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Performance of growing lambs receiving altered plant protein sources with or without probiotics

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Abstract. The study was aimed to examine the response of feeding different plant protein sources on performance of growing *Kajli* lambs. Thirty two lambs, at the age of six months, were randomly divided into eight groups of four animals each in a 4×2 factorial arrangement. Four iso-caloric (70% total digestible nutrients) and iso-nitrogenous (18% crude protein (CP)) diets were formulated using corn gluten meal 30% (CGM), canola meal (CM), cotton seed meal (CSM) and sunflower meal (SFM)) with (50g /ton) or without probiotics. Findings revealed that, dry matter and CP intake was higher in lambs fed CM diet compared to other experimental diets. Addition of probiotics didn't influence (P>0.05) the intake of DM, CP and fiber fractions. Blood urea nitrogen and serum minerals remained unaffected (P>0.05) in lambs fed diet containing different protein sources. Haematological aspects also remained unaltered because of feeding different vegetable protein sources. However, lambs fed CM diets showed higher N intake and N-balance than those fed CGM, CSM and SFM diets and the same was true for weight gain. It is concluded that CM is better protein source for growing lambs than CGM, CSM and SFM and probiotics also influenced the growth performance of growing lambs.

Keywords: Plant protein sources, probiotics, growing lambs.

1. Introduction

Sheep are providing quality animal proteins for human consumption in addition to its significance in socio-economical aspect of Pakistan. *Kajli* is one of the famous sheep breed for its mutton and wool production. Its better carcass yield, quality meat, makes this breed 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 [1,2,3]. These animals graze mainly on weeds, grassy vegetations in fallow lands along with leaves and beans of wild trees. Low productivity of these animals is because of poor quality feed stuff [4,5,6]. Their productivity can be enhanced by feeding them balanced ration. This is possible if we shift from grazing feeding system to stall feeding system. Howevere, data in this regard is very scanty [7,8]. Furthermore, influence of different proteins sources on animal growth and performance can't be denied. Furthermore, ruminant animal performance can be enhanced by addition some additives like probiotics which have been reported to accelerate rumen fermentation [9]. Keeping in view the scenario, the present study was planned to examine the effects of different protein sources with or without probiotics on performance of growing male *Kajli* male lambs.

2. Materials and Methods

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 different protein sources with or without probiotics (Protexin®) on the nutrients intake and their digestibility, growth performance, blood chemistry and N-balance. The two factors were protein sources (Canola meal, cotton seed meal, corn gluten meal 30% and sunflower meal) and probiotic level (0 and 50g/ton).The lambs were fed isocaloric (70% TDN) and isonitrogenous (18% CP) diets formulated using different protein sources according to NRC [10] requirements (Table.1). The diets containing CGM, CM, CSM and 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. Study lasted for 90 days including a fifteen days feed adjustment period. Lambs were offered experimental diets twice a day. Feed intake was recorded daily and the residues were collected and weighed. Lambs were weighed weekly before morning feeding. Feed conversion ratio was worked out by dividing the feed intake (g/d) by weight gain (g/d). The DM, CP, NDF and ADF intake was also calculated. Nitrogen balance was calculated by using the equation described by NRC [10]. Blood sample (10 mL from each lamb) was collected by puncturing jugular vein; 2mL was collected into the vaccutainers each containing 81µL 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 minutes of collection. Blood samples were analyzed for blood urea nitrogen [11] while serum minerals (Na, K, Cl, Ca and Mg) were determined following procedures described by AOAC [12] whereas P was analyzed by using spectrophotometer. Haematological analysis (Complete blood count) was undertaken using blood analyzer (NIHON KOHDEN Japan).

3. Statistical Analysis

The data thus collected were analyzed using the GLM procedure of SAS [13] 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 50g /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 (LSD) option of GLM procedure and declared significant at P < 0.05.

4. Results

Dry matter, CP, NDF and ADF intake was different (P<0.05) in lambs fed diets containing different protein source (Table 2). 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. Blood urea nitrogen (Table 2) were not affected (P>0.05) by protein source or probiotics level. The interaction between protein source and probiotics was non-significant for BUN. Serum minerals Ca, P, Na, Cl, K and Mg were not influenced (P>0.05) by any dietary treatments(Table 2).

Nitrogen intake in lambs was different (P<0.05) due to protein sources. Higher N-intake was noticed in lambs fed CM diet followed by CGM, SFM and CSM. Probiotics supplementation didn't influence N-intake in lambs. Protein source and probiotics level interaction was non-significant for N-intake.

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(Table 2)..

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(Table 2)..

Haemoglobin, PCV, MCHbC, TWBCC, PMWBC, lymphocytes, monocytes, eosinophils and basophils were not affected (P>0.05) by different protein sources. However, TRBC, MCHb, MCV, neutrophils and platelets differed significantly (P<0.05). Haemoglobin, PCV, MCV, MCHb, MCHbC, TWBCC, PMWBC, neutrophils, lymphocytes, monocytes, eosinophils, basophils and platelets were not affected (P>0.05) due to probiotics supplementation (Table 4.5). However, TRBC was influenced (P<0.05) by the addition of probiotics. Interaction between protein source and probiotics level was observed (P<0.05) for total red blood cells, MCV, MCHb, neutrophils and platelets. but not for Hb, PCV, MCHbC, TWBCC, lymphocytes, monocytes, eosinophils, PMWBC and basophils remained unaltered (Table 3).

Table 1. Composition of Experimental diets for growing lambs

Items (g/kg)	Diets[1] CGM		CM		CSM		SFM	
	P_0	P_1	P_0	P_1	P_0	P_1	P_0	P_1
Maize	300	300	300	300	300	300	300	300
Rice polishing	20	20	80	80	40	40	41	41
Wheat Bran	111	111	120	120	140	140	90	90
Wheat Straw	130	130	130	130	130	130	120	120
Sunflower meal	0	0	0	0	0	0	340	340
Canola meal	0	0	280	280	0	0	0	0
Cotton seed meal	0	0	0	0	290	290	0	0
Maize Oil	0	0	0	0	10	10	30	30
Corn gluten meal ,30%	340	340	0	0	0	0	0	0
Molasses	40	40	40	40	40	40	20	20
Urea	9	9	0	0	0	0	9	9
Sodium bi-carbonate	20	20	20	20	20	20	20	20
Common salt	10	10	10	10	10	10	10	10
Di-calcium phosphate	20	20	20	20	20	20	20	20
Protexin®	0	0.05	0	0.05	0	0.05	0	0.05
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 $P_{1=}$ 50g/ton probiotics.

Table 2. Effect of different protein sources with or without probiotics on nutrient intake, blood urea nitrogen and mineral profile in growing Kajli male lambs

Items	Diets[1] CGM		CM		CSM		SFM		SE	Significance A	B	AB
	P_0	P_1	P_0	P_1	P_0	P_1	P_0	P_1				
Dry matter, g/d	1226	1256	1271	1283	1246	1278	1207	1213	13.5	*	NS	NS
Crude protein, g/d	221.0	226.0	228.8	230.9	206.0	212.0	217.0	218.0	2.4	*	NS	NS
Neutral detergent fibre, g/d	188.0	193.0	294.2	297.0	294.0	302.0	460.0	462.0	3.0	*	NS	NS
Acid detergent fibre, g/d	135.0	138.0	178.7	180.4	107.0	110.0	222.0	223.0	1.8	*	NS	NS
Blood urea nitrogen, mg/dl	19.6	19.8	19.3	18.5	19.1	19.5	17.8	18.5	1.8	NS	NS	NS
Nitrogen balance, g/d	24.0	24.8	26.2	26.5	22.6	23.4	23.7	23.9	0.6	*	NS	NS
Daily gain (g)	187.8	194.4	198.6	209.9	181.1	188.9	171.7	180.0	12.0	*	NS	NS
Mineral profile, mg/dl												
Calcium	8.6	8.1	8.8	7.7	8.7	8.0	8.7	7.5	0.9	NS	NS	NS
Phosphorus	6.8	6.7	6.4	6.5	6.7	6.6	6.3	6.6	0.6	NS	NS	NS
Sodium	133.1	132.3	138.8	136.8	143.0	131.1	141.0	134.5	9.7	NS	NS	NS
Chloride	101.6	101.1	100.3	100.8	101.3	102.1	102.3	101.8	4.7	NS	NS	NS
Potassium	4.4	4.2	4.0	4.5	4.8	4.7	4.3	4.8	0.6	NS	NS	NS
Magnesium	2.1	2.4	2.2	2.3	2.0	2.1	2.2	2.3	0.4	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. 3. Effect of different protein sources with or without probiotics on haematology in growing Kajli male 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				
Haemoglobin (g/dl)	8.4	8.5	8.8	8.9	8.1	8.3	8.7	8.8	1.7	NS	NS	NS
Haematocrit (%)	25.7	27.2	25.0	27.7	23.2	24.8	24.9	26.3	1.6	NS	NS	NS
TRBC (m/µl)	6.4	6.8	7.1	10.1	8.3	9.9	8.1	8.1	0.6	*	*	*
MCV (F1)	43.0	43.4	30.8	38.2	38.1	40.6	36.1	37.9	2.5	*	NS	*
MCHb (P_g)	11.7	12.4	8.5	12.0	10.5	12.3	8.6	9.5	0.9	*	NS	*
MCHbC (g/dl)	25.6	26.4	26.0	26.4	28.0	28.9	24.3	27.3	1.7	NS	NS	NS
T WBCC (k/µl)	10.0	10.6	9.0	13.3	9.9	11.1	9.1	9.8	1.4	NS	NS	NS
Neutrophils (%)	43.9	53.3	39.5	47.3	53.8	51.0	52.3	49.5	2.3	*	NS	*
Lymphocytes (%)	40.0	50.8	45.5	55.3	38.5	42.3	42.3	43.8	4.3	NS	NS	NS
Monocytes (%)	0.252	0.787	0.257	0.762	0.252	0.263	0.287	0.32	0.24	NS	NS	NS
Eosinophils (%)	0.5	0.43	0.75	0.56	0.6	0.59	0.62	0.57	0.28	NS	NS	NS
PM WBC (%)	0.235	0.267	0.24	0.259	0.217	0.267	0.267	0.314	0.23	NS	NS	NS
Basophils (%)	0.267	0.285	0.254	0.267	0.175	0.215	0.247	0.285	0.23	NS	NS	NS
Platelets (k/µl)	516	665	615	748	661	676	586	637	36.04	*	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. TRBC=Total Red Blood Cell, MCV=Mean Corpuscular Volume, MCHb=Mean Corpuscular Haemoglobin, TWBCC=Total white Blood Cell Count, MCHbC= Mean Corpuscular Haemoglobin Concentration, PMWBC= Premature white blood cells. 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).

5. Discussion

Higher DMI in lambs fed CM diet has also been supported by the findings of Wiese et al. [14], who reported higher DMI in lambs fed CM diet than those fed lupin and urea diet as dietary protein sources. 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 fed CM diet is better digestibility [15], which might have increased digestion and passage rates [16]. Lower DMI in lambs fed SFM diet might be due to high dietary NDF and ADF content that may limit DMI [17], while higher NDF and ADF intake by lambs fed SFM is due to higher dietary NDF and ADF contents [18]. The results of the present study are supported by other researchers [19,20], who reported different DMI in lambs fed different protein sources. The DMI was not influenced by probiotic supplementation.

The unaltered BUN in lambs fed different protein source diets was supported by the findings of Carro et al. [21]. Unaltered BUN concentration observed in lambs fed diets containing probiotics was also supported by Antunovic et al.[22], who reported no change in BUN concentration in lambs fed diets containing probiotic.

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 [23]. Better amino acid profile of CM may have resulted in better utilization of amino acids for anabolic activity. Lower N-balance in lambs fed SFM diet may possibly because of reduced DM and CP digestibility [24].

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 and increased postruminal flow of N [25]. No change in N-intake, N-balance, fecal and urinary N in lambs fed diets supplemented with probiotics are in concordance with findings of other researchers.

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 [26]. The results of the present study are in concordance with Khan et al. [7] who reported higher weight gain in lambs fed SBM and CM diets as compared with the ones fed CSM diet suggesting that CM and SBM are better protein sources for growing lamb ration than the CSM. Another possible reason may be that, the CM diet contain higher sulfur contents (0.46 Vs. 0.25, 0.22 and 0.19%) that may improved the microbial growth, 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 [27] that are for the most part involved in protein synthesis and thereby reflecting better growth performance. Lower average weight gain noticed in lambs given SFM diet might be due to the higher NDF contents of SFM. Difference in weight gains could have been an outcome of varied rumen degradation of proteins, which in turn leads to different amino acid supplies.

Lack of response in serum minerals (Ca, P, K, Na, Mg and Cl) levels in lambs fed different protein source diets are supported by the findings of other researchers [28]. Unaltered serum minerals in lambs fed diets supplemented with probiotics are in agreement with the results of other researchers [22].

No effect on Hb, PCV, TWBCC, basophils, monocytes, eosinophils and PMWBC has indicated that different protein sources at same CP levels have no effect on the blood profile. The results of the present study are supported by Nelson and Watkins [29], who reported that blood profile remianed unaffected by protein sources indicating that homeostatic mechanism might not be influenced by different protein sources. The PCV, Hb, lymphocytes, WBC, TRBC, MCV, MCHb in animals fed different protein sourcce diets [30]. TRBC, MCV, MCHb, neutrophils and platelets were differed in the present study, but the values were in normal range. Unchanged Hb, PCV, MCV, MCHb, MCHBC, TWBC, PMWBC, lymphocytes, eosinophils, basophils and platelets in lambs fed diets with or without probiotics are supported by the findings of other researcher [31].

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