Rumenal pH and ammonia nitrogen (NH<sub>3</sub>-N) were immediately determined by Conway (1963). Frozen rumen liquor samples were

analyzed for total volatile fatty acids (TVFA's) by steam distillation according to Abou-Akkada and Osman (1967).

Blood samples were taken from each animal at the end of the collection period of each trial before morning meal. Blood samples were collected in vacuoner tubes and left at room temperature for 2hrs, centrifuged at 3500 r.p.m. for 15 minutes to obtain serum, which was stored at  $-20^{\circ}\text{C}$  till analysis. Blood serum was analyzed using special kits for urea (Henery, 1974), total protein (Cannon, 1974), Albumin (Doumas et al., 1975) and GOT and GPI (Reitman and Frankel, 1957).

### Feeding trial

Twenty-four Ossami lambs aged (7 months) were taken from the station herd and were divided into our similar groups, according to weight. Average initial live body -weight was  $20,0 \pm 0.2$  kg/head and animal groups were fed the four respective rations in 2 meals/day for 120 days. All lambs in the trial were given maintenance requirements according to (NRP, 1985) recommendation and rice straw either treated or Untreated were fed *adlibitum*

### Statistical analysis

The data were statistically analyzed according to Sendecor and Cochran (1980) using SAS (1985). The difference between means was tested by Duncan's multiple range test (1955). One-way analysis of variance as the mathematical model:

$$Y_{ijk} = \mu + T_i + e_{ijk}$$

where:  $Y_{ijk}$  = Represents observations.  $\mu$  = Overall mean.  $T_i$  = Treatments.  $e_{ijk}$  = experimental error.

### RESULTS AND DISCUSSION

The use of molasses as carbon source for fungal growth

In Egypt, one of the most import raw materials produced from the sugar industry is the sugar cane molasses. It was found valuable to Study the use of Egyptian sugar cane molasses in fimgal growth.

Table (I) shows that increasing the concentration of molasses from 5 to 10% resulted in increasing ( $P < 0.05$ ) the growth of *Pieurotus ostreatus*. At 10% molasses concentration the mycelia fresh weight reached its maximum being  $15.06 \text{ gl}^{-1}$ . A decrease in mycelia fresh. weight was

noticed at 20 and 30% molasses concentration being 4.39 and 3.73 g l<sup>-1</sup>. So, the addition of 10% molasses concentration to rice straw was used in the following treatments.

### **Chemical composition of rice straw and experimental rations**

Chemical composition of experimental rice straw are presented in Table (2) revealed that rice straw treated with ZAD (T1) rice straw treated with fungus (T2) and rice straw treated with ZAD and fungus (T3) resulted in decreasing, ( $p < 0.05$ ) dry matter (DM) being 90.51, 88.15 and 90.02%, respectively. Substantial increase ( $P < 0.05$ ) in Crude protein. (CP) of the fungal treated rice straw against other treatments was found being 9.78, 6.08, 8.07 and 3.41% for ZAP, fungus, ZAD treated with fungus and control, respectively.

Similar trend was observed by (Gupta. and Singh 1991).

Abdel-Aziz and Ismail (2001) found that NPF, APF and cellulose contents of fungus treated rice straw decreased by 77.67, 48.81 and 39.73%, respectively.

The degradation of various fiber fractions decreased during the ensilage period with the decreasing level of hemicellulose. It was noticed that, the effect of treatment of ZAD, fungus and ZAD with fungus were greater on, NDF, ADF, ADL, cellulose and hemicellulose indicating the greater influence on hemicellulose breakdown as effect of the treatment. These result were in agreement with Gado, (1997); Mahrous, and Abou Ammou (2005) and Bassuny et al., (2003).

### **In vitro degradation of dry matter**

Table (1) Effect of different concentration of molasses on fungal fresh weight.

Molasses concentration	Fungal fresh weight $gl^{-1}$	$\pm SE$
5 (control)	6.55b	0.38
10	15.06a	0.41
20	4.39c	0.26
30	3.73d	0.11

a,b,c and d Means with different superscripts in the same row differ significantly ( $P < 0.05$ )

Table (2) : chemical composition (% on DM basis) of untreated, treated rice straw and concentrate feed mixture.

Item	T1	T2	T3	T4	CFM
DM	91.13	90.51	88.15	90.02	88.80
OM	85.55	82.01	83.65	82.19	87.34
CP	3.41	6.08	9.78	8.07	16.20
CF	38.33	31.08	33.35	35.04	13.04
EE	1.20	1.78	1.57	1.37	3.24
NFE	42.61	43.07	38.95	38.44	54.86
Ash	14.45	17.99	16.35	17.08	12.66
NDF	77.09	73.18	74.29	75.02	32.75
ADF	45.28	43.13	43.21	44.54	10.46
ADL	9.89	9.01	9.32	9.62	3.48
Cellulose	35.39	34.12	33.89	34.92	6.98
Hemicellulose	31.81	30.05	31.08	30.48	22.29

T1 : Untreated rice straw (control), T2: Rice straw +ZAD, T3:Rice straw + fungus, T4: Rice straw + ZAD + fungus, CFM : Concentrate feed mixture

Table 3 : In vitro rate of degradation of dry matter (extent 48 hrs.).

Ensiling Periods (days)	T1	T2	T3	T4	$\pm SE$
30	0.0234d	0.0278	0.0252b	0.0242c	0.003
45	0.0223d	0.0276a	0.0248b	0.0236c	0.002
60	0.0212d	0.0271a	0.0240b	0.0227c	0.003

a,b,c and d Means with different superscripts in the same row differ significantly ( $P < 0.05$ )

T1 : Untreated rice straw (control)

T2 : Rice straw + ZAD

T3 : Rice straw + fungus

Results in Table (3) showed an increase ( $P < 0.05$ ) in the in vitro rate of degradation in treated rice straw in comparison with the control treatment. Also, the biological treatments has the effects; to predigest the cellulose, and to release carbohydrates for fermentation. Biological treatments of roughages reduced log time (Adebowale and Nakashima, 1992 and Gado, 1999). Chalupa and Lee (1966) reported from in vitro cellulose digestion studies, that log time was evident at 6, 12. and 18 hrs. Whereas intubation, after 24 hrs. represented the extent of digestion. The 18 hrs. value was used as index of in vitro cellulose digestion and the 30 hrs. value as a measure of total cellulose digestion. in general, the best improvement in in vitro rate of degradation was found with ZAD followed by fungus and ZAD with fungus under different incubation periods than that control. These results were in agreement with Bassuny et al, (2005)

### **Digestibility coefficients and nutritive value**

Nutrient digestibility coefficients and nutritive value have been affected by biological treatments and presented in table (4). The, DM digestibility of sheep fed rations containing treated

rice straw was significantly -higher ( $p < 0.05$ ) than those of untreated rice straw. This result might be due to the better palatability of treated rice straw than untreated and better utilization by the host animal.

Rice straw treated with fungus had the highest value of crude protein digestibility being 69.5% as shown in Table (4). Digestibility coefficient of CF was significantly ( $P < 0.05$ ) higher for rice straw treated with ZAD being 63.7% and the lowest digestibility coefficient was recorded for control group (45.0%). The improvement in DM, several authors (Gado, 1997; Fouad et al., 1998 and Deraz and Ismail, 2001; Mahrous and Abou Ammou, 2005; Bassuny et al 2003c and Bassuny et al., 2005) observed CP and CF digestibility coefficients over a wide range of low quality roughages by ZAD and fungus treatments.

Digestibility of NDF for both ZAD and fungus treatment was significantly higher ( $P < 0.05$ ) than those of ZAD with fungus and the control group. The ADF digestibility of both ZAD and fungus treatments were significantly higher ( $P < 0.05$ ) than the control treatment. Cellulose digestibility coefficient of rice straw treated by ZAD (64,91%) and fungus

(57,32%) was higher than ZAD with fungus (50.39%) which increased significantly ( $P<0,05$ ) than the control group (47.73%) Hemicellulose digestibility was also the highest value (78.53%) for ZAD treatment followed by fungus (71.55%) and ZAP with fungus (63.81%) and the lowest value was recorded for the control treatment (60.60%). Gado (1997), Fouad et al., (1998.), Deraz and Ismail (2001) reported similar results and Abdel-Aziz and Ismail

(2001); Bassuny et al, (2003b) and :Basuny et at, Who reported that the ZAD and fungus treatments had the effect of loosening lignocellulose bonds and solubilize some the hemicellulose content.

The nutritive value of treatments calculated as TDN were the highest value (61.9%) by ZAD treatment followed by fungus treatment (59.4%) and both were significantly higher value ( $P<0.05$ ) than the control. Also, DCP

Table (4) : Effect of biological treatments on digestibility and nutritive value by experimental animals.

Item	T1	T2	T3	T4	CFM
Digestibility					
DM	54.66b	68.47a	63.71a	55.39b	1.38
OM	56.74d	70.12a	68.68b	65.41c	0.08
CP	44.74c	50.27b	69.48a	54.29b	0.47
CF	45.04c	63.74a	60.71a	52.63b	0.96
EE	62.31c	72.71a	67.32b	65.51b	0.43
NFE	60.31c	74.48a	72.36a	67.21b	1.51
NDF	48.68d	72.97a	58.16b	52.47c	1.13
ADF	39.04d	65.49a	54.18b	46.44c	0.27
ADL	27.42c	47.91a	33.73b	31.07b	0.78
Cellulose	47.73d	64.91a	57.32b	50.39c	0.26
Hemicellulose	60.60d	78.53a	71.55b	63.81c	1.20
Nutritive value %					
TDN	50.50d	61.89a	59.40	55.81c	0.62
DCP	4.32d	6.24c	9.39a	7.40b	1.23

a,b,c and d Means with different superscripts in the same row differ significantly ( $P<0.05$ )

T1 : Untreated rice straw (control)

T2 : Rice straw + ZAD

T3 : Rice straw + fungus

Table (5): Effect of biological treatment on nitrogen balance by experimental animals.

Item	T1	T2	T3	T4	±SE
Nitrogen intake (NI)(g/h/d)	14.13d	17.76c	22.99a	20.09b	0.08
Urinary Nitrogen (UN) (g/h/d)	8.57d	8.73c	9.56b	10.05a	0.63
Feces Nitrogen (FN) (g/h/d)	4.54b	3.67c	5.43a	3.48d	0.65
Digested Nitrogen (DN) (g/h/d)	9.58d	14.09c	17.55a	16.61b	0.14
Nitrogen Balance (NB) (g/h/d)	+1.01d	+5.36c	+7.45a	+6.56b	0.29
NB/NI	7.16c	30.18c	33.69a	32.62a	0.07
NB/ND	10.56d	38.05b	44.12a	39.47b	1.17

a,b,c and d Means with different superscripts in the same row differ significantly (P<0.05)

T1 : Untreated rice straw (control)

T2 : Rice straw + ZAD

T3 : Rice straw + fungus

was the highest for fungus treatment and for ZAP with fungus treatment and both were significantly higher (P<0.05) than the control.

those obtained by Abdel-Aziz and Ismail (2001); Fouad et al, (1998); Deraz and Ismail (2001) and Bassuny et al., 2005)

### Nitrogen Balance (NB)

Nitrogen balance, calculation is shown Table (5). Nitrogen intake (g/h/d) was clearly superior for all animals fed the biological treatment than the control. Therefore, digested N for fungus and ZAD with fungus treatments were significantly higher (P<0.05) than the control treatment. Results also, showed that the NB, (g/h/d) for fungus ZAD with fungus and ZAD treatment were significantly higher (P<0.05) than the control. These results are in agreement with

### Rumen liquor and blood parameters

The rumen liquor parameters, are shown in Table (6). Ammonia-N (NH<sub>3</sub>-N) concentration reached the maximum after 3 hours of feeding in all groups. However, NH<sub>3</sub> concentrations were significantly higher (P<0.05) in the created groups than the control group. After 6 hours of feeding NH<sub>3</sub>-N concentration tended to decrease in all groups. This agree with the results reported by Williams and Newbold 1990) who

reported that the reduction ammonia-N in the rumen liquor appear to be the result of 'increased incorporation of ammonia-N into microbial protein and it was considered a direct result to stimulated microbial activity.

Rice straw treated with fungus maintained the highest value of ruminal VFA's after 3 hr. of feeding followed ZAD treatment ZAD with fungus and the lowest values recorded for the control groups. These results agree with those obtained by Henics (1987) who found that the level of ruminal TVFA's reached its maximum at 3hr. after feeding for lambs fed ad. Libitum. These results of biological treatments which might be related to the more utilization of the dietary energy and positive fermentation in the rumen. On the contrary, the lowest pH values were recorded after 3hr. of feeding for the different treatments.

The values of blood serum urea, total protein, albumin, globulin, albumin/globulin ratio, GOT and GPT for the animals fed rice straw treated with ZAD, fungus, ZAD with fungus and untreated are presented in Table (6) Treatments 2, 3 and 4 which were

fed ration contained biological treatments rice, straw resulted in decreasing non significantly blood serum urea concentration than the animal fed the untreated rice straw (T1). The blood serum total protein and its fractionations were within the normal. values. The range of GOT was 20.36 to 20.52 U/L and for GPT was 33.59 to 33.71 U/L, which are within those normal ranges. These results of blood metabolism are in agreement with those reported by Fouad *et al.*, (1998) and Deraz and Ismail (2001); Bassuny et al., (2003b) and Bassuny et.al.,(2005).

### **Feeding trial**

The average DM intake expressed as (g/h/d), average daily body gain and feed conversion of the experiment groups are presented in Table (7), result revealed that the DMI as (g/h/d), of lambs during 120 days of the experimental period was higher for lambs fed ZAD treatment (103701 g/h/d) followed by fungus treatment (990g/h/d ginid) and ZAD with fungus. (896 g/h/d) than the control (786g/h/d).

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Table (6): Effect of biological treatment on nitrogen balance by experimental animals.

Item	time	T1	T2	T3	T4	±SE
<b>Rumen parameters</b>						
pH	0	5.56c	6.31b	6.85a	5.52c	0.64
	3	5.17c	5.81b	6.56a	5.10c	0.60
	6	5.46c	6.46b	6.97a	5.49c	0.80
NH <sub>3</sub> -N (mg/100ml)	0	11.86a	10.86b	10.91b	10.85b	0.02
	3	17.89d	20.64c	25.83a	22.04b	0.03
	6	13.28d	17.62c	20.44a	19.76b	0.02
TVFA's (meq/100ml)	0	9.54d	10.36a	10.11b	10.02c	0.52
	3	12.52d	13.54b	14.44a	13.04c	0.82
	6	9.25d	9.77a	9.64b	9.44c	0.13
<b>Blood parameters:</b>						
Urea (mg/100ml)		25.20	14.85	24.79	24.90	NS
Total protein (gm/100ml)		7.32	7.40	7.35	7.45	NS
Albumin (gm/100ml)		3.90	3.84	3.78	3.80	NS
Globulin (gm/100ml)		3.60	3.56	3.57	3.65	NS
A/G ratio		1.08	1.07	1.05	1.04	NS
GOT (U/L)		20.46	20.52	20.40	20.36	NS
GPT (U/L)		33.67	33.71	33.59	33.62	NS

a,b,c and d Means with different superscripts in the same row differ significantly (P<0.05)

T1 : Untreated rice straw (control)                      T2 : Rice straw + ZAD

T3 : Rice straw + fungus T4: Rice straw + ZAD + fungus

Table (7): Effect of biological treatment on nitrogen balance by experimental animals. (n=6 Exp. Period 120 days).

Item	T1	T2	T3	T4
Initial weight (kg)	20.1	20.2	20.3	20.2
Final weight (kg)	31.2	40.5	39.9	36.3
Total gain (kg)	11.1	20.3	19.6	16.1
Average daily gain (ADG) (g)	92.5	169.1	163.3	134.3
DMI (g/d)	786	1037	990	896
DMI (g/W <sup>0.75</sup> )	69.0	80.2	77.0	73.1
Concentrate feed mixture	406	406	406	406
Concentrate feed mixture (g/W <sup>0.75</sup> )	35.6	31.3	31.6	33.1
Concentrate feed mixture %	51.6	39.1	41.0	45.3
Roughages	380	631	584	490

Roughages (g/W <sup>0.75</sup> )	33.4	48.8	45.4	40.0
Roughages %	48.4	60.9	59.0	54.6
Feed conversion	8.5	6.1	6.0	6.6

T1 : Untreated rice straw (control)                      T2 : Rice straw + ZAD

T3 : Rice straw + fungus T4: Rice straw + ZAD + fungus

of feed conversion (g DM/g gain) showed that the fungus treatment recorded the best value followed by the ZAD treatment and ZAD with fungus treatment than the control. These results were in agreement with MaMous and Abou Amrnou (2005) and Bassuny et al. (2003b) and Bassuny et al. (2005). Mohamed et al. (1998) indicated that the feed conversion of treated rice straw was better compared with untreated rice straw.

It could be concluded that the biological treatments of rice straw by D, fungus and ZAD with fungus increased protein content, protein digestibility and fiber fractions digestibility. The recycling of agricultural wastes is important to raise its nutritional value and can be used in the ruminants feeding. Biological treatments can utilize lignin along with cellulose and other components of the substrate, these organisms grow slowly and degrade the structural carbohydrates of crop residues. In addition, biological

treatments as a result of molecular biology are preferable in terms of being a biological treatment, rather than the other treatments such as chemical and physical treatments for better and clear environment.

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