Scientific Paper

Evaluation of three *Lolium perenne* L. cultivars with dairy cows, in the high tropic of Nariño-Colombia

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Abstract

The objective of the study was to evaluate three ryegrass (*Lolium perenne* L.) cultivars with dairy cows, in the high tropic of Nariño-Colombia, in two localities: Pasto (PAS) and Cumbal (CUM). In each locality nine cows were used, in a Latin square design (3 x 3) with three treatments (T), corresponding to each cultivar to be evaluated: T1: ryegrass Columbia, T2: ryegrass Samson, T3: ryegrass Ohau. The dry matter intake (DMI) and milk production (MP) were estimated, and samples were collected to analyze: fat, protein, total solids and milk ureic nitrogen (MUN). There was no statistical difference in the DMI among the ryegrasses in the localities. In PAS significant differences were found among cultivars in the variable milk production ($p < 0,05$); with Samson and Ohau higher production was obtained (17,5 and 17,1 kg/cow/day) with regards to Columbia (15,5 kg/cow/day). In the content of fat and non-fatty solids in the milk, there were no differences among the treatments in any of the two localities. However, significant difference ($p < 0,05$) was found in the milk protein in PAS (3,48; 3,61 and 3,47 % for Columbia, Samson and Ohau, respectively). For the variable MUN (mg/dL), significant difference ($p < 0,05$) was observed in the PAS locality. It is concluded that *L. perenne* showed similar nutritional characteristics in the two localities where they were established. The response in milk production and compositional quality was considered good, taking into consideration that the animals did not receive supplementation and the trial was conducted in the dry season, in which the pasture yields decrease.

Keywords: Adaptation, intake, milk production

Introduction

Forage-based animal husbandry systems play a key role in the rural economy of developing countries, and it is stated that productivity depends on the feed availability and to a lower extent on the nutritional requirements of the animals (Enciso *et al.*, 2018).

In the high Colombian tropic the rainfall periods and intense summers are marked, climate variability that affects directly the forage and nutrient offer for the animal. The effects of the climate variations lead to the so called «forage seasonality», and impact negatively on the productivity of animal husbandry systems (Gerber *et al.*, 2013). Animal husbandry systems must focus their efforts on the utilization of forage and animal species resilient to climate change, and on sustaining productivity from the social, environmental and economic points of view (Vargas *et al.*, 2018a).

Milk production in the high tropic of Nariño is developed in different agroecological zones, located between 2 400 and 3 100 m.a.s.l. and influenced by well-defined agrophysical conditions (Guerrero, 1998). In these sites the dairy production of the department is concentrated, mostly under a smallholding productive system which is mostly affected by deficient management practices and low automation in the use of grasslands (Mejía, 2012).

In Nariño production systems are mostly based on naturalized pastures (Carulla, 2016), such as kikuyu [*Cenchrus clandestinus* (Hochst. ex Chiov.) *Morrone*] and Yorkshire fog (*Holcus lanatus* L.). They are both susceptible to adverse climate conditions, such as droughts or frosts, phenomena that affect the zone regularly; which leads to the reduction of the forage and nutrient offer for cattle (Pedraza, 2017).

To guarantee forage production in quantity and quality throughout the year, it is necessary that in milk production zones cultivated pasture varieties are established, higher in production, nutritional quality and resistance, compared with the naturalized pastures (Rao *et al.*, 2015).

In this sense, ryegrass (*Lolium perenne* L.) cultivars originate from species of the *Lolium* genus, which, through crossing and selection, originate different hybrids and varieties, and are classified according to their persistence (annual, perennial) and ploidy (diploid, tetraploid) (Posada *et al*., 2013).

From a research conducted in Nariño, Cadena-Guerrero *et al*. (2019) reported high persistence for perennial ryegrass, as well as a production of up to 14,6 t/ha/year (with nine cuts per year), tolerance to frosts, and resistance to pests, such as *Pyricularia* spp., of high incidence on the zone of this study (Pasto and Cumbal).

In countries like New Zealand, ryegrass represents the forage basis for milk production, which is manifested in high indexes of compositional quality and produced volumes (Burchill *et al*., 2014). Although ryegrass is considered a high-quality pasture, it has been proven that there are genetic variations among the cultivars, which influence their yield and quality and are shown in milk production (Gowen *et al*., 2003).

The objective of this study was to evaluate three ryegrass cultivars with dairy cows in the high tropic of Nariño-Colombia.

Materials and Methods

Location and edaphoclimatic conditions of the experimental area. The study was conducted in July and August, 2018, in two animal husbandry farms located in different milk-producing localities in the Nariño department (Colombia), specifically in the Pasto and Cumbal municipalities. Both localities are located in regions that correspond to the life zone of low mountain dry forest (LMDF), according to Holdrige (2000). During the study, rainfall was low in both zones. Table 1 shows the data concerning the location, as well as the environmental, topographical and edaphic characteristics of the study sites.

Experimental animals. In each locality nine lactating cows in optimum health conditions were selected, whose productive characteristics before the beginning of the essay are related in table 2.

After each milking, the cows had access to grazing at will. In the Pasto farm, the cows were transferred from the paddock to the milking parlor and vice versa; while in Cumbal the milking was carried out at the grazing site. In both localities

Table 1. Climate and edaphic characteristics of the farms.

Indicator	Locality	
	Pasto	Cumbal
Coordinates	N: 1º 11′ 29,6″ W: 77º 18′ 47,9 ″	N: 1º 88.918″ W: 77º 306.083″
Height, m.a.s.l.	2 600	3 000
Mean temperature, ºC	10	8
Topography	Undulated	Flat-undulated
Soil type	Sandy loam	Silty loam
Average rainfall during the experimental period, mm	39,8	50

Table 2. Characterization of the lactating cows selected in the Pasto and Cumbal localities.

Indicator	Locality	
	Pasto	Cumbal
Number of animal	9	9
Breed	Kiwi Cross x Holstein (F1)	Holstein x Simental (75 %/25 %)
Days into milk, d	97	85
Number of parturitions	1	3,5
Body weight, kg	457 (47)	496 (95)
Body condition[1]	3,27 (0,26)	3,36 (0,28)
Milking time	4:30 a. m. y 2:30 p. m.	5:30 a. m. y 3:00 p. m.
Milking type	Mechanical	Manual

[1] Body condition in a scale from 1 to 5.
( ) Values between parentheses standard deviation.

the animals had ad libitum access to fresh water, through automatic drinking troughs in the paddock. In Pasto the salt was supplied before the afternoon milking, in group feeding troughs (average of 150 g/cow/day). In Cumbal, the salt was provided in the salteries located in the paddocks; and it was offered, as average, at a rate of 100 g/cow/day.

Pasture establishment and management. In each farm 3-ha lots were selected, with a degraded plant cover in which the following species prevailed: kikuyu (*C. clandestinus*), Yorkshire fog (*H. lanatus*) and cock's foot (*Dactylis glomerata* L.). The chemical composition of soils for each farm is shown in table 3.

Table 3. Soil chemical analysis.

Indicator	Unit	Locality	
		Pasto	Cumbal
OM	g/100 g	5,1	11,7
pH	pH	6,1	5,
Phosphorus (P)	mg/kg	24,3	8,2
Calcium (Ca)	cmol/kg	8,6	10,2
Potassium (K)	cmol/kg	1,0	1,3
Sulfur (S)	cmol/kg	4,9	9,3
CEC[1]	cmol/kg	11,8	14,9

[1] CEC: cation exchange capacity.

In the prairies commercial herbicide Roundup (active principle: glyphosate) was applied, at a rate of 4 L/ha; eight days after fumigation the lots were prepared with a tractor, by crossed chisel and two harrow passes. According to the recommendations made by Bernier and Alfaro (2006), 60 days were let by for such material to be incorporated to the soil; finally, the area was delimited and divided into three lots (one for each ryegrass variety) of one hectare each.

According to the agronomic evaluations previously carried out in the Obonuco research center (Agrosavia), three ryegrass cultivars were selected: Columbia (perennial hybrid), Samson (diploid perennial) and Ohau (tetraploid perennial). These materials were planted by drilling, at a rate of 50 kg/ha, in each locality. In addition, diammonium phosphate DAP (100 kg/ha) was applied once; and one maintenance fertilization with urea was performed, at a rate of 100 kg/ha, after the second grazing.

Before evaluating the pastures with cattle, in each locality two grazings were performed: the first was a homogenization one, at 90 ± 5 days post-establishment, and the second one, 35 days later, both with breeding heifers (average weight of 250 kg/animal). Before the third grazing (experimental), the lots were subdivided into strips, according to the estimated carrying capacity, and a daily strip was assigned with one day of occupation, in order to guarantee a resting period with 35 days for the following times.

The grazing management was carried out with an electrical tape (electroplastic tape of five conductors, Lhaura trademark, Colombia). For the farm located in Pasto, according to the habitual management, the strip was assigned at three moments: 06:00, 12:00 and 16:00 hours. For the Cumbal farm, the strip was offered at two moments after each milking: 5:30 and 15:00 hours. The animals from both localities had access at will to the drinking water throughout the study time. The cattle did not receive supplementation before or during the trial, in correspondence with the habitual practice in the zone.

The availability was, as average, of 2,2; 2,3; 2,1 and 2,0; 2,1 and 1,9 t DM/ha/rotation for Columbia, Samson and Ohau, respectively, in Pasto and Cumbal.

Experimental design. A Latin square design (3 x 3) was used, with crossover arrangement; three treatments, which corresponded to each of the cultivars: T1: ryegrass Columbia; T2: ryegrass Samson, T3: ryegrass Ohau; and three evaluation periods. Each period lasted 14 days: 7 of adaptation and 7 of measurement, in which the dry matter intake and milk production in the milkings were measured. In the last three days of each period milk samples were collected for the compositional analysis.

Forage intake. The dry matter intake (DMI) of the forage from each ryegrass variety was estimated through the agronomic method (input and output); thus, it was assumed that the difference between the input and output availability was the quantity of forage consumed by the animals. For estimating the availability of ryegrass species the double-sampling methodology was used (Haydock and Shaw, 1975).

Body condition. At the end of each period, the cows were weighed and their body weight was determined; in Pasto the animals were weighed with a scale (Medigan Scales SAS, Colombia), and in Cumbal, by a cattle weight band (weight band OVNI Inalmet, Colombia).

Chemical composition of the pasture. In the measurement periods subsample of the pasture at the count moment. The samples were dried in an oven at temperature of 65 ºC, during 72 h; afterwards, they were sent to the animal nutrition laboratory of the Tibaitata research center of Agrosavia (Bogota, Colombia), where the respective bromatological analyses were conducted to determine: dry matter (DM); crude protein (CP); neutral detergent fiber (NDF); acid detergent fiber (ADF); hemicellulose (HEM); total digestible nutrients (TDN); dry matter digestibility (DMDG); ethereal extract (EE); crude energy (CE); net lactation energy (NLE); and ash, calcium and phosphorus, through the near infrared reflectance spectroscopy (NIRS) technique (Ariza *et al*., 2017), with equipment NIRS DS 2500-FOSS Analytical A/S, Denmark.

Milk production and compositional quality. In each locality milk production was recorded during the seven days of measurement; for Pasto, the production (kg/cow/day) was directly taken from a milk meter (Tru-Test Milk Meters, New Zealand), at 05:00 and 15:00 h, in the mechanical milking parlor. In Cambal a digital scale (Portable Electronic Scale MSC, Spain) was used for weighing at 04:00 and 14:00 h, after manual milking with bucket. During the last three days of each period individual milk samples were taken, which were later deposited in a plastic container with Bronopol® preservative. On the collected samples fat (%, F), protein (%, C), total solids (TS) were determined through the infrared spectroscopy method (AOAC 972.16, of 2015) and ureic nitrogen (MUN) by the infrared method (IR spectrophotometry). The samples were processed in the milk laboratory of the Obonuco-Agrosavia research center with equipment FOSS Milkoscan TM 7RM, FOSS Analytical A/S, Denmark). The milk correction at 4 % of fat (FCM) was done according to the National Research Council (FCM = 0,4 x kg milk + 15 x kg fat).

Statistical analysis. The data of dry matter intake and milk production and compositional quality were subject to variance analysis, using the statistical software SAS Enterprise Guide 9.4. A significance level of 0,05 was considered. In case of rejecting the hypothesis of equality among means, Tukey's test was used to identify the difference among treatments.

Results and Discussion

Bromatological composition. Table 4 shows the bromatological composition of the cultivars at 35 days regrowth. The average DM content of the cultivars was 16,1 and 14,4 % for PAS and CUM, respectively. In both localities cv. Samson showed the highest DM contents.

According to Posada *et al*. (2013), the DM percentage of ryegrass could be influenced by

Table 4. Bromatological composition of the ryegrass cultivars.

Indicator	Pasto			Cumbal		
	Columbia	Samson	Ohao	Columbia	Samson	Ohao
DM, %	15,1	18,0	15,3	14,8	15,5	13,0
CP, %	19,9	22,5	20,9	20,2	20,1	22,1
NDF, %	48,2	48,6	48,0	47,3	47,2	43,6
ADF, %	23,7	24,6	24,4	22,4	22,1	20,1
HEM, %	24,5	23,9	23,5	24,9	25,2	23,5
Lignin, %	3,6	4,3	4,1	3,7	3,7	3,6
TDN, %	62,1	63,7	62,6	62,4	62,8	64,7
DMDG, %	67,9	69,7	68,5	68,5	68,6	70,7
EE, %	2,7	2,8	2,6	2,1	2,5	2,4
CE, Mcal/ kg	4,2	4,2	4,2	4,0	4,1	4,1
NLE, Mcal/ kg	1,4	1,4	1,4	1,4	1,4	1,4
Ash, %	10,0	10,1	10,5	11,8	11,4	11,9
Ca, %	0,6	0,5	0,5	0,5	0,6	0,5
P, %	0,3	0,4	0,4	0,4	0,5	0,5
NSC, %	16,8	16,4	18,4	18,4	18,8	18,9

environmental variables. Within the optimum agroclimate characteristics to obtain good yields of the species, a temperature from 15 to 22 ºC and height between 1 800 and 3 600 m.a.s.l. are considered; above 3 000 m.a.s.l., according to these authors, the yield decreases.

In spite of the edaphoclimatic differences of the zones, the ryegrass cultivars showed similar protein contents to the ones reported by Cardona *et al.* (2017) and Duque *et al.* (2017): 17,2 % and 18,2 % CP, respectively, for samples of kikuyu pasture in several regions of the high tropic of Colombia, including Nariño.

In turn, the CP values were higher than those reported for perennial ryegrass (35 days) by Vargas *et al.* (2018a, 2018b), under similar agroecological and management conditions to the ones in this study, who obtained 15,5 % CP in evaluated materials in the high tropic of Cundinamarca, Colombia.

The NDF and ADF contents provide an estimate of a pasture quality. The NDF controls the passage rate of the rumen and stimulates saliva production as rumen pH buffer (Harper, 2015).

NRC (2001) recommends 25 and 17 % (on DM basis) as the minimum concentrations of NDF and ADF, respectively, in the rations for dairy cows. In this study the NDF content had a similar value in the cultivars of both localities (48 % in PAS and 46 % in CUM). Similar performance was observed in the ADF content: 24 and 22 % for PAS and CUM, respectively.

Villalobos and Sánchez (2010) state that the NSC proportion varies inversely to the NDF content; generally, at lower cell wall (NDF) DM digestibility and energy content of the forage increase.

At rumen level, NSCs are a fast-availability energy source for rumen microorganisms, and their content is related to the utilization efficiency of degradable protein and later synthesis of the microbial protein (Montoya *et al.*, 2004). In this study the NSC content was similar for the three ryegrass cultivars in the two localities: 17,2 and 18,7 % for PAS and CUM, respectively.

According to López *et al.* (2012), the basis for producing a good quantity of milk depends on the NLE concentration in the diet of dairy cows. The NLE content and digestibility of a pasture vary, mainly, according to the species, harvest season and plant age (Chaves *et al.*, 2006). In this regard, Silva *et al.* (2015) stated that NLE decreased in perennial ryegrass at different ages: 1,34; 1,26 and 1,24 Mcal/kg DM at 40, 50 and 60 days, respectively.

In this study, the NLE at 35 days of age was similar for the cultivars in both localities, with values of 1,42 and 1,43 Mcal/kg DM for PAS and CUM, respectively. In high tropic and hillside zone, with maintenance fertilization, Vargas *et al.* (2018b) found 1,40 Mcal de NLE/kg DM for perennial ryegrass cultivars. In temperate zones the NLE could vary between 1,5 and 1,7 Mcal/kg DM (Clark and Kanneganti, 1998), higher values than those generally found for *L. perenne* in Colombia.

Regarding the DM digestibility, it was similar among the cultivars and in the two localities. The digestibility values were higher than those reported for the kikuyu pastures in Colombia (60 and 63 % at 45 and 35 days, respectively), by Posada-Ochoa *et al.* (2014) and Cardona *et al.* (2017).

In this sense, Castro *et al.* (2017) found up to 71 % digestibility 28 days after cutting the forage in *L. perenne*, and related this value to a possible increase of DMI and higher efficiency in the synthesis of microbial protein in rumen.

The average Ca and P contents in the perennial ryegrass cultivars were similar in all the cultivars for both localities and higher than the ones reported by Cedeño *et al.* (2011) in perennial ryegrass and kikuyu pastures, in the high tropic of the Nariño department (Colombia).

Dry matter intake (DMI). The DM intake was similar in all the cultivars for both localities (table 5). These results do not coincide with those obtained by Riquelme and Pulido (2008), who reported intakes between 15,6 and 17,6 kg DM/animal/day; but they are higher than the ones reported by Mejía *et al.* (2017), of 10,91 kg DM/animal/day, when evaluating this indicator in Holstein cows with kikuyu grasslands.

Table 5. Effect of the ryegrass cultivar on DM intake.

Locality	Ryegrass intake kg DM/day			SE ±	P-value
	Columbia	Samson	Ohau		
Pasto	13,1	14,5	12,3	0,724	0,1290
Cumbal	15,2	15,5	14,4	0,500	0,2615

Milk production and compositional quality. Tables 6 and 7 show average milk production and corrected milk at 4 %, of cows which consumed the different *L. perenne* cultivars in the two localities.

In the PAS locality there were statistical differences ($p < 0,05$) among the ryegrass cultivars, regarding milk production (liters/cow/day) and FCM. Cvs. Samson and Ohau were the ones with higher FCM (16,8 and 16,3 kg/cow/day). On the other hand, in the CUM locality there was no difference in milk production among the three cultivars (table 6).

The average milk production for both localities based on *L. perenne* (16,7 and 13,3 kg milk/cow/day for PAS and CUM) was over the expected result only with kikuyu. In this regard, Carulla *et al.* (2004) indicated that kikuyu pasture can sustain milk productions between 8 and 12 liters/cow/day, without supplementation, and that the limiting factors to enhancing production depend on low DMI and the NLE content of this grass. In general, such values were higher than the expected ones (10 kg/milk/day) in cows whose diet was based on *Lolium multiflorum* (Posada *et al.*, 2013).

Lascano *et al.* (2017), when evaluating milk production in two different cow biotypes: F1 (Jersey x New Zealand Holstein) and American Holstein, also reported a production of 16,2 and 12,5 kg milk/cow/day, respectively. The authors ascribed such results to a higher productive efficiency of the New Zealand biotype, compared with the American one.

No significant difference was found for the fat content in milk among the evaluated ryegrass varieties, in any of the two localities. In PAS the fat average was 3,6 % and in CUM, 3,3 %. These contents were lower than the ones reported by Gallego *et al.* (2017), but similar to those reported by Valencia (2013) for cows with predominance of Holstein,

Table 6. Effect of the ryegrass cultivar on milk production and compositional quality, in the Pasto locality.

Indicator	Ryegrass			SE ±	P-value
	Columbia	Samson	Ohau		
MP, kg/cow/day	15,6^{b}	17,6^{a}	17,0^{a}	0,285	0,0004
FCM, kg/cow/day	14,4^{b}	16,8^{a}	16,3^{a}	0,382	0,0010
Fat content, %	3,6	3,8	3,8	0,009	0,2024
Protein content, %	3,5^{b}	3,6^{a}	3,5^{b}	0,030	0,0074
Fat/protein ratio	1,0	1,0	1,1	0,024	0,1958
Content of total solids, %	12,4	12,6	12,5	0,073	0,1055
MUN, mg/dL	9,4^{b}	11,8^{a}	8,1^{b}	0,38	0,0001

a, b, c: values with different superscripts in the same row differ at $p < 0,05$ (Tukey).
FCM: fat corrected milk, F/P: fat/protein ratio, MUN: milk ureic nitrogen.

Table 7. Effect of the ryegrass cultivar on milk production and compositional quality, in the Cumbal locality.

Indicator	Ryegrass			SE ±	P-value
	Columbia	Samson	Ohau		
MP, kg/cow/day	13,0	14,0	13,0	0,542	0,2916
FCM, kg/cow/day	11,4	11,9	11,5	0,457	0,7056
Fat content, %	3,4	3,2	3,3	0,088	0,3137
Protein content, %	3,2	3,3	3,4	0,53	0,2843
F/P ratio	1,0	0,9	0,9	0,02	0,0838
Content of total solids, %	11,9	11,6	12,0	0,14	0,4774
MUN, mg/dL	15,0	15,0	13,3	0,77	0,2386

FCM: Fat corrected milk. F/P: fat/protein ratio. MUN: milk ureic nitrogen.

which grazed in kikuyu grasslands in the high Colombian tropic.

The fat contents in PAS were higher than those from CUM. In PAS the cow biotype Kiwi Cross x Holstein (F1) was managed, and the presence of the Kiwi cross breed –which originated from Holstein-Friesian x Jersey crossbreed– confers the animal higher production of total solids, compared with such breeds as Holstein, due to its higher efficiency in transforming the ingested nutrients into milk nutrients (Holmes, 2006).

Besides the genetic component, fiber is important for dairy fat synthesis. The NDF in the grass, for the two localities, is adjusted to the suggestion by the NRC and comes completely from the forage (100 %). According to the report by Banakar *et al.* (2018), when there is an adequate effective fiber intake rumination and salivation are improved, and adequate pH at rumen level is obtained, which favors fat synthesis in milk (Andrade *et al.*, 2017).

Regarding the protein content in milk, there was statistical difference among the ryegrass varieties in the PAS locality, with values of 3,48; 3,61 and 3,47 % for Columbia, Samson and Ohau, respectively; while in the CUM locality there were no differences (tables 6 and 7).

In the PAS locality, when the cows grazed the cv. Samson, the highest protein concentration in milk was found, besides the highest average forage intake (14,7 kg DM/cow/day) with regards to the cvs. Columbia and Ohau.

The average values of protein in milk (3,51 %) in the PAS locality were higher than the ones reported by Gallego *et al.* (2017) in Holstein cows, in the high tropic of Colombia.

In both localities there was no difference for the variable TS in milk; the TS average in PAS was 12,5 % and in CUM, 11,9 %. In the PAS locality the milk could be classified as excellent, due to its fat protein and total solid content, according to the milk quality classification stipulated in Colombia (Calderón *et al.*, 2016).

According to this classification, if the fat and protein values are higher than 3,5 and 3,2 %, respectively, and the TS higher than 12,2 %, the milk is excellent in compositional quality. The milk in CUM could be classified as good, because the fat values varied between 3,3 and 3,5 %, protein between 2,8 and 3,2 and TS between 11,8 and 12,0 %.

Regarding the variable MUN, significant differences were found among the cultivars in the PAS locality, with values of 11,83; 9,38 and 8,1 mg/dL for Ohau, Columbia and Samson, respectively; in the CUM locality there were no differences (tables 6 and 7).

According to Doo-Hong (2013), between 9 and 12 mg MUN/dL is considered adequate and there is good nitrogen use. MUN is an important tool to try to evaluate protein and energy balance and utilization in the rumen.

The MUN results for the PAS locality showed that the only ryegrass intake that affected MUN in the milk was that of Ohau (8,1 mg/dL), which could have been related to the fact that it was the cultivar with lower DMI by the cows and lower CP percentage, but the one with higher NSC in the forage, with regards to the other cultivars. According to Meléndez (2009), a MUN content lower than 9 mg/dL would indicate a low degradable protein content, compared with the rumen energy availability.

The determination of the MUN in milk is a practical tool with which the farmer can make decisions and avoid nutritional excesses or deficiencies. In dairy cows of the high tropic, due to the nutritional management, almost always a high total protein content, combined with low energy concentration in the diet, is responsible for the urea in milk (Cortes *et al.*, 2018).

Conclusions

The *L. perenne* cultivars showed similar nutritional characteristics in the two localities where they were established. The response in milk production and compositional quality was considered good, taking into consideration that the animals did not receive supplementation and the trial was conducted in the dry season, in which the pasture yields decrease.

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