



Moringa Leaf Meal in Diet of Layers

Rajesh et al.

## Dietary Inclusion of *Moringa Oleifera* Leaf Meal with Enzyme Supplementation on Egg Production and Egg Quality of Laying Hens

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### ABSTRACT

The objective of the study was to evaluate the effect of *Moringa oleifera* leaf meal (MOLM) on production performance of layers. Two hundred, 34-week-old BV 300 White Leghorn layer birds were randomly allotted to 50 replicates with 4 birds in each replicate and these replicates were in turn allotted to 5 dietary groups. T1 (Control: Corn-soybean meal Basal diet (BD)); T2: 5% MOLM in BD diet without enzyme supplementation; T3: 7.5% MOLM without enzyme supplementation; T4: 10% MOLM in BD diet without enzyme supplementation; T5: 10% MOLM with enzyme supplementation. The results revealed that egg production was significantly higher ( $P < 0.01$ ) in 10% MOLM with enzyme supplementation. Significantly higher ( $P < 0.01$ ) feed conversion ratio (FCR) was observed in T4 whereas lower ( $P < 0.01$ ) FCR was observed in T1, T2 and T5. The egg weight was significantly higher ( $P < 0.01$ ) in T5 compared to the other treatments. There was no significant difference recorded in feed intake and egg density among the treatments. Feed cost per egg was significantly ( $P < 0.01$ ) lower for 10% MOLM fed birds with and without enzyme supplementation. Egg quality parameters like yolk color, shell strength, haugh unit, albumen length, shell percentage, shell thickness, shell weight, yolk height, yolk width, albumen height and albumen width were all improved ( $P < 0.01$ ). It can be concluded that *Moringa oleifera* leaf meal (MOLM) can be included upto 10 % level with enzyme supplementation in the diets of commercial layers for better production performance

**Key words:** Egg production, Egg weight, Enzymes, Egg quality, Moringa leaf meal

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### INTRODUCTION

The high cost of protein sources for poultry is an ongoing issue in developing countries. Prices of traditional protein sources have risen so high in recent years that using them in chicken feeds has become uneconomical, and to alleviate this problem, several unconventional feed ingredients are incorporated, among them tree leaves are most promising substitutes with high nutritional values and available at cheaper rates (Kakengi et al., 2005). Among the leaf meals, *Moringa oleifera* leaf meal (MOLM) have high pepsin soluble nitrogen (82-92%) and the low acid detergent insoluble protein (1-2%) values suggest that most of the protein in the meal is available to most animals (Makkar and Becker, 1999). *M. oleifera* leaves have a negligible

content of tannins and have no trypsin and amylase inhibitors or cyanogenic glucosides (Makkar and Becker, 1999). *Moringa* foliage are potential inexpensive protein sources for poultry feeding. The MOLM could replace sunflower seed meal and can be added in the layers ration (Kakengi et al., 2005).

Despite the high nutritional content of MOLM, it has 12.2% crude fibre content (Abo-State et al., 2014). In layers, *Moringa* leaf meal generally lowers laying performance and feed efficiency at high inclusion rates (15-20%) (Kakengi et al., 2005), but also sometimes at lower (5 to 7.5%) levels (Ebenebe et al., 2013). There is little information regarding its utilization in poultry feeding as a protein source in the layer ration. Thus, the study is aimed to investigate the effects of including MOLM as a

protein source on productive performance in commercial layer diets and to evaluate the effect of enzyme supplementation in MOLM based diets at higher inclusion levels, owing to its high crude fibre content.

## MATERIALS AND METHODS

### Birds and diets

The experiment was conducted at the Poultry Experimental Station, Livestock Farm Complex, College of Veterinary Science, Rajendranagar, Hyderabad. Two hundred, 34-week-old BV 300 White Leghorn layer birds were randomly allotted to 50 replicates with 4 birds in each replicate and these replicates were in turn allotted to 5 dietary groups. The birds were raised in cages under uniform management and fed the respective diets from 34th to 49<sup>th</sup> weeks of age. Birds were fed the experimental diet at 120 g/bird per day but this amount was adjusted with regard to the level of their production throughout the experimental periods (NRC, 1994). Leaf was harvested from young Moringa oleifera trees of about four years of age from forage farm under supervision of CVR food products. The harvested leaves from the trees were spread out on a concrete floor and allowed to dry for a period of three days under shade and aerated conditions then run through a hammer mill sieve with a size of five mm to produce the leaf meal.

Five treatment diets include T1 (Control: Corn-soybean meal Basal diet (BD); T2: 5% MOLM in BD diet without enzyme supplementation; T3: 7.5% MOLM in BD diet without enzyme supplementation; T4: 10% MOLM in BD diet without enzyme supplementation; T5: 10% MOLM in BD diet with enzyme supplementation was formulated with MOLM substituting SBM (Table 1). The cocktail enzyme contains (Cellulase: 1,50,255 CMCU/G, Phytase: 2,225 FYT/

G, Beta mannanase: 2,00,000 IU/G, Protease: 7,00,115 IU/G, Alpha Amylase: 70,000 U/G, Lipase: 18,000 U/G, Xylanase: 2,80,000 IU/G, Arabinase: 2120 IU/G, Beta Galactosidase: 25,000 IU/G). The Treatment diets were formulated to be iso-caloric and iso-nitrogenous, to meet the ME and CP requirement of laying hens according to NRC (1994).

### Egg production, egg quality and cost

Feed consumption of each replicate was recorded weekly and feed conversion ratio was calculated period wise and also on cumulative for entire study period. Feed conversion ratio was calculated as feed intake per egg produced. Hen day egg production in per cent was calculated by dividing the total number of eggs laid every day by number of hens survived during each day. The egg weight was recorded to the nearest 0.1 g accuracy in each replicate. The egg density was measured by using digital density balance (LCGC – AS220/X) with 0.0001 g/cm<sup>3</sup> accuracy. The feed cost per egg was calculated on overall, according to the feed intake, FCR and egg weight per bird. The experiment was conducted from December 2017 to April 2018. The feed cost Rs. per kg of all the diets are as follows; T1:20.38, T2:19.68, T3: 19.32 T4: 18.94 T5: 19.31. During the last three consecutive days of each period, one fifty eggs were collected randomly i.e. during 37th, 41st, 45th and 49th week of age to assess the egg quality traits. The proximate analysis of feed was performed as per the procedures described by AOAC (2012).

Data analyzed for mean, standard errors and analysis of variance as per method of Snedecor and Cochran (1989) and comparison of means were done using Duncan (1955) using software of Statistical Package for Social Sciences (SPSS) 15.0 version and significance was considered at P<0.05.

Table 1. Proportion of ingredients used for formulating experimental diets(%)

Ingredients	T1	T2	T3	T4	T5
Maize	59.8	56.5	54.9	53.4	53.4
Soybean meal	21.5	19.8	18.915	17.915	17.915
Deoiled rice bran	7.0	7.0	7.0	7.0	7.0
MOLM	0	5	7.5	10	10
Limestone powder	10	10	10	10	10
DL-Methionine	0.16	0.16	0.16	0.16	0.16
Common salt	0.15	0.15	0.15	0.15	0.15
Vitamin Premix*	0.05	0.05	0.05	0.05	0.05
Trace mineral mixture**	0.1	0.1	0.1	0.1	0.1
Choline Chloride	0.05	0.05	0.05	0.05	0.05
Toxin binder	0.05	0.05	0.05	0.05	0.05
Dicalcium phosphate	1.09	1.09	1.09	1.09	1.09
Enzyme mixture	0	0	0	0	0.025
L-Lysine HCl	0.08	0.08	0.08	0.08	0.08
Total	100	100	100	100	100
Calculated nutrient composition					
CP(%)	15	15	15	15	15
ME					
(kcal/kg DM)	2500	2500	2500	2500	2500
Calcium (%)	4.0	4.0	4.0	4.0	4.0
Available Phosphorus(%)	0.34	0.34	0.34	0.34	0.34
Lysine(%)	0.68	0.68	0.68	0.68	0.68
Methionine(%)	0.32	0.32	0.32	0.32	0.32
Sodium(%)	0.18	0.18	0.18	0.18	0.18
Chlorine(%)	0.22	0.22	0.22	0.22	0.22
Crude fibre(%)	7.52	7.58	7.53	7.68	7.85

\* Vitamin premix provided per kg diet: Vitamin A 200000 IU, Vitamin B2 25 mg, Vitamin D3 3000IU, Vitamin K 2 mg, Riboflavin 25 mg, Vitamin B1 1mg, Vitamin B6 2 mg, Vitamin B12 40 mg and Niacin 15 mg.

\*\* Trace mineral provided per kg diet: Manganese 120 mg, Zinc 80 mg, Iron 25 mg, Copper 10 mg, Iodine 1 mg and Selenium 0.1mg.

## RESULTS AND DISCUSSION

### Proximate composition of MOLM

The proximate composition of MOLM used in this experiment was(% DM basis): moisture of 6.2, crude protein of 24.2, crude fiber 10.4, ether extract 2.50, nitrogen free extract 53.1, ash 9.8, calcium 2.5, phosphorus 0.36.

### Egg production (%)

The egg production was significantly higher ( $P < 0.05$ ) in MOLM included diets as compared to the control during the period I (Table 2). Whereas, in the period II, egg production was significantly higher ( $P < 0.01$ ) in T1, T2, and T5. However, lower egg production was noticed in the T4. In the period III, egg production was significantly higher ( $P <$

0.01) in T5 as compared to other treatments and the lowest egg production was recorded for T4. In the period IV, the highest egg production was observed in T5. Overall, the 10% MOLM (T5) with enzyme supplementation group had higher egg production, followed by 5% MOLM (T2) fed group.

The higher egg production in layers fed with MOLM could be due to the improvement in balanced nutrient supply by MOLM in the diet. The MOLM contains lysine, methionine and a combination of other amino acids, which might supply the required amount of essential nutrients for better production (Sohail et al., 2003). In accordance with the present finding, Uma (2000) reported that methionine and lysine levels in poultry diets have positive correlation with egg production. The higher egg production in the birds fed 10% MOLM with enzyme supplementation was due to the presence of enzyme action on proteins, and metabolism of amino acids for increase in egg production. Wei Lu et al. (2016) reported that, the dietary supplementation of 5% MOLM increased the egg production. Gayathri et al. (2020) reported that MOLM up to 1% improved the production performance of Vanaraja laying hens. Similarly, Nabila et al. (2015) noticed low dose levels of Moringa leaves powder in layers diets improved the laying percentage. Similar results were also reported by Swain et al. (2017) with MOLM in Vanaraja

laying hens. On the contrary, Olugbemi et al. (2010) did not find any significant effect on egg production with MOLM in laying hens. Decrease in egg production at higher (7.5% and 10%) levels of MOLM without enzyme supplementation was attributed to low digestibility of energy and protein (Kakengi et al., 2005).

**Feed intake**

In period I, II and overall period there was no significant (P>0.01) difference in the feed intake of birds among the treatments (Table 2). In period III, the feed intake was significantly higher (P<0.05) in the birds fed MOLM included diets as compared to the control. In period IV, the feed intake in T2, T3 and T4 was significantly higher (P<0.01) when compared to control and T5. These findings are similar with Etalem et al. (2013) who noted that addition of MOLM in layers up to 10% had no effect on feed intake of hens. Sarker et al. (2017) also found that addition of MOLM in the diets of broilers had no significant effect on feed intake. Similar results were also reported by Adejumo et al. (2016). In contrast, Olugbemi et al. (2010) reported addition of 10% and 20% MOLM to the laying hen diet increased the feed intake. In period III, the feed intake was higher in birds fed Moringa leaf meal included diets probably due to increased bulk and decreased metabolizable concentration.

Table 2. Effect of dietary inclusion of Moringa oleifera leaf meal on hen day egg production, feed intake and feed conversion ratio in layers

Diet	Hen day egg production					Feed intake (g/day)					Feed conversion ratio (Feed intake (g)/egg)				
	Period I 34-37wk	Period II 38-41wk	Period III 42-45wk	Period IV 46-49wk	Overall 34-49wk	Period I 34-37wk	Period II 38-41wk	Period III 42-45wk	Period IV 46-49wk	Overall 34-49wk	Period I 34-37wk	Period II 38-41wk	Period III 42-45wk	Period IV 46-49wk	Overall 34-49wk
T1	83.1 <sup>b</sup>	90.1 <sup>a</sup>	90.3 <sup>b</sup>	86.5 <sup>b</sup>	88.0 <sup>b</sup>	116	113	104 <sup>b</sup>	101 <sup>b</sup>	109	140	123 <sup>c</sup>	115 <sup>d</sup>	117 <sup>c</sup>	124 <sup>c</sup>
T2	89.1 <sup>a</sup>	91.3 <sup>a</sup>	90.9 <sup>b</sup>	87.1 <sup>b</sup>	89.6 <sup>b</sup>	118	113	109 <sup>a</sup>	106 <sup>a</sup>	111	133	124 <sup>c</sup>	120 <sup>c</sup>	122 <sup>b</sup>	125 <sup>c</sup>
T3	89.9 <sup>a</sup>	82.6 <sup>b</sup>	86.7 <sup>c</sup>	80.6 <sup>c</sup>	85.0 <sup>c</sup>	118	113	108 <sup>a</sup>	106 <sup>a</sup>	111	132	137 <sup>b</sup>	125 <sup>b</sup>	132 <sup>a</sup>	131 <sup>b</sup>
T4	90.2 <sup>a</sup>	75.4 <sup>c</sup>	83.3 <sup>d</sup>	79.5 <sup>c</sup>	82.1 <sup>d</sup>	118	113	109 <sup>a</sup>	106 <sup>a</sup>	112	132	151 <sup>a</sup>	131 <sup>a</sup>	133 <sup>a</sup>	137 <sup>a</sup>
T5	92.5 <sup>a</sup>	92.2 <sup>a</sup>	94.7 <sup>a</sup>	88.5 <sup>a</sup>	91.5 <sup>a</sup>	119	115	108 <sup>a</sup>	101 <sup>b</sup>	111	129	127 <sup>c</sup>	114 <sup>d</sup>	114 <sup>c</sup>	121 <sup>c</sup>
P Value	0.037	0.001	0.001	0.001	0.001	0.915	0.946	0.007	0.001	0.244	0.197	0.001	0.001	0.001	0.001
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
SEM	1.004	1.097	0.666	0.553	0.556	0.820	0.667	0.571	0.587	0.476	1.616	1.977	1.150	1.251	1.048

Table 3. Effect of dietary inclusion of *Moringa oleifera* leaf meal on egg weight (g) in layers

Diet	Period I	Period II	Period III	Period IV	Overall
	34-37wk	38-41wk	42-45wk	46-49wk	34-49wk
T1	51.3 <sup>c</sup>	52.5 <sup>c</sup>	51.9 <sup>c</sup>	54.7 <sup>d</sup>	52.6 <sup>d</sup>
T2	54.8 <sup>b</sup>	56.2 <sup>b</sup>	57.2 <sup>a</sup>	58.0 <sup>b</sup>	56.6 <sup>b</sup>
T3	55.3 <sup>b</sup>	56.5 <sup>b</sup>	53.8 <sup>b</sup>	56.2 <sup>c</sup>	55.4 <sup>c</sup>
T4	54.8 <sup>b</sup>	55.3 <sup>b</sup>	49.9 <sup>b</sup>	53.4 <sup>e</sup>	53.4 <sup>d</sup>
T5	59.9 <sup>a</sup>	60.0 <sup>a</sup>	58.5 <sup>a</sup>	59.1 <sup>a</sup>	59.4 <sup>a</sup>
P Value	0.001	0.001	0.001	0.001	0.001
N	10.0	10.0	10.0	10.0	10.0
SEM	0.532	0.422	0.510	0.320	0.381

The means with different superscripts in a column differ significantly (P<0.01),(P<0.05)

### Egg weight

In period I and II, the egg weight was significantly higher (P<0.01) in T5 and lower in control birds (Table 3). In period III, the egg weights were significantly higher (P<0.01) in T2 and T5, whereas lower egg weight was recorded in control birds. In period IV, egg weight was significantly (P<0.01) differed in the order of T5>T2>T3>T1>T4. On overall the egg weight was significantly higher in T5 compared to other treatments. The hens in T1 had lower (P<0.01) egg weight than T2 and T3 but did not significantly differ (P>0.01) with T4. The result of the present study is also in consistent with Raphael et al. (2016) who noted a significant effect on egg weight in 5% MOLM diet fed birds. Swain et al. (2017) and Nabila et al. (2015) reported a significant increase in egg weight in Moringa leaf meal included diets. On contrast, Olabode and Okelola (2014) did not find positive effect on egg weight with MOLM.

### Feed conversion ratio

Inclusion of Moringa leaf meal in the layer diets had shown a significant increase in Feed conversion ratio (FCR) among the treatments except T5 in which 10% MOLM was supplemented with enzyme

treatments (Table 2). This was similar with the finding of Olugbemi et al. (2010) who noted that addition of 10% and 20% MOLM to the laying hen diet increased the FCR. In the present study, the improvement of FCR in T5 might be due to the enzyme action attributed to rich content of nutrients in MOLM (Sarwatt et al., 2004) and antimicrobial properties of Moringa (Fahey et al., 2005). Increase in FCR with increase in MOLM inclusion levels in the present study could be due to increase in the fibre content of the diet, which might have impaired nutrient digestibility and absorption (Onu, 2010). Divya et al. (2014) observed no significant difference in FCR of the broiler chicken fed with Moringa leaves powder.

### Feed cost per egg

The feed cost per egg was significantly higher in the control birds when compared to MOLM included diets except T2 with 5% MOLM (Table 4). The significant decrease in the feed cost per egg might be due to the similar feed intake among the birds as well 5.2%, 7.06% and 5.25% decrease of the feed cost per kg of the diet in T3, T4 and T5 diets, respectively when compared to control. Similar results also reported by Swain et al. (2017) with Moringa leaf meal in laying birds.

Table 4. Effect of dietary inclusion of *Moringa oleifera* leaf meal on feed cost per egg (Rs) in layers

Diet	Feed intake (g)	FCR	Egg weight (g)	Feed cost (Rs)
T1	109	124 <sup>c</sup>	52.6 <sup>d</sup>	2.22 <sup>a</sup>
T2	111	125 <sup>c</sup>	56.6 <sup>b</sup>	2.20 <sup>ab</sup>
T3	111	131 <sup>b</sup>	55.4 <sup>c</sup>	2.16 <sup>bc</sup>
T4	112	137 <sup>a</sup>	53.4 <sup>d</sup>	2.12 <sup>c</sup>
T5	111	121 <sup>c</sup>	59.4 <sup>a</sup>	2.13 <sup>c</sup>
P-Value	0.244	0.000	0.000	0.002
N	10	10	10	10
SEM	0.476	1.048	0.381	0.01

The means with different superscripts in a column differ significantly ( $P < 0.01$ ), ( $P < 0.05$ )

### Egg density

Dietary supplementation of MOLM meal in the layer diets did not influence the egg density during the overall experimental period (Table 5). These results are in accordance with the values of Rama Rao et al. (2013), who reported that the dietary inclusion of guar meal up to 15% did not affect the egg density.

### Egg quality parameters

Egg qualities in the present study were affected positively by the dietary inclusion of MOLM at different levels from 34 to 49 weeks of age (Table 5 and 6). Yolk colour, shell strength, haugh unit (HU), albumen length, shell percentage, shell thickness, shell weight, yolk height, yolk width, albumen height and albumen width were all improved especially at 5% and 10% MOLM with addition of enzyme diet. This was similar with the findings of Etalem et al. (2013), Wei Lu et al. (2016), Wubalem et al. (2016) and Gayathri et al. (2020) who noted improvement in egg quality traits with MOLM included diet. Farhana et al. (2021) indicated that addition of 1.5% MOLM improved the egg quality parameters of layers.

The results of the present study showed that egg yolk index, HU and egg shell thickness except in T5, linearly decreased as MOLM levels increased with the lowest values recorded in the egg of hens fed the highest MOLM level and these results are similar to the findings of Ahmad et al. (2018) who noted that egg quality parameters decreased with the increase of MOLM in the diet. Limitations in the use of plant based feed additives are due to anti-nutritional factors, as in *Moringa oleifera* there is high content of fiber, saponins, phytoestrogens and many other compounds (Makkar and Becker, 1999). Higher levels of plant based feed additives hinder the normal metabolism and affect the production, shell thickness and overall egg production (El-Sheikh et al., 2015). Whereas in T5 group, due to the addition of enzymes, the digestibility and the availability of fiber and the other nutrients might have improved which reflected in the egg quality parameters. The shell percentage, shell weight and shell thickness which represents the shell quality were better for MOLM included diets when compared to control. The calcium content in the Moringa leaf meal used in the present study was 2.5% which was higher and contributed to the better shell quality.

Table 5. Effect of dietary inclusion of *Moringa oleifera* leaf meal on egg quality of layers from 34 to 49 weeks of age

Diet	Egg Density (g)	Yolk color	Shell strength (N)	Haugh Unit score	Shell thickness (mm)	Shell percentage	Shell weight (g)
T1	1.09	5.97 <sup>d</sup>	21.9 <sup>d</sup>	70.0 <sup>e</sup>	0.43 <sup>d</sup>	9.28 <sup>d</sup>	4.85 <sup>c</sup>
T2	1.09	8.14 <sup>c</sup>	28.5 <sup>b</sup>	85.4 <sup>b</sup>	0.44 <sup>b</sup>	9.71 <sup>c</sup>	5.43 <sup>b</sup>
T3	1.09	8.55 <sup>b</sup>	25.7 <sup>c</sup>	78.8 <sup>c</sup>	0.48 <sup>c</sup>	9.81 <sup>bc</sup>	5.47 <sup>b</sup>
T4	1.09	8.74 <sup>b</sup>	25.0 <sup>c</sup>	75.6 <sup>d</sup>	0.48 <sup>c</sup>	10.3 <sup>a</sup>	5.45 <sup>b</sup>
T5	1.09	8.92 <sup>a</sup>	35.1 <sup>a</sup>	87.6 <sup>a</sup>	0.43 <sup>a</sup>	10.1 <sup>ab</sup>	5.98 <sup>a</sup>
P Value	0.117	0.001	0.001	0.001	0.001	0.001	0.001
N	10.0	10.0	10.0	10.0	10.0	10.0	10.0
SEM	0.0001	0.158	0.697	0.409	0.004	0.053	0.052

Means with different superscripts in a column differ significantly (P<0.01),(P<0.05).

Table 6. Effect of dietary inclusion of *Moringa oleifera* leaf meal on yolk and albumin quality of layers from 34 to 49 weeks of age

Diet	Yolk index	Yolk height (mm)	Yolk width (mm)	Albumen height (mm)	Albumen width (mm)	Albumen length (mm)	Albumen index
T <sub>1</sub>	1.09	5.97 <sup>d</sup>	21.9 <sup>d</sup>	70.0 <sup>e</sup>	0.43 <sup>d</sup>	9.28 <sup>d</sup>	4.85 <sup>c</sup>
T <sub>2</sub>	1.09	8.14 <sup>c</sup>	28.5 <sup>b</sup>	85.4 <sup>b</sup>	0.44 <sup>b</sup>	9.71 <sup>c</sup>	5.43 <sup>b</sup>
T <sub>3</sub>	1.09	8.55 <sup>b</sup>	25.7 <sup>c</sup>	78.8 <sup>c</sup>	0.48 <sup>c</sup>	9.81 <sup>bc</sup>	5.47 <sup>b</sup>
T <sub>4</sub>	1.09	8.74 <sup>b</sup>	25.0 <sup>c</sup>	75.6 <sup>d</sup>	0.48 <sup>c</sup>	10.3 <sup>a</sup>	5.45 <sup>b</sup>
T <sub>5</sub>	1.09	8.92 <sup>a</sup>	35.1 <sup>a</sup>	87.6 <sup>a</sup>	0.43 <sup>a</sup>	10.1 <sup>ab</sup>	5.98 <sup>a</sup>
P Value	0.117	0.001	0.001	0.001	0.001	0.001	0.001
N	10	10	10	10	10	10	10
SEM	0.0001	0.158	0.697	0.409	0.004	0.053	0.052

Means with different superscripts in a column differ significantly (P<0.01), (P<0.05).

## CONCLUSION

It can be concluded that *Moringa oleifera* leaf meal (MOLM) could be included up to 10 % level with enzyme supplementation in the diets of commercial layers without affecting the egg production, feed intake, feed conversion ratio and egg quality parameters. Besides, addition 10% MOLM with enzyme supplementation decreased the feed cost per egg.

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