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Response of Growing Lambs Fed on Different Vegetable Protein Sources with or without Probiotics

MUHAMMAD FAROOQ KHALID[1], MUHAMMAD SARWAR[1], MAHR-UN-NISA AND ZIA-UR-REHMAN†
Institute of Animal Nutrition and Feed Technology, University of Agriculture, Faisalabad, Pakistan
†*Department of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan*
[1]Corresponding author's e-mails: drms01@gmail.com; farooq325@gmail.com

ABSTRACT

The study was planned to examine the effects of different protein sources with or without probiotics on the nutrients intake and their digestibility, nitrogen (N) balance, blood metabolites and growth performance. For this purpose, thirty two six month old lambs were randomly divided into eight groups, four animals in each, in a 4×2 factorial arrangement. Four iso-caloric and iso-nitrogenous diets were formulated using different protein sources [corn gluten meal 30% (CGM), canola meal (CM), cotton seed meal (CSM) and sunflower meal (SFM)] with (50 g/ton) or without probiotics. Dry matter (DM) and crude protein (CP) intake were higher (5.54%) in lambs fed CM diet, whereas neutral detergent fiber (NDF) and Acid detergent fiber (ADF) intake were higher in animals fed SFM diet. Dry matter, CP, NDF and ADF digestibility was not affected (P>0.05) in lambs fed diets containing different protein sources. Addition of probiotics increased (P<0.05) NDF and ADF digestibility (9.0 & 9.56%, respectively). Blood glucose was significantly (P<0.05) higher (18.67%) in lambs fed CM diets. The N-intake and N-balance were different (P<0.05) in lambs fed diets containing different protein sources. Highest weight gain was observed in lambs fed CM diet, and lowest in lambs fed SFM diet. Feed conversion ratio was similar (P>0.05) across all the treatments. It is concluded that CM is better protein source for growing lambs than CGM, CSM and SFM. Moreover, diets containing probiotics significantly influenced the growth performance of growing experimental lambs. © 2011 Friends Science Publishers

Key Words: Protein sources; Nitrogen balance; Blood metabolities; Growing lambs

INTRODUCTION

Sheep are numerically and socio-economically important animals in Pakistan and provide quality animal proteins for human consumption (Aldomy *et al.*, 2009; Shahzad *et al.,* 2010). More than 20 sheep breeds are found in Pakistan (Bhutto *et al*., 1993; Sarwar *et al.,* 2010a). *Kajli* is one of the famous sheep breed for its mutton and wool production. Its better carcass yield, quality meat, in addition to being a good-looking animal makes it an animal of choice among farmers and consumers. Unlike Australia and New Zealand, the countries with well maintained grazing pastures and grasslands, Pakistan doesn't have much to offer for grazing of these animals. These animals graze mainly on weeds, grassy vegetations in fallow lands along with leaves and beans of wild trees. A low productivity of these animals is because of poor quality feed stuff (Sarwar *et al*., 2002; Shahzad *et al.,* 2009a). Their productivity can be enhanced by feeding them balanced ration. This is possible if we shift from grazing feeding system to stall feeding system.

Some sporadic studies have been reported in which sheep were maintained under stall feeding (Khan *et al*., 1997; Irshaid *et al*., 2003; Sarwar *et al*., 2010b). Sheep have the ability to produce quality carcass at the age of six months if fed diets with high proteins. Different protein sources are known to increase nutrient intake and digestibility, enhanced rumen microbial enzyme production leading to improved weight gain (William *et al*., 1991).

The nutrient utilization can further be improved by accelerating the rumen function through the use of feed additives like probiotics (Ding *et al*., 2008). Probiotics have also been reported to increase daily weight gains in lambs by improving nutrient utilization in the rumen due to their positive effects on rumen microflora (Abd El-Ghani, 2004). The present study was planned to investigate the effect of different protein sources with or without probiotics on nutrient intakes and their digestibility, growth performance, blood chemistry and nitrogen (N) balance in 6 month old *Kajli* male lambs.

MATERIALS AND METHODS

Experimental diets and lambs husbandry: Thirty two male *Kajli* lambs of six months age used in the previous trial were randomly divided into eight groups of four animals each in a 4×2 factorial arrangement to study the effect of

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different protein sources with or without probiotics (Protexin®) on the nutrients intake and their digestibility, growth performance, blood chemistry and N-balance. Two factors were protein sources (Canola meal, cotton seed meal, corn gluten meal 30% & sunflower meal) and probiotic level (0 & 50 g/ton). The lambs were fed isocaloric (70% TDN) and isonitrogenous (18% CP) diets formulated using different protein sources according to NRC (1985) requirements (Table I). The diets containing cotton seed meal (CGM), canola meal (CM), cotton seed meal (CSM) and sunflower meal (SFM) were supplemented with either 0 or 50 g/ton of Protexin®. All the lambs were maintained in individual pens and fresh and clean drinking water was made available round the clock. Lambs were vaccinated against local prevalent diseases.

Growth performance study: The growth trial lasted for 90 days including a 10 days feed adjustment period. Lambs were offered experimental diets twice a daily. Feed intake was recorded daily and the residues were collected and weighed. The lambs were weighed weekly before morning feeding. Feed conversion ratio was worked out by dividing the feed intake (g/day) by weight gain (g/day). The dry matter (DM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) intake was also calculated. All the lambs were shifted to metabolic cages, which were specially designed for urine and feces collection, separately to determine digestibility by total collection method. After adaptation period, 5 days served as collection period and the same was repeated after every 20 days thereafter. Four digestibility trials were conducted during the whole experimental period. Urine was collected in the removable plastic bowls set beneath the metabolic cages. The urine samples collected daily in plastic bowls were acidified with 50% H_2SO_4 to prevent any N losses (Nisa *et al*., 2004). Total feces and urine voided by individual animal was weighed daily and composited by animal and these stored at -20°C for further analysis. At the end of each collection period, the samples were thawed. Fecal samples were dried at 55°C and ground through 1 mm screen. Feed and fecal samples were analyzed for ADF and NDF following the procedure laid down by Van Soest *et al*. (1991) and DM and nitrogen contents using Kjeldahl method described by AOAC (1990). The nitrogen balance was calculated by using the equation described by NRC (1985).

Blood sampling and biochemical analysis: At the end of this study, blood samples were collected six hours post feeding. Blood sample (10 mL from each lamb) was collected by puncturing jugular vein; 2 mL was collected into the vaccutainers each containing 81 µL of 15% EDTA (anticoagulant) solution, while 8 mL was collected in test tube to harvest the serum for further analysis. Plasma samples were separated and frozen at -20°C within 60 min. of collection. Blood samples were analyzed for plasma glucose, blood urea nitrogen (BUN; Bull *et al*., 1991) and creatinine according to method described by Davies *et al*. (2007).

Statistical analysis: The data thus collected were analyzed using the GLM procedure of SAS (1998) using 4×2 factorial arrangement in a completely randomized design. The Model statement was:

$$Y = \mu + PS_i + PR_j + (PS \times PR)_{ij} + e_{ijk}$$

Where Y = any of dependent variable tested in the study; μ = overall mean, PS_i = protein sources: either CM, CGF, CSM or SFM; PR_j = level of probiotic either 0 or 50 g /ton; $(PS \times PR)_{ij}$ = interaction between protein source and probiotic level; and e_{ijk} = residual error.

The means were compared using the Least Significant Difference option of General Linear Model procedure and declared significant at $P < 0.05$.

RESULTS

Nutrient intake and digestibility: Dry matter, CP, NDF and ADF intake was different (P<0.05) in lambs fed diets containing different protein source (Table II). Higher DM and CP intake was observed in CM diet and NDF and ADF intake was higher (P<0.05) in lambs fed SFM diet. Dry matter, CP and NDF digestibility was higher in lambs fed CM diet, and ADF digestibility was higher in CSM diet (Table III). Addition of probiotics did not influence (P>0.05) DM, CP, NDF and ADF intake. but improved (P<0.05) NDF and ADF digestibility. No interaction was observed between protein source and probiotics level on DM, CP, NDF and ADF intake or their digestibility (Table II & III).

Blood metabolites: Blood urea nitrogen and creatinine (Table IV) were not affected (P>0.05) by protein source or probiotics level Blood glucose concentration differed (P<0.05) across all protein source diets (Table IV). Higher blood glucose concentration was observed in lambs fed CM diet, but was not affected due to probiotics supplementation The interaction between protein source and probiotics was non-significant (P>0.05) for BUN and creatinine, however, blood glucose concentration behaved differently(P<0.05).

Nitrogen balance: Nitrogen intake in lambs was different (P<0.05) due to protein sources (Table V). Higher N-intake was noticed in lambs fed CM diet followed by CGM, SFM and CSM. Probiotics supplementation did not influence N-intake in lambs. Protein source and probiotics level interaction was non-significant (P>0.05) for N-intake. Fecal and urinary N remained unaffected (P>0.05) in lambs due to all the dietary treatments (Table V). Nitrogen balance was different (P<0.05) due to different protein sources. Highest N was retained by lambs fed CM diet, and lowest in lambs fed SFM diet. Probiotics supplementation had no influence on N-balance.

Growth performance: Total weight gain by lambs was different (P<0.05) due to different protein sources. Total weight gain was highest in lambs fed CM diet and lowest in lambs fed SFM diet. Similarly, probiotic supplementation improved (P<0.05) total weight gain. The FCR was not affected (P>0.05) by any of the dietary treatments (Table VI).

Table I: Ingredient composition of experimental diets

Ingredients	Diets[1]							
	CGM		CM		CSM		SFM	
	P_0	P_1	P_0	P_1	P_0	P_1	P_0	P_1
Maize	30	30	30	30	30	30	30	30
Rice polishing	2	2	8	8	4	4	4.1	4.1
Wheat Bran	11.1	11.1	12	12	14	14	9	9
Wheat Straw	13	13	13	13	13	13	12	12
Sunflower meal	0	0	0	0	0	0	34	34
Canola meal	0	0	28	28	0	0	0	0
Cotton seed meal	0	0	0	0	29	29	0	0
Maize Oil	0	0	0	0	1	1	3	3
Corn gluten meal (30%)	34	34	0	0	0	0	0	0
Molasses	4	4	4	4	4	4	2	2
Urea	0.9	0.9	0	0	0	0	0.9	0.9
Sodium bi-carbonate	2	2	2	2	2	2	2	2
Common salt	1	1	1	1	1	1	1	1
Di-calcium phosphate	2	2	2	2	2	2	2	2
Protexin®	0	0.005	0	0.005	0	0.005	0	0.005
Chemical composition (%)								
Crude protein	17.98	17.98	17.94	17.94	18.01	18.01	17.95	17.95
Dry matter	90.1	90.1	90.34	90.34	90.67	90.67	91.04	91.04
Total digestible nutrients	71.85	71.85	71.31	71.31	71.88	71.88	69.94	69.94
Neutral detergent fiber	15.37	15.37	23.15	23.15	25.65	25.65	38.11	38.11
Acid detergent fiber	10.98	10.98	14.06	14.06	9.37	9.37	18.4	18.4
Calcium	0.57	0.57	0.74	0.74	0.59	0.59	0.65	0.65
Phosphorus	0.83	0.83	1.06	1.06	1.07	1.07	0.97	0.97
Potassium	0.69	0.69	1.07	1.07	1.18	1.18	0.94	0.94
Chloride	0.79	0.79	0.80	0.80	0.79	0.79	0.77	0.77
Sulfur	0.25	0.25	0.46	0.46	0.22	0.22	0.19	0.19

[1]CGM, CM, CSM and SFM stand for corn gluten meal, canola meal, cotton seed meal and sunflower meal, respectively. $P_{0=}$ 0 probiotics and $P_{1=}$ 50 g/ton probiotics

Table II: Effect of different protein sources with or without probiotics on nutrient intake in growing *Kajli* male lambs

Nutrients	Diets[1]								SE	Significance		
	CGM		CM		CSM		SFM			A	B	AB
	P_0	P_1	P_0	P_1	P_0	P_1	P_0	P_1				
Nutrients Intake, g/d												
Dry matter	1226	1256	1271	1283	1246	1278	1207	1213	13.5	*	NS	NS
Crude protein	221.0	226.0	228.8	230.9	206.0	212.0	217.0	218.0	2.4	*	NS	NS
Neutral detergent fibre	188.0	193.0	294.2	297.0	294.0	302.0	460.0	462.0	3.0	*	NS	NS
Acid detergent fibre	135.0	138.0	178.7	180.4	107.0	110.0	222.0	223.0	1.8	*	NS	NS

[1]CGM, CM, CSM and SFM stand for corn gluten meal, canola meal, cotton seed meal and sunflower meal, respectively. $P_{0=}$ 0 probiotics and $P_{1=}$ 50g/ton probiotics. SE = standard error. Factor A= Protein source, Factor B= Probiotic level and AB= Interaction of protein source with probiotic level. NS= Non Significant and *= Significant ($P<0.05$)

Table III: Effect of different protein sources with or without probiotics on nutrients digestibilities in growing *Kajli* male lambs

Nutrients	Diets[1]								SE	Significance		
	CGM		CM		CSM		SFM			A	B	AB
	P_0	P_1	P_0	P_1	P_0	P_1	P_0	P_1				
Nutrient digestibility, %												
Dry matter	66.0	68.5	69.5	70.5	65.3	66.5	60.7	61.9	2.4	NS	NS	NS
Crude protein	68.6	69.3	69.8	69.9	69.5	69.8	67.9	68.3	1.3	NS	NS	NS
Neutral detergent fibre	50.4	56.2	52.4	56.7	51.1	54.9	47.3	51.5	1.9	NS	*	NS
Acid detergent fibre	42.9	48.2	43.8	47.6	45.5	48.0	42.4	47.5	2.2	NS	*	NS

[1]CGM, CM, CSM and SFM stand for corn gluten meal, canola meal, cotton seed meal and sunflower meal, respectively. $P_{0=}$ 0 probiotics and $P_{1=}$ 50g/ton probiotics. SE = standard error. Factor A= Protein source, Factor B= Probiotic level and AB= Interaction of protein source with probiotic level. NS= Non Significant and *= Significant ($P<0.05$)

DISCUSSION

Higher dry matter intake (DMI) in lambs fed CM diet is supported by the findings of (Wiese *et al*., 2003), who reported higher DMI in lambs fed CM diet than those fed lupin and urea diet. This may be the result of better availability of nutrients and their readily digestion by rumen microbes. Another plausible reason for higher DMI in lambs

Table IV: Effect of different protein sources with or without probiotics on blood metabolites in growing *Kajli* male lambs

Items (mg/dL)	Diets[1]								SE	Significance		
	CGM		CM		CSM		SFM			A	B	AB
	P_0	P_1	P_0	P_1	P_0	P_1	P_0	P_1				
Blood urea nitrogen	19.6	19.8	19.3	18.5	19.1	19.5	17.8	18.5	1.8	NS	NS	NS
Blood Glucose	60.1	60.8	60.0	63.3	49.7	54.2	58.0	58.8	1.6	*	NS	*
Creatinine	0.75	0.78	0.68	0.70	0.72	0.77	0.83	0.78	0.74	NS	NS	NS

[1]CGM, CM, CSM and SFM stand for corn gluten meal, canola meal, cotton seed meal and sunflower meal, respectively. P_0= 0 probiotics and P_1= 50g/ton probiotics. SE = standard error. Factor A= Protein source, Factor B= Probiotic level and AB= Interaction of protein source with probiotic level. NS= Non Significant and *= Significant (P<0.05)

Table V: Effect of different protein sources with or without probiotics on nitrogen balance in growing *Kajli* male lambs

Items (g/day)	Diets[1]								SE	Significance		
	CGM		CM		CSM		SFM			A	B	AB
	P_0	P_1	P_0	P_1	P_0	P_1	P_0	P_1				
N intake	35.4	36.2	36.6	37.0	33.0	33.9	34.7	34.9	0.4	*	NS	NS
Faecal N	11.08	11.10	10.18	10.25	10.08	10.26	10.80	10.73	0.48	NS	NS	NS
Urinary N	0.255	0.247	0.253	0.246	0.254	0.245	0.256	0.248	0.02	NS	NS	NS
N Balance	24.0	24.8	26.2	26.5	22.6	23.4	23.7	23.9	0.6	*	NS	NS

[1]CGM, CM, CSM and SFM stand for corn gluten meal, canola meal, cotton seed meal and sunflower meal, respectively. P_0= 0 probiotics and P_1= 50g/ton probiotics. SE = standard error. Factor A= Protein source, Factor B= Probiotic level and AB= Interaction of protein source with probiotic level. NS= Non Significant and *= Significant (P<0.05)

Table VI: Effect of different protein sources with or without probiotics on growth performance in growing *Kajli* lambs

Items	Diets[1]								SE	Significance		
	CGM		CM		CSM		SFM			A	B	AB
	P_0	P_1	P_0	P_1	P_0	P_1	P_0	P_1				
Initial Wt (kg)	28.2	24.3	24.0	23.1	24.9	27.6	25.9	24.2	0.9	*	*	NS
Final Wt (kg)	45.1	41.8	41.9	42.0	41.2	44.6	41.4	40.4	0.9	*	*	NS
Daily gain (g)	187.8	194.4	198.6	209.9	181.1	188.9	171.7	180.0	12.0	*	NS	NS
Weight gain (kg)	16.9	17.5	17.9	18.9	16.3	17.0	15.5	16.2	0.7	*	*	NS
Feed conversion ratio	6.53	6.46	6.4	6.11	6.88	6.77	7.03	6.74	0.65	NS	NS	NS

[1]CGM, CM, CSM and SFM stand for corn gluten meal, canola meal, cotton seed meal and sunflower meal, respectively. P_0= 0 probiotics and P_1= 50g/ton probiotics. SE = standard error. Factor A= Protein source, Factor B= Probiotic level and AB= Interaction of protein source with probiotic level. NS= Non Significant and *= Significant (P<0.05)

fed CM diet is better digestibility (Baile & Forbes, 1972; Shahzad *et al.,* 2009b), which might increase digestion and passage rates (Mertens, 1977). Lower DMI in lambs fed SFM diet might be due to high dietary NDF and ADF content that may limit DMI (Klopfenstein, 1991), while higher NDF and ADF intake by lambs fed SFM is due to higher dietary NDF and ADF contents (Carneiro *et al*., 2006). The results of the present study are supported by other researchers (Chakeredza *et al*., 2003; Ponnampalam *et al*., 2005), who reported different DMI in lambs fed different protein sources.

The DMI was not influenced by probiotic supplementation. This may be either due to the nutrient rich diets that were supplying sufficient nutrient to microbes (Begum *et al.*, 2010; Javaid *et al*., 2011) or the inability of supplemented probiotics to alter the morphology of intestinal mucosa (Buts *et al*., 1986), which is strongly diet dependent (Auclair, 2000). The results of the present study are in agreement with the findings of other researchers (Antunovic, 2005, 2006; Chaucheyras-Durand *et al*., 2008; Whitley *et al.,* 2009) who reported no change in DMI by animals fed diets containing probiotics. They further stated that absence of change in DMI may be due to less adhesion of microbial cells of probiotics that is required for desirable changes in ruminal fermentation.

The absence of change in DM digestibility in lambs fed different protein sources is supported by the findings of other researchers (Stern *et al*., 1983; Khan *et al*., 1997; Phillips & Rao, 2001). Higher DM digestibility by lambs fed CM diet might be the result of more availability of S at ruminal level to synthesize S-containing amino acids and vitamins (Khan *et al*., 1997) that may increased the microbial growth (Sniffen & Robinson, 1987) leading to more production of microbial enzymes per unit time and thus improved DM digestibility (Edward *et al*., 2008). Lower DM digestibility in SFM diets may be due to more dietary NDF (Staples *et al*., 1984). Higher NDF and ADF digestibility in probiotics supplemented diets may be attributed to increased rumen pH, which ultimately improved the colonies of fibrolytic bacteria (Chaucheyras-Durand *et al*., 2008). Furthermore, probiotics

supplementation has a positive effect on various digestive processes especially cellulolysis and synthesis of microbial proteins (Yoon & Stern, 1995). The results of the present study are corroborated by other researchers (Krehbiel *et al*., 2003; El-Waziry & Ibrahim, 2007), who reported higher fiber digestibility of feed supplemented with probiotics.

Among the blood metabolites, unaltered BUN in lambs fed different protein source diets was supported by Carro *et al*. (2006) and Davies *et al*. (2007). Unaltered BUN concentration observed in lambs fed diets containing probiotics was also supported by Antunovic *et al*. (2005) and Masek *et al*. (2008), who reported no change in BUN concentration in lambs fed diets containing probiotic.

The higher concentration of blood glucose in lambs fed CM diet might be due to more gluconeogenesis and raised levels of hepatic enzymes responsible for glucose synthesis (Kaneko *et al*., 1997). The lower blood glucose concentration in lambs fed SFM diet may be attributed to high rumen degradability resulting in lesser availability of glucogenic amino acids (Sano *et al*., 2007). Unaltered blood glucose levels in response to probiotic supplementation are supported by Antunovic *et al*. (2005) who reported no marked glucose concentration in lambs fed diets supplemented with or without probiotics. Likewise, no change in serum creatinine due to different protein source is supported by Street (2001) who observed no effect of dietary protein source on creatinine. Unaltered serum creatinine in lambs fed diets with probiotics is in agreement with the results of Antunovic *et al*. (2006).

Higher N intake by lambs fed CM diet might be due to higher DM and CP intake. Higher N balance by lambs fed CM diet might have resulted from higher N intake and its digestibility. Furthermore, higher N-balance may be an outcome of more available N at ruminal level enhancing microbial fermentation (Legleiter *et al*., 2005). Better amino acid profile of CM might have resulted in better utilization of amino acids for anabolic activity (Brown & Johnson, 1991). The results of the present study are contrary to the results of other researchers (Merchen *et al*., 1987; Milis *et al*., 2005) who reported that dietary protein source had no effect on N-balance. Lower N-balance in lambs fed SFM diet may possibly because of reduced DM and CP digestibility (Woods *et al*., 1962).

The increased tendency in N-balance in lambs fed probiotic-supplemented diets is due to higher N digestibility and better utilization of dietary N, resulting in improved ruminal bacterial growth (Yoon & Stern, 1996) and increased postruminal flow of N (Putnam *et al*., 1997). Unaltered nitrogen concentration of nitrogen in urine and faeces of lambs fed on diets containing probiotics are in concordance with findings of other researchers (Ahmed & Salah, 2002; Hernandez *et al*., 2009).

Better weight gain in lambs fed CM diet is due to higher nutrient intake and their digestibility and N-balance, improved microbial activities, substrate availability and microbial protein synthesis (Khan *et al*., 1997; Atti *et al*., 2004). Another possible reason may be that, the CM diet contain higher sulfur contents (0.46 vs. 0.25, 0.22 & 0.19%) that may improved the microbial growth (Sniffen & Robinson, 1987), leading to more digestibility of nutrients and VFAs production that ultimately provided energy for muscle mass accretion. Furthermore, sulfur is also required by the ruminal microbes for the synthesis of sulfur containing amino acids and vitamins (NRC, 1984) that are for the most part involved in protein synthesis and thereby reflecting better growth performance (Brown & Johnson, 1991). Lower average weight gain noticed in lambs given SFM diet might be due to the higher NDF contents of SFM (Staples *et al*., 1984). Difference in weight gains could have been an outcome of varied rumen degradation of proteins, which in turn leads to different amino acid supplies (Urbaniak, 1995). Better FCR by lambs fed CM diet may be a function of better nutrient utilization efficiency in comparison with other groups. The results of present study are also supported by the findings of Waller *et al*. (1980) and Klopfenstein *et al*. (1978). Khan *et al*. (1997) also reported improved FCR in lambs fed CM and SBM diets as compared to those fed CSM diet.

Higher weight gains in lambs fed diets containing probiotics might possibly have resulted from higher consumption of DM, better nutrient digestibility and N-balance. Furthermore, probiotics supplementation may enhance the digestion and feed efficiency, thus leading to a greater weight gain in small ruminants (Robinson, 2002). However, the results of the present study are not supported by the findings of other researchers (Titi *et al*., 2008; Jang *et al*., 2009). Better FCR in response to probiotics supplementation may be the consequence of improved cellulolysis and digestibility of nutrients (Yoon & Stern, 1995).

The findings of the present study imply that experimental lambs fed on diet containing CM as vegetable protein source performed better than those fed on diets containing CGM, CSM and SFM as vegetable protein sources. Furthermore, probiotic supplementation also significantly influenced the growth performance of growing experimental lambs.

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