

EFFECTIVENESS OF DIFFERENT TYPES OF TOTAL MIXED RATIONS (TMR) WITH THREE PROTEIN LEVELS ON GROWTH PERFORMANCE IN MALE LOHI SHEEP

MUHAMMAD ARIF *

Department of Livestock Production and Management, Faculty of Veterinary and Animal Sciences, PMAS-Arid Agriculture University, Rawalpindi, Pakistan. *Corresponding Author Email: doc.marif@gmail.com

MUHAMMAD FAROOQ IQBAL

Department of Livestock Production and Management, Faculty of Veterinary and Animal Sciences, PMAS-Arid Agriculture University, Rawalpindi, Pakistan.

TANVEER AHMAD

Department of Livestock Production and Management, Faculty of Veterinary and Animal Sciences, PMAS-Arid Agriculture University, Rawalpindi, Pakistan.

KASHIF ISHAQ

Department of Livestock Production and Management, Faculty of Veterinary and Animal Sciences, PMAS-Arid Agriculture University, Rawalpindi, Pakistan.

Abstract

The effectiveness of different types of TMR with three protein levels on growth performance and carcass traits in male Lohi sheep was determined. Eighteen male Lohi sheep were selected for 90-days feeding trial and the estimated age of each individual was 120 ± 15 days. Initial body weights of these individuals were 15.00 ± 0.13 (kg). These individuals were randomized into six replications using a 2×3 factors factorial arrangement. Maize silage and maize hay were used in total mixed rations formulations as wet (W) and dry (D) total mixed rations (TMR), respectively. Three protein levels viz., Low crude protein (LCP), normal (control) crude protein (NCP) and high crude protein (HCP) were used for making different dietary treatments. Six dietary treatments WTMR: LCP, WTMR: NCP, WTMR: HCP, DTMR: LCP, DTMR: NCP and DTMR: LCP and HCP were used in the current experiment. Data for growth performance parameters, carcass characteristics, meat quality and blood parameters of male Lohi sheep fed on different treatments were recorded. Individuals fed on wet TMR had shown overall the best performance ($P < 0.05$) as compared with the individuals fed on dry TMR. Highest body weight was observed in treatment WTMR: HCP (33.20 Kg) followed by lambs fed on WTMR: NCP (31.90 Kg) and WTMR: LCP (28.70 Kg; $P < 0.04$). The best performance of average daily gain (ADG) was estimated around 200 g/day, which was observed in individuals fed WTMR: HCP ($P < 0.02$). An interaction effect for dry matter intake (DMI) was also noted in lambs fed dry and wet TMR ($P < 0.04$). Highest DMI (831.1 g/day) was found in lambs fed wet TMR: HCP. An interaction effect for meat crude protein chemical composition, acetic acid, propionic acid and butyric acid were also reported for lambs fed on wet TMR: HCP. It was concluded that inclusion of high crude protein in wet TMR had significantly improved growth performance parameters, fermentation characteristics and carcass traits in male Lohi sheep. Therefore, it is recommended that farmers may use high crude protein with wet TMR for better growth performance of small ruminants.

Keywords: Total Mixed Ration, Acetic Acid, Propionic Acid, Dry Matter Intake, Growth Performance And Carcass Traits.

INTRODUCTION

In Pakistan, the productivity and growth performance of small ruminants especially sheep and lambs are very low due to the lack of proper dietary nutrition and feedstuffs both in quality and quantity (Ishaq et al. 2016). Due to scarcity of natural feeding resources/pasture in the semi-arid and arid climatic zones, sheep production systems need permanent evolution to meet the nutritional requirements of animal flocks (Beigh et al. 2017). Mostly farmers are using nutritive deficit feedstuffs, which do not fulfill the nutrition requirements of sheep.

This production approach is impeding animal productivity and causing significant financial losses for small-scale farming communities that directly impact the country's economy and farmer's interest in future farming (Ishaq et al. 2016). The Lohi is a well-known breed of thin-tailed sheep that originates in Pakistan and it is responsible for up to forty percent of the Punjab province's entire mutton production (Sharif et al. 2022).

Farmers are providing concentrate rations to raise Lohi sheep, however these rations are very unbalanced and insufficient in energy and minerals, making it impossible to accomplish the aims of high productivity and good meat quality (Ishaq et al. 2016; Beigh et al. 2017).

However, in other countries, the production of sheep and lambs has increased significantly in recent decades, largely due to the adoption of varied feeds. Small ruminants feeding habits and feed intake on various fodders vary, which may have an impact on their growth and meat quality. Feeding mixed rations to livestock saves work, lowers feed refusal, and gives the animals nutritional benefits (Zhao et al. 2016; Oliveria et al. 2020; Mendes et al. 2021).

Total mixed ration (TMR) is widely used for sheep, dairy and beef animals because it is very effective not only in improving the health of animals but also increases the production of these animals (Zhao et al. 2016; Oliveria et al. 2020). The growth performance and carcass characteristics of animals are primarily determined by their feed intake, as well as the quality, quantity, and total digestible nutrient content of the feed they consume (Alhidary et al. 2016; Oliveira et al. 2020).

The inclusion of an adequate amount of crude protein in sheep diets influences growth performance, carcass traits, digestibility and blood metabolites (Nudda et al. 2013; Meehan et al. 2021; Sileshi et al. 2021). Many factors affect feed intake, including the animal (weight, production level, sex and age of the animal), ration (crude protein, calories, amount, crude fiber), chewing time, feeding environment and space needed (Abdullah & Musallam; Zhong et al. 2018; Mendes et al. 2021; Askar et al. 2023).

Nutrient digestibility and growth performance of animals are directly linked with the availability of protein in sheep and lamb's diets (Wang et al. 2020; Obeidat et al. 2020). The growth performance, feed efficiency and carcass traits of sheep and lambs are improved with the addition of dietary protein (Hajji et al. 2016; Wang, et al. 2020; Raducuta et al. 2023). So, appropriate levels of protein in diets would be desirable for

fattening of sheep and lamb (Obeidat et al. 2019; Sileshi et al. 2021). Therefore, desirable growth and fattening of sheep depends on rations, especially total mixed rations with proper dietary protein levels.

However, the insufficient amount of data is available that is limited regarding the amount of protein supplementation which is administered in wet and dry TMR to growing animals, particularly male Lohi sheep lambs. The utilization of wet and dry total mixed rations with appropriate protein levels during the growing phase of male Lohi sheep was hypothesized to improve feed efficiency, growth performance, fermentation characteristics and enhance nutrient consumption.

MATERIALS AND METHODS

Chemical composition of feed ingredients:

In the current study, proximate analysis of feed ingredients (Table 1) was carried out using the method of AOAC (2003) at the Animal Nutrition Laboratory, Department of Livestock Production and Management, Faculty of Veterinary and Animal Sciences, PMAS-Arid Agriculture University, Rawalpindi. Gross energy was measured by bomb calorimeter at the Central Laboratory, Ravi Campus University of Veterinary and Animal Sciences, Lahore.

Experimental animals and treatments

Eighteen male Lohi sheep (120±15 days old) with average weight 15.00 (± 0.13) kg were randomly assigned using 2 × 3 factors factorial arrangement under complete randomized design and each treatment was replicated three times. All animals were kept in individual pens and had the same animal husbandry management for ninety days research trail with 10-days adaptation time. Experimental trail was carried out at University Research Farm (URF), Koont, PMAS-Arid Agriculture University Rawalpindi (PMAS-AAUR) during October 2020 to January 2021.

All individuals were dewormed and vaccinated per local vaccination schedule. The present experiment used normal treatments in wet and dry total mixed rations based on crude protein (CP) requirement as described by National Research Council (NRC, 2007), USA. Three diets viz. low CP (10 % lower CP as compared to NRC, 2007 requirement; LCP), normal CP (NRC, 2007 CP requirement; NCP), and high CP (10 % higher CP than NRC, 2007 requirement; HCP) were prepared.

Each of three diets was used in Wet TMR (maize silage based; WTMR) and Dry TMR (maize hay based; DTMR) experimental diets. In total six dietary treatments were formulated and assigned to animals in a way that each diet was fed to 3 animals. These treatments were designated as T₁ (WTMR: LCP), T₂ (WTMR: NCP), T₃ (WTMR: HCP), T₄ (DTMR: LCP), T₅ (DTMR: NCP) and T₆ (DTMR: HCP). All the wet and dry total mixed rations fed to male Lohi lambs were iso caloric.

The calculated amount of total mixed ration for each individual was given twice a day. Each individual was fed during morning (7.00 to 8.00 AM) and in the evening (6.00 to 7.00

PM). Clean and fresh water was provided ad libitum. The feed ingredients and chemical composition of diets are shown in Table 2.

Table 1: Chemical analysis of maize silage and hay fed to growing lambs

Chemical composition (%)	Maize Silage	Maize Hay
Metabolizable energy(M cal/kg)	2.3	2.1
Dry matter	25.47	87.21
Ether extract	3.5	2.7
Crude protein	9.9	8.6
Ash	4.6	5.3
Neutral detergent fiber	51.24	60.93
Acid detergent fiber	28.53	34.47
Cellulose	22.16	28.33
Hemicellulose	22.71	26.46
Acid insoluble ash	3.89	5.13

Data Collection

All individuals were managed by providing standardized husbandry conditions. Data for growth performance, blood analyses and slaughter traits of Lohi sheep lambs were recorded to measure effects of different treatments. The line border weigh each Lohi sheep lamb was recorded as initial body weight (IBW). Individually, the Lohi sheep lambs were fed twice daily.

The daily needed amount of total mixed ration to each animal was divided into two halves. Each individual was fed during morning (7.00 to 8.00 AM) and in evening (6.00 to 7.00 PM). Every morning, the leftover feed was collected.

Actual intake of the treatment was recorded as the difference between the weight of the feed supplied to each lamb and the leftovers left in the feeding utensils. For each treatment, weight, feed intake, average daily gain (ADG or DWG), average daily feed intake (ADFI or DMI) and weekly weight increase (Sobrinho *et al.* 2003; Kadim *et al.* 2013), feed conversion efficiency of each individual (Sen *et al.* 2004) were recorded at the end of experimental trail.

Apparent digestibility of each feed was determined by using an inert marker (chromium oxide) in all treatments for last three days of experiment (Guzman-Cedillo *et al.* 2017). Last day of experimental trail, blood samples of each individual from each group were collected two hours before morning meal and these blood samples were analyzed (Nudda *et al.* 2013).

Table 2: Ingredients (%) and chemical composition used in the diets fed to growing lambs (on DM basis); where TMR = Total Mixed Ration; NCP = Normal crude protein, HCP = High crude protein, LCP = Low crude protein

Ingredient (%) used for different treatments on a DM basis						
Ingredients % diet	Wet TMR			Dry TMR		
	LCP	NCP	HCP	LCP	NCP	HCP
Molasses	-	-	-	6	6	6
Maize Grain	21	13	6	9	2	2
Soybean Meal	-	-	-	-	-	11
Corn gluten (30%)	15	18	18	18	18	18
Wheat Bran	3	3	3	3	4	3
Sunflower Meal	2	6	13	4	10	-
Megalac	1	2	2	2	2	2
DCP	1.5	1.5	1.5	1.5	1.5	1.5
Min. Mixture	1.5	1.5	1.5	1.5	1.5	1.5
Silage/Hay (%)	55	55	55	55	55	55
Total (Kg)	100	100	100	100	100	100
Chemical composition of different treatments						
Ingredients % diet	Wet TMR			Dry TMR		
	LCP	NCP	HCP	LCP	NCP	HCP
Metabolizable energy (M. Cal./kg)	2.86	2.87	2.88	2.87	2.87	2.88
Crude protein	13	14.5	16	13	14.5	16
Ash	7.1	6.9	7.4	7.5	7.7	7.3
Neutral detergent fiber	32.1	35.7	33.4	33.3	33.1	32.5
Acid detergent fiber	21.5	22.1	21.7	21.9	21.7	21
Cellulose	18.5	18.1	18.3	19.7	19.9	20.9
Hemi-cellulose	10.6	11.6	11.7	11.4	11.4	11.5
Acid insoluble ash	3.7	3.1	2.9	4.1	3.9	3.5

Different parameters such as blood urea nitrogen, triglyceride, blood sugar, total cholesterol, creatinine, very low density lipoprotein, blood glucose (G), total protein (TP), blood urea nitrogen, cholesterol and blood serum creatinine were assessed under different treatments. Experimental diets and fecal material under various treatments were evaluated for detailed chemical composition through proximate analyses method (AOAC, 2003). NDF (neutral detergent fiber), ADF (acid detergent fibre), hemicellulose and cellulose were measured by using different techniques as developed by Van Soest *et al.* (1994).

At the end of feeding experiments, two lambs from each group were transported to Hilal Meat Processing (Pvt) Ltd, Jand (Kohat) Road, Bhal Sayedan, Tehsil Fateh Jhang District Attock, Pakistan. Individuals were given overnight rest and were not provided food, so these individuals were off feed approximately for 12 hrs before slaughtering and had access to drinking water. Slaughter live weight (SLW) was measured and individuals were slaughtered following Halal (Muslim) method. Non-carcass components (head, trotter, pluck and liver) were weight just after slaughtering of each individual as described by Bonvillani *et al.* (2010). Within one hour of slaughtering, dressed as hot carcass weight (HCW) and kept it immediately in a chiller room at 4 °C for 24 hrs at Hilal Meat Processing

(Pvt) Ltd. After 24 hours of chilling, cold carcasses were weighed again as cold carcasses weight (CCW). Hot carcass yield or dressing percentage (DP), cooling loss index, cold carcass yield were calculated on the basis of HCW and CCW as described by Bonvillani et al. 2010 and Kadim et al. 2013. Water holding capacity was measured as described by Das *et al.* (2010). Drip Loss (DL %) was determined using technique defined by Earl et al. 1996. Ruminal and intestinal fluid samples were taken shortly after the animals were slaughtered and pH was measured from the fluid. Portable pH-meter was used for this purpose (Jasmin et al. 2011). Volatile fatty acids (VFAs) were measured by the procedure explained by Filípek et al. 2009 and Cottyn and Boucque, 1968. *In vitro* true digestibility of dry matter, fiber (neutral detergent fiber (NDF) and acid detergent fiber (ADF) and hemicelluloses was determined by using daisy incubator and methods described by Tassone et al. 2020. Estimates of the percentages of meat dry matter, crude protein, and crude fat were obtained from minced muscles by following the procedures by AOAC, 2003.

Statistical analysis: All collected data were analyzed using linear model procedures with R statistical software (4.3.0). When the effects of treatments on response variables were found significant, Tukey's HSD test was performed for comparing means (Team, R Core 2020).

RESULTS

The interaction effects of wet and dry total mixed rations with different protein levels on growth performance, slaughter, carcass and meat quality traits (Table 3), meat chemical composition, nutrient digestibility, gas and fatty acid concentration, serum mineral chemistry and nitrogen balance are shown in Table 4. Main effects of wet and dry total mixed rations with different protein levels on slaughter, carcass and meat quality traits (Table 5) meat chemical composition and nutrient digestibility, blood metabolites, serum blood profile and serum mineral chemistry of male Lohi sheep are shown in Table 6. The details of each section are explained below.

Growth Performance

In the present study, final body weight was differed significantly in the present study among all groups ($P < 0.04$). It was observed that highest final body weight (FBW) was retrieved in wet TMR: HCP (33.20 Kg) and was observed lowest in lambs fed wet TMR: LCP (28.70 Kg) as shown in Table 3. These results had demonstrated that lambs fed on both wet and dry TMR groups with different protein levels had a significant impact on animal performance traits. The diet rich in protein had higher body weight. Average daily gain data showed that lambs fed wet TMR has a more significant effect on performance of animals as compared to lambs fed dry TMR. Average daily gain data showed the interaction effect for both (wet and dry) treatments (Table 3) and were differed statistically significantly among groups ($P < 0.04$). However, wet TMR (silage based) groups showed overall the best performance as compared with that of dry TMR (hay based) groups. The best average daily gain (ADG) performance (200 g/day) was observed in lambs fed wet

TMR: HCP ($P < 0.02$). The lowest ADG performance (113.7 g/day) was observed in dry TMR: LCP.

An interaction effect for dry matter intake (DMI) was also observed in lambs fed dry and wet TMR ($P < 0.04$). Highest DMI (831.1 g/day) was found in lambs fed wet TMR: HCP. Lowest DMI (535.7 g/day) was spotted in lambs fed dry TMR: LCP (Table 3). There was also found statistically an interaction effect of feed to gain ratio (FGR) for both treatments ($P < 0.04$; Table 3). Lowest feed to gain ratio were found as 4.02 and 4.07 in lambs fed dry TMR with NCP and HCP, respectively. FGR (4.95) was observed highest in wet TMR: LCP.

Slaughter traits

The interaction effects of wet and dry total mixed rations with different protein levels on slaughter traits of male Lohi sheep are shown in Table 3. Main effects of wet and dry total mixed rations with different protein levels on slaughter traits of male Lohi sheep are shown in Table 5. Details of each slaughter trait are given below.

There was found non-significant ($P > 0.05$) effect of feeding wet and dry TMR to lambs supplemented with different levels of crude proteins on fasting weight (Kg) as shown in Table 5. The values of fasting weight (Kg) and trotter weight (Kg) were found higher in lambs fed wet total mixed rations (TMR's) based on maize silage as compared to lambs fed dry total mixed rations (TMR's) based on maize hay as shown in Table 5. There was significant ($P < 0.001$; Table 5) main effect for fasting weight (Kg), in lambs fed wet and dry TMR's supplemented with different CP levels. Trend for fasting weight was found linearly increased with the rise in CP levels for both wet and dry TMR's feeding.

There was also noted significant ($P < 0.02$) main effect for head weight (Kg) in lambs fed wet and dry TMR's supplemented with different CP levels and trend for head weight (Kg) was found linearly increased i.e., increased head weight (Kg) in lambs with increasing CP levels (Table 5).

There was significant interaction ($P < 0.001$) for intestinal weight (Kg) in lambs fed wet and dry TMR's supplemented with different CP levels and trend for intestinal weight (Kg) was found linearly increased with the increase in energy level (Table 3).

The best pluck weight (1.55 Kg) was observed in lambs fed wet TMR supplemented with 16 % crude protein followed by lambs fed wet TMR supplemented with 14.5 % CP and 13.0 % CP. Lowest PW (1.05 Kg) was observed in dry TMR feeding group supplemented with CP 13.0 %. There was linear rising trend of PW in lambs fed dry TMR supplemented with CP 14.5 % and 16.0 % respectively ($P < 0.001$; Table 3).

This shows that lambs fed wet TMR have more significant effect on non-carcass traits performance as compared to lambs fed dry TMR. Similar trend was observed in dressing percentage of lambs fed wet and dry TMR supplemented with different CP levels. In the present study, hot carcass weight was significantly higher in rations with high protein levels.

Carcass traits

Hot carcass weight (Kg) were found higher in lambs fed wet total mixed rations (TMR's) based on maize silage as compared to lambs fed dry total mixed rations (TMR's) based on maize hay as shown in Table 5. Furthermore, there was also noted significant ($P < 0.02$) main effect of CP levels on hot carcass weight (Table 5).

Cold carcass weight (CCW) was found non significantly higher ($P > 0.05$) in lambs fed wet TMR's as compared to lambs fed dry TMR's. There was found statistically significant ($P < 0.007$) increasing trend for CCW of lambs fed wet and dry treatment with increasing CP levels (Table 5). An interaction effect for chest weight (CW), was observed statistically significant in lambs fed dry and wet TMR ($P < 0.04$: Table 3). Highest CW (18.59 %) was found in lambs fed wet TMR: HCP and increased in linear trend in lambs fed wet TMR: LCP (17.19 %) and wet TMR: NCP (17.52 %) respectively. The lowest chest weight (16.37) was spotted in lambs fed dry TMR: NCP and rest of dry TMR groups were having statistically similar values of CW (%) as shown in Table 3. An interaction effect for foreleg weight (FLW), was observed statistically significant in lambs fed dry and wet TMR ($P < 0.001$). The best FLW (20.86 %) was observed in lambs fed dry TMR: HCP followed by lambs fed dry TMR: NCP and dry TMR: LCP. Lowest FLW (20.47%) was observed in dry TMR feeding group supplemented with 14.5 % CP (Table 3). There was also found an interaction effect of muscle fat weight (MFW) as shown in Table 3 for both treatments ($P < 0.001$). There was found statistically significant ($P < 0.001$) increasing trend for RSCW of lambs fed both treatments with increasing CP levels (Table 5). Left side carcass weight (LSCW %) showed a statistically similar significant trend in Table 3 as that of final body weight (FBW) and fasting body weight (FBW) ($P < 0.04$). Highest LSCW (%) was found in lambs fed wet TMR (53.56 %) and dry TMR (52.23 %) supplemented with 16 % CP. The lowest LSCW (%) was found in lambs fed wet TMR (51.30 %) and dry TMR (51.05 %) supplemented with 13 % CP. Overall in both wet and dry TMR groups there was a rising trend in LSCW (%) supplemented with 13 % CP, 14.5 % CP and 16 % CP. This difference in LSCW (%) vs RSCW (%) was found due to the presence of heart on left side of lamb's chest cavity or cage (Table 5). Thigh weight (THW), and liver weight (LW) were found non significantly higher ($P > 0.05$;) in lambs fed wet TMR's as compared to lambs fed dry TMR's. Loin weight (LLW), meat muscle weight (MMW), and bone weight (BONW) were found non significantly higher ($P > 0.05$) in lambs fed dry TMR's as compared to lambs fed wet TMR's.

Carcass characteristics plays crucial role in livestock rearing and production of different rations (Ebrahimi et al. 2007; Wang et al. 2020). In the present study, lambs fed wet TMR have more significant effect on carcass traits performance as compared to lambs fed dry TMR with increasing crude protein levels.

Meat Quality Traits

The interaction (Table 3) and main (Table 5) effects of type of feed and three protein levels for meat quality traits are described below:

Interaction effect for hot carcass yield or dressing percentage, cold carcass yield and drip loss ($P < 0.01$, $P < 0.01$, $P < 0.04$) was observed for both treatments as shown in Table 3. Highest HCY (%) was found in lambs fed on WTMR: HCP and DTMR: LCP (54.44 % and 51.13 %), respectively and similar trend was observed for CCY (%) in aforementioned groups. An interaction effect for drip loss (DL) was noted statistically significant in lambs fed dry and wet TMR ($P < 0.04$). DL (%) was found lowest as 3.23 % in lambs fed on WTMR: HCP and increased in non-linear trend in lambs fed wet TMR: NCP (5.95 %) and WTMR: LCP (8.23 %) respectively. Cooling loss index (CLI) showed a statistically similar significant trend in Table 3 as that of cold carcass yield (CCY) ($P < 0.01$). Highest CLI (%) was found in lambs fed on WTMR: LCP and DTMR: NCP (3.99 % and 3.77 %), respectively. Water holding capacity (%), muscle to bone ratio and muscle to fat ratio were found non significantly high ($P > 0.05$) in lambs fed dry TMR's as compared to lambs fed wet TMR's as shown in Table 3. Cold carcass yield (%) was found non significantly high ($P > 0.05$) in lambs fed wet TMR's as compared to lambs fed wet TMR's. There was found non-significant ($P < 0.05$) results for water holding capacity (%), cold carcass yield (%), muscle to bone ratio and muscle to fat ratio depending on CP levels after feeding both treatments.

Meat Chemical Composition

The interaction (Table 4) and main (Table 6) effects of type of feed and three protein levels for meat chemical composition are described below:

There was an interaction effect of meat crude protein (MCP) as shown in Table 4 for both treatments ($P < 0.04$). Highest protein level in wet and dry TMR had shown significantly highest percent MCP. The highest MCP (%) was found as 20.47 % and 19.50 % in lambs fed wet and dry TMR supplemented with 16 % CP. MCP (%) was increased linearly in lambs fed wet TMR supplemented with 13% CP (18.25 %) and 14.5 CP (19.06 %) respectively. Meat dry matter (MDM) was found non-significantly higher ($P > 0.05$) in lambs fed dry TMR's as compared to lambs fed wet TMR's with varying CP levels. There was found statistically significant main effect ($P > 0.05$; Table 6) results for MDM (%) based on CP levels and types of feed. MDM (%) was found in increasing trend as 23.49 %, 24.23 % and 24.64 % respectively, in lambs with increasing CP levels i.e., 13 % CP, 14.5 % CP and 16 % CP. Meat crude fat (MCF) was found significantly higher ($P > 0.05$) main effect in lambs fed wet TMR (3.06 %) as compared to lambs fed dry TMR (2.65 %). There was found statistically significant ($P < 0.06$) results for MCF (%) based on CP levels and both treatments. MCF (%) was found highest as 3.11 % with CP level 14.5 %.

Apparent Digestibility

The main effects of feeding wet and dry TMR was found in apparent digestibility of crude protein, crude fiber and crude fat in male Lohi sheep are given in Table 6 are described as under

There was non-significant ($P < 0.05$) effect of feeding wet and dry TMR to lambs supplemented with different levels of crude proteins on crude protein digestibility (CPD) (Table 6). Crude fiber digestibility (CFD %) was increased linearly in lambs fed dry TMR

supplemented with 13% CP, 14.5 % CP and CP 16 % level. CFTD (%) was increased linearly in lambs fed varying levels of CP for both types of feed.

Gas, IGF-1 and Volatile Fatty Acids Concentration

The interaction effect of feeding wet and dry TMR was seen in volatile fatty acids concentration, methane (CH₄) concentration and IGF-1 concentration in male Lohi sheep ruminal fluid and blood serum samples are given in Table 4, which are described as under.

There was highly significant ($P < 0.001$) interaction effect in acetic acid (AA) concentration, propionic acid (PA) concentration and butyric acid (BA) concentration ($P < 0.04$) as shown in Table 4 in ruminal fluid of male Lohi sheep for both treatments. AA (mmol/L) concentration was found as 98.25 mmol/L and 59.45 mmol/L in ruminal fluid of male Lohi sheep lambs fed wet and dry TMR supplemented with 16 % CP. AA (mmol/L) concentration was increased linearly in in ruminal fluid of lambs fed wet TMR supplemented with 13% CP (42.90 mmol/L) and 14.5 CP (55.05 mmol/L) respectively. AA (mmol/L) concentration was increased linearly in in ruminal fluid of lambs fed dry TMR supplemented with 13% CP (38.45mmol/L) and 14.5 % CP (44.60 mmol/L) respectively. Highest PA (mmol/L) concentration was found as 38.5 mmol/L and 26.1 mmol/L in in ruminal fluid of lambs fed wet and dry TMR supplemented with 16 % CP. PA (mmol/L) concentration was increased linearly in ruminal fluid of lambs fed wet TMR supplemented with 13% CP (20.1 mmol/L) and 14.5 CP (22.9 mmol/L) respectively. PA (mmol/L) concentration was increased linearly in in ruminal fluid of lambs fed dry TMR supplemented with 13% CP (17.95 mmol/L) and 14.5 % CP (22.6 mmol/L) respectively. Highest BA (mmol/L) concentration was found as 17.40 mmol/L and 10.05 mmol/L in in ruminal fluid of lambs fed wet and dry TMR supplemented with 16 % CP. BA (mmol/L) concentration was increased linearly in in ruminal fluid of lambs fed wet TMR supplemented with 13% CP (10.35 mmol/L) and 14.5 CP (12.50 mmol/L) respectively. BA (mmol/L) concentration was increased linearly in ruminal fluid of lambs fed dry TMR supplemented with 13% CP (7.65 mmol/L) and 14.5 % CP (9.60 mmol/L) respectively.

An interaction effect for methane gas production (CH₄) was noted statistically significant in in ruminal fluid of lambs fed dry and wet TMR ($P < 0.001$). Highest CH₄ (mmol/L) concentration was found as 48.20 mmol/L and 28.23 mmol/L in ruminal fluid of lambs fed wet and dry TMR supplemented with 16 % CP. CH₄ (mmol/L) concentration was increased linearly in in ruminal fluid in lambs fed wet TMR supplemented with 13% CP (21.60 mmol/L) and 14.5 CP (28.05 mmol/L) respectively. CH₄ (mmol/L) concentration was increased linearly in ruminal fluid of lambs fed dry TMR supplemented with 13% CP (18.56 mmol/L) and 14.5 % CP (21.45 mmol/L) respectively. There was significant ($P < 0.001$) interaction effect was observed in IGF-1 concentration ($P < 0.04$) as shown in Table 4 in blood serum of male Lohi sheep for both treatments. IGF-1 concentration (ng/mL) was found as 58.81 ng/mL and 50.59 ng/mL in ruminal fluid of male Lohi sheep lambs fed wet and dry TMR supplemented with 16 % CP. IGF-1 concentration (ng/mL) was increased linearly in lambs fed wet TMR supplemented with 13% CP (46.15 ng/mL) and 14.5 CP (51.05 ng/mL) respectively. IGF-1 concentration (ng/mL) was also increased

linearly in lambs fed dry TMR supplemented with 13% CP (40.49 ng/mL) and 14.5 % CP (47.10 ng/mL) respectively. Hence it was concluded that wet TMR feeding show good fermentation characteristics supplemented with 16 % CP and is triggering growth of male Lohi sheep.

Serum Metabolites

The blood glucose level (mg/dL) of lambs that were fed dry TMRs was found to be non-significantly higher ($P>0.05$) than the blood glucose level of lambs that were fed wet TMRs. The results of our study were similar with the finding of the Wang et al. (2020), where change in diet type did not influence the serum metabolites such as total protein thus all the individuals had normal physiological process and normal metabolism. In lambs with increasing CP levels, there was shown to be a significant main effect ($P>0.05$) in the direction of an increasing trend for total protein (g/dL). The levels of blood urea nitrogen (BUN) (mg/dL), serum creatinine (mg/dL), and total protein (g/dL) were found to be non-significantly greater ($P>0.05$; Table 6) in lambs who were fed wet TMRs as opposed to lambs that were fed dry TMRs. It was discovered that the major effect for blood glucose (mg/dL), blood urea nitrogen (mg/dL), and serum creatinine (mg/dL) in lambs with varied CP levels were not found significant ($P>0.05$).

Serum Lipid Profile

The levels of cholesterol (mg/dL), triglycerides (mg/dL), and very-low-density lipoprotein (VLDL) cholesterol (mg/dL) were found to be non-significantly higher ($P>0.05$; Table 6) in lambs who were fed dry TMRs as opposed to lambs that were given wet TMRs. When comparing lambs with different CP levels, it was discovered that the major effect for cholesterol (mg/dL), triglyceride (mg/dL), and very-low-density lipoprotein (VLDL) cholesterol (mg/dL) was found non-significant ($P>0.05$).

Serum Minerals Chemistry

The interaction effect of feeding wet and dry TMR was found in sodium (mEq/dL), potassium (mEq/dL), calcium (mg/dL) and main effect due to type of feed and CP levels was observed in chloride (mEq/dL) in male Lohi sheep blood samples are given in Table 4 and Table 6. There was found statistically highly significant ($P<0.001$) interaction effect of sodium (Na) as shown in Table 4 in serum samples of male Lohi sheep for both treatments. Na (mEq/dL) was found highest as 155.10 mEq/dL and 157.95 mEq/dL in blood samples of male Lohi sheep lambs fed wet and dry TMR supplemented with 16 % CP. Na (mEq/dL) was increased linearly in lambs fed wet TMR supplemented with 13% CP (153.25 mEq/dL) and 14.5 CP (151.40 mEq/dL) respectively. Na (mEq/dL) was increased linearly in blood samples of lambs fed dry TMR supplemented with 13% CP (149.85 mEq/dL) and 14.5 % CP (154.50 mEq/dL) respectively. K (mEq/dL) concentration was found highest as 5.22 mEq/dL and 4.80 mEq/dL in serum samples of male Lohi sheep lambs fed wet and dry TMR supplemented with 16 % CP. K (mEq/dL) concentration was increased linearly in lamb's serum sample fed wet TMR supplemented with 13% CP (4.44 mEq/dL) and 14.5 CP (4.56 mEq/dL) respectively (Table 4). K (mEq/dL) concentration was increased linearly in serum samples of lambs fed dry TMR

supplemented with 13% CP (4.16 mEq/dL) and 14.5 % CP (4.54 mEq/dL) respectively. Calcium (mg/dL) conc. was found non significantly higher ($P>0.05$) in lambs fed wet TMR's as compared to lambs fed dry TMR's. Chloride (mEq/dL) was found non significantly higher ($P>0.05$) in lambs fed dry TMR's as compared to lambs fed wet TMR's. There was non-significant ($P>0.05$) main effect for blood glucose (mg/dL), There was significant ($P>0.05$) main effect in chloride (mEq/dL) of lambs with increasing CP levels.

Table 3: Interaction effect of wet and dry total mixed rations with different crude protein levels on growth performance, slaughter, carcass, meat quality traits of male Lohi sheep (n=3), where TMR = Total Mixed Ration; LCP = Low crude protein, NCP = Normal crude protein, HCP =High crude protein

Response Variables/ factors	Parameters (Units)	Wet TMR (Silage based)			Dry TMR (Hay based)			p- Value	SEM
		LCP	NCP	HCP	LCP	NCP	HCP		
Growth performance	Final body weight (Kg)	28.70 ^c	31.90 ^b	33.20 ^a	24.52 ^d	26.70 ^{cd}	28.45 ^c	0.04	0.42
	Daily weight gain (g/day)	148.15 ^c	180.74 ^b	200.0 ^a	113.70 ^d	134.15 ^c	141.56 ^c	0.02	4.98
	Dry matter intake (g/day)	732.89 ^b	788.8 ^{ab}	831.1 ^a	535.7 ^d	539.4 ^d	576.8 ^c	0.04	14.70
	Feed to gain ratio	4.95 ^a	4.37 ^b	4.15 ^b	4.72 ^a	4.02 ^b	4.07 ^b	0.04	0.14
Slaughter traits	Intestine weight (Kg)	1.94 ^b	2.82 ^a	2.87 ^a	1.54 ^c	1.59 ^c	1.49 ^c	0.001	0.08
	Pluck weight (Kg)	1.28 ^{ab}	1.38 ^{ab}	1.55 ^a	1.05 ^b	1.19 ^b	1.30 ^{ab}	0.04	0.07
	Testes weight (g)	70.0 ^b	79.5 ^a	81.5 ^a	49.5 ^d	68.5 ^{bc}	60.0 ^c	0.04	4.80
Carcass traits	Left side carcass weight (%)	51.30 ^b	51.85 ^{ab}	53.56 ^a	51.05 ^b	51.79 ^b	52.23 ^{ab}	0.04	0.43
	Chest weight (%)	17.19 ^b	17.52 ^{ab}	18.59 ^a	17.80 ^{ab}	16.37 ^b	17.81 ^{ab}	0.03	0.36
	Foreleg weight (%)	20.81 ^a	20.54 ^a	20.70 ^a	19.24 ^b	20.47 ^a	20.86 ^a	0.001	0.27
	Muscle fat weight (%)	9.46 ^b	10.17 ^b	11.39 ^a	7.33 ^c	10.54 ^a	10.87 ^a	0.001	0.49
	Hot carcass yield (%)	50.52	50.05	54.44	51.13	50.52	48.49	0.01	1.26
	Cold carcass yield (%)	48.51	48.65	52.50	49.92	48.62	46.94	0.01	1.40
Meat quality traits	Drip loss (%)	8.23 ^a	5.95 ^{ab}	3.23 ^c	5.26 ^{ab}	6.34 ^{ab}	4.94 ^{bc}	0.04	0.67
	Cooling loss index (%)	3.99 ^a	2.82 ^b	3.56 ^a	2.36 ^c	3.77 ^a	3.19 ^{bc}	0.01	0.45

Table 4: Interaction effect of wet and dry total mixed rations with different crude protein levels on meat quality composition, nutrient digestibility, Gas and fatty acid concentration, serum mineral chemistry and nitrogen balance of male Lohi sheep (n=3), where TMR = Total Mixed Ratio; LCP = Low crude protein, NCP = Normal crude protein, HCP =High crude protein

Response Variables/factors	Parameters (Units)	Wet TMR (Silage based)			Dry TMR (Hay based)			p-Value	SEM
		LCP	NCP	HCP	LCP	NCP	HCP		
Meat chemical composition	Meat crude protein (%)	18.25 ^b	19.06 ^{ab}	20.47 ^a	18.72 ^b	18.91 ^b	19.50 ^a _b	0.04	0.36
Nutrient digestibility (%)	<i>In vitro</i> true DM digestibility	68.48 ^b	71.57 ^{ab}	71.36 ^{ab}	61.35 ^a	70.13 ^{ab}	75.0 ^a	0.04	2.71
VFA, IGF-1 and methane concentration	Acetic acid (mmol/L)	42.90 ^c	55.05 ^b	98.25 ^a	38.45 ^c	44.60 ^c	59.45 ^b	0.001	2.50
	Propionic acid (mmol/L)	20.1 ^c	22.9 ^c	38.5 ^a	17.95 ^d	22.6 ^c	26.1 ^b	0.001	1.18
	Butyric acid (mmol/L)	10.35 ^{bc}	12.50 ^b	17.40 ^a	7.65 ^c	9.60 ^{bc}	10.05 ^b _c	0.04	1.12
	IGF-1 (ng/mL) conc.	46.15 ^c	51.05 ^b	58.81 ^a	40.94 ^{dc}	47.10 ^c	50.95 ^b	0.001	0.61
	Methane (mmol/L) conc.	21.60 ^c	28.05 ^b	48.20 ^a	18.56 ^d	21.45 ^c	28.23 ^b	0.001	0.98
Serum mineral chemistry	Na (mEq/dL) conc.	153.25 ^{bc}	151.40 ^{bc}	155.10 ^{ab}	149.85 ^c	154.50 ^{ab}	157.9 ^{5a}	0.001	1.04
	K (mEq/dL) conc.	4.44 ^{ab}	4.56 ^{ab}	5.22 ^a	4.16 ^b	4.54 ^{ab}	4.80 ^{ab}	0.02	0.27
Nitrogen balance	Nitrogen balance (g/day)	7.03 ^b	9.69 ^b	16.64 ^a	11.40 ^{ab}	11.29 ^{ab}	12.73 ^a _b	0.03	1.68

Table 5: Main effects of types of feed and crude protein (CP) levels on slaughter, carcass and meat quality traits of male Lohi sheep (n=3), where LCP = Low crude protein, NCP = Normal crude protein, HCP =High crude protein

Response Variables/factors	Parameters (Units)	Type of Feed		P-value	SEM	CP Levels			P-value	SEM
		Silage	Hay			LCP	NCP	HCP		
Slaughter traits	Fasting weight (Kg)	30.63	26.13	p>0.05	0.41	26.12 ^c	28.86 ^b	30.16 ^a	0.001	0.51
	Head weight (Kg)	2.03	1.90	p>0.05	0.06	1.79	2.03	2.07	0.02	0.077
	Trotter weight (g)	0.985	0.863	p>0.05	0.07	0.810	0.979	0.983	0.15	0.087
Carcass traits	Cold carcass weight (Kg)	15.30	12.65	p>0.05	0.36	12.84	14.04	15.05	0.007	0.44
	Hot carcass weight (Kg)	15.85	13.06	p>0.05	0.35	13.27 ^c	14.52 ^{ab}	15.58 ^a	0.004	0.42
	Dressing percentage (%)	49.62	49.25	p>0.05	0.64	49.91	49.54	48.85	0.44	0.78

	Right side carcass weight (%)	48.31	47.77	p>0.05	0.24	47.11 ^b	48.18 ^a	48.83 ^a	0.001	0.31
	Thigh weight (%)	30.12	29.54	p>0.05	0.32	28.91 ^b	29.49 ^b	31.08 ^a	0.001	0.55
	Loin weight (%)	29.46	29.67	p>0.05	0.24	28.76 ^b	29.28 ^b	30.66 ^a	0.001	0.41
	Kidney weight (Kg)	0.079	0.073	p>0.05	0.20	0.084	0.069	0.075	0.81	0.02
	Liver weight (g)	0.470	0.430	p>0.05	0.037	0.420	0.486	0.444	0.40	0.046
	Meat muscle weight (%)	56.78	58.31	p>0.05	1.06	50.58 ^c	56.94 ^b	65.03 ^a	0.01	1.29
	Bone weight (%)	32.54	33.28	p>0.05	1.28	22.84 ^c	32.71 ^b	43.18 ^a	0.04	1.56
Meat quality traits	Water holding capacity (%)	6.19	6.39	p>0.05	0.35	6.83	6.24	5.80	0.13	0.43
	Muscle to bone ratio	19.16	19.34	p>0.05	0.45	18.06	19.83	19.86	0.66	0.55
	Muscle to fat ratio	2.67	2.85	p>0.05	0.17	2.64	2.82	2.82	0.66	0.21

Table 6: Main effects of types of feed and crude protein (CP) levels on meat chemical composition, apparent digestibility, Blood metabolites, serum lipid profile and serum mineral chemistry of male Lohi sheep (n=3), where LCP = Low crude protein, NCP = Normal crude protein, HCP =High crude protein. VLDL= Very-low-density lipoprotein

Response Variables/factors	Parameters (Units)	Type of Feed		P-value	SEM	CP Levels			P-value	SEM
		Silage	Hay			LCP	NCP	HCP		
Meat chemical composition	Meat dry matter (%)	23.96	24.28	p>0.05	0.46	23.49	24.23	24.64	0.07	0.56
	Meat crude fat (%)	3.06	2.65	p>0.05	0.44	2.60	3.11	2.86	0.06	0.54
Apparent digestibility (%)	Crude protein digestibility	87.41	87.79	p>0.05	1.54	84.18 ^c	87.16 ^b	91.46 ^a	0.02	1.88
	Crude fat digestibility	87.31	87.32	p>0.05	1.21	85.98	86.48	89.50	0.11	1.49
	Crude fiber digestibility	85.46	85.47	p>0.05	0.78	83.32	86.56	88.00	0.007	0.96
Blood metabolites	Blood glucose (mg/dL)	74.33	96.17	p>0.05	10.42	86.50	84.25	85.00	0.98	12.77
	Blood urea nitrogen (mg/dL)	14.83	10.87	p>0.05	3.09	14.63	11.18	12.75	0.67	3.79
	Serum creatinine (mg/dL)	0.70	0.63	p>0.05	0.08	0.58	0.67	0.75	0.28	0.10
Serum lipid profile	Cholesterol (mg/dL)	54.50	60.33	p>0.05	10.69	48.50	55.50	68.25	0.37	13.09

	Triglyceride (mg/dL)	40.5	46.0	p>0.05	10.79	38.00	42.50	49.25	0.70	13.2 2
	VLDL Cholesterol (mg/dL)	13.03	14.12	p>0.05	2.13	12.55	13.43	14.75	0.71	2.61
Serum mineral chemistry	Ca (mg/dL) conc.	5.61	5.39	p>0.05	0.09	5.32 ^c	5.71 ^a	5.46 ^b	0.03	0.11
	Chloride (mEq/dL)	114.7 3	115.6 7	p>0.05	1.21	113.63 ^b	114.00 ^b	117.98 ^a	0.04	1.47

DISCUSSION

Less availability of macro and micronutrients to the animal's blood stream hinders, delays and impedes growth of animals (Ishaq et al. 2016; Obeidat et al. 2019; Obeidat and Obeidat, 2022). In the present study, the highest final body weight of lambs was reported in wet total mixed ration with high crude protein contents. Similar results were observed by Hajji (2016) where the rations with high protein level increased the body weight of individuals and high protein promoted fat deposition in three sheep breeds. Our results were in consistence with the results of various scientists (Wang et al. 2020; Sileshi et al. 2021; Raducuta et al. 2023), where they had observed that the individuals fed on diet with highest protein levels had significantly increased final body weights in Tibetan sheep. Reason for this greater total gain might be the availability of high protein level in proper diet, which enhanced protein availability to animals and increase absorption of amino acids ultimately improved the growth of animal (Estrada-Angulo et al. 2018; Wang et al. 2020). Shen et al. (2018) provided another similar explanation for increase in final BWs (body weights) with the increase in dietary protein which might be due to the greater intake of protein enhanced the quantity of non-degradable protein in rumen of lamb and was turned into amino acid and peptides, which were absorbed and utilized directly in small intestine. Higher protein levels increases the activity and number of microorganisms in rumen and these microorganism maximized ruminal fermentation (Lv et al. 2020) and ultimately enhanced the body weight of lamb (Raducuta et al. 2023).

Average daily gain data showed that lambs fed wet TMR (silage) with high crude protein had significant effect on performance of animals as compared to lambs fed dry TMR. The results of ADG of current study were matching with the results of reported by Wang et al. (2020). Wang et al. (2023) reported that silage group has higher ADG as compared to hay groups. Thus diet rich in protein had higher ADG, which is due to the higher absorption of amino acids, which ultimately enhanced ADG of lambs (Estrada-Angulo et al. 2018; Wang et al. 2020). Wang et al. (2020) had observed similar results where the individuals fed on diet with highest protein levels had significantly increased ADG in Tibetan sheep. Thus, high protein based supplement in rations increased ADG in lambs and approved as more efficient feed conversion. Similar findings were observed by Sileshi et al. (2021) and Raducuta et al. (2023), where the dietary protein supplement increased the ADG significantly which was due to the improvement in protein and dry matter consumption in lambs. However, Dabiri and Thonney, (2004) reported very small difference in ADG among lambs when fed with dietary protein supplement ranges

between 15 or 17% crude protein. Some other studies did not find any increase in ADG with the increase CP level in diets (Kaya et al. 2009).

Highest dry matter intake (DMI) found in lambs fed wet TMR with high crude protein. Similar results were found by various researchers (Dove and Milne, 2006; Sileshi et al. 2021), where increasing protein levels in diets facilitated the high DMI and high growth rate of lambs, which was due to balanced diet with high protein levels. Some other studies did not find any increase in DM intake with the increase CP level in diets (Kaya et al. 2009). In the present study, the lowest feed to gain ratio were found in lambs fed dry TMR with normal and high crude protein levels. The lambs fed wet and dry TMR supplemented with high crude protein (16 %) in this experiment showed better FGR respectively.

Slaughter and carcass characteristics plays crucial role in livestock rearing and production of different rations (Ebrahimi et al. 2007; Wang et al. 2020). In the present study, the slaughter and carcass traits were reported significantly higher in wet TMR with high energy levels. The fasting and final weight, left side carcass weight, pluck, trotter, hot carcass, chest, foreleg and muscle fat weight were found higher in lambs fed wet TMR with high protein level. However, other carcass traits such as right side carcass weight, thigh weight and liver weight were similar in lambs fed wet TMR's as compared to lambs fed dry TMR's but showed highest weight in rations with high crude protein level. Similarly, trend for fasting weight was found linearly increased with the rise in CP levels for both wet and dry TMR's feeding. There was significant main effect for head weight and intestinal (Kg) in lambs fed wet and dry TMR's supplemented with different CP levels and trend for head weight (Kg) was found linearly increased with increasing crude protein levels in lamb diet. The cold carcass weight did not differ among both types of TMRs however it increased significantly in lambs fed wet and dry treatment with high CP levels. Thus, the current study reported the best slaughter and carcass traits in wet TMR with high crude protein level. Similar results were reported by Ebrahimi et al. (2007), where this study has reported better slaughter and carcass traits of lambs fed on higher CP rations. Wang et al. (2020) reported similar results where they had reported that high protein diets significantly improved weight of hot carcass of sheep. In another study found that higher CP levels did not improve carcass traits (Raducuta et al. 2023).

In the present study, lambs fed wet TMR have more significant effect on carcass traits performance as compared to lambs fed dry TMR with increasing crude protein levels. In the present study, cold carcass weight and other carcass parameters were significantly greater in diets with higher protein levels. Similar results were reported by Ebrahimi et al. (2007), where this study has reported higher cold carcass weight of lambs fed on higher CP rations. Wang et al. (2021) reported similar results where they had reported that high protein diets significantly improved bone and net meat weight of sheep. However, other studies did not find significant difference in carcass characteristics and slaughter parameters with higher dietary protein levels (Ebrahimi et al. 2007; Raducuta et al. 2023). Similar results were reported by various scientists (Raducuta et al. 2023) where higher CP levels in rations did not significantly improve the amount of carcass muscle or muscle surface area.

Varying pH in rumen caused by feeding excessive concentrates and roughages either based on protein or energy in the diet, affects cellulolytic bacteria and rumen protozoa count ultimately effect pH, nutrient digestibility, VFA's production and nutrients absorption in gastro-intestinal tract of lamb (Wang et al. 2020). However, in the present study, rumen pH, small intestine pH and large intestine pH did not change with the change in type of ration, however, large intestine pH of lambs decreased when fed both rations in high crude protein level but there was similar effect for Rumen pH and small intestine pH of lambs fed wet and dry TMR's with varying CP levels.

Cooling loss index (CLI), water holding capacity (%), muscle to bone ratio and muscle to fat ratio were similar in lambs fed dry TMR's as compared to lambs fed wet TMR's with change in protein level. Similar results were obtained by Prima et al. (2019) and reported a non-significant effect of CP levels on weight of meat, fat, meat to fat ratio, bone weight, meat to bone ratio. In the present study, the highest protein level in wet and dry TMR had shown significantly highest percent meat crude protein and meat crude fat. Same results were reported by Wang et al. (2020) and they had linked the high amount of MCP with the highest protein level in lamb diet. Meat dry matter (MDM) also changed with the rations containing different amounts of protein. Thus, our study and previous studies has reported that the dietary protein levels play a major role in the meat composition of sheep (Wang et al. 2020; De Marzo et al. 2023).

In the present study, crude protein digestibility was reported similar on both type of rations, however, crude fiber digestibility, dry matter digestibility, neutral detergent fiber (NDF) and acid detergent fiber (ADF) digestibility (%) were higher in both TMRs with high protein level. Similar results were reported by Obeidat et al. (2019), where high protein level improved the digestion of diets and digestibility of NDF, ADF was higher in rations with highest protein level. Thus, the digestibility of feed in lamb was improved with the increase in amount of protein level in the rations, where NDF and ADF digestibility increased with the higher protein levels.

In the present study, the volatile fatty acid and methane gas and IGF-1 concentration were reported highest for both types of rations at higher protein level. The acetic acid (AA) concentration, propionic acid (PA) concentration and butyric acid (BA) concentration) in ruminal fluid of male Lohi sheep varies with the type of rations and were reported highest in silage based rations as compared with hay based diets. The results of current study had shown that the volatile fatty acids (VFA) concentration was matching with the results of Wang et al. (2020) and their study showed that silage based feeding group had significantly increased in VFA as compared to hay based feeding group. The reason behind might be the decreased pH in rumen of silage feeding groups. The current study had shown that the higher amount of fatty acids were produced in diets with high protein level. These results were in line with the findings of Cui et al. (2019) and results of their study showed that the amount of VFA was lower in protein deficient diets and higher amount of fatty acid were produced in diets with high protein level. Thus, based on results of current study and previous study, wet TMR feeding was triggering growth of male Lohi sheep supplemented with 16 % CP.

The blood glucose, total protein, blood urea nitrogen, serum creatinine level of lambs that were fed dry TMRs was found similar to lambs that were fed wet TMRs. The results of our study was similar with the finding of the Wang et al. (2020), where change in diet type did not influence the serum metabolites such as total protein thus all the individuals had normal physiological process and normal metabolism. In the present study, the serum metabolite levels did not change with the change in crude protein levels. However, Wang et al. (2020), reported that the BUN, serum creatinine and blood glucose significantly changed with the change in protein levels and could affect metabolism and translocation of sugar in Hu sheep. In the present study, the levels of cholesterol, triglycerides, and very-low-density lipoprotein cholesterol did not change with the change in type of rations and protein levels. These results showed that serum lipid profile was reported normal and all individuals had normal metabolic activities for normal physiological function (Wang et al. 2020). These results for TSH and TG serum lipid profile were reported consistent with the findings of Wang et al. (2020), where high dietary protein level did not significantly influence the serum lipid metabolites in Tibetan sheep. Similar results for very-low-density lipoprotein (VLDL) cholesterol were reported by (Wang et al. 2020) and reported non-significant effects of high protein level on VLDL cholesterol in Hu male lambs.

In the present study, serum minerals such as sodium, potassium, calcium amount varies with the change in TMRs and protein levels Amount of sodium and potassium in serum of lambs fed on rations with higher amount of protein level. These results were similar with the finds of Wang et al. (2020) where serum concentrations were significantly higher at the greater protein thus it improves the function of growth hormone in Tibetan sheep and increase the growth performance and improved the carcass characteristics of Tibetan sheep. Thus, improvement of carcass characteristics in our study was also linked with higher serum minerals concentration in higher CP rations.

CONCLUSION

It is concluded from the results obtained by the current trial that male Lohi Sheep lambs responded well to the treatment of the high crude protein level in wet TMR based on maize silage. Crude protein was shown to increase the muscle mass in sheep and clearly reduced total fat and increment the lean muscle. The ADG of lamb was obtained highest when fed wet TMR with CP16 %. DMI and feed to gain ratio was improved with increasing CP levels in both treatments. A dietary plan based on wet TMR based on maize silage supplemented with 16 % CP not only enhances the growth performance but also improves carcass characteristics with good impact on blood metabolites and meat quality of male Lohi sheep.

Acknowledgment: We acknowledge animal nutrition laboratory, livestock production and management PMAS-AAUR and central laboratory University of veterinary and animal sciences, Lahore for technical support.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- 1) Abdullah, A. Y., & Musallam, H. S. (2007). Effect of different levels of energy on carcass composition and meat quality of male black goats kids. *Livestock Science*, 107, 70–80.
- 2) Alhidary, I. A., Abdelrahman, M. M., Alyemni, A. H., Khan, R. U., Al-Saiady, M. Y., Amran, R. A., & Alshamiry, F. A. (2016). Effect of alfalfa hay on growth performance, carcass characteristics, and meat quality of growing lambs with ad libitum access to total mixed rations. *Revista Brasileira de Zootecnia*, 45, 302–308.
- 3) Alhidary, I., Abdelrahman, M. M., Alyemni, A. H., Khan, R. U., Al-Mubarak, A. H., & Albaadani, H. H. (2016). Characteristics of rumen in Naemi lamb: Morphological changes in response to altered feeding regimen. *Acta Histochemica*, 118, 331–337.
- 4) AOAC. 2003. *Official Methods of Analysis of AOAC International*, 17th Ed. Tech. Rep. Gaithersburg, MD, USA.
- 5) Askar, A. R., Allam, M. A., Kewan, K. Z., Darwesh, R., Lamara, M., Sabra, E. A., . . . Rabee, A. E. (2023). Effect of concentrates level on digestibility, ruminal fermentation, and bacterial community in growing camels. *Animal Biotechnology*, 34, 4500–4509.
- 6) Beigh, Y. A., Ganai, A. M., & Ahmad, H. A. (2017). Prospects of complete feed system in ruminant feeding: A review. *Veterinary world*, 10, 424.
- 7) Bonvillani, A., Peña, F., Domenech, V., Polvillo, O., García, P. T., & Casal, J. J. (2010). Meat quality of Criollo Cordobes goat kids produced under extensive feeding conditions. Effects of sex and age/weight at slaughter. *Spanish Journal of Agricultural Research*, 8, 116–125.
- 8) Cottyn, B. G., & Boucque, C. V. (1968). Rapid method for the gas-chromatographic determination of volatile fatty acids in rumen fluid. *Journal of Agricultural and Food Chemistry*, 16, 105–107.
- 9) Cui, K., Qi, M., Wang, S., Diao, Q., & Zhang, N. (2019). Dietary energy and protein levels influenced the growth performance, ruminal morphology and fermentation and microbial diversity of lambs. *Scientific Reports*, 9, 16612.
- 10) Dabiri, N., & Thonney, M. L. (2004). Source and level of supplemental protein for growing lambs. *Journal of Animal Science*, 82, 3237–3244.
- 11) De Marzo, D., Losacco, C., Laudadio, V., Tufarelli, V., & Xiong, Y. L. (2023). Influence of Dietary Protein Source and Level on Histological Properties of Muscle and Adipose Tissue of Lambs. *Foods*, 12, 1284.
- 12) Dove, H., & Milne, J. A. (2006). Intake and productivity of lambs grazing leafy or stemmy forage rape and the effect of energy or protein supplements. *Australian Journal of Experimental Agriculture*, 46, 763–769.
- 13) Earl, L. A., King, A. J., Fitzpatrick, D. P., & Cooper, J. E. (1996). A modification of a method to determine expressible moisture in ground, dark poultry meat. *Poultry science*, 75, 1433–1436.
- 14) Ebrahimi, R., Ahmadi, H. R., Zamiri, M. J., & Rowghani, E. (2007). Effect of energy and protein levels on feedlot performance and carcass characteristics of Mehraban ram lambs. *Pakistan Journal of Biological Sciences: PJBS*, 10, 1679–1684.
- 15) Estrada-Angulo, A., Castro-Pérez, B. I., Urfas-Estrada, J. D., Ríos-Rincón, F. G., Arteaga-Wences, Y. J., Barreras, A.,... Zinn, R. A. (2018). Influence of protein level on growth performance, dietary energetics and carcass characteristics of Pelibueyx Katahdin lambs finished with isocaloric diets. *Small Ruminant Research*, 160, 59–64.

- 16) Filípek, J., Dvořák, R., & others. (2009). Determination of the volatile fatty acid content in the rumen liquid: comparison of gas chromatography and capillary isotachopheresis. *Acta Veterinaria Brno*, 78, 627–633.
- 17) Guzman-Cedillo, A. E., Corona, L., Castrejon-Pineda, F., Rosiles-Martínez, R., & Gonzalez-Ronquillo, M. (2017). Evaluation of chromium oxide and titanium dioxide as inert markers for calculating apparent digestibility in sheep. *Journal of Applied Animal Research*, 45, 275–279.
- 18) Hajji, H., Joy, M., Ripoll, G., Smeti, S., Mekki, I., Gahete, F. M.,... Atti, N. (2016). Meat physicochemical properties, fatty acid profile, lipid oxidation and sensory characteristics from three North African lamb breeds, as influenced by concentrate or pasture finishing diets. *Journal of Food composition and Analysis*, 48, 102–110.
- 19) Ishaq, K., Younas, M., Riaz, M., & Ali, M. (2016). EFFECTIVENESS OF HIGH INPUT FEEDING SYSTEM IN RELATION TO GROWTH AND CARCASS QUALITY OF VARIOUS CLASSES OF BEETAL KIDS. *Pakistan Journal of Agricultural Sciences*, 53.
- 20) Jasmin, B. H., Boston, R. C., Modesto, R. B., & Schaer, T. P. (2011). Perioperative ruminal pH changes in domestic sheep (*Ovis aries*) housed in a biomedical research setting. *Journal of the American Association for Laboratory Animal Science*, 50, 27–32.
- 21) Kadim, I. T., Al-Karousi, A., Mahgoub, O., Al-Marzooqi, W., Khalaf, S. K., Al-Maqbali, R. S., . . . Raiymbek, G. (2013). Chemical composition, quality and histochemical characteristics of individual dromedary camel (*Camelus dromedarius*) muscles. *Meat science*, 93, 564–571.
- 22) Kaya, I., Unal, Y., Sahin, T., & Elmali, D. (2009). Effect of different protein levels on fattening performance, digestibility and rumen parameters in finishing lambs. *J Anim Vet Adv*, 8, 309–312.
- 23) Lv, X., Cui, K., Qi, M., Wang, S., Diao, Q., & Zhang, N. (2020). Ruminal microbiota and fermentation in response to dietary protein and energy levels in weaned lambs. *Animals*, 10, 109.
- 24) Meehan, D. J., Cabrita, A. R., Maia, M. R., & Fonseca, A. J. (2021). Energy: protein ratio in ruminants: insights from the intragastric infusion technique. *Animals*, 11, 2700.
- 25) Mendes, M. S., Souza, J. G., Herbster, C. J., Brito Neto, A. S., Silva, L. P., Rodrigues, J. P., . . . Pereira, E. S. (2021). Maintenance and growth requirements in male Dorperx Santa Ines lambs. *Frontiers in Veterinary Science*, 8, 676956.
- 26) Nudda, A., Battaccone, G., Atzori, A. S., Dimauro, C., Rassu, S. P., Nicolussi, P., . . . Pulina, G. (2013). Effect of extruded linseed supplementation on blood metabolic profile and milk performance of Saanen goats. *Animal*, 7, 1464–1471.
- 27) Obeidat, B. S., Subih, H. S., & Ata, M. (2019). Protein supplementation improves performance of lambs fed low-quality forage. *Animals*, 10, 51.
- 28) Obeidat, M. D., & Obeidat, B. S. (2022). Fattening performance, nutrient digestibility, and carcass traits of two fat-tailed sheep breeds. *Tropical Animal Health and Production*, 54, 375.
- 29) Oliveira, P. P., Berndt, A., Pedroso, A. F., Alves, T. C., Pezzopane, J. R., Sakamoto, L. S., . . . Rodrigues, P. H. (2020). Greenhouse gas balance and carbon footprint of pasture-based beef cattle production systems in the tropical region (Atlantic Forest biome). *Animal*, 14, s427–s437.
- 30) Nutrient Requirements of Small Ruminants, N. R., Council, N. R., on the Nutrient Requirements of Small Ruminants, C., on Agriculture, B., on Earth, D., & Studies, L. (2007). Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids. 中国法制出版社.
- 31) Prima, A., Purbowati, E., Rianto, E., & Purnomoadi, A. (2019). The effect of dietary protein levels on body weight gain, carcass production, nitrogen emission, and efficiency of productions related to emissions in thin-tailed lambs. *Veterinary world*, 12, 72.

- 32) Raducuta, I., Marin, M., Custura, I., & Maftei, M. (2023). Effects of different protein levels on fattening performance and meat parameters in merino lambs. *Indian Journal of Animal Research*, 57, 749–753.
- 33) Sen, A. R., Santra, A., & Karim, S. A. (2004). Carcass yield, composition and meat quality attributes of sheep and goat under semiarid conditions. *Meat science*, 66, 757–763.
- 34) Sharif, N., Ali, A., Dawood, M., Khan, M. I.-u.-R., & Do, D. N. (2022). Environmental effects and genetic parameters for growth traits of Lohi sheep. *Animals*, 12, 3590.
- 35) Shen, J., Chen, Y., Moraes, L. E., Yu, Z., & Zhu, W. (2018). Effects of dietary protein sources and nisin on rumen fermentation, nutrient digestion, plasma metabolites, nitrogen utilization, and growth performance in growing lambs. *Journal of Animal Science*, 96, 1929–1938.
- 36) Sileshi, G., Mitiku, E., Mengistu, U., Adugna, T., & Fekede, F. (2021). Effects of dietary energy and protein levels on nutrient intake, digestibility, and body weight change in Hararghe highland and Afar sheep breeds of Ethiopia. *Journal of Advanced Veterinary and Animal Research*, 8, 185.
- 37) Sobrinho, A. S., Kadim, I. T., & Purchas, R. W. (2003). Effect of genotypes and age on carcass and meat quality characteristics of ram lambs. *Agricultural and Marine Sciences*, 8, 73–78.
- 38) Tassone, S., Fortina, R., & Peiretti, P. G. (2020). In Vitro techniques using the DaisyII incubator for the assessment of digestibility: A review. *Animals*, 10, 775.
- 39) Tassone, S., Masoero, G., & Peiretti, P. G. (2014). Vibrational spectroscopy to predict in vitro digestibility and the maturity index of different forage crops during the growing cycle and after freeze-or oven-drying treatment. *Animal Feed Science and Technology*, 194, 12–25.
- 40) Team, R. C. (2020). RA language and environment for statistical computing, R Foundation for Statistical. Computing.
- 41) Van Soest, P. J. (1994). Nutritional ecology of the ruminant. Cornell university press.
- 42) Wang, Q., Wang, Y., Hussain, T., Dai, C., Li, J., Huang, P., . . . others. (2020). Effects of dietary energy level on growth performance, blood parameters and meat quality in fattening male Hu lambs. *Journal of animal physiology and animal nutrition*, 104, 418–430.
- 43) Wang, S., Li, D., Zhang, K., Ma, Y., Liu, F., Li, Z., . . . Du, L. (2023). Effects of initial volatile fatty acid concentrations on process characteristics, microbial communities, and metabolic pathways on solid-state anaerobic digestion. *Bioresource Technology*, 369, 128461.
- 44) Wang, W., Ungerfeld, E. M., Degen, A. A., Jing, X., Guo, W., Zhou, J., . . . others. (2020). Ratios of rumen inoculum from Tibetan and Small-tailed Han sheep influenced in vitro fermentation and digestibility. *Animal Feed Science and Technology*, 267, 114562.
- 45) Zhao, J., Ma, X., Jin, Y., Su, R., Liu, W., Ren, Y., . . . Zhang, J. (2016). Energy requirements for the maintenance and growth of Dorper-Jinzhong crossbred ram lambs. *Italian Journal of Animal Science*, 15, 94–102.
- 46) Zhao, X., Zuo, S., Guo, Y., Zhang, C., Wang, Y., Peng, S., . . . Luo, H. (2023). Carcass meat quality, volatile compound profile, and gene expression in Tan sheep under different feeding regimes. *Food Bioscience*, 56, 103213.
- 47) Zhong, R. Z., Fang, Y., Zhou, D. W., Sun, X. Z., Zhou, C. S., & He, Y. Q. (2018). Pelleted total mixed ration improves growth performance of fattening lambs. *Animal Feed Science and Technology*, 242, 127–134.