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Effect of Different Protein Sources on Microbial Protein Synthesis in Sheep Feed Maize Based Diets

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Abstract: In this study, the effects of different protein sources on microbial protein synthesis were examined. Four Kivircik X Morkaraman (G1), wether fed with maize silage based diets, fistulated with duodenal and ruminal canula and weighing 54.22 kg in average were used in 4×4 Latin Square experimental design. The research was completed in 4 periods each lasted 22 days. The diets were prepared in izocaloric and izonitrogenic on dry matter basis, Soybean Meal (SM), Vetch (V), Chickling Vetch (CV) and maize Gluten Meal (GM) were used in rations as protein source. The animals were housed in individual pen and adlibitum fed. Animals consumed fresh water freely as they needed. Dry Matter (DM), Crude Protein (CP), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Organic Matter (OM), Ammonia Nitrogen (NH_3-N) in the duodenal samples and microbial purin, Volatile Fatty Acids (VFA), CP, NH_3 levels in the ruminal samples were determined in present study. The amounts of Microbial Crude Protein (MCP), CP sourced from feedstuff (By-protein), ammonia nitrogen (NH_3-N) and digestion rates of nutrients in the duodenal digesta were calculated using different equations. The indicator methods, CrO_2 absorbed NDF and microbial purin were used to determine the digestion rates of nutrients and microbial protein in the duodenal digest. The differences among the group means were significant ($p<0.05$) for amounts of acetic butric and propionic acids but not significant for the time of the post feeding. The amounts of the acetic, butric and propionic acids groups were 45.04, 4.32, 9.35 mmol L^{-1} for SM; 82.96, 7.71, 17.81 mmol L^{-1} for V 64.17, 7.72, 14.48 mmol L^{-1} for CV and 65.72, 7.67, 20.56 mmol L^{-1} for GM. The differences among the groups were significant ($p<0.05$) for rumen NH_3 concentration but not significant for pH value. The differences in NH_3 concentration between 2nd and other post feeding times (h) and the differences in terms of the pH value between 0th and other post feeding times were significant ($p<0.05$). The NH_3 concentrations and the pH values for the groups were 16.98, 14.27, 14.58, 14.18 mg 100 mL^{-1} and 6.25, 6.24, 6.11, 6.09, respectively. The difference among the groups were significant ($p<0.05$) for the digestible CP but not significant for true digestibility of the DM, CP, NDF, ADF and OM. Digestibility rates of the nutrients were calculated as 55.71, 57.83, 57.83, 51.33, 62.58% for SM, 63.02, 60.43, 52.13, 40.93, 78.66% for GP, 77.37, 76.81, 69.54, 57.84, 78.66% for BB, 72.72, 64.87, 45.07, 33.70, 66.19% for GM, respectively. The differences among the groups in the duodenal digest was significant for by-pass-N ($p<0.05$) but not significant for NH_3-N and MCP. The amounts of by-pass-N, MCP and NH_3-N were as 8.81, 5.71, 5.06, 9.83 g; 32.41, 16.91, 21.96, 51.25 g; 105.79, 98.56, 115.53, 102.63 g, respectively.

Key words: Ruminant, microbial protein synthesis, soybean meal, vetch, chickling vetch, maize gluten meal

INTRODUCTION

For prevent the protein insufficient, which occurrence with forbidden animals protein sources in ruminant feeding the Legume protein sources have recently take an important interest. The vetch and chickling vetch seeds are good sources of protein supplement. They are also used as silage materials, wet and dry grass for ruminants. The vetch and chickling vetch seeds, which their proteins digested completely in the rumen are cheaper than the oil cake. To estimate the efficiency of the microbial protein synthesis of these feeds, which have highly degradable proteins in rumen brings up the possibility of usage of these feeds in ruminant feeding (Bolat, 1985; Ergül, 1993; Ozen *et al*., 1999; Acikgoz, 2001).

Maize gluten meal is a good by pass protein sources resistant in rumen fermentation and highly digestible in the abomasum and the ileum for especially highly milky cows (Clark *et al*., 1987; Ensminger *et al*., 1990; Piepenbrink and Schingoethe, 1998). By pass proteins rate

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of the maize gluten mea is 55% of total proteins (Clark *et al*., 1987; Ensminger *et al*., 1990). Soybean meal has been used as a protein sources in the animal feeding in a long time (Schingoethe *et al*., 1976). Soybean meal's proteins are higher digestible than maize gluten meal's. By pass proteins rate of this feed is approximately 26-28% of the total proteins (Clark *et al*., 1987). Using crude proteins rate of the feeds is usually not sufficient criterion for protein necessity of the ruminant animals. For this reason, estimation of the microbial protein synthesis rate in the rumen has recently get importance (Karsli, 1998). The term of the microbial protein is used for the protein originated microbes that pass to the dodemum with in the total proteins (Dewhurst *et al*., 2000). The amount of daily synthesis microbial protein proportion to amount of daily degraded organic matter in the rumen uses for the determination efficiency of protein synthesis (Stern and Hoover, 1979). The value for a feed is the contribution to the amino acids profiles pass to the ileum (Madsen and Hvelplund, 1985). Microbial protein synthesis is a function of the microbial mass increase and microbial mass increase speed (Owens and Zinn, 1988; Dewhurst *et al*., 2000). The proportion of the amino acids originate microbial synthesis in total amino acids absorbed from the ileum is >50-75% (Karsli, 1998; Dewhurst *et al*., 2000). As a resul of many researches, Karsli (1998) declereted that the average microbial protein synthesis is 13.0 g/100 g true degredible organic matter in the rumen.

MATERIALS AND METHODS

In this investigation four Morkaraman x Kivircik (G1) rams average 54,22 kg weighted, ruminaly canulated (Dougherty, 1981) and procsimal dodenal T kanulated (Komarek, 1981) have been used. Soybean meal, vetch, chickling vetch and maize gluten meal were used as the protein sources. The investigation was designed in 4×4 Latin Square Method. The groups are soybean meal (the 1st. group), vethc (2nd. group), chickling vetch (3rd group) and maize gluten meal (4th. group) (Düzgüneş *et al*., 1987). Rations were prepared izocaloric and izonitrogenic. The rations had average 15,52% crude protein and 6,14 MJ kg^{-1} (NEL) energy. Dried Sugar Pulps (DSP), Barley (B), Wheat Bran (WB) and Cotton Seed Meal (CSM) were used to make the rations izocaloric and izonitrogenic. The experiment was completed in four periods longed 22 days. Each period included two different periods the former period lasted 9 days (training) and subsequent period lasted 13 days (experimental). Animals were feed 5% of the adlibitum consumption. Feed and feed stuff consumptions were calculated according to this consumption. The nutrient analysis were made according to Akyildiz (1969) and Van Soest (1963) in rumen, duodenum, faces content, feeds and left material from the feeds. Duodenum samples were collected two times in a day during the last four days of the experimental period and the samples times were moved ahead 2 h for each sampling times every day. Duodenum samples were collected in the main period. NH_3 and DM analysis were made according to Markham (1942) and Akyildiz (1969). The ruminal samples were collected six times before the feeding and two hours interval after the feeding in the 22nd day of the every samples period of the research. Total 50 mL sample was collected every samples time in which pH was measured immediately after that 1 mL. Total (%) (w/v) H_2SO_4 was added to the samples and totally 300 mL ruminal liquid was collected. NH_3 N (Markham, 1942) and VFA analysis (Shimadzu Gas Chromaograph GC-14B%10 Ds-1200/%1 H_3DO_4, 80/100 chromosorb WAG, 2×2mm ID9 colon) were made in these samples, The remaining samples were unified and dried (Karsli, 1998) in which crude protein (Akyildiz, 1969) and RNA (microbial purin) analysis were made according to Zinn and Owen (1986).

Chromium analyses were made according to Williams *et al*. (1962) in faces and duodenal samples. The purin analyses were made according to Zinn and Owen (1986) in ruminal and duodenal samples. NH_3-N analyses in ruminal liquid were made by Markham Distilation Method adapted to the Micro Kjeldahl Method. Dry matter and crude protein analysis in the faces, ruminal and duodenal samples were made according to Wendee Method (Akyildiz, 1969). The statistic analysis and the differences between the groups were analyzed in the SAS Institute Inc. (1993) packet program.

RESULTS AND DISCUSSION

The daily feed stuff consumptions in the groups are given in Table 1. The other data obtained from research are summered in the Table 2-5. The results that come to light by this investigation were discussed from different

Table 1: The feed stuff rate (%) and metabolic energy value (in DM) used in the rations

FEED	DM (%)	CP (%)	CF (%)	EE (%)	ASH (%)	NFE (%)	NEL*
CV	90.44	28.44	8.45	1.81	3.41	57.89	8.77
V	89.09	32.93	7.17	1.07	4.25	54.59	8.67
SM	89.58	48.69	11.26	3.21	7.08	29.76	9.31
GM	90.06	62.95	0.65	2.11	1.33	32.95	8.30
DSP	89.80	10.67	20.56	0.99	5.40	62.38	7.72
B	91.23	11.96	4.53	1.86	2.24	79.41	7.10
WB	88.47	15.76	8.13	5.69	5.28	65.14	9.54
CSM	95.27	25.33	20.05	7.31	6.39	40.92	5.54
MS	24.25	6.53	26.45	1.25	6.29	59.48	4.72

NEL values were calculated according to Meyer

Table 2: The true digestible rate of the feed stuff

Feed	*DM (%)	*OM (%)	**CP (%)	*NDF (%)	*ADF (%)
	$\bar{X} \pm S\bar{X}$				
SM	69.84±6.27	81.24±4.96	64.38±7.25ab	56.37±10.46	58.74±11.82
V	69.32±6.27	73.81±4.96	83.21±7.25ab	55.01±10.46	40.50±11.82
CV	75.18±6.27	79.52±4.96	88.79±7.25a	65.87±10.46	55.57±11.82
GM	68.31±6.27	77.56±4.96	56.80±7.25b	58.35±10.46	55.67±11.82
General	70.66	78.03	73.29	58.90	52.61
SEM	3.24	2.56	3.74	5.40	6.10

*The differences are not significant, ab: the differences between value that have same latter in the same column are significant, **p<0.05

Table 3: The daily feed stuff consumption in the groups

Feed	*DM (%)	*OM (%)	*CP (%)	*NDF (%)	*ADF (%)
	$\bar{X} \pm S\bar{X}$				
SM	1.208±0.26	1.148±0.30	0.136±0.36	0.485±0.14	0.325±0.83
V	1.240±0.26	1.182±0.30	0.141±0.36	0.577±0.14	0.329±0.83
CV	1.705±0.26	1.630±0.30	0.186±0.36	0.792±0.14	0.448±0.83
GM	1.253±0.26	1.177±0.30	0.136±0.36	0.521±0.14	0.303±0.83
General	1.3519	1.2841	0.14987	0.59395	0.35131
SEM	167.28	156.92	18.56	74.13	42.92

*The differences are not significant

Table 4: The amount of the daily mass (g/day)and the content (%) of the mass to pass duodenum duodenum

Feed	DM g day^{-1}	*OM (%)	**CP (%)	*NDF (%)	*ADF (%)
	$\bar{X} \pm S\bar{X}$				
SM	593.06±25.96	72.94±2.41	25.77±1.21ab	35.12±4.26	22.73±1.91b
V	652.07±25.96	79.17±2.41	21.30±1.21b	42.99±4.26	33.18±1.91a
CV	646.74±25.96	81.14±2.41	24.23±1.21b	38.33±4.26	30.93±1.91a
GM	602.90±25.96	77.99±2.41	26.51±1.21a	34.33±4.26	21.15±1.91b
GENERAL	635.22	77.81	23,20	37.69	27.00
SEM	13.40	1.25	0.63	2.20	0.99

*The differences are not significant, ab: The differences between value that have same latter in the same column are significant, **p<0.05

Table 5: The amount of Mic-CP synthesis in the rumen, by pass protein and NH3-N rate in the mass passed to the duodenum

Feed	*Mic-CP g day^{-1}	**By pass protein g day^{-1}	*NH^3-N g day^{-1}	EMPS***
	$\bar{X} \pm S\bar{X}$			
SM	105.79±19.17	32.41±7.38ab	8.81±1.98	12.70±4.49
V	98.56±19.17	16.91±7.38b	5.71±1.98	15.56±4.49
CV	115.53±19.17	21.96±7.38b	5.07±1.98	13.10± 4.49
GM	102.63±19.17	51.26±7.38a	9.83±1.98	10.80±4.49
GENERAL	105.63	30.64	7.36	13.04
SEM	9.90	3.81	1.02	2.32

*The differences are not significant, **p<0.05, ***Mic-CPg/100g TDOM, ab: the differences between value that have same latter in the same column are significant

aspect. This investigation investigates the microbial protein synthesis efficiency of the Soybean Meal (SB), Vetch (V), Chcklen Vetch (CV) and maize Gluten Meal (GM) in the rumen. There are no different DM, OM, CP, NDF and ADF consumption in the groups. But DM consumption, first of all and other parameters are numerically the highest in the V group. The numerical differences, which were observed in this group were thought from the anti nutritional factors (Roy, 1981; Mangan, 1988; Liener, 1989; Dixon and Hosking, 1992), different structure of the carbohydrate and protein in the feed (Dixon and Hosking, 1992; Hanbury *et al*., 2000) and the difference flavor of the feed dependent upon these factors (Kilic, 1985). Beside all these factors, the digestibility of the DM and other feed stuffs may be caused these differences. Likewise the results to get in this research concerning total and ruminal digestibility rate of DM and other nutrient have also same results. SThe maximum total digestibility rate of the OM was observed for CV and the minimum for V in the feed groups. These differences observed between the groups in the total digestibility of DM and OM was not significant. The results observed in the digestibility of DM and OM are similar the literature (Hanbury *et al*., 2000). Although, the statistical differences weren't found in the groups for OM and DM, the numerical differences found in the groups were caused by the constructions of the feed stuf and anti nutritional factors in the feed (Huisman and Jansman, 1991; Hanbury *et al*., 2000) and by the different degradations of these feeds observed in rumen (Table 2). We know that the anti nutritional factors, which have negative effect on the digestibility

and utilization of energy and nutrients (McSweeney *et al*., 2001; Seabra *et al*., 2001) are not wholly eleminated (Dixon and Hosking, 1992) also most of them eliminated in the rumen (Majak, 1992).

There are not any differences digestibility rates of the CP in the groups. As DM and OM, the highest digestibility rate of CP have been observed with 76.81% in the V. The reasons of the high digestibility observed in CP in the V are probably that the vetch have high rate solubility proteins, low tannin rate, which high in other groups and even so the effects of β-(N-oxalylamino) alanin = ODAP, the undistracted amino acid an anti nutritional factor in V decreased with rapidly digestion rate in the rumen (Hanbury *et al*., 2000).

The highest digestibility rate of the NDF with 69,54% was obtained in CV and the lowest rate with 52.13% in the V. The differences obtained in the groups are significant ($p<0.05$).

The highest digestibility rate of the ADF with 57.84% was obtained in CV too but lowest rate with 33.70% was obtained in GM and the differences obtained in the groups are significant ($p<0.05$).

When the results obtained in the rate of the NDF and ADF digestibility (Table 3) have been controlled, the essential differences have obtained in between GM and CV groups. According to this, both NDF digestibility and ADF digestibility in the GM were lower than CV and the differences between these groups were significant ($p<0.05$). Cotton seed meal, dry sugar beep pulp and wheat bran formed these reasons because the CV included these feeds less than GM in total ration. GM included the KSM, DSBP and WB approximately 75% rate in it. Because the NDF and ADF in DM intake were high in GM group and low in CV group, the fiber digestibility is low in GM group and high in CV group and these results are very classical knowledge.

When the results of the true digestibility rate of the DM, OM, NDF and ADF were investigated, any statistical significant were not obtained in the groups. But the true digestibility rates of the CP were significant in the groups. While the highest rate was obtained CV group, the lowest rate was obtained in the GM group. The differences between the groups in the true digestibility rate of the CP were significant ($p<0.05$). The highest rate of the CP digestibility was in the CV group and the lowest in GM group. The reason of the high digestibility in the CV group is that the CV group has highly solubility protein in the water for why the protein of the CV mostly demolished in the rumen (Boulter and Derbyshire, 1976; Nocek and Russell, 1988; Ensminger *et al*., 1990; Dixon and Hosking, 1992; Hanbury *et al*., 2000). Even if there was no significant difference between V and other groups in which include GM and SM, in the same way V group has highly soluble protein in the rumen and digestibility rate of the CP of the V similar to CV. This situation came into existence from same reason.

We quests that the cause of the low digestibility of the CP of the GM group in the rumen is that this group has non soluble protein in water and by pass protein in higher amount than other groups (Boulter and Derbyshire, 1976; Nocek and Russell, 1988; Ensminger *et al*., 1990; Dixon and Hosking, 1992; Hanbury *et al*., 2000).

True digestibility rate of the feed stuff expresses total digestibility of this feed stuff in the rumen. True digestibility rate is a ratio of the feed stuff passed to duodenum, which is not include the microbial mass to total consumption of the feed stuff (Karsli and Russell, 2000). The real digestion rate observed in the ration groups was higher than the total digestion rate (Table 2 and 3) because of the incomplete digestion of the microbial mass in small intestines and the proportional increase in the amount of feces with the addition of the endogenous nutrients due to the growth of both the rumen and colon originated microorganisms in the content (Holden *et al*., 1994; Karsli and Russell, 2000).

The amounts of acetic, propyonic and butyric acids formed in the rumen were found to be significantly ($p<0.05$) different among the ration groups. The main difference among the ration groups for these three acids was mainly observed between the CSM groups and the others. Although, the starch level of the ration is high especially in the groups C and CV, it has been thought that the reason for the especially high acetic acid amounts in these granule rations has been caused by the high levels of polysaccharides other than starch (De Visser *et al*., 1992; Erasmus *et al*., 1994).

There was no significant difference observed on the alterations of the acetic, propyonic and butyric acids among the ration groups in time. As seen from the Table 4, the means of butyric and propyonic acids were low in amount at the time of 0 h but were high in amount at the second h. Acetic acid, one of the Volatile Fatty Acids (VFG), was the highest in amount at the time of 2 h, but lowest in amount at the time of 8 h (Table 4). The data obtained for VFGs was in agreement with the literature. The VFG amount formed in the rumen due to rapid breakage of nutrients with the increasing microbial activity soon after the feeding increased from the first half h to the 4th h and consequently the rumen pH decreased because of the same reason (Church, 1979; Hoover and Stokes, 1991). There was no significant difference for pH among the ration groups. By investigating the interactions between the time period and the ration type (Table 4), the significant ($p<0.05$) differences were

determined for the pH values in the ration groups between the 0 h and the 2, 4, 6, 8 and 10 h but not for the other h. As shown from the Table 4, the data obtained for the time period after the feeding are in line with the literature. The pH of the rumen dropped during the first period after feeding (the first half hour to the forth hour) due to the VFG production; then, in the later hours, the pH elevated to its normal level because of the absorptions of these acids from the rumen (Kilic, 1985; Hoover, 1986; Owens and Goetsch, 1988). The differences observed among the mean NH_3 values of the ration groups were significant ($p<0.05$). As seen from the Table 4, the significant ($p<0.05$) differences were determined for the NH_3 values in the ration groups between the 2 h and 0, 4, 6, 8 and 10 h but not for the other hours. The NH_3 amounts detected for the ration groups were higher than the normal values, 3-8 mg NH_3 100 mL^{-1}. It is thought that the observed high NH_3 level in the groups was not only due to high total protein levels in the rations but also due to high levels of easily soluble protein levels in the rations. It has been thought that the C, CV and GM groups had lower NH_3 levels than the CSM group because the microbial protein synthesis increase in the C and CV groups due to the high starch level and the gluten from corn in the GM group is less washable in the rumen (Madsen and Hvelplund, 1985). The higher amounts of NH_3 and MPSE in the groups CV and C compared to the groups CSM and GM were due to the high soluble levels of proteins as starches in the former groups having high levels of starches (Karsli, 1998). The NH_3 level of in the group GM was low because the proteins of this ration were less washable in the rumen.

There was no significant difference for the dry matter content of the substances passing to duodenum. As shown in Table 5, the highest second value for the dry matter content of the substances passing to duodenum was obtained from the group CV, where the dry matter consumption was higher (Table 1). It was observed that breakage level of organic matters in the rumen was higher in the group CV than the other groups (Table 1 and 5). The amounts detected among the different ration groups were close to each other for this trait.

There was no significant difference for the daily dry matter amount of the substances passing to duodenum and for the OM and NDF levels of this dry matter. On the other hand, There were significant ($p<0.05$) differences among the ration groups for the CP and ADF levels of this dry matter because the proteins found in the C and CV groups could not be broken in the rumen due to their structural properties and there was elevated loss of nitrogen in these groups having lower bypassing rates than the CSM and GM groups (Tamminga, 1979).

There was significantly ($p<0.05$) higher amounts of ADF levels passing to duodenum in the groups C and CV than the groups CSM and GM not only because of the higher consumed amounts of ADF in the former groups but also because of the less staying time of these rations having fast fermentations in the rumen (Blank *et al*., 1998). There was also similar but insignificant cases for the NDF amount (Table 5).

There was no significant difference for the amounts and rates of synthesized microbial proteins among the ration groups. The group CV had the highest crude microbial protein amount. It has been reported that the 50-85% of the CP passing to duodenum is Mic-CP (Karsli, 1998; Dewhurst *et al*., 2000). The highest (81%) and lowest (61%) microbial protein syntheses (Table 5) detected in the present study were in agreement with the findings of other researchers by Cole *et al*. (1976), Stokes *et al*. (1991), Erasmus *et al*. (1986) and Karsli (1998). The differences observed among the ration based protein amounts (by-passes) were significant ($p<0.05$) among the ration groups. However, there was no significant difference for the amounts of NH_3 N passing to duodenum and Microbial Protein Synthesis Efficiency (MPSE) among the ration groups (Table 5).

The group CV had the highest dry matter consumption and microbial proteins synthesized in the rumen of the all the ration groups studied. Therefore, it was thought that one of the reasons for the higher microbial protein amount was most probably due to the higher dry matter consumption. Microbial proteins synthesis and MPSE increase linearly with the dry matter consumption (Karsli, 1998). It has been reported that the CP amount of the ration should range from 11-13% in order to obtain the optimal Microbial proteins synthesis (Hume, 1970; Satter and Roffler, 1975; Karsli and Russell, 2000). However, these proteins should be formed with the digestible ones in the rumen (Ludden and Cecava, 1995). It could be said that the amount of the microbial proteins synthesis decreased because of the lower dry matter consumption in the group C. The higher MPSE in this group could be explained with the better balance between the energy and the nitrogen and with the higher wash of them in the rumen. The reason of the lower MPSE and microbial proteins synthesis in the group GM was due to retardation of microbial growth with the higher by-pass protein rate detected in this ration (Satter and Roffler, 1975; Ensminger *et al*., 1990; Karsli and Russell, 2000). The first consideration for the group GM based on the isocaloric and isonitrogenic sides of the rations is that its nitrogen source is not suitable for the protein synthesis (Ensminger *et al*., 1990). The GM group followed by the group CSM had higher by-pass protein amount passing

duodenum than the groups C and CV (Table 4 and 5). The present finding observed in the GM group was in line with the literature (Stokes *et al*., 1991; Erasmus *et al*., 1994; Henning *et al*., 1993; Ludden and Cecava, 1995). It has been thought that this is because of the higher solubility of leguminous proteins and lower solubility of cereal proteins in the rumen. It has been also thought that the high levels of by-pass proteins in the CSM group is because of the heat process applied during the CSM production which cause proteins to denature (Boulter and Derbyshire, 1976; Nocek and Russell, 1988; Erasmus *et al*., 1986; Dixon and Hosking, 1992).

CONCLUSION

As a result, the present study using vicia, lathyrus and corn glutamine instead of soybean pulp states generally that for sheep:

- There was no negative effect of leguminous seed feeds (vicia and lathyrus) and corn glutamine on the feed and nutrient consumption
- There was no negative effect of these feeding materials on the dry matter and OM and the total digestibility of CP, NDF and ADF or their digestibility in the rumen
- These feeding materials increase the production of VFG such as acetic, propyonic and butyric acids but cause no difference on the rumen pH
- There is no significant difference between these feeding materials and soybean pulp for the microbial proteins synthesized in the rumen and MPSE

The more extended future studies should be conducted in order to investigate the relevant subjects in detail.

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