# Optimizing the utilization of maize silage in forage blends-based rations to improve production performance and reduce methane emissions from fattening calves

Khalid Farooq<sup>1</sup>, Asad Sultan<sup>1</sup>, Assar Ali Shah<sup>1</sup>, Rifat Ullah Khan<sup>1</sup>, Rajwali Khan<sup>1</sup>, Ghazala Yasmeen<sup>2</sup>, Nazir Ahmad Khan<sup>1\*</sup>

(1. Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar 25130, Pakistan; 2. Pakistan Scientific and Technological Information Center (PASTIC), Islamabad 44000, Pakistan)

**Abstract:** This research investigated the effect of various mixtures of maize silage and ryegrass fodder on nutrient digestibility, production performance, and methane (CH<sub>4</sub>) emission of fattening calves. Twenty-four fattening calves ((228±10) kg body weight (BW), (280±5) d of age) were allocated to 8 diets for 84 d, according to a randomized complete block design, and the blocks were balanced for BW, age, and sex. The diets consisted of 4 levels of maize silages, i.e., 40% (MS40), 50% (MS50), 60% (MS60), and 70% (MS70) in the maize silage and ryegrass fodder mixture on a dry matter (DM) basis, and each of the mixture was supplemented with either low (LC, 1.0% BW) or high (HC, 1.5% BW) levels of fattening concentrate. The results revealed greater (p<0.05) intakes of DM (8.35 kg/d), organic matter (OM, 7.85 kg/d), and metabolizable energy (ME, 12.00 Mcal/d) in calves fed with MS70-LC diet. The highest (p<0.05) digestibility (g/100 g) of DM (65.2), OM (67.3), crude protein (69.1), and neutral detergent fibre (56.3) was recorded for MS40-HC diet. The maximum (p<0.05) average daily gain (ADG, 571 g/d) was recorded for diet MS70-LC. The lowest value (p<0.05) of CH<sub>4</sub> emission was recorded in MS70-LC, MS70-HC, and MS60-HC as compared to other diets. Including 70% maize silage in the ryegrass-maize silage based diet improved ADG by 174 g/d with LC feeding. It is concluded that the optimum inclusion level of maize silage and ryegrass in the fattening ration can improve animal production performance and reduces CH<sub>4</sub> production and concentrate requirements of fattening calves.

**Keywords:** average daily gain, farm-grown forages, forage mixture, fattening calves, maize silage, ryegrass fodder **DOI:** 10.25165/j.ijabe.20241703.7899

**Citation:** Farooq K, Sultan A, Shah A A, Khan R U, Khan R, Yasmeen G, et al. Optimizing the utilization of maize silage in forage blends-based rations to improve production performance and reduce methane emissions from fattening calves. Int J Agric & Biol Eng, 2024; 17(3): 75–81.

#### 1 Introduction

Optimum utilization of farm-grown forages in ruminant rations can reduce the requirement of concentrate ingredients, optimize local recycling and re-utilization of nutrients, and provide long-term sustainability to the global livestock production systems<sup>[1-3]</sup>. Over the last few years, there has been a rapid increase in the prices of concentrate ingredients, resulting in growing research on the optimum utilization of feeds, mainly forage biomass. Forages are natural, low-cost, and a significant source of nutrients for ruminants<sup>[3,4]</sup>, and feeding of improved quality of forages can increase profitability and productivity of dairy and fattening animals in many production systems, particularly in developing countries<sup>[5,7]</sup>. In this context, the introduction of high biomass producing and

Biographies: Khalid Farooq, PhD candidate, research interest: forage production, Email: drkhalidfarooq@gmail.com; Asad Sultan, Associate Professor, research interest: poultry nutrition, Email: asadzia2003@yahoo.com; Assar Ali Shah, Assistnat Professor, research interest: animal nutrition, nutritional valve of insects for animals, Email: assaralishah@yahoo.com; Rifat Ullah Khan, Associate Professor, research interest: animal nutrition and fertility, Email: rukhan@aup.edu.pk; Rajwali Khan, Assitant Professor, research interest: beef cattle production, Email: rajwalikhan@aup.edu.pk; Ghazala Yasmeen, Additional Director, research interest: nutritional and phytochemical value of medicinal plants; Email: malikghazala@gmail.com.

\*Corresponding author: Nazir Ahmad Khan, Professor, research interest: ruminant nutrition, forage quality evluaiton. Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar 25130, Pakistan. Tel: +92-3451235646, Email: nazir.khan@aup.edu.pk.

highly digestible fodder varieties, and optimizing their utilization by balancing the protein and energy supply to fattening animals through suitable forage mixes has been identified as one of the main cross-cutting issues to be researched.

Research has established that including maize silage in the forage mixture can reduce concentrate requirements and methane (CH<sub>4</sub>) emission of dairy and beef cattle<sup>[8,9]</sup>. The grains in maize silage contain a high proportion of starch that rapidly ferments in the rumen and results in lower CH<sub>4</sub> production<sup>[6]</sup>. Feeding maize in combination with ryegrass can play a crucial role in the sustainable supply of dietary nutrients and metabolizable energy (ME) to the fattening animals, as these forages have high and stable biomass yield under a wide variety of environmental and agronomical conditions, high ME, and supports high dry matter (DM) intake (DMI) and animal productivity<sup>[9,10]</sup>. Maize silage is particularly rich in ME and supports higher intake. However, the crude protein (CP) concentration is lower (<8%). It has been shown that lower (<10%) CP content can impair rumen fermentation efficiency and microbial protein synthesis[11]. Feeding high CP-containing concentrate ingredients can correct this deficiency; however, concentrates are getting expensive, and importing concentrate ingredients puts a lot of pressure on farmers' profitability and the local economies. Alternatively, the CP concentration of the maize silage-based diet can be balanced by incorporating CP-rich forages, such as ryegrass, into the diet<sup>[9]</sup>. Ryegrass has a high DM digestibility (>65%), high CP content (>15%), and many vitamins and minerals[12,13]. These features of ryegrass make it suitable for supporting the high growth rate of fattening animals in combination with maize silage. Feeding a blend of maize silage and ryegrass to fattening animals can provide a high amount of ME and an adequate amount of CP for optimum rumen fermentation and animal performance.

The proportion of maize silage in the forge fraction of the diet, the nutritional quality of other forages, and the amount of concentrate have shown significant influence on animal productivity, production efficiency, and profitability<sup>[8,14,15]</sup>. Therefore, this study was carried out to investigate the effect of different proportions of maize silage and ryegrass in the forage blend on the intake and digestibility of DM, dietary nutrients and the growth performance of fattening calves. The second aim was to compare these forage mixtures for CH<sub>4</sub> emission and the concentrate sparing potential.

#### 2 Materials and methods

# 2.1 Location of the trial and forage production

The fattening trial was conducted at SJK commercial fattening farm (34.8498°N, 72.2689°E) in Northern Pakistan. Ryegrass was grown near the farm using recommended agronomic, crop management, and irrigation practices. For the feeding trial, first regrowth (7 weeks mature) of ryegrass was used. The first growth was not used, due to the large variability in yield and nutrition value. To maintain uniformity in the maturity stage of ryegrass throughout the experimental period, the ryegrass was sown on different dates in different fields, after calculating the requirements and estimated yield. For maize silage production at the farm, cv. P30K08 was planted at a seed rate of 66 000/hm<sup>2</sup>, and standard agronomic, irrigation, weed control and management practices were applied. For silage production maize crop was harvested at proper maturity stage (35.0% DM content), chopped (1.0-1.5 cm particle size), and ensiled in bunker silos. To improve silage fermentation quality, homofermentative inoculants (2 g/t of fresh forage) was used to supply Lactobacillus plantarum at the rate of 1×105 cfu/g[16]. Compaction was done layer after layer using a heavy-weight tractor and a wheel loader to ensure optimal fermentation and lower energy loss. The silage was air-tight and sealed with double layer of polyethene sheets, followed by covering with 20 cm thick sand load and tyres.

## 2.2 Experimental design, animals and diets

For the fattening trial, twenty-four fattening calves were selected from the herd on the basis of body weight ((BW) (228± 10) kg), age ((280±5) d), and sex. A randomized complete block design was used to assign claves to eight experimental diets. The blocks (dietary groups) were balanced for age, sex, and BW. The eight diets have a factorial combination of four levels of maize silage in the forage mixtures and two levels of concentrate. The mixtures of maize silage and ryegrass were as follows: 40% maize silage and 60% ryegrass (MS40); 50% maize silage and 50% ryegrass (MS50); 60% maize silage and 40% ryegrass (MS60); and 70% maize silage and 30% ryegrass (MS60). Each of the four forage blends was either supplemented with a high level of concentrate (HC, 1.5% of BW) or a low level of concentrate (LC, 1.0% of BW). The eight diets were MS40-LC, MS40-HC, MS50-LC, MS50-HC, MS60-LC, MS60-HC, MS70-LC, and MS70-HC. The chemical composition of the concentrate is listed in Table 1. The forage blends (ad-libitum) and concentrate were fed individually, and all calves had free access to clean drinking water throughout the experimental period. Samples of ryegrass and maize silage were collected during each experimental week for two consecutive days, pooled, and representative samples were analysed for nutrient composition, fibre profile, and DM digestibility (in

*vitro*). The ME was estimated using the mathematical model of National Research Council (NRC)<sup>[16]</sup>.

#### 2.3 Sampling and data collection

Data on the DMI of the individual calf was recorded daily. Representative samples of each forage (1 kg) and concentrate (0.5 kg) were collected every week for two consecutive days. All samples were immediately transferred to cooling boxes, transported to laboratory and immediately analysed for DM content. The weekly samples were pooled by feed type, thoroughly mixed, and representative samples (0.5 kg) were air-dried, ground to 1 mm particle size using Welly Mill (Model 4, Thomas Co., Philadelphia, PA, USA), and stored in plastic bottles for laboratory analysis.

Table 1 Proximate chemical composition, protein chemical profile, carbohydrate chemical profile, energy values and digestibility of the main feed ingredients fed to experimental calves

experimental carves						
Measurements	Maize silage (Mean±SD)	Ryegrass (Mean±SD)	Concentrate (Mean±SD)			
Dry matter (DM) % fresh matter	33.8±1.01	19.2±1.81	89.1±1.20			
Proximate chemical profile (% DM)						
Ash	4.01±0.35	6.12±0.44	$5.62\pm0.82$			
Ether extract	$3.52\pm0.62$	$3.85\pm1.22$	4.87±1.25			
Crude protein (CP)	$7.56\pm0.33$	15.1±1.84	$13.1 \pm 0.88$			
Protein chemical profile (% CP)						
Soluble CP	40.5±1.00	44.2±1.32	40.12±0.77			
NDICP	$10.9 \pm 0.82$	$13.66 \pm 0.65$	$6.23\pm0.32$			
ADICP	$4.81\pm0.53$	$5.52\pm0.52$	$2.23\pm0.30$			
Rumen degraded protein	$67.4 \pm 0.85$	73.8±1.09	$67.88\pm0.96$			
Rumen undegraded protein	$33.6 \pm 0.85$	26.2±1.09	32.12±0.96			
Carbohydrate chemical profile (%	DM)					
Acid detergent lignin	$3.11\pm0.38$	10.05±0.65	04.4±0.27			
Acid detergent fibre	25.9±1.31	34.7±1.78	20.3±1.66			
Neutral detergent fibre	42.3±1.80	54.8±2.70	28.2±1.78			
Starch	$32.8 \pm 0.92$	$1.70\pm0.29$	41.2±1.23			
Non-fibre carbohydrates	40.4±0.90	22.20±0.22	44.2±0.23			
Energy values						
Total digestibility nutrients (%)	72.43±0.90	57 .1±0.98	73.5±1.19			
Metabolizable energy (Mcal/kg)	2.57±0.41	$2.13\pm0.43$	$2.85\pm0.37$			
Digestibility						
DMD (in vitro) (% DM)	70.8±1.52	62.9±1.99	83.2±2.22			
Ffermentation quality						
рН	4.01±0.11	-	-			
NH <sub>3</sub> -N (% of total N)	$8.32\pm1.10$	-	-			

Note: ADICP, acid detergent insoluble crude protein (CP); DMD, dry matter digestibility; NDICP, neutral detergent insoluble CP, NH<sub>3</sub>-N, ammonia-N; SD, standard deviation

# 2.4 Laboratory analysis

The whole crop maize silage, ryegrass, and concentrate samples were analysed for contents of proximate chemical components, fibre composition, and in vitro DM digestibility (DMD). The content of acid detergent fibre (ADF), CP, ash, ether extract (EE), DM, and acid detergent lignin (ADL), were analyzed according to Association of Official Analytical Chemists (AOAC)<sup>[17]</sup>. The method of Van Soest et al.<sup>[18]</sup> was used for neutral detergent fibre (NDF) analysis. The method of Lacitra et al.<sup>[19]</sup> was used for determination of acid detergent insoluble CP (ADICP) and neutral detergent insoluble CP (NDICP) contents. Total carbohydrate (CHO) and non-fibre carbohydrates (NFC) contents were determined according to NRC<sup>[16]</sup>. The starch kits (Catalog # K-TSTA, K-AMYL, K-BGLU) of Megazyme (Wicklow, Ireland) were used for analysis of starch content. The DMD (in vitro) was analysed using Tilley and Terry's two-step in vitro procedure<sup>[20]</sup>.

The in-situ technique as reported earlier<sup>[21]</sup> was used for determination of CP degradation kinetics. The CP effective rumen degradable (RDP) and bypass (RUP) fractions were determined using equations of NRC<sup>[16]</sup>. The ME values and total digestibility nutrients (TDN) were quantified according to mathematical models of NRC<sup>[16]</sup> using the chemical profile data.

For the determination of enteric  $CH_4$  production, sulfur hexafluoride (SF6) tracer technique.  $CH_4$  concentrations in the intake air and eructating gas were analyzed using a dispersive infrared  $CH_4$  analyser (model VIA-500; Horiba, Kyoto, Japan). The data from the analyser were entered at 1-min intervals into a computer (NEC, Tokyo, Japan), and subsequently standardized automatically to 101.3 kPa, 0°C, and 0 water vapor pressure. The following equation was used for measurement of  $CH_4$  production from the data

$$CH_{4}\left(\frac{g}{d}\right) = \frac{SF6 \text{ release rate}\left(\frac{g}{d}\right) \text{ from permeation tubes}\left[CH_{4}\left(\frac{\mu g}{m^{3}}\right)\right]}{\left(SF6\left(\frac{\mu g}{m^{3}}\right) \text{ in collected samples}\right)} \tag{1}$$

The correction was made for the concentration of ambient gases. The average daily  $\mathrm{CH_4}$  production for each animal within each period was used as a single value for statistical analysis of the results.

#### 2.5 Body weight and body condition score

All experimental calves were weighed for two consecutives days at the start of the experiment and then after each data collection week before morning feeding, using electronic cattle weighing system. The body wight and body condition score (BCS) were were determined as reported earlier<sup>[16]</sup>.

Statistical analysis

The PROC MIXED procedure of Statistical Analysis System (SAS) was used to determine the effects of forage blend composition on DM intake, average daily gain (ADG), final BW, and BCS. Experimental weeks were included in the model as a repeated effect on the individual cow. Fixed effects in the model were forage-blends, level of concentrate, and weeks of the experiment, and the random effect was the replications. The model was as given below:

$$Y_{ijk} = \mu + MSR_i + LC_j + MSR_i \times LC_j + C_{ijk}$$
 (2)

where,  $Y_{ijk}$  is the response variable;  $\mu$  is the general mean; MSR<sub>i</sub> is the fixed effect of the forage blends; LC<sub>j</sub> is the fixed effect of the level of concentrate; MSR<sub>i</sub>×LC<sub>j</sub> is the interaction of the forage blends and level of concentrate;  $\mathcal{E}_{ijk}$  is a random error. For significant effects, post-hoc analyses (Tukey-Kramer test) were conducted to determine the pair-wise differences in the respective means using the for parameters with significant effects (p<0.05) of forage blends, level of concentrate, or their interactions. The "pdmix 800 SAS macro software" (SAS Institute Inc., Cary, NC, USA) was used to obtain means with different letters.

## 3 Results

# 3.1 Chemical profile of forages and concentrate

Table 1 summarizes data on proximate chemical profile, CP chemical profile, carbohydrate chemical profile, energy values and digestibility of forages and concentrate used in the experimental rations. Maize silage had a DM content of 33.8%, near to the optimal targeted DM content. The maize silage had excellent nutritional and fermentation quality, as reflected by the high starch (32.8% DM), DMD (72.4%), TDN (72.4%) and ME (2.57 Mcal/kg),

and lower NDF (42.3% DM) and pH value (4.01). The nutritional value of ryegrass was also optimal, as reflected by high CP content (15.1% DM), TDN (57.1%), in vitro DMD (62.9% DM) and ME (2.13 Mcal/kg).

### 3.2 Intake of dry matter, nutrients, and metabolizable energy

Data on the effect of various mixtures of maize silage and ryegrass supplemented with high and low levels of concentrate on the intake of DM, NDF, organic matter (OM) and ME of the fattening calves are summarized in Table 2. Except for CP, the diet composition altered the intakes of DM, CP, NDF and ME (p<0.001). Comparison of the experimental diets revealed that the MS70-LC diet, supported significantly higher (p<0.05) intakes of DM (8.35 kg/d), OM (7.85 kg/d) and ME (12.00 Mcal/kg). Whilst, the MS40-LC diet supported the lowest (p<0.05) intake of DM (6.06 kg/d), OM (5.69 kg/d), and ME (9.04 Mcal/kg). Calves fed with MS60-LC diet had the highest (p<0.05) intake (3.37 kg/d) of NDF, while the lowest NDF intake (2.47 kg/d) was recorded for the MS40-LC diet.

Table 2 Effects of various blends of maize silage and ryegrass, supplemented with low or high levels of concentrate on intakes of dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fibre (NDF) and metabolizable energy (ME) content of the fattening calves

Diets -		ME/			
	DM	OM	CP	NDF	Mcal·kg <sup>-1</sup>
MS40-LC	$6.060^{d}$	5.690 <sup>d</sup>	0.870	2.470b	$9.040^{d}$
MS40-HC	$6.790^{\circ}$	$6.320^{\circ}$	1.000	$2.790^{\rm ab}$	$10.110^{\rm cd}$
MS50-LC	$6.930^{\circ}$	6.510°	0.980	$2.810^{\rm ab}$	10.300°
MS50-HC	$7.590^{b}$	$7.060^{b}$	1.100	$3.110^{a}$	11.270 <sup>b</sup>
MS60-LC	7.570 <sup>b</sup>	7.120b	1.060	$3.060^{a}$	11.23 <sup>b</sup>
MS60-HC	$7.910^{\rm ab}$	$7.360^{\rm ab}$	1.160	$3.370^{a}$	11.890°
MS70-LC	8.350ª	7.850ª	1.110	$3.050^{a}$	12.000°
MS70-HC	$7.800^{\mathrm{ab}}$	$7.250^{\mathrm{ab}}$	1.120	$3.180^{a}$	11.560°
SEM	0.332	0.254	0.310	0.365	0.805
Significance	***	***	NS	*	***

Note: Mean with different superscript letters within columns are different at p<0.05. The MS70, MS60, MS50, MS40 denotes 70%, 60% 50% and 40% maize silage in the maize silage and ryegrass fodder in forage blends on DM basis; HC denotes high level (1.5% of body weight) of concentrate; LC denotes low level (1% of body weight) of concentrate; SEM, standard error of the means; \*\*\*, p<0.001; \*, p<0.005; NS, non-significant (p>0.05)

# 3.3 Digestibility of dry matter, nutrients, and metabolizable energy

Data on the effect of various blends of maize silage and ryegrass supplemented with high and low levels of concentrate on digestibility of DM, OM, CP, and NDF is listed in Table 3. The diets composition altered the digestibility of DM (p<0.001), OM (p<0.001), CP (p<0.05), and NDF (p<0.001). The highest (p<0.05) digestibly of DM (65.2%), OM (67.3%), CP (69.1%), and NDF (56.3%) was recorded for MS40-LC diet. Whilst, the lowest (p<0.05) digestibly of DM (62.2%), OM (64.4%), CP (63.7%), and NDF (50.4%) was recorded for MS70-LC diet.

# 3.4 Effects of forage blends and concentrates levels on growth performance of fattening calves

Table 4 summarizes the data on the effects of various blends of maize silage and ryegrass supplemented with high and low levels of concentrate on growth performance and BCS of fattening calves. The initial BW did not differ (p>0.05) among the dietary groups, ranging from 211 to 213 kg. Final body weight of the calves significantly altered (p<0.001) due to the diet composition. The lowest (p<0.05) final BW (245 kg) was recorded for MS40-LC diet, and the highest (p<0.05) final BW (261 kg) was recorded for MS70-LC diet. Among the diets containing the same level of concentrate,

the final BW of the calves was consistently increased (p<0.05) with an increasing level of maize silage (40%-70%) in the forage blends, except for MS70-HC. Similarly, with the increasing level of maize silage (40%-70%) in the forage blends, the BCS consistently increased (p<0.05). The maximum (p<0.05) BCS (6.50) was supported by MS70-LC and the minimum (p<0.05) BCS (5.00) was supported by MS40-LC. Figure 1 shows the data on the weekly mean BW changes of experimental calves fed with various blends of maize silage and ryegrass supplemented with high and low levels of concentrate.

Table 3 Effects of various blends of maize silage and ryegrass, supplement with low or high levels of concentrate on digestibility of dry matter (DM), organic matter (OM), crude protein (CP) and neutral detergent fibre (NDF) the fattening calves

fattening carves						
Diets -	Digestibility/%					
Diets	DM	OM	СР	NDF		
MS40-LC	64.390ab	66.650ab	67.940ab	54.840 <sup>b</sup>		
MS40-HC	65.210 <sup>a</sup>	67.290°	69.070°	56.340°		
MS50-LC	63.500 <sup>b</sup>	65.730 <sup>b</sup>	$65.680^{\mathrm{ab}}$	52.220°		
MS50-HC	$64.420^{\mathrm{ab}}$	66.680°	68.770°	53.960bc		
MS60-LC	$62.700^{bc}$	65.110 <sup>b</sup>	64.740 <sup>b</sup>	$51.030^{\rm cd}$		
MS60-HC	63.500 <sup>b</sup>	65.630 <sup>b</sup>	$65.810^{\mathrm{ab}}$	52.210°		
MS70-LC	62.220°	64.400 <sup>b</sup>	63.710 <sup>b</sup>	$50.430^{d}$		
MS70-HC	$63.010^{bc}$	65.220 <sup>b</sup>	64.650 <sup>b</sup>	$51.270^{\rm cd}$		
SEM	1.020	0.680	0.780	0.470		
Significance	***	***	*	***		

Note: Mean with different superscript letters within columns are different at P<0.05. The MS70, MS60, MS50, MS40 denotes 70%, 60%, 50%, and 40% maize silage in the maize silage and ryegrass fodder in forage blends on DM basis; HC denotes high level (1.5% of body weight) of concentrate; LC denotes low level (1% of body weight) of concentrate; SEM, standard error of the means; \*, p<0.05; \*\*\*, p<0.001

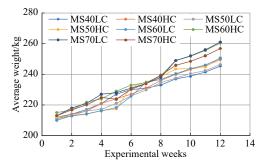
Table 4 Effects of various blends of maize silage and ryegrass, supplemented with low or high levels of concentrate on body weight changes, body condition score (BCS) and methane emission

Diets	Body weight/kg		BCS	Methane production			
Diets	Initial	Final	Gain	DCS	$g \cdot d^{\scriptscriptstyle -1}$	$g \cdot kg^{-1} DMI$	$g{\cdot}kg^{{\scriptscriptstyle -1}}\;ADG$
MS40-LC	212.200	245.300 <sup>d</sup>	33.100 <sup>d</sup>	5.000°	145.700a	19.840ª	302.900ª
MS40-HC	211.400	249.600 <sup>cd</sup>	$38.300^{\circ}$	$5.200^{\text{bc}}$	127.600°	$18.790^{ab}$	277.300°
MS50-LC	211.200	$246.300^{\scriptscriptstyle d}$	35.100 <sup>cd</sup>	5.500 <sup>b</sup>	136.900 <sup>b</sup>	$18.780^{\mathrm{ab}}$	$309.100^{a}$
MS50-HC	212.300	$250.800^{\circ}$	38.500 <sup>b</sup>	5.500 <sup>b</sup>	130.100°	$18.030^{\mathrm{ab}}$	$274.800^{\circ}$
MS60-LC	210.500	$250.400^{\circ}$	39.900bc	5.750 <sup>b</sup>	142.300ab	$18.230^{ab}$	286.800 <sup>b</sup>
MS60-HC	213.100	258.300ab	$45.200^{\mathtt{a}}$	$6.000^{\text{ab}}$	138.000 <sup>b</sup>	17.990 <sup>b</sup>	$263.500^{\text{cd}}$
MS70-LC	212.900	$261.000^{\mathtt{a}}$	$48.100^{\mathrm{a}}$	$6.500^{\rm a}$	120.300 <sup>d</sup>	17.450b	$255.200^{d}$
MS70-HC	213.000	$256.800^{\scriptscriptstyle b}$	43.800ab	$6.000^{\rm ab}$	115.700 <sup>d</sup>	16.170 <sup>b</sup>	$252.000^{d}$
SEM	4.080	2.650	2.790	0.500	2.703	0.445	5.384
Significance	NS	***	***	*	*	*	**

Note: Mean with different superscript letters within columns are different at p<0.05. MS40, MS50, MS60 and MS70 contained 40%, 50%, 60%, and 70% maize silage in ryegrass and maize silage blend on DM basis; HC mean high level (1.5% of body weight) of concentrate; HC means high level (1.5% of body weight) of concentrate; LC means low level (1% of body weight) of concentrate; SEM, standard error of the means; \*\*\*, p<0.001; \*, p<0.05.

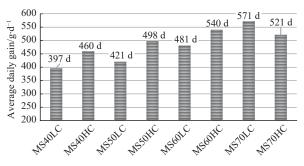
# 3.5 Effects of forage blends and concentrates levels on and methane emission of fattening calves

The effects of forage blends and concentrate levels on ADG of calves is shown in Figure 2. The greatest (p<0.05; 571 g/d) ADG was recorded for MS70-LC diet, while the lowest (p<0.05; 397 g/d) ADG was recorded for MS40-LC diet. Within the same level of concentrate supplementation, increasing the level of maize silage (40%-70%) in the forage blends increased the ADG of the calves.



Note: The MS70, MS60, MS50, MS40 denotes 70%, 60%, 50%, and 40% maize silage in the maize silage and ryegrass fodder in forage blends on DM basis.

Figure 1 Weekly mean body weight changes of experimental calves fed with various blends of maize silage and ryegrass and supplemented with high (HC) or low levels (LC) of concentrate



Note: The MS70, MS60, MS50, MS40 denotes 70%, 60%, 50%, and 40% maize silage in the maize silage and ryegrass fodder in forage blends on DM basi.

Figure 2 Effects of various blends of maize silage and ryegrass, supplement with high (HC) or low (LC) levels of concentrates on average daily weight gain of calves.

The lowest methane emission in terms of grams per day was recorded (p<0.05) for MS70-LC, MS70-HC, and MS60-HC as compared to other diets (Table 5). Regarding grams per kg DMI, the highest emission was noted for MS40-LC, and the lowest (p<0.05) values were recorded for MS70-LC, MS70-HC, and MS60-HC. The minimum (p<0.05) methane emission in terms of grams per kg of average daily gain was recorded for MS70-LC and MS70-HC as compared to other diets.

Table 5 Effect of different blends of maize silage (MS) and ryegrass with low and high levels of concentrate on methane emission

Diets —		Methane production	on
	g·d <sup>-1</sup>	g·kg⁻¹ DMI	g·kg <sup>-1</sup> ADG
MS40-LC	145.7ª	19.84ª	302.9ª
MS40-HC	127.6°	18.79ab	277.3°
MS50-LC	136.9b	$18.78^{ab}$	309.1ª
MS50-HC	130.1°	18.03ab	274.8°
MS60-LC	142.3ab	18.23 <sup>ab</sup>	286.8b
MS60-HC	138.0 <sup>b</sup>	17.99 <sup>b</sup>	$263.5^{cd}$
MS70-LC	120.3 <sup>d</sup>	17.45 <sup>b</sup>	255.2 <sup>d</sup>
MS70-HC	115.7 <sup>d</sup>	16.17 <sup>b</sup>	$252.0^{d}$
SEM	2.703	0.445	5.384
Significance	*	*	**

Note: Mean with different superscript letters within columns are different at p < 0.05. The MS70, MS60, MS50, MS40 denotes 70%, 60%, 50%, and 40% maize silage in the maize silage and ryegrass fodder in forage blends on DM basis; HC denotes high level (1.5% of body weight) of concentrate; LC denotes low level (1% of body weight) of concentrate; ADG, average daily gain; DMI, dry matter intake; SEM, standard error of the means; \*, p < 0.05; \*\*, p < 0.01.

## 4 Discussion

One of the significant constraints in improving the productivity of farm animals in many tropical countries is the poor availability of forages in terms of quantity and quality[1,3]. Research has demonstrated that the inclusion of good quality forages in the diets can improve the animal productivity under small scale production systems<sup>[22,23]</sup>. Moreover, the optimal use of good quality forages such as maize silage and ryegrass with high energy densities, and high intake potentials can also improve the feed use efficiency and the profitability of commercial farms and reduce the requirements for concentrate ingredients<sup>[8,14]</sup>. In this context, this study reports the first dataset on the effect of different blends of maize silage and ryegrass fodder on the intake and digestibility of dietary components, CH<sub>4</sub> emissions, and growth performance of calves, and concentrates sparing potential of the different blends. The results revealed that the inclusion of 70% maize silage in the ryegrassbased diet improved the ADG by 111 g/d and minimized the requirements of concentrate feed.

The nutritional quality of maize silages used in the present study had a good nutritional and fermentation quality, as reflected by the high starch (32.8%) and lower NDF (42.3%) contents, and high DMD (72.4%), TDN (72.4%), ME (2.57 Mcal/kg) and pH value (4.01)<sup>[8]</sup>. The ryegrass forage also had a good nutritional value, as reflected by the high ME (2.13 Mcal/kg) and CP content (15.1% DM), which are consistent with the literature values<sup>[12,24,]</sup>.

The intake of all nutrients increased with the increasing inclusion levels of maize silage from 40%-70% in the forage blends in the diets supplemented with a low level of concentrate. The inclusion of maize silage in grass/legume-based diets increases DM intake due to higher contents of starch, lower NDF content, and smaller particle size<sup>[8,25]</sup>. The consistent increase in DM intake with the increasing inclusion levels of maize silage in the forage blends is an exciting finding of this study. Ryegrass is a good quality grass with high digestibility; even then, the DM intake and ADG increased with the inclusion of maize silage in a ration, which highlights the scope of maize silage for supporting optimum growth of fattening animals. The increased intake of nutrients with an increasing level of maize silage in the diet can be due to the lower fiber contents, smaller particle size, and higher concentrations of fermentable nutrients such as starch in maize silage[26,27]. It is also suggested that a more steady and balanced supply of readily available carbohydrates and NH<sub>3</sub>-N from the blend of ryegrass, maize silage, and concentrate improved the digestibility and DM intake<sup>[28]</sup>. In the present study, feeding 70% maize silage in the ryegrass-based diet supported maximum weight gain and spared concentration. This could be related to the highest intakes of DM (8.35 kg/d) and ME (12.00 Mcal/kg) recorded for a diet containing 70% MS and supplemented with a low level of concentrate. When using high-quality forages with high energy densities or high intake potentials, the forages can account for a large proportion of the diet<sup>[29]</sup>, and enable the animal to achieve optimal productivity. Even further, concentrates cannot fully compensate for the low forage digestibility; thus, highly digestible forage is essential for a high growth rate, independent of concentrate level<sup>[29]</sup>. Notably, in diets supplemented with a high level of concentrate, the intake of DM, OM, NDF, and ME increased with the increasing inclusion level of maize silage from 40% to 60%, and decreased with the further increase in maize silage level up to 70%. A negative effect of the combination of a high proportion of maize silage and a high level of

rapidly degradable concentrate on rumen fermentation and dairy cow performance has been reported earlier<sup>[8,30]</sup>.

In the present study, the highest digestibly of DM (65.2%), OM (67.3%), CP (69.1%), and NDF (56.3%) were recorded for diets containing 60% ryegrass in the forage blends and supplemented with a high level of concentration. While the lowest digestibly of DM (62.2), OM (64.4%), CP (63.7%), and NDF (50.4%) was recorded for diets containing 70% maize silage in the forage blends and supplement with a low level of concentrate. Ryegrass has excellent forage quality for livestock[13], when harvested in the vegetative to early boot stage of maturity, it contains highly digestible rumen degradable CP, and fibre[12,13]. However, the high digestibility of the diet containing a high proportion of ryegrass does not support high DMI in the present study. Generally, a positive relationship exists between forage digestibility and forage intake<sup>[29,30]</sup>. Despite higher DM and NDF digestibility of ryegrass, the DMI of calves increased with the increase in replacing ryegrass with maize silage. This suggests that, compared to the grass silages, the rate of digestion and the passage of digesta from the rumen is faster with the maize silages. The high starch content and smaller particle size of maize silages could partly explain the more rapid degradation and clearance in the rumen<sup>[8]</sup>. The decreased retention time in the rumen results in a less distension and higher DMI.

On the other hand, the larger particle size, greater NDF content, and slower rate of fermentation of ryegrass increases the duration of particles buoyant in the rumen, which increase the filling effect of NDF over time<sup>[29]</sup>. The lower DMI observed for ryegrass compared with the maize silages suggests that the increased filling effect of grass NDF is a potential limitation for feeding a high proportion of ryegrasses to high-producing animals. On the other hand, the high intake potential of maize silage is essential for supporting the higher productivity levels, particularly during the finishing period. Multiple mechanisms regulate the forage DMI, such as forage NDF content, rate of rumen degradability, and rate of rumen passage<sup>[29,30]</sup>. The higher intake characteristics of maize silage can be attributed to lower NDF and higher starch or energy content, smaller particle size, and faster degradation and clearance in the rumen<sup>[8]</sup>.

Similar to the response in DMI, the maximum final BW (261 kg), average daily gain (571 g/d), and BCS (6.50) were recorded for a diet containing 70% maize silage in the forage blend and supplemented with a low level of concentrate. In the present study, feeding 70% maize silage in the diet improved daily weight gain by 111 g/(calf·d) and reduced concentrate requirement from 1.5% to 1% BW. Using high-quality forages with high energy densities and high intake potentials in the fattening ration minimizes the need for concentration in the diet[8], and enables the animal to achieve optimal productivity. Even further, concentrates cannot fully compensate for the low forage digestibility; thus, highly digestible forage supporting higher intake is essential for a high growth rate, independent of concentrate level[28,29]. As discussed earlier, maize silage supports higher intake compared with the ryegrass, and thus, the proportion of maize silage in the forge fraction and the amount of concentrate have a more significant effect on animal production performance<sup>[5,8,14]</sup>. In agreement with our findings, O'Mara et al.<sup>[31]</sup> reported that maximum DMI and milk yield were achieved when 75% of good-quality grass was replaced with high-quality maize silage.

The increasing level of maize silage in the forage blend decreased CH<sub>4</sub> production when expressed in DMI and ADG. Similar results have been shown by Burke et al.<sup>[32]</sup>, who reported that increasing the inclusion of maize silage in the diet by replacing

other forages led to a decrease in CH<sub>4</sub> emission per unit of DM and ME intake. This study's results align with the results of Benchaar et al.<sup>[33]</sup> and Hassanat et al.<sup>[10]</sup>, who reported that with an increasing ratio of maize silage in the forage blend, the ADG increased and CH<sub>4</sub> production decreases. Hassanat et al.<sup>[10]</sup> found that a 50:50 proportion of maize silage and alfalfa caused a lower CH<sub>4</sub> output per kg milk yield. Kasuya and Takahashi<sup>[34]</sup> recorded lower CH<sub>4</sub> production in dairy cows when grass or legume silage was included in the forage blend.

This well-designed, systematic study showed that the inclusion of a proper blend of good quality forages in fattening rations improves animal growth performance and reduces concentrate requirements and the environmental footprint of beef production, presenting prospects for the much-needed long-term sustainability of the fattening farms in the face of growing shortage and increasing prices of concentrate ingredients.

### 5 Conclusions

This study revealed that the optimum inclusion level of maize silage and ryegrass in fattening ration improves the animal production performance and reduces the CH<sub>4</sub> production and concentrate the requirements of fattening calves. The results showed that the diet containing 70% maize silage and a low level of concentrate supported higher intakes of DM [8.35 kg/(calf·d)], OM [7.85 kg/(calf·d)] and ME (12.00 Mcal/kg), resulting in higher average daily gain (571 g/d). In contrast, the lowest values of the intakes of DM [6.06 kg/(calf·d)], OM [5.69 kg/(calf·d)], ME (9.04 Mcal/kg) and average daily gain (397 g/d) were recorded for a diet containing 40% maize silage and supplement with a low level of concentrate. With a high level of concentrate supplementation, the daily weight gain increased with an increasing proportion of maize silage, up to 60% of the forage blend. A more detailed comparison of the different inclusion levels revealed that feeding 70% maize silage in the ryegrass-based diet improved the daily weight gain by 111 g/d and reduced the concentrate requirements from 1.5% to 1.0% of the body weight.

# Acknowledgements

This work was supported by the Department of Animal Nutrition, University of Agriculture Peshawar, Khyber Pakhtunkhwa, Pakistan. And this study was financially supported by Higher Education Commission (HEC) of Pakistan through (HEC Indigenous Scholarship) grant. The experiment was approved by the ethical committee (Grant No. FAH&VS 19-23) of the Faculty of Animal Husbandry and Veterinary Science, The University of Agriculture Peshawar, Khyber Pakhtunkhwa Pakistan. All authors of this article hereby declare that there is no conflict of interest in its publication.

# [References]

- Khan N A, Rahman S U, Cone J W. Chemical composition, ruminal degradation kinetics and methane production (in vitro) potential of local and exotic grass species grown in Peshawar. Pakistan Journal of Botany, 2020; 52(1): 161–166.
- [2] Sufyan A, Ahmad N, Shahzad F, Embaby M G, AbuGhazaleh A, Khan NA. Improving the nutritional value and digestibility of wheat straw, rice straw, and corn cob through solid state fermentation using different Pleurotus species. Journal of the Science of Food and Agriculture, 2022; 102(6): 2445–2453.
- [3] Khan N A, Sulaiman S M, Hashmi M S, Rahman S U, Cone J W. Chemical composition, ruminal degradation kinetics, and methane production (in vitro) of winter grass species. Journal of the Science of Food and

- Agriculture, 2021; 101(1): 179-184.
- [4] Habib G, Khan NA, Sultan A, Ali M. Nutritive value of common tree leaves for livestock in the semi-arid and arid range lands of northern Pakistan. Livest Sci, 2016; 184: 64–70.
- [5] Keady T W J, Kilpatrick D J, Mayne C S, Gordon F J. Effects of replacing grass silage with maize silages, differing in maturity, on performance and potential concentrate sparing effect of dairy cows offered two feed value grass silages. Livestock Science, 2008; 119(1-3): 1–11.
- [6] Hart K J, Huntington J A, Wilkinson R G, Bartram C G, Sinclair L A. The influence of grass silage-to-maize silage ratio and concentrate composition on methane emissions, performance and milk composition of dairy cows. Animal, 2015; 9(6): 983–991.
- [7] Khan M T, Ahmad N, Khan N A, Ullah A H, Ullah S. Inclusion of the fungus *Pleurotus florida* in the diet affects performance and feed efficiency traits in calves: a case study on Ravi buffalo. Trop Anim Health Prod, 2023: 55: 390.
- [8] Khan N A, Yu P Q, Ali M, Cone J W, Hendriks W H. Nutritive value of maize silage in relation to dairy cow performance and milk quality. Journal of the Science of Food and Agriculture, 2015; 95(2): 238–252.
- [9] Dillon P. Achieving high dry-matter intake from pasture with grazing dairy cows. In: Elgersma A (Ed.). Fresh Herbage for Dairy Cattl. Berlin: Springer. 2006; pp.1–26.
- [10] Hassanat F, Gervais R, Julien C, Massé D I, Lettat A, Chouinard P Y, et al. Replacing alfalfa silage with corn silage in dairy cow diets: Effects on enteric methane production, ruminal fermentation, digestion, N balance, and milk production. Journal of Dairy Science, 2013; 96(7): 4553–4567.
- [11] Refat B, Prates L L, Khan N A, Lei Y G, Christensen D A, McKinnon J J, et al. Physiochemical characteristics and molecular structures for digestible carbohydrates of silages. J Agric Food Chem, 2017; 65: 8979–8991.
- [12] Taweel H Z. Perennial ryegrass for dairy cows: grazing behavior, rumen function and performance. PhD dissertation. Wageningen: Wageningen University, 2004; 09. 156p.
- [13] Lumes R. Utilization of annual ryegrass ryegrass. 2009; Available: http://msucares.com/crops/forages/newsletters/09/1.pdf. Accessed on [2024-01-03].
- [14] Liu Z L, Yang D P, Chen P, Lin S B, Jiang X Y, Zhao W S, et al. Effect of dietary sources of roasted oilseeds on blood parameters and milk fatty acid composition. Czech Journal of Animal Science, 2008; 53(5): 219–226.
- [15] Khan N A, Khan N, Tang S X, Tan Z L. Optimizing corn silage quality during hot summer conditions of the tropics: investigating the effect of additives on in-silo fermentation characteristics, nutrient profiles, digestibility and post-ensiling stability. Front Plant Sci, 2023; 14: 1305999.
- [16] National Research Council. Growth and body Reserves. Nutrient Requriment of Beef Cattle. 1996; Available: https://nap.nationalacademies.org/catalog/9791/nutrient-requirements-of-beef-cattle-seventh-revised-edition-update-2000. Accessed on [2024-01-03].
- [17] Association of Official Analytical Chemists. Methods of analysis arlington. TX: Association of Official Analytical Chemists. 1997. doi: 10.1371/journal.pone.0094363.
- [18] Van Soest P J, Robertson J B, Lewis B A. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 1991; 74: 3583–3597.
- [19] Lacitra G, Hernandez T M, Van Soest P J. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim Feed Sci Technol, 1996; 57(4): 347–358.
- [20] Tilley J M A, Terry D R. A two stage technique for the in vitro digestion of forage crops. Grass and Forage Science, 1963; 18(2): 104–111.
- [21] Khan N A, Booker H, Yu P Q. Molecular structures and metabolic characteristics of protein in brown and yellow flaxseed with altered nutrient traits. J Agri Food Chem, 2014; 62: 6556–6564.
- [22] Khan N A, Habib G, Ullah G. Chemical composition, rumen degradability, protein utilization and lactation response to selected tree leaves as substitute of cottonseed cake in the diet of dairy goats. Animal Feed Science and Technology, 2009; 154: 160–168.
- [23] Khan M T, Khan N A, Bezabih M, Qureshi M S, Rahman A. The nutritional value of peanut hay (*Arachis hypogaea* L.). as an alternate forage source for sheep. Trop Anim Health Prod, 2013; 45: 849–853.
- [24] Baldinger L, Baumung R, Zollitsch W, Knaus WF. Italian ryegrass silage in winter feeding of organic dairy cows: forage intake, milk yield and composition. Journal of the Science of Food and Agriculture, 2011; 91(3): 435–442.

- [25] Jung H G, Allen M S. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. Journal of Animal Science, 1995; 73(9): 2774–2790.
- [26] Jiang M M, Ma Y L, Khan N, Khan M Z, Akbar A, Khan R U, et al. Effect of spring maize genotypes on fermentation and nutritional value of whole plant maize silage in northern Pakistan. Fermentation, 2022; 8(11): 587.
- [27] Khan NA, Tewoldebrhan T A, Zom R L G, Cone J W, Hendriks W H. Effect of corn silage harvest maturity and concentrate type on milk fatty acid composition of dairy cows. J Dairy Sci, 2012; 95(3): 1472–1483.
- [28] Abeysekara S, Khan N A, Yu P Q. Relationship between protein molecular structural makeup and metabolizable protein supply to dairy cattle from new cool-season forage corn cultivars. Spectrochim Acta A Mol Biomol Spectrosc, 2018; 191: 303–314.
- [29] Allen M S. Relationship between forage quality and dairy cattle production. Animal Feed Science and Technology, 1996; 59(1-3): 51-60.
- [30] Abrahamse P A, Vlaeminck B, Tamminga S, Dijkstra J. The effect of silage and concentrate type on intake behavior, rumen function, and milk

- production in dairy cows in early and late lactation. Journal of Dairy Science, 2008; 91(12): 4778–4792.
- [31] O'mara F P, Fitzgerald J J, Murphy J J, Rath M. The effect on milk production of replacing grass silage with maize silage in the diet of dairy cows. Livestock Production Science, 1998; 55(1): 79–87.
- [32] Burke F, Murphy J J, O'donovan M A, O'mara F P, Kavanagh S, Mulligan F J. Comparative evaluation of alternative forages to grass silage in the diet of early lactation dairy cows. Journal of Dairy Science, 2007; 90(2): 908–917.
- [33] Benchaar C, Hassanat F, Gervais R, Chouinard P Y, Petit H V, Massé D I. Methane production, digestion, ruminal fermentation, nitrogen balance, and milk production of cows fed corn silage-or barley silage-based diets. Journal of Dairy Science, 2014; 97(2): 961–974.
- [34] Kasuya H, Takahashi J. Methane emissions from dry cows fed grass or legume silage. Asian-Australasian Journal of Animal Sciences, 2010; 23(5): 563–566.