Ruminal pH and temperature, papilla characteristics, and animal performance of fattening calves fed concentrate or maize silage-based diets

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Feeding systems can play an important role, not only in beef farm profitability but also in animal health and performance. Fourteen Avileña-Negra Ibérica bulls, with an initial weight of 270 kg (SE 22.6 kg) and aged 223 d (SE 16.2) were used to study the effect of two feeding systems on ruminal pH and temperature and animal performance when calves were kept in loose housing conditions. Feeding systems were barley (*Hordeum vulgare* L.) grain-based concentrate plus barley straw (CONC) and maize (*Zea mays* L.) silage-based total mixed ration (TMR). Internal wireless boluses were used to collect pH and temperature values every 10 min throughout the measurement period (15 d). Diet did not modify (P > 0.10) average daily gain, carcass weight, dressing percentage, ruminal mucosa color, or papilla counts. Papilla width and papilla width/lamina propria thickness were significantly lower (P < 0.05) in TMR than in CONC animals. Time spent below ruminal pH thresholds of 7.0, 6.6, 6.2, and 5.8 and the corresponding areas under the curve were higher (P < 0.05) for animals fed under the TMR system. No significant changes were observed between experimental treatments in parameters related to ruminal temperature or estimated number of times that the animals were drinking during the day (P > 0.10). Although animal performance is not affected, feeding fattening calves on a concentrate plus barley straw diet can result in better rumen conditions than using maize silage-based TMR.

Key words: Acidosis, feeding system, monitoring, rumen, total mixed ration.

INTRODUCTION

Fattening cattle are fed under different feeding systems depending on farm facilities, consumer preferences, and economic circumstances. While some of these systems are mainly based on pasture and silage, others are based on the supply of concentrate feed and forage, both *ad libitum*. Some farmers consider the possibility of increasing productivity by increasing the amount of concentrate offered in the last months of the fattening period (Cooke et al., 2004; O'Kiely, 2011). On the other hand, given the sudden and constant rise in the prices of ingredients for concentrates, farmers are more interested in reducing the use of concentrates and turning to forage-based diets (Casasús et al., 2012).

The effects of these types of diets (forage- and concentrate-based) on animal performance and carcass and meat characteristics have been extensively studied. Concentrate-based rations allow faster, more controlled, and predictable growth rates than forage-based diets (Cooke et al., 2004; Walsh et al., 2008; O'Kiely, 2011). Animals can achieve similar growth rates when they are fed forage-based diets, but these are associated with higher feed intake and worse feed to gain ratios, while still being economically competitive (Casasús et al., 2012).

Feeding management can affect not only animal performance but also animal welfare. Using concentrate-based rations has been criticized for its consequences on ruminal pH and the development of subacute ruminal acidosis processes (González et al., 2012). Using concentrates and decreasing ruminal pH values are two closely interrelated factors and there is no clear discrimination between the two (Calsamiglia et al., 2012). It is necessary to focus not only on pH control, but also on how this is affected by the type of feed consumed by animals and the way this feed is supplied and consumed.

The development of subacute acidosis cannot be associated to early clinical symptoms (Nagaraja and Titgemeyer, 2007; Wahrmund et al., 2012). While early detection methods were based on using a wired pH probe placed in the rumen through a ruminal cannula, the more advanced methods are based on wireless pH probes, which end up in the reticulo-rumen for the duration of the animal's life. They allow accurate pH and temperature

readings in real time. These probes are used in dairy cattle herds to provide early detection of management practices that increase the risk of sub-clinical acidosis, thus helping to prevent its negative consequences.

The aim of this research was to study the effect of two feeding management systems (concentrate feed plus barley straw in separate feeding troughs vs. maize silage-based total mixed ration –TMR) on ruminal pH and temperature dynamics, papilla characteristics, and animal performance of Avileña-Negra Ibérica calves kept in loose housing conditions.

MATERIALS AND METHODS

Animals and diets
Fourteen Avileña-Negra Ibérica bulls with an initial body weight of 270 kg (SE 22.6 kg) and aged 223 d (SE 16.2) were included in the study. Seven animals received barley (*Hordeum vulgare* L.) grain-based concentrate feed from a hopper feeder (space allowance 15 cm per head) plus barley straw in long form (unchopped) (CONC group), whereas the other seven received a maize (*Zea mays* L.) silage-based total mixed ration (TMR) in a bunk feeder (space allowance 45 cm per head) (TMR group). Ingredients and chemical composition of diets are shown in Table 1.

Animals were housed in groups under the cover of feedlot facilities in Riocabado (40°49'48" N 4°48'11" W, 906 m a.s.l., Ávila, Spain). The CONC treatment received 2 kg barley straw (as-fed basis in a bunk feeder) per animal and per day plus *ad libitum* fresh concentrate available from a hopper feeder, while TMR was delivered to the bunk feeder for the TMR group once a day (at 10:00 h) after cleaning feed refusal from the previous day (approximately 10%). Clean fresh water was always available for both groups.

Table 1. Ingredients and chemical composition of two diets offered to loose-housed fattening calves.

	CONC[1]	TMR
Ingredients (as-fed basis), g kg^{-1}		
Maize silage	-	740
Barley grain	335	11
Maize grain	300	132
Dried maize distillers grains	150	49
Soybean meal 44% crude protein	20	50
By-pass fat	10	9
Palm oil	20	-
Sugar beet pulp	20	-
Palm kernel expeller	20	-
Rapeseed expeller	52	-
Wheat middlings	53	-
Vitamin mineral premix	20	9
Chemical composition (DM basis), g kg^{-1}		
Dry matter (as-fed basis)	890	473
Crude protein	152	139
Neutral detergent fiber	201	280
Ash	60	58
Net energy for fattening, kcal kg^{-1} DM	1438	1308

[1]CONC animals also received 2 kg barley straw (92 g moisture, 32 g crude protein, 928 g neutral detergent fiber, 559 g acid detergent fiber, 1.7 g ether extract, 51 g ash, and 352 kcal net energy per kg DM) per animal per day. TMR: total mixed ration.

Animal handling and management was conducted according to Directive 2010/63/EU (Official Journal of the European Union, 2010) for the protection of animals used for scientific purposes.

Ruminal pH and temperature monitoring
When the animals had been fed the same diet for approximately 3 mo, internal wireless smaXtec® boluses (smaXtec animal care sales GMBH, Graz, Austria) were used to collect ruminal pH and temperature data every 10 min. Each bolus ended up in the reticulum (where it remained until the animal was slaughtered) after being calibrated (pH 4 and 7) and introduced with an oral balling gun following manufacturer instructions. Data were recorded for a period of 15 d.

Animal performance and rumen wall characteristics
Animals were slaughtered when they reached the body weight at which they are usually sent to abattoir on this commercial farm (between 525 and 550 kg, in this case, 535 kg, SE 8.8 kg). The following animal performance data were recorded: weight and age at slaughter, duration of fattening period, total weight gain (animals were weighed once a month), and average daily gain (ADG) during the fattening period, carcass weight, and dressing percentage (carcass weight/weight at slaughter).

Once the animal was eviscerated, the rumen was opened and washed, the color of the mucosa was always visually evaluated (1, clear; 2, dark) by the same person, and the bolus was recovered. Samples from the dorsal sac of the rumen were taken (5 × 5 cm) for histological examination. Samples were fixed by immersion in buffered formaldehyde (4%) for at least 24 h. After fixation, digital photographs were taken to evaluate mucosal epithelium color (red, blue, and green indices and gray scale) as an indicator of the degree of keratinization (Benavides et al., 2013) with ImageJ 1.43 software (ImageJ, National Institute of Mental Health, Bethesda, Maryland, USA) to process the pictures.

Subsequently, samples were included in cassettes and dehydrated by alcohol ascending scales and then embedded in paraffin (Automatic Tissue Processor TP1020, Leica Microsystems, Nussloch, Germany). Sections, 5 μm thick (microtome RM2255, Leica), were stained with hematoxylin-eosin and Masson trichrome. Photomicrographs were taken (Advanced Research Microscope Eclipse 80i, shooting program ACT-1, Nikon Instruments, Tokyo, Japan) of each sample stained with hematoxylin-eosin at 40X to assess papilla width (total papilla width, AT) and at 200X to measure lamina propria thickness (ALP).

Analytical procedures
Procedures described by AOAC (2003) were used to determine DM (AOAC official method 934.01), ash (AOAC official method 942.05), and Kjeldahl N

(AOAC official method 976.06). Neutral detergent fiber (expressed as including residual ash) was determined by the Van Soest et al. (1991) method by adding sodium sulfite to the solution.

Statistical analysis
Animal performance data were subjected to one-way ANOVA with the diet as the only source of variation.

Ruminal pH and temperature data first were averaged for each day: maximum, minimum, and mean, area under the curve, and time spent below pH threshold of 7.0, 6.6, 6.2, 5.8, and 5.4. The area under the curve was calculated by multiplying the absolute value of the deviation in pH by the time (min) pH is below the threshold, which is expressed as pH units per min. The average pH value for each hour of each day was also calculated for each animal.

Temperature data were summarized as maximum, minimum, mean, and time above 39.0, 39.2, 39.4, and 39.6 °C. The area under the curve was calculated as indicated for pH. Temperature data for the water intake times of each animal were identified: Temperature shows a sharp decrease followed by a slow increase to approximately pre-water intake temperature (Dye and Richards, 2008). The start of a drinking event was identified when ruminal temperature decreased more than 0.28 °C from the previous measurement. The end of a drinking event was considered when the temperature was above 38.4 °C or when it stopped increasing in a period of 10 min.

The pH and temperature data were subjected to analysis of repeated measures; the covariance structure (first-order autoregressive) was selected by considering the Schwarz Bayesian and Akaike criteria. Diet was considered as a fixed effect, day as a repeated measure, and the animal in the treatment as the subject. A correlation matrix between performance and pH and temperature data was built. All the analyses were performed with SPSS 16.0 for Windows (IBM Corp., New York, USA).

RESULTS

Animal performance traits are presented in Table 2. Diet did not modify (P > 0.10) average daily gain, carcass weight, or dressing percentage.

Table 3 shows values of ruminal mucosa characteristics. Neither mucosa color nor papilla counts were affected by diet (P > 0.10). However, papilla width and the relationship between papillae and their lamina propria thickness were significantly lower (P < 0.05) in animals receiving TMR than those fed CONC.

Table 4 shows mean, maximum, and minimum daily pH and temperature values, time spent below ruminal pH thresholds, and areas under the curve. Daily pH values were higher for CONC than for TMR animals (P < 0.01). All the computed values (daily time at pH and area under curve), with the exception of time and area under the curve at pH below 5.4, were higher (P < 0.05) for TMR

Table 2. The effect of offering fattening calves straw + *ad libitum* concentrate (CONC) as compared with maize silage-based total mixed ration (TMR) on animal performance parameters.

Animal performance parameters	Diet			
	CONC	TMR	SED	P-value
Age at slaughter, d	405	431	35.0	0.475
Average daily gain, kg d^{-1}	1.40	1.31	0.097	0.390
Carcass weight, kg	289	298	10.9	0.464
Dressing percentage	54.3	55.4	0.68	0.131

SED: Standard error of difference.

Table 3. The effect of offering fattening calves straw + *ad libitum* concentrate (CONC) as compared with maize silage-based total mixed ration (TMR) on ruminal mucosa characteristics.

Ruminal mucosa characteristics	Diet			
	CONC	TMR	SED	P-value
Ruminal mucosa color				
Subjective (1: clear; 2: dark)	1.71	2.00	0.184	0.147
R (red index)	77.0	75.5	13.02	0.909
G (green index)	65.0	63.1	10.38	0.859
B (blue index)	48.0	49.1	8.06	0.900
Gray (gray scale)	66.7	64.9	10.86	0.876
Papillae cm^{-2}	70.0	59.5	13.16	0.452
Papilla width (AT), μm	423	356	30.2	0.046
Lamina propria thickness (ALP), μm	118	123	8.4	0.593
Internal width (AT - ALP)/2, μm	152	117	14.3	0.028

SED: Standard error of difference.

animals. The diet × day interaction observed for some of the studied parameters could indicate that the effect can vary throughout the day and between days.

Besides the day effect, no significant changes were observed between experimental groups in those parameters related to temperature or the estimated number of times that the animals were drinking per day (P > 0.10) (Table 4).

DISCUSSION

Animal performance
Daily gain data observed in the present study are similar to those published by Casasús et al. (2012) for animals fed under similar feeding systems at similar ages, and performance data fell within the range expected for the Avileña-Negra Ibérica breed according to Fernández-Perea and Alenda Jiménez (2004).

Casasús et al. (2012) reported no changes in growth rate, weight, and carcass yield when comparing diets consisting of concentrate or total mixed ration. The absence of differences in performance parameters during the fattening period can be indicative of the adequacy of both rations to meet the animals' needs and achieve optimal growth rates (Moya et al., 2011). Likewise, maize silage, despite being forage, provides high energy content compared with other forages (e.g., grass silage) or grazing, which can result in lower growth rates (Keane et al., 2006).

Ruminal mucosa and pH
The lack of effect on ruminal mucosa must be highlighted. The slight color change noted by subjective assessment could not be confirmed when objective measures were

Table 4. The effect of offering fattening calves straw + *ad libitum* concentrate (CONC) as compared with maize silage-based total mixed ration (TMR) on least square means of ruminal pH and temperature parameters.

	Diet			P-value		
	CONC	TMR	SED	Diet	Day	Diet × Day
Daily pH values						
Mean	6.72	6.35	0.058	0.000	0.161	0.035
Maximum	7.19	6.92	0.054	0.002	0.043	0.006
Minimum	6.14	5.78	0.080	0.001	0.633	0.448
Daily time (min) at pH						
< 7.0	1130	1381	57.4	0.011	0.955	1.000
< 6.6	481	1005	89.5	0.000	0.424	0.146
< 6.2	136	406	79.4	0.007	0.068	0.028
< 5.8	19	235	46.7	0.001	0.001	0.002
< 5.4	0	16	13.6	0.127	0.228	0.283
Area under the curve (min·pH per day)						
< 7.0	455	941	78.7	0.000	0.137	0.025
< 6.6	137	435	64.1	0.001	0.080	0.034
< 6.2	27	177	36.3	0.001	0.015	0.036
< 5.8	0	48	13.8	0.003	0.040	0.080
< 5.4	0	1	1.6	0.555	0.682	0.683
Daily temperature values						
Mean	38.76	38.78	0.153	0.936	0.015	0.746
Maximum	34.68	34.57	0.297	0.720	0.028	0.849
Minimum	39.68	39.78	0.154	0.538	0.259	0.507
Daily time (min) at temperature (°C)						
> 39.0	643	675	185.8	0.869	0.468	0.293
> 39.2	435	439	174.1	0.984	0.478	0.461
> 39.4	215	159	111.0	0.631	0.538	0.986
> 39.6	93	49	58.8	0.484	0.652	0.936
Area under the curve (min·°C per day)						
> 39.0	232	205	90.8	0.778	0.649	0.980
> 39.2	120	90	56.5	0.610	0.664	0.994
> 39.4	51	31	28.5	0.492	0.632	0.989
> 39.6	19	10	12.1	0.460	0.520	0.978
Number of drinks per animal per day	4.96	4.09	0.623	0.193	0.130	0.081

SED: Standard error of difference.

taken. Papilla counts were numerically higher (15%), while papillae were 19% wider and had 30% more internal width ((AT - ALP)/2) in the CONC than in the TMR group. These differences can be related to increases in VFA concentration that determine an increase in absorption surface (Shen et al., 2004; Suárez et al., 2006). In this respect, Resende-Junior et al. (2006) reported that an increase in feeding frequency determined an increase in papilla length and width to promote nutrient absorption.

Over and above economic considerations, diet has clear implications for rumen health and animal welfare. In previous experiments, animals receiving TMR usually showed higher ruminal pH values than concentrate-fed animals (Bach et al., 2007; Moya et al., 2011). Nevertheless, in the current experiment, the TMR group had lower pH values than CONC animals. Decreases in ruminal pH are directly related not only to feed composition ('high-concentrate syndrome') but also to feeding management (space availability in the bunk feeder) and the animals' feeding behavior (number of meals per day, amount of feed eaten at each meal) (Calsamiglia et al., 2012). The pH values reported here are higher than those reported by Moya et al. (2011) (5.75 and 6.25 for concentrate and silage-based diets, respectively), Bach et al. (2007), and Blanch et al. (2010) (5.49 and 5.92, respectively for concentrate-based dairy rations). These differences can be related to the type of animals and feed composition.

Animals in the CONC group had free access all day to a hopper feeder supplying feed *ad libitum*, while feed was freshly distributed in the bunk feeder early in the morning to animals in the TMR group where it was available until the next day. This method of feed delivery can determine differences in the food intake pattern between animals although total intake could result unaffected (Schwartzkopf-Genswein et al., 2003). Thus, it is expected that a larger number of animals will try to maximize intake when feed is freshly delivered by going to the bunk feeder in the case of the TMR group, while the animals in the CONC group are expected to spread meals throughout the day. Reduced feeding frequency (once a day) in competitive social conditions can lead to competition between animals, thus reducing the number of meals per day and increasing the amount of feed ingested at each meal (Livshin et al., 1995; Robles et al., 2007; González et al., 2008); this results in a deregulation of the mechanisms to maintain rumen conditions (Schwartzkopf-Genswein et al., 2003). Robles et al. (2007) observed that increasing the frequency of concentrate distribution (from once to twice a day) resulted in more stable rumen conditions and increased maximum pH. Likewise, Soto-Navarro et al. (2000) noted a tendency for pH to be lower when animals were fed a concentrate diet once a day instead of twice. Taking into account the availability of fresh food throughout the day, our results are consistent with these authors' suggestions. Moreover, it must be taken into account that CONC animals had free access of up to 2 kg of long barley straw, which could also have helped for a higher fiber intake in CONC compared to TMR animals

and thus increase rumination and saliva production. These circumstances can also have accounted for the better rumen conditions found in CONC animals (González et al., 2012).

Daily pH variations

The feed delivery system can also affect the efficiency of feed utilization. Thus, delivering feed once a day (TMR group) can lead to wasting feed regardless of the amount of feed consumed by the animals, and distributing feed to ensure total consumption can be a cost-effective alternative (Pritchard and Bruns, 2003). Likewise, some feeding patterns can cause excessive consumption at a certain time of day, which would result in an increased incidence of digestive problems. In this respect, hopper feeders supply fresh feed as it is consumed, thus ensuring a consistent and constant consumption over time in the CONC group. Decreases in pH would be related to large food intake in the hours prior to this. When the evolution of mean pH throughout the day for each experimental group is analyzed (Figure 1), hourly pH values are significantly different (P < 0.05) during the day.

As the amount of fermentable matter that reaches the rumen is higher, pH is lower (Bach et al., 2007). The pH values tend to peak in the moments before feed intake, begin to fall afterward, and reach the minimum between 3 and 6 h after feed intake depending on the ration (Duffield et al., 2004; Marden et al., 2005; Palmonari et al., 2010). As acid production decreases, volatile fatty acid absorption and rumen buffer capacity (mainly from saliva) increase, and pH begins to recover initial values; this process is estimated to last approximately 18 h (Whitelaw et al., 1970; Maekawa et al., 2002; Palmonari et al., 2010). The pH pattern observed in TMR animals suggests that most of the feed is consumed as soon as it is delivered. In the present study, animals in the CONC group have a more uniform pattern of pH decline (more stable mean pH) than those in the TMR group (Figure 1). In any case, pH
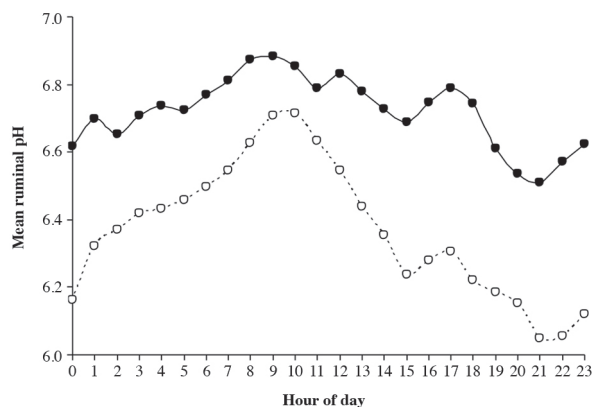
rises during the night and the maximum value is reached around 09:00-10:00 h for both groups, that is, just before feed is delivered to TMR animals. The nadir is reached at 21:00-22:00 h probably as a consequence of an evening meal for all the animals.

Ruminal temperature

The intraruminal temperature measurement as an indicator of body temperature is independent of external disturbing factors and cannot be manipulated from the outside although it is approximately 0.5 °C higher than core body temperature due to the activity of heat-producing rumen microorganisms (Sievers et al., 2004). This makes measuring temperature a suitable and feasible way to detect potential adverse changes in the animals' health in real time (Wahrmund et al., 2012). However, water consumption dramatically reduces ruminal temperature for a period of time proportional to the water temperature and amount of water ingested. It will take between 20 min and 2 h for ruminal temperature to recover (Yamada et al., 2001; Bewley et al., 2008).

In this study, no significant linear relationship between ruminal pH and temperature was observed. Sudden changes in the diet involving rapid and continuous drops in ruminal pH can lead to increases in ruminal temperature (AlZahal et al., 2007; 2008). In fact, these authors found a significant linear relationship between ruminal pH and temperature (AlZahal et al., 2008; Wahrmund et al., 2012), which led them to suggest that ruminal temperature can be used to diagnose subacute ruminal acidosis (AlZahal et al., 2008). However, in the present study, no relationship between ruminal pH and temperature was observed. The fact that animals were not in a ruminal acidosis situation (periods of more than 3 h at pH < 5.5, Wahrmund et al., 2012) and the wide range of pH and temperature values found in the current experiment compared with the narrow ranges cited by these authors (pH 5 to 5.6 and 39-41 °C, AlZahal et al., 2007; Wahrmund et al., 2012) may have accounted for this lack of relationship.

CONCLUSIONS

The results of the present study indicate that although animal performance is not affected, feeding fattening calves a concentrate plus barley straw diet can produce better rumen conditions than a maize silage-based TMR.

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