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Research Article

# Soyhulls, Corn or Barley as Supplements of a High-Quality Pasture Silage: Effects on *In Vitro* Ruminal Fermentation

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## Abstract

Two experiments were conducted to study the in vitro effects of combining different supplement concentrates with high quality pasture silage. In Experiment I, the effect of increasing the proportion of soyhulls, corn or barley in mixtures with pasture silage from 0 to 100% on in vitro gas production parameters was evaluated. Experiment II determined fermentation activity of inocula from heifers consuming pasture silage alone or silage supplemented with soyhulls, corn or barley at 1% of BW; the rumen fluids were incubated in vitro with forages or concentrates. The gas data were fitted to a simple exponential model with lag phase. Increasing the level of concentrates enhanced the gas volume and decreased its production rate ($P< 0.001$), but these responses were different for each concentrate. Lag phase was longer as inclusion levels of corn and barley were increased ($P < 0.001$), but it did not change with the inclusion of soyhulls. No differences were determined between the inocula of heifers supplemented with soyhulls, corn or barley when forages were used as substrates, but differences were evident when the substrates were concentrates. Inoculum from the animals supplemented with barley had a rapid and short fermentation, while the inoculum from non-supplemented animals led to the highest gas production and lag time. Supplementation with corn and soyhulls generated similar responses, despite the differences in carbohydrate composition between the two. The diet of the donor determined the inoculum activity, which subsequently interacted with the type of substrate incubated, leading to important effects on gas production profiles. Therefore, the interpretation of gas production outcomes of mixed feedstuffs or diets should account for these factors.

**Keywords:** Concentrates; Silage; Ruminal Digestion

## Introduction

The inclusion of concentrates in ruminant forage-based diets often causes associative effects due to digestive and metabolic interactions. Positive effects can occur when the combination supplies a limiting nutrient either to the ruminant or to the ruminal microbiota, as is typical when limited quantities of concentrates are added to low quality forages, and these combinations are linked to higher OM digestibility in the resulting mixtures [1]. Negative effects generally occur when a larger proportion of highly fermentable concentrates are included, and these mixtures often lead to lower forage intake and digestion, due to a reduction in fiber degradation rate [2]. It is difficult to predict whether an associative effect will be positive or negative, and errors in mixing practices usually lead to undesirable animal responses and the misuse of feed resources.

It is also known that the inclusion of grains in diets changes the species composition of the ruminal bacterial community [3,4] and that supplementation with different types of cereals leads to distinct rumen microbial ecosystems [5]. However, the literature contains no studies comparing rumen responses as concentrate inclusion in a forage-based diet increases.

The in vitro gas production technique is a useful tool to evaluate several combinations of feedstuffs at the same time [6-8], but the technique has some limitations. For example, Rymer et al. [9] report that the gas production profiles of a particular feedstuff may be distinctive only if the inoculum is harvested from animals fed the same feedstuff. Therefore, changes in the inoculum may not be expressed in the same way when it is incubated on different feeds.

The present study aims to determine the effects on in vitro ruminal fermentation of the inclusion of different types of concentrates in a high-quality forage diet. In order to cover different aspects of the matter, two complementary approaches were used, and two experiments were performed: the first studied the fermentation characteristics of mixtures composed of high quality pasture silage and increasing levels of soyhulls, corn or barley incubated with the same inoculum (Experiment I), while the second studied the fermentation activity of inocula from donors fed high quality pasture silage and supplemented with soyhulls, corn or barley over different substrates (Experiment II).

## Materials and Methods

The two experiments were conducted at the Experimental Farm of the Veterinary Faculty (Facultad de Veterinaria-UdelaR, San José, Uruguay, 34° 41' S and 56º 32' W). The care and handling of the experimental animals were approved by the Bioethics Committee of the Veterinary Faculty.

### Experiment I

This experiment was conducted to determine the effects of increasing the proportion of different concentrates in the substrate on in vitro gas production parameters. Mixtures of high quality pasture silage with soyhulls, corn or barley were prepared to include each concentrate at levels from 0 to 100% in 10% increments (a total of 31 mixtures) and used as substrates. The chemical composition of the concentrates and the forage used are given in table 1. Fresh ruminal liquor from a lactating Holstein cow (BW = 580 kg), collected from the ventral sac of the rumen through a fistula, was used as inoculum. During the previous 15 d, the donor cow had consumed the same pasture silage used as the substrate as the only food. Each combination of concentrate and level was incubated in triplicate, and 3 fermentation flasks with no substrate were included as inoculum blanks (96 flasks in total).

### Experiment II

To evaluate the effect of the concentrate supplements on the in vitro fermentation activity of the inoculum, 4 sources of ruminal fluid were incubated with 2 types of feedstuffs. Twenty-four Hereford heifers (mean BW = 224.2 kg, S.E.M. = 4.2) were individually housed and randomly assigned to 4 different diets: pasture silage alone (S), pasture silage supplemented with soyhulls (S+SH), pasture silage supplemented with barley (S+B) or pasture silage supplemented with corn (S+C). The silage and supplements were the same as those used in Experiment I (Table 1), and the feed was offered daily from 7:00 until 20:00 h. Pasture silage was offered ad libitum, and the concentrates were consumed prior to this offering at 10 g DM/kg BW once daily. The supplementation level and feeding management were selected to match the commonly used agronomic practices of farmers in the temperate zone of South America. Daily total intake per heifer was measured (offered - refused food) and was similar between groups (mean = 7198 g DM/d, S.E.M. = 249.5, $p$ = 0.078). The average concentrate intake of the supplemented animals reached 31% of the total feed consumed. Daily mean ruminal N-NH3 concentrations were 12.96, 18.53, 15.78 and 18.17 mg/dL for S, S + SH, S + C and S + B diets, respectively (S.E.M. = 0.482, $p < 0.001$).

| Item | Soyhulls | Barley | Corn | Pasture silage | Birds foot trefoil* | Oats forage* |
|---|---|---|---|---|---|---|
| DM | 902.2 | 895.4 | 884.8 | 226.7 | 317.4 | 195.2 |
| OM | 939.3 | 956.6 | 987.3 | 868.9 | 940.7 | 917.1 |
| CP | 154.6 | 949.1 | 86.2 | 167.8 | 108.1 | 84.0 |
| NDF | 570.2 | 229.3 | 121.0 | 449.5 | 478.4 | 576.3 |
| ADF | 390.1 | 87.0 | 34.0 | 319.7 | 303.6 | 294.8 |

**Table 1:** Chemical composition of the feedstuffs used for the study [g/kg, DM basis].

**Note:** * feedstuffs only used in experiment I.

The in vitro fermentation activity of the inocula was evaluated by a gas production trial. After a 21-d adaptation period, 60 mL of rumen fluid were collected from each animal 8 h after the beginning of the meal. Rumen fluid from those animals consuming the same diet was mixed. The mixtures of ruminal fluid (n = 4) were used as inocula for the 2 types of substrates: "forages" (pasture silage, birdsfoot trefoil (L. *corniculatus*) and oats) and "concentrates" (soyhulls, corn and barley), with the understanding that different substrate types would generate different ruminal environments. Each combination of substrate and ruminal fluid was incubated in triplicate, and 3 fermentation flasks with no substrate per rumen fluid source were included as inoculum blanks (84 flasks in total).

### *In vitro* gas production procedure

The same in vitro gas production technique was used in both experiments. The substrates were dried and weighed (0.5 g) into 125 mL fermentation flasks. A total volume of 40.5 mL of N-free medium [10], lacking VFA and vitamin solutions, was added to each flask. The flasks were then stoppered with butyl-rubber septa and refrigerated (4ºC) for 8 h before inoculation to hydrate the substrate. Immediately prior to inoculation, the flasks were randomly placed in a water bath at 39ºC, where they remained for the entire measurement period. Each flask was inoculated with 10 mL of rumen fluid, stoppered with a butyl-rubber septum and sealed with

aluminum crimp. All manipulations were performed under a $CO_2$ stream.

Gas pressure measurements were conducted using a pressure meter with a transducer (Sper Scientific Ltd., Scottsdale, USA) at 2, 4, 6, 8, 10, 12, 18, 24, 48, 72 and 96 h after inoculation. After the pressure readings, the gas was vented. Gas volume in mL was estimated according to the equation V = 4.40 P +0.09 P2 (V: gas volume in mL and P: observed pressure in psi; $R^2$=0.998), which had been obtained in a previous experiment under the same conditions.

### Chemical analyses

Prior to the analyses (except for dry matter), the feedstuffs were oven-dried for 48 h at 60 ºC and ground through a 1-mm screen. Dry matter (DM) was determined by drying the material at 105 ºC to constant weight. Ash and crude protein (CP) were determined using the AOAC [11] methods (942.05 and 984.13, respectively); neutral detergent fiber (NDF) and acid detergent fiber (ADF) were assayed sequentially according to the methods of Robertson and Van Soest [12] using an ANKOM220 fiber analyzer (ANKOM Technology Corp., Macedon, NY, USA) with a heat-stable alpha-amylase and no sodium sulfite, and these values were expressed inclusive of residual ash. Organic matter (OM) was calculated by the following equation: (OM % = 100 - Ash %).

### Calculations and statistical analyses

The gas volume data obtained from each fermentation flask were fitted by nonlinear regression using PROC NLIN of SAS® (version 8.02, SAS Institute Inc., Cary, NC, USA) to the model

$V = a\ (1\text{-}e^{-kd\ (t\text{-}L)})$

where "V" (mL/g OM incubated) is cumulative gas production at time t; "a" (mL/g OM incubated) is potential gas production; "kd" ($h^{-1}$) is the fractional rate of gas production and "L" (h) is the lag time of gas production.

The data from experiment I were analysed using PROC MIXED of SAS® (version 8.02, SAS Institute Inc., Cary, NC, USA) according to the following model

$Yijk = \mu + Ci + Lj + (C*L)ij + \varepsilon ijk$

where $Yijk$ is the observed in vitro gas production parameter, $\mu$ is the overall mean, $Ci$ is the fixed effect of the concentrate $i$ ($i$ = soyhulls, corn or barley), $Lj$ is the fixed effect of the inclusion level $j$ ($j$ = 0 to 100%, in 10% increments), $(C*L)ij$ is the interaction between concentrate $i$ and inclusion level $j$ and $\varepsilon ijk$ is the residual error. The fermentation flask was considered to be the experimental unit.

When interactions between the concentrate and the inclusion level parameter were significant, linear and quadratic regressions were used to describe the impact of the inclusion level of each concentrate on the gas production parameters (experiment I) using PROC GLM of SAS® (version 8.02, SAS Institute Inc., Cary, NC, USA). The fermentation flask was considered the experimental unit. Differences were considered statistically significant at $p < 0.05$ and were significant trending at $0.05 < p < 0.10$.

The data from experiment II (effect of supplementation with different concentrates on ruminal fermentation activity) were analysed using PROC MIXED of SAS® (version 8.02, SAS Institute Inc., Cary, NC, USA) according to the following model:

$Yijk = \mu + Ii + Fj + (I*F)ij + \varepsilon ijk$

where $Yijk$ is the observed in vitro gas production parameter, $\mu$ is the overall mean, $Ii$ is the fixed effect of the inoculum $i$ ($i$ = S, S+SH, S+B or S+C) measured in $k$ replicates (3 flasks), $Fj$ is the fixed effect of the type of feedstuff $j$ ($j$ = forage or concentrate), $(I*F)ij$ is the interaction between inoculum $i$ and type of feedstuff $j$ and $\varepsilon ijk$ is the residual error. Each forage or concentrate was considered the experimental unit. The means of inoculum and feedstuff type were separated using the LSMEANS procedure of SAS® (version 8.02, SAS Institute Inc., Cary, NC, USA). If the interaction I*F was significant, the main simple effect of inoculum was analysed using the "SLICE" option. Differences among means with $p < 0.05$ were considered statistically significant and were significant trending at $0.05 < p < 0.10$.

## Results

### Experiment I

Figure 1 shows the responses of gas volume (1A), gas production rate (1B) and lag time (1C) to the increasing concentrate levels in the substrate. All of the concentrates responded differently to increases in their levels, as significant interactions among them were observed for all parameters. Therefore, the effect of increasing the concentrate level was analysed by linear and quadratic regressions for each concentrate separately (Table 2). Gas volume ("a") rose linearly as the level increased for each of the concentrates, and only the slope differed among them (1.31*x for soyhulls, 1.09*x for corn and 1.05*x for barley, data not shown in the table). As the levels of soybean hulls and barley increased, the rate of gas production ("kd") decreased at decreasing rates (Linear: $p < 0.001$; Quadratic: $p < 0.001$), but increasing the level of corn in the substrate only slowed the rate linearly (Linear: $p < 0.001$). Lag time increased at an increasing rate when the level of corn increased (Linear: $p < 0.001$; Quadratic: $p = 0.011$) and at a constant rate when barley was used (Linear: $p < 0.001$). Lag time did not change with increasing levels of soyhulls.

### Experiment II

The fermentation activities of the inocula were different between forage and concentrate substrate conditions (Table 3), as

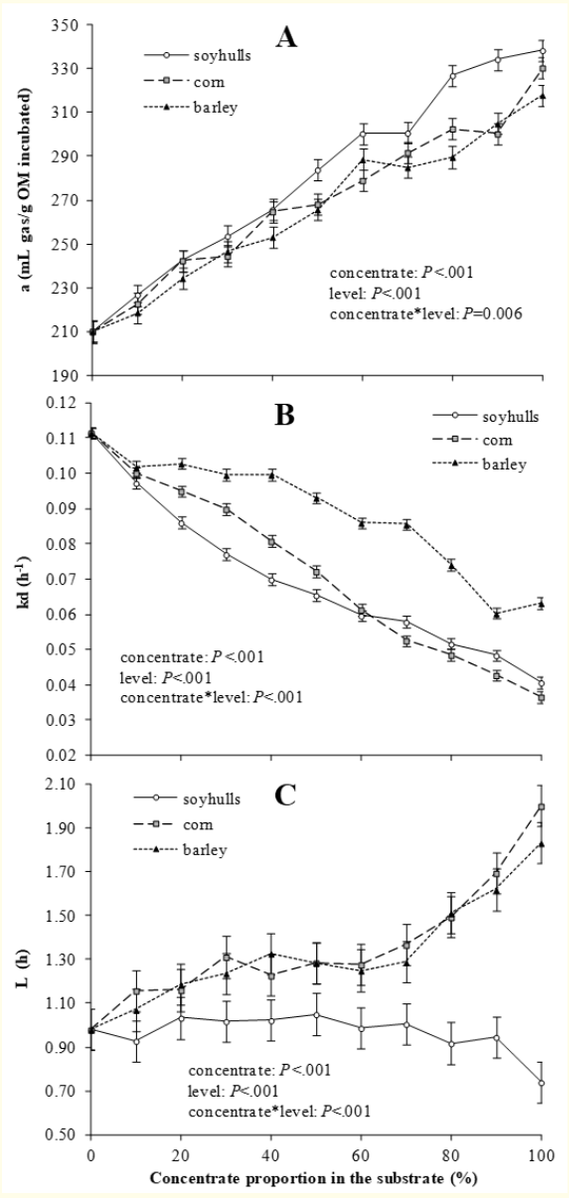


**Figure 1:** In vitro gas production parameters according to proportion of the different concentrates in the substrate. A: Potential gas production ("a") of soyhulls. B: Rate of gas production ("kd"). C: Lag time ("L").

significant interactions between the inoculum and the substrate were detected for gas production ("a") and for lag time ("L") ($p < 0.001$). Only when the substrate was a concentrate did the response in gas production significantly differ between rumen fluids. The lowest gas production ("a") and shortest lag time ("L") were observed when rumen fluid from animals supplemented with barley was used. Meanwhile, the inoculum from non-supplemented animals exhibited the highest gas production and lag times.

| Parameter | Concentrate | Linear | | Quadratic | |
|---|---|---|---|---|---|
| | | p-values | RSD[§] | p-values | RSD |
| a*(ml/g OM incubated) | soyhulls | <.001 | 7.59 | 0.106 | 7.34 |
| | corn | <.001 | 7.54 | 0.492 | 7.60 |
| | barley | <.001 | 8.03 | 0.197 | 7.93 |
| kd†($h^{-1}$) | soyhulls | <.001 | 0.006 | <.001 | 0.004 |
| | corn | <.001 | 0.003 | 0.135 | 0.003 |
| | barley | <.001 | 0.004 | <.001 | 0.003 |
| L‡(h) | soyhulls | 0.125 | 0.166 | 0.057 | 0.157 |
| | corn | <.001 | 0.181 | 0.011 | 0.165 |
| | barley | <.001 | 0.159 | 0.102 | 0.154 |

**Table 2:** Effects of increasing inclusion level of different concentrates on in vitro gas production parameters.

**Notes:** *potential gas production; †fractional rate of gas production; ‡lag time of gas production; §residual standard deviation

The rate of gas production ("kd") was lower for the inoculum from non-supplemented animals ($p = 0.001$). Although the interaction between treatment and substrate was only significant trending ($p = 0.069$), the low rates observed when the concentrates were incubated with the inoculum from non-supplemented animals is notable.

## Discussion

The increases in the concentrate levels generated unexpected responses. Although the increases in gas volume related to the increase of fermentable compounds in the substrates are not surprising, the results of the gas production rate and lag time are unusual. The reduction of the gas production rate was most likely due to the source of the rumen fluid used as inoculum, as it came from an animal fed exclusively pasture silage. However, this decrease was less pronounced when barley was used and may have been partially offset by the rapid rumen degradation of the barley's starch [13]. The longer lag times observed with increasing grain (barley and corn) content suggest that the adhesion of the microbiota to particles was affected by starch inclusion. The increase of soyhulls in the substrate also led to unusual responses, enhancing the gas volume and decreasing the rate of gas production similar to the starchy concentrates but not affecting the lag time. This latter feature may be attributable to the increase of substrates used by cellulolytic bacteria, which would cause higher levels of bacterial attachment to the

| Parameter | Substrate: Forages | | | | Substrate: Concentrates | | | | *SEM*[#] | *p*-values | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *F*[§] | *F+SH*[&] | *F+B*[¶] | *F+C*[∞] | *F* | *F+SH* | *F+B* | *F+C* | | Inoculum | Substrate | Interaction[£] |
| a[*] (mL/g OM incubated) | 213 | 228 | 229 | 225 | 309[x] | 273[yz] | 255[z] | 289[xy] | 5.9 | 0.112 | <.001 | <.001 |
| kd[†] ($h^{-1}$) | 0.07[b] | 0.08[a] | 0.08[a] | 0.07[ab] | 0.04[b] | 0.07[a] | 0.07[a] | 0.06[ab] | 0.005 | 0.020 | 0.001 | 0.069 |
| L[‡] (h) | 1.7 | 1.4 | 1.4 | 2.0 | 3.0[x] | 2.0[y] | 1.2[z] | 1.9[y] | 0.12 | <.001 | 0.002 | <.001 |

**Table 3:** Fermentation activity of the inocula of heifers fed high quality pasture silage alone or silage supplemented with soyhulls, corn or barley, according to the type of substrate used (forages or concentrates).

**Notes:** [*]potential gas production; [†]fractional rate of gas production; [‡]lag time of gas production; [§]inoculum from pasture silage diet; [&]inoculum from pasture silage supplemented with soyhulls diet; [¶]inoculum from pasture silage supplemented with barley grain diet; [∞]inoculum from pasture silage supplemented with corn grain diet; [#]standard error of the means; [£]interaction of inoculum*substrate. [xyz] Within a row, for each substrate, means followed by different letters are significantly different (simple main effects studied using SLICE); [abc] Within a row, means followed by different letters are significantly different (inoculum effect compared by LSmeans).

cell walls. This hypothesis is supported by Barrios-Urdaneta., *et al*. [14], who reported higher levels of bacterial attachment to straw cell walls after 8 and 12 h of incubation in a medium supplemented with a soluble fiber source (pectin) rather than starch and sugars. Additionally, the significant increase in gas production observed with increasing soyhulls content could be due to CH4 production, as this concentrate contains high amounts of degradable fiber.

No differences for gas production were found between the inocula of donors supplemented with soyhulls, corn or barley when the substrates were forages, but differences were present when the concentrates were used as substrates. This result was unexpected, as previous *in vivo* reports mention the detrimental effect of supplementation on fiber fermentation [15]. In a previous study using rumen fluid from cattle supplemented with corn or wheat, Cajarville., *et al*. [16] observed lower *in vitro* digestion rates when wheat was used, with particular impact on fiber digestion. Moreover, rumen fluid from non-supplemented animals in the present work, which theoretically should have contained a higher proportion of cellulolytic bacteria, did not show better fermentation activity on the incubated forages. This response may be related to the high quality of the forages used as substrates, which had NDF and ADF levels of approximately 50 and 30%, respectively. When high quality forages are used, the type of concentrate does not affect ruminal activity, at least at the supplementation levels utilized in the present work.

When the concentrates were used as substrates, however, differences in fermentation activity were apparent between inocula. Trei., *et al.* [17] incubated barley and sorghum for 3 h and found higher gas volumes using rumen fluids from grain-fed steers as compared to hay-fed ones. In our experiment, differences were observed not only among supplemented or unsupplemented animals but also among the types of supplement used. Moreover, the type of starch consumed by the donor influenced the *in vitro* gas production profile. The inoculum from animals supplemented with barley rapidly attacked the substrate, as indicated by short lag times, but yielded small overall gas volumes. These results suggest that the inoculum had a higher microbial activity but also that the microbial environment was negatively affected over time. This response may also be associated with the batch-culture *in vitro* technique employed in this study. Meanwhile, corn supplementation generated rumen fluids exhibiting similar gas volumes and lag times to those of soyhulls supplementation, despite the large differences between these concentrates in terms of carbohydrate composition.

In summary, the unusual results of experiment I may be explained by the lack of adaptation of the microbiota to the concentrates, and the data from experiment II suggest that the interpretations of gas production profiles are tightly associated with the diet of the donor. Despite the fact that fermentation course took place in a strongly buffered environment (characteristic of batch-culture techniques), and consequently only pH-independent effects could be tested; the inocula activities were greatly affected by the donor diets and were differently expressed according to the type of feed used as a substrate. Therefore, the results obtained with this technique cannot be separated from the use of specific rumen fluids.

## Conclusion

Increasing the inclusion level of different concentrates in high quality silage raised gas volume but slowed the rate of gas production. This unusual response may be due to the use of an inoculum provided by an animal fed only forage. The gas production profiles differed according to the concentrate used as the donor's supplement, and differences in this effect were observed between forage and concentrate substrate conditions. Considering the results from both experiments, the diet of the inoculum donor appears to have a major role on gas production, but this effect also depends of the

type of substrate incubated. As a consequence, the interpretation of *in vitro* experiments should consider the interaction between the diet of the donor and the substrate incubated.

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## Bibliography

1. Moore JE., *et al*. "Effects of supplementation on voluntary forage intake, diet digestibility, and animal performance". *Journal of Animal Science* 77 (1999): 122-135.

2. Dixon RM and Stockdale CR. "Associative effects between forages and grains: consequences for feed utilisation". *Australian Journal of Agricultural Research* 50 (1999): 757-773.

3. Van der Linden Y., *et al*. "Influence of supplementation of corn stover with corn grain on the fibrolytic bacteria in the rumen of sheep and their relation to the intake and digestion of fiber". *Journal of Animal Science* 59 (1984): 772-783.

4. Tajima K., *et al*. "Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR". *Applied and Environmental Microbiology* 67.6 (2001): 2766-2774.

5. Martin C., *et al*. "Effect of wheat and corn variety on fiber digestion in beef steers fed high-grain diets". *Journal of Animal Science* 77 (1999): 2269-2278.

6. Sandoval-Castro CA., *et al*. "A mixture simplex design to study associative effects with an in vitro gas production technique". *Animal Feed Science and Technology* 101 (2002): 191-200.

7. Robinson PH., *et al*. "Evaluation of the extent of associative effects of two groups of four feeds using an *in vitro* gas production procedure". *Animal Feed Science and Technology* 150 (2001): 9-17.

8. Kiran D and Krishnamoorty U. "Rumen fermentation and microbial biomass synthesis indices of tropical feedstuffs determined by the *in vitro* gas production technique". *Animal Feed Science and Technology* 134 (2007): 170-179.

9. Rymer C., *et al*. "*In vitro* cumulative gas production techniques: History, methodological considerations and challenges". *Animal Feed Science and Technology* 123-124 (2005): 9-30.

10. Williams BA., *et al*. "An *in vitro* batch culture method to assess potential fermentability of feed ingredients for monogastric diets". *Animal Feed Science and Technology* 123-124 (2005): 445-462.

11. Association of Official Analytical Chemist (AOAC). Official Methods of analysis. 15th edition". Arlington VA, USA (1990).

12. Robertson JB and Van Soest PJ. "The detergent system of analysis and its application to human foods". In: James WPT, Theander O, editors. Analysis of dietary fiber in foods. New York: Marcel Dekker (1981): 123.

13. Offner A., *et al*. "Quantitative review of in situ starch degradation in the rumen". *Animal Feed Science and Technology* 106 (2003): 81-93.

14. Barrios Urdaneta A., *et al*. "*In vitro* microbial digestion of straw cell wall polysaccharides in response to supplementation with different sources of carbohydrates". *Australian Journal of Agricultural Research* 51 (2000): 393-400.

15. Mould FL., *et al*. "Associative effects of mixed feeds. I. effects of types and level of supplementation and the influence of the rumen fluid pH on cellulolysis *in vivo* and dry matter digestion of various roughages". *Animal Feed Science and Technology* 10 (1983): 15-30.

16. Cajarville C., *et al*. "Rumen pH, $NH_3$-N concentration and forage degradation kinetics of cows grazing temperate pastures and supplemented with different sources of grain". *Animal Research* 55 (2006): 511-520.

17. Trei J., *et al*. "Effect of grain processing on in vitro gas production". *Journal of Animal Science* 30 (1970): 825-831.