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COMPARISON OF RUMINAL DEGRADABILITY OF STARCH OF MAIZE GRAIN AND MAIZE SILAGES BY *IN VITRO* METHOD

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Abstract

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The objective of this experiment was to determine the ruminal degradability of starch using the *in vitro* method. Maize silages from whole plants (MS), maize cob silage prepared by ensiling of the cobs with bracts (MCS), and maize grain silages (MGS) were compared with maize grain (MG). The tested feeds differed in total starch contents. Averaged starch contents with standard deviation values were 343.3 ± 18.7 g/kg for MS; 522.0 ± 8.9 g/kg for MCS; 727.9 ± 16.9 g/kg for MGS; and 682.8 ± 22.6 g/kg dry matter for MG. The *in vitro* ruminal degradability of starch was determined by the feed incubation period – for 2, 4, 6, 16 and 24 hours. The higher degradation was observed at the 4-h of incubation for maize silage from whole plants (46.6 ± 4.9 %), maize cob silage (41.2 ± 1.6 %) and maize grain silage (39.7 ± 1.4 %) as compared to maize grain (32.0 ± 1.0 %). Enhanced values of the *in vitro* ruminal degradability of starch continued to increase until the 24-h of incubation, when the differences between silages and maize grain were balanced. A significant difference of the curves of degradability profile was found between silages and maize grain ($P < 0.0001$) but was not significant between MS, MCS and MGS.

maize silage, maize grain, starch; ruminal degradability, *in vitro* method

Starch is the primary energy component of cereal grains, that is the basic nutrient in diets used to promote high levels of production. The efficient utilization of grain by ruminants is a result of ruminal starch digestion (Ørskov, 1986). Cereal grains contain a relatively high amount of starch, with the highest percentage in maize grain. Maize starch has specific characteristics that are not known in other grains. Differences in the chemical and physical structure of starch granules determine the quality of starch in feed and its availability and digestibility. The most important site of maize grain starch digestion is the rumen where 50–95 % of starch is degraded. Studies comparing the ruminal starch degradability of different feeds (Nocek and Tamminga, 1991; Offner et al., 2003) confirmed that with regard to the degradation of starch, maize grain is a highly concentrated feed with a high content of lower degradable starch in the rumen (Philippeau et al., 1999). A lower degradability of starch in the rumen increases the amount of it which has to be digested in the small intestine (Nocek and Tamminga, 1991). For this reason maize with its high nutritional value and slow degradability of starch in the rumen is ideal for the feeding of high-yielding dairy cows. Feed rations composed of silage on maize basis are important sources of energy noted for their medium degradability in the rumen.

Various *in vivo* (Jensen et al., 2005), *in situ* (Michalet-Doreau et al., 1997) procedures, and *in vitro* laboratory techniques (Wester et al., 1992; Huhtanen and Sveinbjornsson, 2006) were used to predict the ruminal degradability and digestibility of starch. The effect of ensiling process, hybrid and maturity on ruminal starch degradability was quantified in *in sacco* and *in vitro* experiments (Philippeau and Michalet-Doreau, 1998; Verbic at al., 1995; Dado and Beek, 1998).

The objective of our study was to determine the ruminal starch degradability of different silages on maize basis and maize grains using the *in vitro* method. The starch degradability of selected feeds was assessed in five time intervals.

MATERIAL AND METHODS

Animals and diet

Two non-lactating cows (Black Pied) fitted with large ruminal cannulas were used for our experiments as donors of ruminal fluid for the *in vitro* method. The diets consisted of lucerne hay (4 kg), maize silage (10 kg) and ground barley (1 kg) with a mixture of vitamins and minerals. The diet was administered in two equal rations daily.

Feeds

The values of the *in vitro* ruminal degradability of starch were determined in 3 maize silage samples from whole plants (MS), 3 samples of maize silage prepared by cobs ensilage with bracts (MCS), 3 samples of maize grain silage (MGS), and 3 samples of ground maize grain (MG).

Analytical methods

The total starch content in the samples was estimated by total of α-linked glucosides, using the method described by MacRae and Armstrong (1968) and modified by Wester et al., (1992). Dry feed samples were milled through a 1 mm screen. Each feed was subject to triple analysis. The glucose was assayed enzymatically using the Bio-la-test, GLU GOD 250, (Lachema, Brno, Czech Republic). The starch content was calculated as 90% of the total glucose content and recalculated per sample of dry matter.

Chemical composition of feeds was analysed according to AOAC (2005).

In vitro ruminal degradability of starch (IVRDS)

The degradable starch content was determined through incubation of the samples in an incubation solution (buffer mixed with ruminal fluid) for 2, 4, 6, 16 and 24 hours. The buffer was prepared as described by Goering and Van Soest (1970). Ruminal fluid was taken from two cannulated cows, mixed, filtered, tempered to 39 °C and saturated with CO_2. Each feed was subject to triple analysis. Dry feed samples were milled through a 1 mm screen. The trial included a blank for each time interval, a glucose standard and a standard sample for elimination of the variability of ruminal fluid. A more detailed method of determining the IVRDS of feeds was described earlier (Tománková and Homolka, 2004).

Statistical analyses

Data analysis was performed using the nonlinear model with mixed effects, procedure of SAS (2001). The model was as follows:

$$\text{Content of starch}_{ij}(t) = 100 \cdot [1 - (b + \beta_i) \cdot e^{-(c + f_1 \cdot MS_i + f_2 \cdot MCS_i + f_3 \cdot MGS_i) \cdot t}] + \varepsilon_{ij},$$

where: t – incubation length,

β_i – random effect of sample i

ε_{ij} – random error of measuring

$b, c, f_1, f_2, f_3, d^2, \sigma^2$ – unknown constants:

Parameter	Estimate ± Standard error
b	1.2369 ± 0.03522
c	0.1368 ± 0.004405
f_1	0.07322 ± 0.008159
f_2	0.07335 ± 0.007801
f_3	0.07047 ± 0.007972
d^2	0.1138 ± 0.01673
σ^2	1.5089 ± 0.2688

The hypothesis about parameters were tested by the likelihood ratio test.

RESULTS AND DISCUSSION

The chemical composition of analyzed feeds and some chemical characteristics of the quality of the fermentation process in silage are given in Tab. I.

The silages differ as to the amount of starch (maize silage from whole plants: 343.3 ± 18.7 g/kg; maize cob silage: 522.0 ± 8.9 g/kg; maize grain silage: 727.9 ± 16.9 g/kg dry matter). Averaged starch content with standard deviation was 682.8 ± 22.6 g/kg for maize grain. The starch content of maize silages is a direct function of plant maturity and the proportion of grains in the whole plant (Huntington, 1997). Determination of starch content in tested feeds is presented in Fig. 1.

The results show that the highest content of starch was found in MGS which contains the highest amount of maize grains. On the other hand, the lowest content of starch was found in silage prepared from whole plants of maize. Mahanna (1994) reported that an increase in the percentage of grain in the silage increased the starch content of maize silage.

Results of *in vitro* starch degradability are presented in Tab. II.

The values of starch degradability for MS, MCS, MGS and MG measured after 2 hours of incubation in our *in vitro* experiments were comparable with results obtained by Doležal and Dvořáček (2000) by *in sacco* method. The *in sacco* and *in vitro* data gave the same ranking of the grains in terms of starch degradability (Herrera-Saldana et al., 1990). This indicates that *in vitro* data can represent starch degradability as it occurs in the rumen.

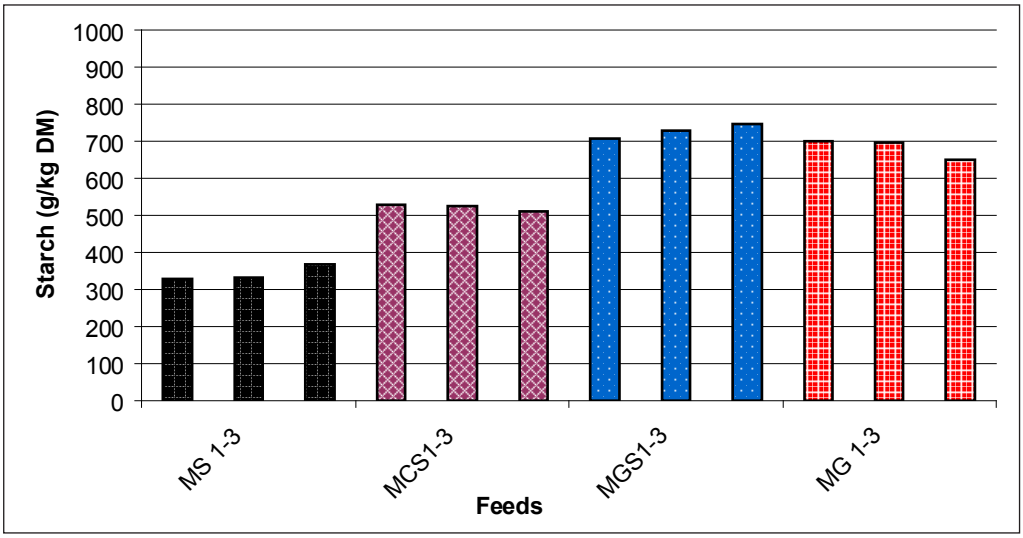
The increase of values IVRDS of silages started earlier than in the maize grain. The higher degradation was observed at the 4-h of incubation for maize silage from whole plants, maize cob silage and maize grain silage (Tab. II) as compared to maize grain where values ranging from 31.1 to 33.4% were found.

The lower values of starch degradation after a 6-h incubation period were found in maize grain (40.6–44.8%) when compared to the IVRDS values of silages (Tab. II). Dado and Beek (1998) found the IVRDS value for a 6-h incubation time in maize grain 39.1%, which corresponds to above-mentioned value. Contrary to our results, Doležal and Dvořáček

I: *Chemical composition of feeds*

Feeds	ODM	CP	Fat	Fibre	Ash	NFE	ADF	NDF	LA	AA	PA	pH
	g/kg	g/kg DM							% (ODM)			
MS_1	385.2	103.5	35.3	198.3	55.8	607.0	284.7	453.7	1.24	0.32	0.07	3.74
MS_2	304.8	92.0	39.5	230.6	49.1	588.7	274.9	456.2	1.32	0.25	0.06	3.80
MS_3	305.4	87.5	34.5	220.7	48.6	608.5	267.3	437.0	1.42	0.23	0.07	3.56
MCS_1	453.0	77.1	36.3	111.4	24.9	750.2	132.1	274.9	1.80	0.47	0.00	3.43
MCS_2	526.4	92.3	46.3	98.2	19.8	743.3	130.3	267.7	1.50	0.36	0.05	3.54
MCS_3	529.1	96.3	40.1	97.2	20.8	754.4	138.8	259.2	1.45	0.50	0.05	3.56
MGS_1	647.6	89.4	46.2	28.3	18.4	817.5	44.3	117.6	1.53	0.22	0.09	3.64
MGS_2	609.4	80.3	43.1	29.7	14.4	832.4	34.6	119.1	0.46	0.08	0.07	4.20
MGS_3	619.6	76.0	36.5	17.9	13.6	855.9	27.7	104.5	0.53	0.06	0.07	4.19
MG_1	898.7	100.4	44.0	22.5	18.1	814.9	48.9	119.7				
MG_2	906.7	90.6	46.1	34.6	14.7	783.1	65.3	102.5				
MG_3	906.2	104.1	57.5	44.8	24.4	769.1	61.4	139.1				

MS – maize silage from whole plants, MCS – maize cob silage with bracts, MGS – maize grain silage, MG – maize grains, ODM – original dry matter, CP – crude protein, Fat – crude fat, Fibre – crude fibre, NFE – nitrogen-free extract, ADF – acid detergent fibre, NDF – neutral detergent fibre, LA – lactic acid, AA – acetic acid, PA – propionic acid.



MS – maize silage from whole plant, MCS – maize cob silage, MGS – maize grain silage, MG – maize grain

1: *Total starch content of MS, MCS, MGS and MG in dry matter*

II: *In vitro degradability of starch (%) in 2, 4, 6, 16 and 24 h incubation of silages (MS, MCS, MGS) and maize grain (MG)*

Incubation length (h)	Feeds											
	MS_1^a	MS_2^a	MS_3^a	MCS_1^a	MCS_2^a	MCS_3^a	MGS_1^a	MGS_2^a	MGS_3^a	MG_1^b	MG_2^b	MG_3^b
2	19.32	22.28	23.98	9.69	20.07	19.03	10.15	15.84	14.89	21.30	16.20	14.09
4	39.70	50.23	49.72	41.02	43.15	39.34	41.06	40.25	37.84	31.59	33.36	31.14
6	58.63	68.33	70.44	63.41	66.04	68.59	66.63	69.35	70.84	40.57	43.16	44.79
16	92.29	96.62	97.34	95.39	97.52	97.45	95.08	95.71	95.27	81.76	85.25	85.46
24	95.88	99.00	99.30	98.90	99.41	99.43	98.58	99.32	98.57	97.34	97.93	96.84

MS – maize silage from whole plants, MCS – maize silage prepared by ensiling cobs, MGS – maize grain silage, MG – maize grain, $^{a-b}$ curves of degradability profile in columns with different letters differ significantly ($P < 0.0001$)

(2000), using the *in sacco* method, found lower values of degraded starch in the maize silages after 8 hours of incubation in the rumen (54% for MCS and 48% for MGS). In our *in vitro* experiments, higher values of degraded starch in silages were found even after the 6-h of incubation (63.4–68.6% for MCS and 66.6–70.8% for MGS). Degradability of ruminal starch can be influenced by a method of feed processing and type of diet. The advantages of *in vitro* method include higher reproducibility in comparison with other methods and quick and inexpensive determination of starch degradability. On the other hand, the *in vi*tro methods do not provide absolute values which are obtained on experimental animals because the kinetic properties intrinsic to the starch are not measuring accurately (Mills, 1999). From this point of view, they are more suitable for measuring the relative differences among the feeds.

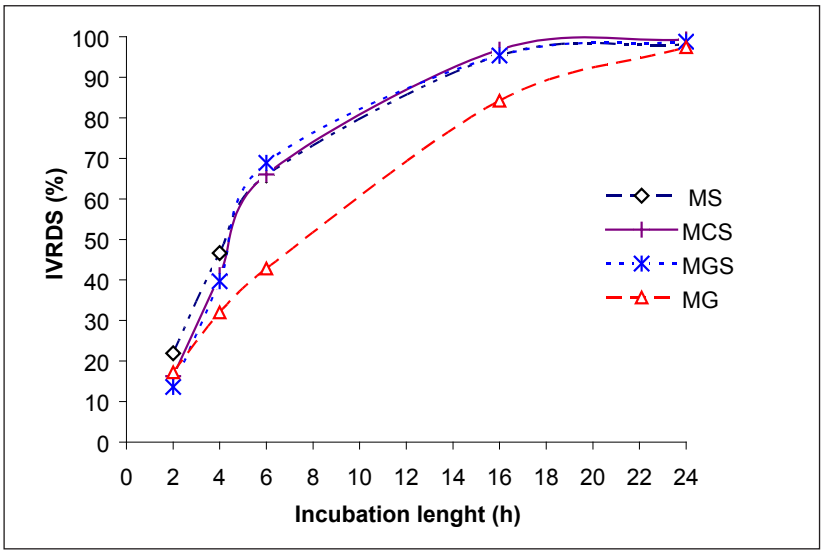
Small differences in IVRDS values were observed among the MS, MCS and MGS except for MG after the 16-h of incubation (Tab. II). We have found the IVRDS values ranging from 81.8 to 85.5% for maize grain. Dado and Beek (1998) observed the IVRDS value 89.2% even after a 12-h incubation period. The authors also pointed out the importance of sample preparation and the size of grain particles for the resulting value of ruminal starch degradability. Maize starch tends to be more slowly degraded within the rumen than other cereals, and it also benefits to a greater extent from physical and chemical processing techniques designed to increase ruminal and total-tract starch digestion (Mills et al., 1999).

In the case of 24-h of incubation only minimal differences in IVRDS values were found among all the feeds, including MG.

Lower values of starch degradability in maize grain in all assayed time intervals support the previous findings that ensiling of maize grain increased starch degradation in the rumen. These results correspond to data published by Doležal and Zeman (2005).

The comparison of curves of the degradability profile of maize silages and maize grain showed that degradability of starch was significantly higher between silages and maize grain ($P < 0.0001$). There where no significant differences for silages among themselves.

From the obtained results is possible conclude that curves of degradability profile of tested silages was nearly identical (Fig. 2).



MS – mean of 3 samples of maize silage from whole plant, MCS – mean of 3 samples of maize cob silage, MGS – mean of 3 samples of maize grain silage, MG – mean of 3 samples of maize grain

2: *In vitro ruminal degradability of starch of maize grain and maize silages*

However, the total content of starch in silage is an important factor in measuring the consequent extent of starch utilization after rumen degradation. These increases of IVRDS were found for silages measured between 4 and 24 hours of incubation intervals. On the contrary of these findings the MG had slower IVRDS. After 24 hour of incubation interval the differences between silages and MG were minimal.

Simple and rapid determination of the starch degradation in laboratory by *in vitro* method becomes useful for practical evaluation of nutritive value and quality of feeds.

SUMMARY

The objective of this experiment was to determine the in vitro ruminal degradability of starch (IVRDS) in the set of maize silages. The set included three types of maize silages made from (1) whole plants (MS), (2) maize cobs together with bracts (MCS), and (3) maize grain (MGS). These maize silages were compared with non-ensiled maize grain (MG). The total starch content was estimated by total of α-linked glucosides. The glucose was assayed enzymatically using the Bio-la-test, GLU GOD 250. The starch content was calculated as 90% of the total glucose. High contents of starch were found in MG (682.8 g/kg dry matter (±22.6 s.d)) and for silages in MGS (727.9 g/kg dry matter (±16.9 s.d.)) and MCS (522.0 g/kg dry matter (±8.9 s.d.)).

The IVRDS was calculated as a reciprocal value of sample residuum obtained after 2, 4, 6, 16 and 24 hours of incubation intervals. The IVRDS values measured after 2 hours of incubation were 17.2 ± 4.7% and 17.2 ± 3.0% for silages and MG, respectively. After 4 hours of incubation, the IVRDS values of silages were 42.5 ± 4.2% while MG showed a slower degradation (32.0 ± 1.0%) of starch, which continued even at 16 hours of incubation (84.2 ± 1.7%) compared to the maize silages (95.8 ± 1.6 %). After 24 hours of incubation only small IVRDS differences between silages (98.7 ± 1.0%) and MG (97.4 ± 0.5%) were observed.

It is possible to conclude that the ensiling of MG increased the rate of the IVRDS. The increased IVRDS were found in the silages measured after 4 to 24 hours of incubation. Contrary of these findings, MG exhibited a lower rate of the IVRDS. After 24 hour of incubation, the differences between silages and MG were minimal.

The *in vitro* estimated degradability of starch differed significantly ($P < 0.0001$) between the analysed silages and maize grain. No significant differences ($P > 0.05$) were, however, detected between the different types of silages.

SOUHRN

Porovnání bachorové degradovatelnosti škrobu kukuřičného zrna a kukuřičných siláží *in vitro* metodou

Cílem práce bylo metodou *in vitro* stanovit bachorovou degradovatelnost škrobu (IVRDS) u souboru kukuřičných siláží. Soubor zahrnoval tři druhy kukuřičné siláže: (1) siláž z celých rostlin (MS), (2) siláž z kukuřičných palic s listeny (MCS) a (3) siláž kukuřičného zrna (MGS) v porovnání s nesilážovaným kukuřičným zrnem (MG). Obsah škrobu byl stanoven pomocí Bio-la-testu, GLU GOD 250 a vypočten jako 90% z celkové glukózy. Vysoký obsah škrobu byl kromě MG (682,8 g/kg sušiny (±22,6 s.d.)) u siláží MGS (727,9 g/kg sušiny (±16,9 s.d)) a MCS (522,0 g/kg sušiny (±8,9 s.d.)).

Degradovatelnost škrobu v bachoru (IVRDS) byla vypočítána z hodnot ve zbytcích krmiv (jako reciproká hodnota) po 2, 4, 6, 16 a 24hodinové inkubaci vzorku. Hodnoty IVRDS se pohybují v rozmezí 17,2 ± 4,7% u siláží po 2 hodinách inkubace. U kukuřičného zrna byla stanovena průměrná hodnota 17,2 ± 3,0%. V případě 4hodinové inkubace jsou průměrné hodnoty IVRDS se směrodatnou odchylkou u všech siláží 42,5 ± 4,2% a zrno kukuřice začíná vykazovat zpomalování degradovatelnosti škrobu (32,0 ± 1,0 %), které pokračuje i při 16hodinové inkubaci (84,2 ± 1,7%) oproti hodnotám IVRDS u siláží (95,8 ± 1,6 %). Po 24hodinové inkubaci jsou rozdíly hodnot IVRDS mezi silážemi (98,7 ± 1,0%) a zrnem kukuřice (97,4 ± 0,5%) minimální.

Závěrem lze říci, že silážováním kukuřice se urychluje degradovatelnost škrobu v bachoru. Na rozdíl od kukuřičného zrna hodnoty IVRDS u siláží začínají stoupat již po 4hodinové inkubaci. Po 24 hodinovém inkubačním intervalu se rozdíly mezi kukuřičnými silážemi a kukuřičným zrnem vyrovnávají. Průkazné rozdíly v degradovavatelnosti škrobu jsou mezi testovanými silážemi a kukuřičným zrnem ($P < 0,0001$) ale mezi silážemi navzájem nebyly zjištěny průkazné rozdíly ($P > 0,05$).

kukuřičná siláž, kukuřičné zrno, škrob, bachorová degradovatelnost, *in vitro* metoda

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REFERENCES

AOAC, 2005: Association of Official Analytical Chemist. Official Methods of Analysis. 18th Edition, Washington, DC.

DADO, R. G., BEEK, S. D., 1998: *In vitro* ruminal starch digestibility in opaque–2 and regular corn hybrid. Anim. Feed Sci. Tech., 73, 151–160.

DOLEŽAL, P., ZEMAN, L., 2005: Effect of different forms of bacterial inoculants on the fermentation process of ensiled crushed maize moisture grains. Czech J. Anim. Sci., 50, (5), 201–207.

DOLEŽAL, P., DVOŘÁČEK, J., 2000: Význam degradovatelnosti škrobu ve výživě vysokoprodukčních dojnic. III.mezinárodní vědecká konference Agro-

region 2000, Jihočeská univerzita České Budějovice, 133–134.
GOERING, H. K., VAN SOEST, P. J., 1970: Forage fibre analyses (apparatus, reagents, procedures, and some applications). Agric. Handbook No 379, ARS, USDA, Washington DC.
HERRERA-SALDANA, R. E., HUBER, J. T., POORE, M. H., 1990: Dry matter, crude protein, and starch degradability of five cereal grains. J. Dairy Sci., 73, 2386–2393.
HUHTANEN P., SVEINBJORNSSON J., 2006: Evaluation of methods for estimating starch digestibility and digestion kinetics in ruminants. Anim. Feed Sci. Tech., 130 (1–2), 95–113.
HUNTINGTON, G. B., 1997: Starch utilization by ruminants: From basics to the bunk. J. Anim. Sci., 75, 852–86.
JENSEN, C., WEISBJERG, M. R., NORGAARD, P., HVELPLUND, T., 2005: Effect of maize silage maturity on site of starch and NDF digestion in lactating dairy cows. Anim. Feed Sci. Tech., 118, 279–294.
MACRAE, J. C., ARMSTRONG, D. G., 1968: Enzyme methods for determination of alpha-linked glucose polymers in biological materials. J. Sci. Food Agric., 19, 578–581.
MAHANNA, B., 1994: Proper management assures high-quality silage, grains. Feedstuffs, 66, 12.
MICHALET-DOREAU, B., PHILIPPEAU, C., DOREAU, M., 1997: *In situ* and *in vitro* ruminal starch degradation of untreated and formaldehyde-treated wheat and maize, Rep. Nutr. Dev., 37, 305–312.
MILLS, J. A. N., FRANCE, J., DIJKSTRA, J., 1999: A review of starch digestion in the lactating dairy cows and proposals for a mechanistic model: 1. Dietary starch characterisation and ruminal starch digestion. J. Anim. Feed Sci., 8, 291–340.
NOCEK, J. E., TAMMINGA, S., 1991: Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. J. Dairy Sci., 74, 3598–3629.
OFFNER, A., BACH, A., SAUVANT, D., 2003: Quantitative review of in situ starch degradation in the rumen. Anim. Feed Sci. Tech., 106, 81–93.
ØRSKOV, E. R., 1986: Starch digestion and utilization in ruminants. J. Anim. Sci., 63, 1624–1633.
PHILIPPEAU, C., LE DESCHAULT DE MORNREDON, F., and MICHALET-DOREAU, B., 1999: Relationship between ruminal starch degradation and the physical characteristics of corn grain. J. Anim. Sci., 77, 238–243.
PHILIPPEAU, C., MICHALET-DOREAU, B., 1998: Influence of genotype and ensiling of corn grain on *in situ* degradation of starch in the rumen. J. Dairy Sci., 81, 2178–2184.
SAS Institute Inc., 2001: Release 8.02 (TS2MO) of the SAS® System for Microsoft® Windows®. SAS Institute Inc., Cary, NC.
TOMÁNKOVÁ, O., HOMOLKA, P., 2004: *In vitro* ruminal degradability of cereal grain starch. Czech J. Anim. Sci., 49, 151–155.
VERBIC, J., STEKAR, J. M. A., RESNIK – CEPON, M., 1995: Rumen degradation characteristics and fibre composition of various morphological parts of different maize hybrids and possible consequences for breeding. Anim. Feed Sci. Tech., 54, 133–148.
WESTER, T. J., GRAMLICH, S. M., BRITTON, R. A., STOCK, R. A., 1992: Effect of grain sorghum hybrid on *in vitro* rate of starch disappearance and finishing performance of ruminants. J. Anim. Sci., 70, 2866–2876.

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