EFFECT OF PROBIOTIC SUPPLEMENTATION AND VARYING DIETARY PROTEIN LEVELS IN CREEP FEED ON GROWTH PERFORMANCE OF SALT RANGE LAMBS

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ABSTRACT: Main aim of this work was to findout the influence of probiotic supplementation and suitable dietary protein level in creep feed for optimum growth performance of Salt Range lambs. Lambs (n = 16; Age = 30±10 days) were chosen and divided into four equal groups. Four dietary treatments; diet-1 (CP 18% with no probiotic), diet-2 (CP 18% with probiotic), diet-3 (CP 20% with no probiotic) and diet-4 (CP 20% with probiotic) were randomly allocated to lambs under Completely Randomized Design. The raw data were analyzed through ANOVA technique using SAS 9.3 software. The growth performance was determined in terms of average daily gain, average daily feed intake, feed conversion ratio, feed cost per kg gain and blood parameters; hemoglobin, blood glucose, blood urea and blood cholesterol. Results indicated that average daily gain of lambs fed diets having 20% protein with and without probiotic supplementation are not different ($P>0.05$), whereas average daily gain of lambs fed 20% protein was found higher ($P<0.05$) than of those fed 18% protein with and without probiotic supplementation. Average daily feed intake was found higher ($P<0.05$) in diet-3 than diet-2 but remained not different in animals fed diet-1 and diet-4. Lambs fed dietary protein 20 percent were found more efficient ($P<0.05$) in terms of feed conversion ratio than that of 18 percent protein. Blood parameters were found not different by various dietary treatments. It was inferred that creep feed with probiotic supplementation couldn't influence growth but higher dietary crude protein 20% in creep feed improved growth performance of Salt Range lambs in pre-weaning phase.

Key words: Creep feed, probiotic supplementation, dietary protein levels, growth and Salt Range lambs.

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INTRODUCTION

Salt Range is well known and single fat tailed sheep breed of Punjab province in Pakistan and famous for mutton production (Afzal and Naqvi, 2003). Mostly it is prevalent in rangeland of Pothowar region. It's delicious meat, better carcass yield and well-adjusted habitat makes this breed quite popular among farmers and consumers. The wide consumption at food restaurants and especially high demand for sacrificial purposes at Eid-ul-Azha festival provides an opportunity to farmers for fetching premium price. However, sheep is facing challenges in terms of low productivity due to high variability of rainfall, over grazing, scanty and low-quality natural pastures (Sarwar *et al*., 2002). Specially during the fodder scarcity period, ewes mostly struggle to feed their neonatal lambs (El-Hag *et al*., 2007). Mis-mothering, prolonged starvation and low birth weight are other non-infectious factors which results in minimum growth, morbidity and mortality of lambs (Mustafa *et al*., 2014).

Creep feeding is one of the promising techniques for successful rearing of nursing lambs whose staple feed is only milk. The creep diet is usually grain protein supplement mixture and can easily be picked as young lambs are much curious in chewing or engulfing. It can trigger rumen development earlier. Different diet plans like protein sources can speed up microbial production of rumen, which results to increase nutrient intake and consequently improve growth (William *et al*., 1991). Feed utilization and nutrients absorption can be more accelerated by triggering the rumen function using feed additives in the form of probiotics (Ding *et al*., 2008). The transition of lambs from liquid diet to solid diet more efficiently with addition of probiotic in feed, which accelerates the microbial activities in rumen (Durand and Fonty, 2001). However, haphazard use of dietary protein and probiotic in lambs nursing solid feed may affect growth and health of lambs. Benefits for dietary manipulation of creep feed is variable in published literature but still need to conduct research on the use of probiotics to clarify the beneficial effects in lambs. That is why this project was designed with main aim to evaluate varying dietary protein levels supplemented with or without probiotic in creep feed for optimum pre-weaning growth performance of Salt Range lambs.

MATERIALS AND METHODS

Experimental site: The study was performed at University Research Farm, Koont Tehsil Gujar Khan District Rawalpindi, Punjab, Pakistan. This experimental site is situated near to Dhudial, famous village of District Chakwal and located at 33°3'52N 72°58'24E about 70 KM south of the capital city Islamabad, Pakistan.

Experimental treatments: Sixteen lambs (Age = 30±10 days) were selected from the Salt Range flock maintained at University Research Farm Koont and divided into four equal groups. Each group had 4 lambs. Four dietary treatments; diet-1 (CP 18% with no probiotic), diet-2 (CP 18% with probiotic), diet-3 (CP 20% with no probiotic) and diet-4 (CP 20% with probiotic) were randomly allocated to lambs under Completely Randomized Design.

All procedures performed involving experimental animals in this study were in accordance with the ethical standards laid down by Faculty of Veterinary and Animal Sciences and Animal welfare policies.

Feeding of lambs: The selected Salt Range lambs were stable and in good health condition as they passed one month with their dams where they could suckle milk. Then experimental animals were reared on creep feeding on *ad libitum* basis. The composition of experimental creep feeds is given in Table 1. The lambs in Group-A were fed diet-1 (CP 18% with no probiotic), whereas lambs in Group-B were fed diet-2 (CP 18% with probiotic). Similarly, animals in Group-C were fed diet-3 (CP 20% with no probiotic) and lambs in Group-D were fed diet-4 (CP 20% with probiotic). The probiotic supplement used in creep feed was Actera-yeast at rate of 3g/day/head. The Actera-yeast was live yeast product available in granular powder form and composed of 20 (billion/g) live yeast cell count. The experiment diets were formulated following NRC (2007) feeding standards for small ruminants. Daily diet was weighed, offered and orts were collected and weighed through digital weighing balance. The diet was given to lambs two times a day at 08:00 and 14:00 h while orts were collected at 07:00 and 13:00 h, respectively. Fresh water was available in buckets round the clock. This feeding trial was carried on for 60 days including 15 days adjustment period under uniform management and housing conditions. The adjustment period in which feeding treatment was gradually changed over to experimental diets so that animals could adjust with their new feed.

Table 1: Composition of creep feeds comprising varying dietary protein levels with and without probiotic supplementation for Salt Range lambs during pre-weaning phase.

S.#	Ingredients	Inclusion level percentage			
		Creep feeds without probiotic		Creep feeds with probiotic	
		CP 18%	CP 20%	CP 18%	CP 20%
1	Probiotic (Acterayeast)*	0	0	3g/d	3g/d
2	Soybean meal	18	20	18	20
3	Cotton seed meal	8	10	8	10
4	Maize gluten 30%	2	3	2	3
5	Maize grain ground	32	30	32	30
6	Wheat bran	9	9	9	9
7	Cracked oat grain	15	15	15	15
8	Rice middling	6	5	6	5
9	Molasses	8	6	8	6
10	Mineral mixture	2	2	2	2
	Total	100	100	100	100
	CP%	18	20	18	20
	TDN%	78.1	77.9	78.1	77.9
	ME (MCal/kg)	2.51	2.50	2.51	2.50

*Acterayeast comprises probiotic yeast *Sacchromyces cerevisiae* (NAWAN Group of Companies)

Parameters studied: The effect of creep feeding with various protein levels and probiotic supplementation was determined in terms of these parameters; dry matters intake (kg/d), average daily gain (g/d), feed conversion ratio, measurement of blood metabolites (hemoglobin, urea, cholesterol & glucose) and feed cost per kg live weight gain (Pak Rupees).

Data collection: Feed offered and orts for each experimental animal were recorded on daily basis. Body weight was noted through digital weighing balance on weekly basis. However, feed conversion ratio and feed cost per kg weight gain was calculated at the end of trial. Blood samples were taken at the start and final day of feeding trial from jugular vein.

Measurement of blood metabolites: From each lamb samples of blood (10 ml) were taken from jugular vein in the morning before feeding. One blood sample was taken in serum separator tube and other blood sample was fetched in tube containing 5 mg of sodium fluoride and 4 mg of potassium oxalate for later-on glucose determination. The collected samples were kept immediately in ice and later-on centrifuged on the same day at 2500 rpm for 15 minutes at 4^{o}C to separate serum and plasma. These samples were then frozen till analyzed for hemoglobin, urea, cholesterol and glucose with the help of commercially available kits.

Statistical analysis: Software SAS 9.3 was used to analyze the data through ANOVA technique following Completely Randomized Design (CRD). The means were compared with post hoc Duncan Multiple Range Test.

RESULTS

The influence of different dietary protein levels and probiotic supplementation on performance of Salt Range lambs was determined through results in terms of following parameters.

Average daily gain: Influence of varying dietary protein levels and probiotic supplementation on average daily gain of Salt Range lambs is given in Table 2. The average daily gain of lambs with diet-4 (CP 20% with probiotic) was found not different (P>0.05) from that of diet-3 (CP 20% with no probiotic). Similarly, average daily gain between diet-2 (CP 18% with probiotic) was not different (P>0.05) with diet-1 (CP 18% with no probiotic). However, Salt Range lambs fed diet with 20% protein gained higher (P<0.05) than of those fed dietary protein 18 percent.

Daily feed intake: Effect of different dietary protein levels and probiotic supplementation on daily feed intake of Salt Range lambs is given in Table 2. The average daily feed intake remained higher (P<0.05) in Salt range lambs with diet-4 (CP 20% with probiotic) than of those fed diet-2 CP 18% with probiotic) but statistically not different with diet-1 (CP 18% with no probiotic) and diet-3 (CP 20% with no probiotic).

Feed conversion ratio (FCR): Effect of varying dietary protein levels and probiotic supplementation on feed conversion ratio of Salt Range lambs is given in Table 2. Salt range lambs fed dietary protein 20 percent were found more efficient (P<0.05) in terms of FCR than of those fed dietary protein 18 percent. The influence of probiotic supplementation on FCR was found not different (P>0.05) among lambs fed diet-3 (CP 20% with no probiotic) and diet-4 (CP 20% with Probiotic), likewise FCR of lambs fed diet-1 (CP 18% with no probiotic) was also found not different (P>0.05) with that of diet-2 (CP 18% with probiotic).

Table 2: Influence of varying dietary protein levels, probiotic supplementation and their interaction in creep feed on growth performance of Salt Range lambs.

Parameters	Dietary protein 18%		Dietary protein 20%	
	(Control) Diet-1: No probiotic	Diet-2: Probiotic	Diet-3: No probiotic	Diet-4: Probiotic
Average daily gain (g/d)	150±5.7^{b}	150±6.3^{b}	175±5.6^{a}	178±5.0^{a}
daily feed intake (Kg/d)	0.626±0.017^{ab}	0.610±0.022^{b}	0.660±0.015^{ab}	0.673±0.015^{a}
Feed conversion ratio	4.183±0.074^{a}	4.070±0.047^{a}	3.775±0.050^{b}	3.778±0.051^{b}

$^{a \text{ and } b}$ Means with different super scripts in a row are significantly different with each other (P<0.05)

Feed cost per kg gain: The calculated feed cost per kg gain in Salt Range lambs was found highest in diet-1 (CP 18% with no probiotic) followed by diet-2 (CP 18% with probiotic), diet-4 (CP 20% with Probiotic) and diet-3 (CP 20% with no probiotic). The feed cost per kg gain were PKR 199.45, 194.05, 180.14 and 179.99 in diet-1, diet-2, diet-4 and diet-3, respectively. Numerically, diet-3 was remained most economical creep feed.

Measurement of blood metabolites: Influence of various dietary protein and probiotic supplementation on measurement of blood metabolites (hemoglobin, urea, cholesterol & glucose) in Salt Range lambs is given in Table 3. All these serum parameters; Hemoglobin, Urea, Cholesterol and Glucose were found not different (P>0.01) among various dietary treatment.

Table 3: Influence of various dietary protein, probiotic supplementation, and their interaction on blood metabolites in Salt Range lambs.

Blood Metabolites	Dietary protein 18%		Dietary protein 20%		SEM	P Value
	(Control) Diet-1: No probiotic	Diet-2: Probiotic	Diet-3: No probiotic	Diet-4: Probiotic		
Haemoglobin (g/dL)	8.550	9.200	8.938	8.287	0.07181	P>0.01
Serum urea (mg/dL)	48.50	70.25	61.25	44.75	0.9843	P>0.01
Cholesterol (mg/dL)	19.00	26.25	22.50	20.75	0.8780	P>0.01
Glucose (mg/dL)	89.25	48.25	70.50	87.50	0.6292	P>0.01

DISCUSSION

Findings of present study depicted that overall probiotic supplementation did not influence (P>0.05) growth performance of Salt Range lambs during pre-weaning phase. Dietary probiotic supplementation didn't change lamb growth performance in pre-weaning stage was also documented by many workers (Robinson, 2002; Kawas *et al*., 2007a; Titi *et al*., 2008; Saleem *et al*., 2017). Absence of dietary probiotic influence might be attributed to the factor of pre-weaning phase when rumen usually not developed enough at this stage. Adjacently in published literature, probiotic supplementation had been known to improve growth performance of finishing lambs (Kawas *et al*., 2007b). This divergence might be attributed to factors of different lamb's production stage, feed type and dose rate of probiotic.

Salt Range lambs fed diet with 20% protein in current study were found more efficient (P<0.05) in terms of FCR, feed consumption and weight gain. This might be due to more likely availability of nutrients in creep feed for lambs during pre-weaning phase. Influence of higher dietary protein on growth of ruminants was also substantiated previously (Hoffman *et al*., 2001; Bohnert *et al*., 2002; Chumpawadee *et al*., 2006; Ali *et al*., 2009). In a study, Vosooghi *et al*. (2014) reported that higher dietary protein enhanced preliminary feed intake and then increased weight gain in lambs. The efficient growth might be attributed to increased availability of amino acids in small intestines where amplitude of nutrients absorption could be greater than ruminal fermentation (Meissner *et al*., 1996). Similarly, Atti *et al*. (2004) strengthened that lambs fed high protein diet during first 6 weeks of pre-weaning phase showed higher growth performance.

Nutrient dense diet in lambs improved feed conversion and weight gain (Majdoub-Mathlouthi *et al*., 2013). Higher dietary CP content encouraged animals for better feed and nutrients intake (Negesse *et al*., 2001; Phengvichith and Ledin, 2007; Li *et al*., 2012). Pre-weaning lamb's might be more crucial when creep feeding was started and rumen in the process of development. Higher dietary protein in creep feed could trigger rumen development process. In a previous study, Cui *et al*. (2019) reported that low protein and energy diet badly affected rumen development in terms of the rumen weight and mucosal thickness while studying influence of dietary nutrients on rumen morphology. Conclusively, it was inferred that young animals might require high protein and energy to boost rumen development.

Conclusion: It was concluded that creep feed with probiotic supplementation could not influence growth but higher dietary crude protein 20% in creep feed improved growth performance of Salt Range lambs in pre-weaning phase.

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