UDC 636.085.52:579.64

doi: 10.15389/agrobiology.2018.2.258eng doi: 10.15389/agrobiology.2018.2.258rus

BIOLOGY OF ALFALFA SILAGE MAKING (review)

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Abstract

Alfalfa dry matter is characterized by the less content of sugar, celluloses and hemicelluloses and more quantity of pectin in comparison to grasses (P. Mc-Donald et al., 1970). The high level of pectin provides increased rate of feed fermentation in a rumen (E.F. Annison, et al., 1962). This leads to improved assimilation of alfalfa silage dry matter by cattle, despite the low energy level unlike to cereal grasses silage (M. Grabov, 2016). As a result, the nutrients intake and productivity of cows increase. However, there are some particularities in qualitative alfalfa silageand haylage-making, such as absence of abundant Enterobacteriaceae bacteria on the alfalfa plants (R.A. Shurchno et al., 2008), unlike cereal grasses (Yu.A. Pobednov et al., 2015). Thereof the basic kind of alfalfa silage and haylage spoilage is butyric (putrid) fermentation. With due regard to this fact, the main principle of alfalfa conservation is based on the known rule of G.W. Wieringa (1963), which tells about increasing of clostridium bacteria sensitivity to active acidity (pH) of feed when dry matter content in plants rises. This allows providing feed preservation under significantly higher pH, than at ensiling the freshly-cut mass (F. Weissbach, 2012). However, fodder must reach fast acidification with determined pH value to eliminate a butyric fermentation at each dry matter content. But this condition especially difficult for performance at alfalfa ensiling, because plants contain much weak-bound water even at 35 % dry matter content, in contrast to cereal grasses and red clover. At weak acidification, it can lead to intensive proteolysis (X.S. Guo et al., 2012) with ammonia accumulation and an increase in buffer capacity of feed. As a result, pH of alfalfa silage does not decline to necessary level for elimination the clostridium bacteria growth during the long period and it causes to accumulation a butyric acid and the products of putrid decay of the proteins. It is possible to reduce the intensity of proteolysis by increased feed acidification with addition of liquid organic acids or inoculants of lactic acid bacteria combined with sugar. Another way is ensiling of alfalfa wilted to ≥ 40 % dry matter content followed by application of the lactic acid bacteria-based inoculants. At this level of dehydration, the content of sugar in dry matter increases 1.6 times (Yu.A. Pobednov et al., 2016), and addition of the bacterial inoculants leads to increasing a degree of feed acidification as well as storage and feed-out stability (F. Weissbach, 2012). Application of enzymes in ensiling alfalfa wilted to ≥ 40 % dry matter is one more advanced method of this forage crop conservation (A.A. Anisimov, 2006). Another effective approach of alfalfa silage-making is using enzyme additives combined with lactic acid bacteria (M. Grabov, 2016).

Keywords: alfalfa, proteolysis, dry matter content, acidification, lactic acid bacteria-based inoculants, enzymes, silage quality

Animals fed with poor silage experience deficit of nutritious matter, which negatively affects their productivity, health, meat and milk production [1]. The problem with production of the high quality feed is particularly acute at procurement of silage and haylage from alfalfa. It is more often applied in the agricultural practice since for many farming units production of the feed with content of up to 22 % raw protein in dry matter (DM) is a real way to increase the economic stability [2]. Besides high levels of raw protein, alfalfa is characterized by the less content of pectin, as well as presence of special structured fiber [3]. High pectin fermentation results in accelerated alfalfa fermentation in a ru-

men. Thus, digestibility of pectin and pectin acid in sheep reaches 90 % [4]. Alfalfa does not follow the known rule that animal consumption of bulky feed dry matter correlates to its nutrient value. Nutritional value of the dry matter in the diet with alfalfa silage is less that in the diet with grass silage (6.9 vs. 7.1 mJ/kg for net energy of lactation), however its daily consumption by cows is higher, 22.1-23.2 and 20.3-21.2 kg [5]. This results in increased consumption of nutrients and animal productivity.

At the same time, high content of raw protein in alfalfa is often associated with its main unfavorable technological attribute of a non-silage culture [6]. Having compared buffering capacity of various plant species containing raw protein, calcium, and magnesium, the researchers have found that the richer the plants in the said compounds, the higher their buffer capacity [7]. However, it is mineral compounds, and not the nitrogenous matter, that play the main role in improvement of buffer capacity. Alfalfa is rich in mineral substances with expressed properties of bases [8, 9]. High buffer capacity of alfalfa entails accumulation of significantly larger quantity of lactic acid than it is required for acidification of grasses and clover, which in the context of sugar deficit is hard to ensure at regular silage making even in sun-cured form. This fact increases the interest in use of lactobacillus medicines at alfalfa silage making.

Sugar deficit and buffer capacity of the biomass leads to necessity for sun-wilting of alfalfa up to ≥ 45 % of DM (10). Chemical content of plants may be improved by their wilting in mowing. Thus, after 6-hour mowing of alfalfa up to 35.1 % of DM, amount of sugars increased 1.3 times from 4.82 to 6.24 % [11]. Simultaneously, sugar-buffer ratio to cured mass increased from 1.0 to 1.4. Consequently, rapid dehydration of alfalfa provides transferring thereof from the category of non-ensilaging plants to the category of hardly-ensilaging plants. However, in alfalfa, unlike the clover and grasses, the procedure has no detectable effect on ensilaging results [12].

Earlier it was erroneously suggested than plants cured up to $\geq 35 \%$ DM are successfully ensilaged regardless of sugar content in them [13]. This suggestion still does not have an experimental confirmation [14, 15]. The concept of silage capacity of vegetable feed should also be clarified. In Russia it is still associated with chemical composition of the ensilaged mass and with misconception that wilting to up to 40 % DM does not have negative effect on the intensity of lactic-acid fermentation [16]. Meanwhile, even poor wilting up to 30 % DM noticeably retains development of lactobacillus resulting in slowing down the speed of feed acidification and, consequently, occurrence of the negative microbiological processes in the feed [15]. Nature of the later is totally dependent on the plant species and the extent of wilting. Because of that, even high concentration of sugar in cured grasses does not warrant production of high quality silage.

Ensilaging also depends on the factors that promote the plant ability to rapid and quiet strong acidification. The most important of them is quantitative and qualitative composition of epiphytic microflora. It is usually favorable at ensilaging of maize, 1 g of which at the middle dough stage contains $\geq 10^5$ CFU of lactobacillus [7]. In the above count they are represented by only highly active hormone-enzyme *Lactobacillus plantarum* [17] well-adapted to bulk fermentation with relatively high content of dry matter. Silage from the chopped maize, regardless of the significant DM content, is rapidly acidified at the middle dough stage to active acidity (pH) ≤ 4.2 that depresses development of all unfavorable bacterium. In perennial herbs, mainly alfalfa, the composition of epiphytic microbes association is often unfavorable [18], but determines not only the dominant type of feed damage, but also ways to avoid spoiling. It is established [19] that alfalfa is not characterized by the presence on coli group usual in large

number in grasses [17]. Mainly, alfalfa silage is damaged due to butyric (septic) fermentation [15]. It leads to the main conservation principle of alfalfa (rule of G.W. Wieringa) that is based on a fact that Clostridia sensitivity to feed acidity significantly increases along with increase of the dry matter in the green biomass [20]. It allows production of the oil acid free silage from the cured alfalfa at significantly higher pH as compared to ensilaging of new-mown grasses.

It should be noted that such rule applies only to ensilaging of highprotein leguminoze grasses, but becomes fully irrelevant at use of sugarcontained cured grasses with other composition of epiphytic microbial flora requiring application of other conservation methods. As apart from the highprotein leguminoze grasses, butyric fermentation is often neither eliminated, nor becomes more manifested (secondary fermentation) in ensilaging of grasses in cured form [14, 15]. This is due to intensive development of coli forms – enterobacterium against the background of slow feed acidification. Enterobacteria, because of ineffective sugar fermentation in grasses, lead to sugar deficit in the ensilaged mass and the butyric fermentation. Not only Clostridia, but also enterobacterium shall be suppressed to stop the butyric fermentation in the silage from cured grasses. Regardless of the wilting extent, grasses shall be acidified to $pH \le 4.3$ for 3 days [21] to exclude growth of enterobacteria. Accounting for the limited lactobacillus fermentation at beginning of ensilaging, the required acidification speed and extent may be achieved only with addition of osmotolerant lactobacillus strains. In lack of them, even easily ensilaging grasses cured up to ≥ 30 % DM are not such in fact [22]. Thus, in the global practice the term "fermentability" is recently used in lieu of "ensilage capacity" [23], the term that is based on the chemical composition of plants and defines their potential ability to acidification.

Note that the rule of G.W. Wieringa [20] is not fully correct for alfalfa. According to the rule, it could be concluded [7] that at sugar-buffer ratio of 0.5-10 the alfalfa shall be cured up to 37-41 % DM. However, it is impossible to produce qualitative feed from alfalfa by spontaneous ensilaging, provided such quantity of dry matter. Green alfalfa mass rich in protein and pectin contains a lot of poorly bound water [24]. Because of acidification delay, plant protein-egrading enzymes remain highly active for a long time [25]. During the first 2 days of ensilaging, proteolysis in alfalfa comprises 25 μ mol of amino acids/h per 1 kg of DM [26]. Wilting up to 35 % DM does not reduce activity thereof. It should be highlighted that protein of clover and grasses is hydrolyzed during ensilaging to significantly lesser extent that alfalfa protein [27].

Due to the rapid sugar consumption, amino acids are subjected to oxidative de-amination with high ammonium production [28] that improves buffer capacity of feed in ensilaging. Otherwise, alfalfa, which due to preliminary wilting acquires the properties of hardly-fermented plants, is again transformed into the non-ensilaging crop within the first 2 days of ensilaging. That is why alfalfa, unlike hardly-fermentable perennial grasses and clover, is usually not ensilaged, but hay-laid by wilting up to $\geq 45 \%$ DM.

It is considered that haylage, as compared to silage, is preserved not due to active acidification, but due to formation of the physiological dryness in cured grasses that warrants lack of access of moisture germs contained in the plants [29, 30]. Besides, even A.A. Zubrilin [31] warned of an obvious mistake to count only dry matter in the green mass at determination of the extent of development of this or other micro germ group. It is known that several products, for instance, jelly contains a lot of water, but it is inaccessible for many microbes. Recently, at determination of micro germ development extent, activity of contained water (Aw). Aw (is expressed in dimensionless units of the scale from 0 to 1) —

is a quantity of non-bound micro germ accessible water in product) is estimated [32]. Even at 45-50 % DM, Aw in plants does not fall below 0.95 [7], whereas pH is not standardized only at Aw of no more than 0.85 [33]. It is significantly lower than it is noted in grasses, even if such grasses are cured up to 60 % DM. To this end, it could be concluded that wilting of alfalfa up to \geq 45 % DM is not only associated with creation of physiological dryness in plants, but also with the necessity for assurance of the high preservation of feed in the context of weak acidification. In fact, feed acidification extent and accumulation of the fermentation acids are standardized at haylaying of the sugar-containing grasses testified in support of such assumption.

According to G. Pahlow at al. [21], at conservation of sugar-containing grasses with $DM \ge 45 \%$, it should be acidified during 3 days to $pH \le 4.5$, and shall accumulate in the DM of at least 3.5 % acetic acid. The latter is required for suppression of yeast fungi development, being the main initiators of aerobic damage of haylage when removed from the storage reservoirs [7]. Just like at ensilaging of cured grasses, it could be achieved only with involvement of lactobacillus. However, medicines based on the heterofermentative lactobacillus strains should be used at preparation of haylage from grasses producing, along with the lactic acid, significant quantity of acetic acid [34]. Accordingly, ensilaging process also flows in the green mass cured up to $\ge 45 \%$ DM.

Regardless of affiliation with non-ensilaging cultures, alfalfa (at ensilaging in small insulated vessels) in the laboratory tests is preserved for a long time even in newly mown form without fermentation and damage thereof. It is explained by presence of the secondary vegetable metabolites with antimicrobial action [35]. It includes several free non-protein amino acids [36], saponines, many phenol compounds, alkaloids [37] and other compounds. Majority of the secondary metabolites are cancerogenous and, thus, hazardous to animals [38]. The role of the secondary vegetable metabolites in preservation of alfalfa silage is so high that results of the laboratory tests could not be transferred in production environment without relevant adjustment. Presence of the secondary metabolites in alfalfa and other legumetized species is also due to the high aerobic stability of produced silage and haylage [35, 39]. However, it is impossible to preserve alfalfa silage and havlage in production environment without proper acidification. It is alfalfa inability to acidify during a continuous time to the extent eliminating development of butyric bacteria mainly causing feed damage. Thus, in one of the lab-based alfalfa ensilaging tests with 39.9 % DM after 3, 7, 15, 30 and 60 days of ensilaging the pH value was 5.85; 5.54; 5.17; 4.85, and 4.57, respectively [11]. It means that acidification had reached value eliminating the butyric fermentation only after 2 months of ensilaging. High preservation ability of feed in the laboratory tests was assured by the secondary vegetable metabolites; however in the production environment such silage would have become useless during the said storage term.

To increase the alfalfa fermentation ability, it is required to suppress proteolysis in process of plant dehydration in the field and their further ensilaging. Such opinion, in particular, is supported by X.S. Guo at al. [40]. In the field it is achieved by the intensive wilting of alfalfa in mowing (within 2-4 hours after mowing content of DM in the green mass achieves 35 % and more) [11]. Proteolysis intensity at ensilaging may be reduced by fast switching of pH to the limits of proteolytic enzyme activities [25, 41], which is hard to achieve at spontaneous fermentation of the cured mass due to its buffer capacity and lack of sugar. The situation becomes more challenged because the optimal proteolytic pH in alfalfa is lower than in clover, by 6.0 and 6.5 [42]. It means that alfalfa shall be acidified faster and more intensively than the clover to avoid proteolysis. At low alfalfa wilting ($\leq 35 \%$ DM), stability of the feed is ensured only at its rapid acidification to pH ≤ 4.6 , which could not be reached even with the use of lactobacillus. It is established that administration of lactobacillus medicines at ensilaging of alfalfa (36 % DM) did not result in notable increase of the feed acidification (pH 4.34-4.40 against 4.42 in regular silage) [43]. Several researchers believe that qualitative silage could be produced at ensilaging of alfalfa even with the use of lactobacillus biologicals in combination with sugar additives [44]. However, their results show that such approach relates to significant loss of nutrient compounds (up to 30 %) and is useless in practice. According to other data, effect of molasses with lactobacillus medicines on reduction of proteolysis at ensilaging of alfalfa has been growing with increase of the dry matter content from 20 to 37 % [45]. Use of molasses had resulted in increase of the acetic acid in feed that evidences on activation of unfavorable microbial flora.

To elevate sugar content, the cured alfalfa ensilaging with fermentative additives is considered. Thus, in favorable weather conditions Ferkon fermentative medicine upon ensilaging of alfalfa cured up to 30 % DM was as effective as formic acid [46]. However, at detailed analysis, both medicines were ineffective. Just like at administration of Ferkon, and at introduction of 0.5 % muratic acid, pH of feed comprised 4.72-4.70 and accumulation of ammonium in dry matter of silage was 0.43-0.41 %. It means that neither of the used medicines ensured creation of the active acidification that is able to prevent butyric fermentation. Upon ensilaging of clover meadow cured to the same content of dry matter and Ferkon medicine, formic acid, and content of ammonium in the feed comprising accordingly 0.09 and 0.10 %, pH of silage was 4.14 and 4.37. There is a report on the use of common method for satisfactory ensilaging of alfalfa-grass mixture cured up to 35-40 % DM, with share of alfalfa from 50 to 75 % [47]. At ensilaging of net alfalfa with Ferkon and formic acid, positive results were obtained only at wilting to up $\geq 40 \%$ DM [12]. In such a case, silage produced in the production environment is acidified to pH of 4.47 and 4.33, which ensured its high storage stability.

L. Kung at al. [48] had achieved good results by ensilaging of alfalfa with the same content of dry matter upon the use of heterofermentative *Lactobacillus buchneri* with β -glucanase, α -amilase, xylanase and galactomanase. Such ensilaging (along with better preservation and quality of feed) had resulted in notable increase of its aerobic stability. It was certain [35, 39] that haylage and silage from the cured legumetized grasses are quiet resistant to aerobic fall, elimination issue of which arises only at preparation of silage from the maize at middle dough stage [49, 50], sorgo [51] and haylage from grain crops and grasses [52, 53].

Alfalfa and lactobacillus-based ensilaging technology shall be used since even in the southern regions of Russia it is often impossible to cure the mass to the haylage moisture [54] resulting in low quality feed. In Volgograd Region, more than half of the prepared haylage is annually associated with the low grade feed [55]. At favorable wilting conditions, when content of DM in alfalfa rapidly goes up to value ≥ 45 %, stimulation of lactobacillus fermentation by the use of the lactic fermentating agents provides significant effect. According to O.M. Kurnaeva [56], haylaying of alfalfa in the productive environment with introduction of Litosil lactobacillus medicine had reduced accumulation of ammonium in the feed from 26.4 to 10.1 mg%, with loss of DM from 17.6 to 13.2 %, and had ensured stability of haylage at storage. At haylaying of alfalfa (with up to 50 % DM) in tranches, feed pH following 6 and 12 months of storage remained high and comprised accordingly 5.21 and 5.06 [56]. For this reason, accumulation of the oil acid in the natural feed kept growing: after 6 and 12 months of storage it comprised 0.06 and 0.11 %. During the entire storage term the oil acid was not accumulated in the haylage where, due to administration of Litosil medicine, feed pH had rapidly decreased to the extent limiting the growth of butyric acid bacteria. Upon haylaying of the same mass in roll under films, feed pH after 1 and 6 months of storage comprised accordingly 5.08 and 5.32, while content of butyric acid comprised 0.11 and 0.21 %, accordingly. Use of Litosil in this case also ensured production of the feed free from butyric acid. Consequently, even upon wilting up to 50 % DM warranted preservation of feed both at storage in tranches and rolls is ensured only upon use of lactobacillus medicines.

Upon reduction of DM in alfalfa, instability of silage at storage is significantly increased. To solve the problem, we must first of all increase the lactobacillus-butyric acid bacteria competition, which to a significant extent is promoted by alfalfa wilting up to $\geq 40 \%$ DM. In this case, lactic acid is accumulated more than in ensilaging of newly mown alfalfa [15]. At slow acidification, it is important that butyric acid bacteria are present in plants as spores [18]. The dryer the plants, the slower the spores of butyric acid bacteria are growing; and lactobacillus (even at low activity) manage to acidify the feed to the required pH value. Thus, the alfalfa ensilaging mechanism cured up to $\geq 45 \%$ DM is applied recently [33]. This is also associated with the requirement of DM preservation within the range from 40 to 50 % (i.e. the acceptable deviation from the recommended value is no more than 5 %) [7], which significantly challenges haylaying of alfalfa in the production context and accounts for the feasibility of use of lactobacillus fermentations accelerating acidification of the mass and increasing the feed stability at storage and removal.

It is understood that upper wilting limit of the ensilaged alfalfa mass is determined by its technical properties, namely the compression ability. At placing of alfalfa for storage in tranches, content of DM shall not exceed 50 %, provided careful grinding of plants and their absolute air insulation [7]. The upper wilting limit is related not only to the ability of the mass to rapid acidification under the effect of biological preparations to the state excluding development of butyric acid bacteria. The more the green mass is cured, the more effective is application of lactobacillus medicines at wilting. Thus, at ensilaging of alfalfa cured up to 41.1 % DM by lactobacillus medicine Ecosyl, the feed is acidified to pH 4.45 vs. to 4.64 in regular silage [57]. Herewith, it is pH at beginning of ensilaging process suppressing the proteolysis that is important rather than final active acidity of the feed [58]. It is indicated by reduction of the share of nitrogen ammonia in the total feed nitrogen (with 11.2 to 7.6 %) in the silage prepared by lactobacillus fermentation.

At less dry matter in the green mass, effectiveness of Ecosyl use is notably decreased. For instance, at ensilaging of alfalfa with 38.1 % of DM, pH of the trial and control feed comprised 4.73 and 4.53, accordingly. Share of nitrogen ammonia in the total nitrogen had decreased from 12.7 to 9.4 %. At ensilaging of alfalfa with 22.6 % DM, pH in control and test sample comprised 5.23 and 4.93; share of nitrogen ammonia in the total nitrogen was 17.2 and 14.5 %. The above data testifies that upon increase of the quantity of DM in the ensilaged alfalfa from 22.6 to 41.1 %, share of nitrogen ammonia in the total nitrogen of feed had practically decreased in two times — from 14.5 to 7.6 %, and pH had decreased from 4.93 to 4.45. Accordingly, ensilaging of alfalfa cured up to \geq 40 % DM with lactobacillus not only promotes acceleration, but also more acidification of feed that improves its stability at storage.

According to some researchers, use of lactobacillus is the main reason of ammonia growth in the dry matter of alfalfa silage [59]. Such conclusion is based on the assumption that at sugar deficit in the ensilaged green mass, lactic bacteria uses carbon skeleton of amino acids for energetic metabolism. It hap-

pens in cases when use of lactobacillus medicines does not promote any more intensive acidification of the feed. Thus, in one of alfalfa ensilaging tests (Taisia variety) with 31.3 % DM by regular way and with introduction of Biotrof preparation, pH in finished silage comprised accordingly 5.06 and 5.02, ammonia content in the dry matter comprised 0.54 and 0.62 % [11]. At the same time, at ensilaging of alfalfa Pastbischnaya 88 variety rich in sugar, even at regular ensilaging of plants with 24.5 % DM the feed was acidified to pH 4.70. Biotrof preparation did not have any significant effect on the acidification extent of the finished silage (pH 4.68), but had accelerated such process. Dry matter of the control silage sample contained 0.17 % of oil acid, while it was not found in the trial sample. Intensification of acidification had resulted in accumulation of ammonia in dry matter of feed from 0.30 to 0.26 %. Accordingly, at development of the effective technology of ensilaging of the cured alfalfa by lactobacillus medicine it is important to account for the possible significant role of the kind differences in plants.

Interesting results were obtained by Hungarian researchers [60] at ensilaging of alfalfa (32.3 % DM) upon use of maize flour, in which 89 % of starch was hydrolyzed to simple sugars. Maize flour was added accounting for 1.0 % to the green mass. According to authors, it promoted production of high quality feed with good ratio of lactic and acetic acids. At the same time, addition of cellulosolythic enzymes and their combinations with lactobacillus at ensilaging of alfalfa with 34 % DM was ineffective just as in the above-described tests [61]. Ensilaging of alfalfa cured up to 33.40 and 53.00% DM with addition of tannin was rested with positive results [62]. Importantly, in this case the effect of tannin use for protheolysis limitation had increased by increasing the content of dry matter in the green mass. Disadvantage of the above method is that high tannin dosages (≥ 2.0 % of DM) reduce digestibility of the nutrient components in the feed.

The most important property of alfalfa silage produced with addition of lactobacillus medicine is its high productive action validly exceeding the productive action of spontaneous fermentation silage. At difference of total 0.3 kg in consumption of DM rations with alfalfa silage produced in regular manner and with addition of lactobacillus, the difference in the average daily milk yield was 0.8 kg (40.7 vs. 39.9 kg) at higher milk fat (3.43 vs. to 3.37 %) in animals of the test group [48]. The literature sources yet lack clear explanation of such case. Some researchers associate it with increase of the mass of rumen microbial flora, which serves a source of complete protein for high productive cows [63, 64].

Let us also consider the factors which, besides decrease of protheolysis in alfalfa green mass cured up to ≥ 40 % DM, may be the reason for more intense acidification of the feed at addition of lactobacillus enzymes. First of all, it promotes more homofermentative type of lactobacillus fermentation in the silage [65, 66]. Lactic acid which is dissociated to ions more than other fermentation acids [7, 67] promotes higher acidity in the feed. Important condition of successful ensilaging of alfalfa in deeply cured form is creation of sugar both at rapid wilting of plants up to ≥ 40 % DM [11, 15], as well as in fermentation process of the cured mass in anaerobic conditions. It is due to content of up to 5 % of starch [23] in the dry matter of alfalfa which is hydrolyzed under the effect of fermentation acids to simple sugars. Besides, alfalfa contains saponine (C₂₇H₃₇O₁₆) that is fermented into sapogenin, glucose, and unknown tart substance [68]. It may happen that another important message is that up to 2 % of apple acid is accumulated in the dry matter of alfalfa cured up to ≥ 40 % DM, which, according to certain authors [69], may be fermented by lactobacillus.

Thus, alfalfa is characterized, along with sugar deficit, by very high buffer capacity exceeding 1.6-2.3 times the buffer capacity of other fodder plants.

The higher the buffer capacity, the more fermentation acids are neutralized, which strongly suppresses feed acidification, especially during the first ensilaging phase. At high protein and pectin contents, alfalfa biomass, even when wilted up to ≥ 35 % dry matter (DM), still contains significant amount of weak-bound water which at slow acidification leads to high proteolytic activity and protein hydrolysis. It is accompanied by high accumulation of ammonium in silage and further increase in buffer capacity. Thence, acidification of wilted alfalfa to the pH suppressing clostridia may take more time, even in use of lactobacilli or their combinations with enzymes, resulting in feed damage. In this, it is required i) to reduce proteolysis in the ensilaged green mass, ii) to slow down growth of butyric acid bacteria during the first phase of ensilaging with intensification of lactobacillus fermentation, and iii) to increase the pH to that critical for butyric acid bacteria. To this end, alfalfa mass shall be rapidly wilted to ≥ 40 % DM and subjected to ensilaging with the use of osmotolerant lactobacillus strains.

REFERENCES

- Laptev G.Yu., Novikova N.I., Il'ina L.A., Yyldyrym E.A., Soldatova V.V., Nikonov I.N., Filippova V.A., Brazhnik E.A., Sokolova O.N. Dynamics of mycotoxin accumulation in silage during storage. *Agricultural Biology*, 2014, 6: 123-130 (doi: 10.15389/agrobiology.2014.6.123eng).
- 2. Chub O. Zhivotnovodstvo Rossii, 2015, 10: 55-56 (in Russ.).
- 3. Mak-Donal'd P., Edvards R., Grinkhaldzh Dzh. *Pitanie zhivotnykh* [Animal feeding]. Moscow, 1970 (in Russ.).
- 4. Ennison E.F., L'yuis D. Obmen veshchestv v rubtse [Rumen metabolism]. Moscow, 1962 (in Russ.).
- 5. Grabov M. Tsenovik, 2016, 5: 43 (in Russ.).
- Albrecht K.A., Beauchemin K.A. Alfalfa and other perennial legume. In: Silage science and technology, Agronomy Monograph 42. D.R. Buxton, R.E. Muck, J.H. Harrison (eds.). ASA, CSSA, and SSSA, Madison, 2003: 633-664 (doi: 10.2134/agronmonogr42.c14).
- 7. Vaisbakh F. Problemy biologii produktivnykh zhivotnykh, 2012, 2: 49-70 (in Russ.).
- Sosnowski J., Jankowski K., Wiśniewska-Kadźajan B., Jankowska J., Kolczarek R. Effect of the extract from *Ecklonia maxima* on selected micro- and macroelements in aerial biomass of hybrid alfalfa. *J. Elementol.*, 2014, 19(1): 209-217 (doi: 10.5601/jelem.2014.19.1.608).
- 9. Hancock D.W., Buntin G.D., Ely L.O., Lacy R.C., Heusner G.L., Stewart R.L. Alfalfa management in Georgia. Athens, 2005.
- Pobednov Yu.A., Kosolapov V.M., Bondarev V.A., Akhlamov Yu.D., Mamaev A.A., Klimenko V.P., Otroshko S.A., Shevtsov A.V. *Silosovanie i senazhirovanie kormov (rekomendatsii)* [Silage and hay making]. Moscow, 2012 (in Russ.).
- 11. Pobednov Yu.A., Mamaev A.A., Ivanova M.S. V sbornike nauchnukh trudov: *Zhuchenkovskie cht-eniya II* [II Zhuchenko Readings. Iss. 11(59)]. Moscow, 2016. Vypusk 11(59): 180-188 (in Russ.).
- 12. Anisimov A.A. Vash sel'skii konsul'tant, 2006, 4: 28-30 (in Russ.).
- 13. Vasin V.G., Zotikov V.I., Vasina A.A. *Proizvodstvo kormov dlya molochnykh kompleksov* [Feed production for dairy commercial farms]. Orel, 2012 (in Russ.).
- 14. Weissbach F., Honig H. Über die Vorhersage und Steurung des Gärungsverlaufs bei der Silierung von Grünfutter aus extensiven Anbau. *Landbauforschung Völkenrode*, 1996, 1: 10-17.
- 15. Pobednov Yu.A. *Teoreticheskie predstavleniya i sposoby konservirovaniya kukuruzy i trav na osnove regulirovaniya mikrobiologicheskikh protsessov* [Theoretical aspects and methods for preserving maize and herbs based on the regulation of microbiological processes]. St. Petersburg, 2017 (in Russ.).
- 16. Bondarev V.A., Kosolapov V.M., Klimenko V.P., Krichevskii A.N. *Prigotovlenie silosa i senazha s primeneniem otechestvennykh biologicheskikh preparatov* [Domestic biologicals in silage and hay making].Moscow, 2016 (in Russ.).
- 17. Fehrmann E., Müller Th. Jaresverlauf des epiphytischen Mikrobenbesatzes auf einen Graslandstandort. *Das Wirtschaftseigene Futter*, 1990, 36(1): 66-78.
- 18. Shmidt V., Vetterau G. Proizvodstvo silosa [Silage making]. Moscow, 1975 (in Russ.).
- 19. Shurkhno R.A., Norina O.S., Tagirov M.Sh., Naumova R.P. Doklady Rossiiskoi akademii sel'skokhozyaistvennykh nauk, 2008, 6: 23-26 (in Russ.).
- Viringa Dzh. Materialy 8-go Mezhdunarodnogo lugopastbishchnogo kongressa (11-21 iyulya, 1960 g., g. Reding, Angliya) (perevod s angliiskogo) [Proc. 8th Int. Grassland Congress, 11-21 July, 1960, Reading. England]. Moscow, 1963: 334-343 (in Russ.).
- Pahlow G., Weissbach F. New aspects of evaluation and application of silage additives. Landbauforschung Völkenrode, 1999, 206: 141-158.

- 22. Pobednov Yu.A. Problemy biologii produktivnykh zhivotnykh, 2009, 3: 89-100 (in Russ.).
- 23. *Proizvodstvo grubykh kormov. Kniga 1* /Pod redaktsiei D. Shpaara [Coarse fodder production. Book 1. D. Shpaar (ed.)]. Torzhok, 2002 (in Russ.).
- 24. Pobednov Yu.A. Adaptivnoe kormoproizvodstvo, 2016, 2: 21-37 (in Russ.).
- 25. Charmley E., Veira D.M. Inhibition of proteolysis at harvest using heat in alfalfa silages: effect on silage composition and digestion by sheep. *J. Anim. Sci.*, 1990, 68(3): 758-766 (doi: 10.2527/1990.683758x).
- 26. McKersie B., Buchanan-Smith J. Changes in the levels of proteolytic enzymes in ensiled alfalfa forage. *Canadian Journal of Plant Science*, 1982, 62(1): 111-116 (doi: 10.4141/cjps82-017).
- 27. Purwin C., Pysera B., Fijałkowska M., Antoszkiewicz Z., Piwczynski D., Wyzlic I., Lipinski K. The influence of ensiling method on the composition of nitrogen fractions in red clover, alfalfa and red fescue silage. *Proc. XVI International Silage Conference*. Hämeenlinna, 2012: 256-257.
- Ulit'ko V.E., Pykhtina L.A., Desyatov O.A. Povyshenie produktivnogo deistviya kormov pri proizvodstve moloka i myasa v Srednevolzhskom regione [Increasing feed effect on milk and meat production in the Middle Volga region]. Ul'yanovsk, 2016 (in Russ.).
- 29. Li S.S., Pshenichnikova E.N., Kroneval'd E.A. Vestnik Altaiskogo gosudarstvennogo agrarnogo universiteta, 2014, 2(112): 98-102 (in Russ.).
- 30. Makarov S.A. *Mezhdunarodnyi nauchno-issledovatel'skii zhurnal*, 2016, 3(45-3): 109-112 (doi: 10.18454/IRJ.2016.45.035) (in Russ.).
- 31. Zubrilin A.A. *Konservirovanie zelenykh kormov* [Green forage preserving]. Moscow, 1938 (in Russ.).
- 32. Gorelikova G.A. *Osnovy sovremennoi pishchevoi biotekhnologii* [Fundamentals of modern food biotechnology]. Kemerovo, 2004 (in Russ.).
- 33. Pobednov Yu.A. Problemy biologii produktivnykh zhivotnykh, 2016, 2: 42-54 (in Russ.).
- Driehuis F., Oude Elferink S.J.W.H., Van Wikselaar P.G. Fermentation characteristics and aerobic stability of grass silage inoculated with *Lactobacillus buchneri*, with or without homofermentative lactis acid bacteria. *Grass Forage Sci.*, 2001, 56(4): 330-343 (doi: 10.1046/j.1365-2494.2001.00282.x).
- 35. Weissbach F. Consequences of grassland de-intensification for ensilability and feeding value of herbage. *Landbauforschung Völkenrode*, 1999, 206(Sonderheft): 41-53.
- Sagiyan A.S. Enantiomerno chistye nebelkovye aminokisloty. Sposoby polucheniya [Enantiomeric pure non-protein amino acids — synthesis, isolation, purification technique]. Moscow, 2010 (in Russ.).
- 37. Luckner M. *Vtorichnyi metabolizm u mikroorganizmov, rastenii i zhivotnykh* [Secondary metabolism in microorganisms, pants, and animals]. Moscow, 1979 (in Russ.).
- 38. Heldt H.-W. Biokhimiya rastenii [Plant biochemistry]. Moscow, 2014 (in Russ.).
- 39. Davies D.R., Fychan R., Jones R. Aerobic deterioration of silage: causes and controls. *Proc. Alltech's 23rd Annual Symposium «Nutritional Biotechnology in the Feed and Food Industries»*. Nottingham, 2007: 227-238.
- 40. Guo KH.S., Cheng W., Tao L., Zhu Yu., Zhou H. Contribution of endo and exopeptidases to formation of nonprotein nitrogen during ensiling of alfalfa. *Proc. KHVI International Silage Conference*. Hämeenlinna, 2012: 58-59.
- McKersie B.D. Effect of pH on proteolysis in ensiled legume forage. *Agron. J.*, 1983, 77(1): 81-86 (doi: 10.2134/agronj1985.00021962007700010019x).
- Tao L., Guo X.S., Zhou H., Undersander D.J., Nandety A. Short communication: characteristics of proteolytic activities of endo- and exopeptidases in alfalfa herbage and their implications for proteolysis in silage. *J. Dairy Sci.*, 2012, 95(8): 4591-4595 (doi: 10.3168/jds.2012-5383).
- 43. Filya I., Muck R.E., Contreras-Govea F.E. Inoculant effects on alfalfa silage: fermentation products and nutritive value. *J. Dairy Sci.*, 2007, 90: 5108-5114 (doi: 10.3168/jds.2006-877).
- 44. Shifer K., SHtainkhefel' O., Nad' B. Novoe sel'skoe khozyaistvo, 2007, 4: 74-78 (in Russ.).
- 45. Hashemzadeh-Cigari F., Khorvash M., Chorbani G.R., Taghizadeh A. The effects of wilting, molasses and inoculants on the fermentation quality and nutritive value of lucerne silage. *S. Afr. J. Anim. Sci.*, 2011, 41(4): 377-388 (doi: 10.4314/sajas.v41i4.8).
- 46. Kosolapov V.M., Bondarev V.A., Panov A.A., Akhlamov Yu.D., Udalova E.V., Isaenkov N.I., Anisimov A.A., Otroshko S.A., Klimenko V.P. *Tekhnologiya silosovaniya vysokobelkovykh* mnogoletnikh bobovykh trav s polifermentnym preparatom Ferkon (rekomendatsii) [Silaging of highprotein perennial legumes using multi enzyme preparation Ferkon – recommendation]. Moscow, 2008 (in Russ.).
- 47. Smitt K.-O., Pratz H. Mit Luzerne die Futtergrudlage. Rheinische Bauer Zeitung, 1996, 5: 20.
- Kung L.J., Taylor C.C., Lynch M.P., Neylon J.M. The effect of treating alfalfa with *Lactobacillus buchneri* 40788 on silage fermentation, aerobic stability, and nutritive value for lactating dairy cows. J. Dairy Sc., 2003, 86: 336-343 (doi: 10.3168/jds.S0022-0302(03)73611-X).
- 49. Ranjit N.K., Taylor C.C., Kung L. Effect of *Lactobacillus buchneri* 40788 on the fermentation, aerobic stability, and nutritive value of maize silage. *Grass Forage Sci.*, 2002, 57: 72-81 (doi: 10.1046/j.1365-2494.2002.00304.x).
- 50. Kristensen N.B., Sloth K.H., Højberg O., Spliid N.H., Jensen C., Thøgersen R. Effects of micro-

bial inoculants on corn silage fermentation, microbial contents, aerobic stability, and milk production under field conditions. J. Dairy Sci., 2010, 93: 3764-3774 (doi: 10.3168/jds.2010-3136).

- 51. Tabacco E., Righi F., Quarantelli A., Borreani G. Dry matter and nutritional losses during aerobic deterioration of corn and sorghum silages as influenced by different lactic acid bacteria inocula. *J. Dairy Sci.*, 2011, 94: 1409-1419 (doi: 10.3168/jds.2010-3538).
- 52. Shah A.A., Xianjun Y., Zhihao D., Siran W., Tao S. Effect of lactic acid bacteria on ensiling characteristics, chemical composition and aerobic stability of king grass. *Journal of Animal & Plant Sciences*, 2017, 27: 747-755.
- Randby A.T., Gismervik K., Andersen A., Skaar I. Effect of invasive slug populations (*Arion vulgaris*) on grass silage: I. Fermentation quality, in-silo losses and aerobic stability. *Anim. Feed Sci. Tech.*, 2015, 199: 10-19 (doi: 10.1016/j.anifeedsci.2014.09.026).
- Marchenko F.Yu., Zabashta N.N., Golovko E.N. V sbornike nauchnykh trudov Severo-Kavkazskogo nauchno-issledovatel'skogo instituta zhivotnovodstva [In: Scientific papers of North-Caucasian Research Institute of Animal Husbandry]. Krasnodar, 2016: 182-188 (in Russ.).
- 55. Amerkhanov Kh.A., Tyapugin E.A., Simonov G.A., Tyapugin S.E. *Effektivnost' vedeniya mo*lochnogo skotovodstva v usloviyakh Evropeiskogo Severa Rossii [Effective dairy cattle farming in the European North of Russia]. Moscow, 2001 (in Russ.).
- 56. Kurnaev O.M. Vplyv tekhnologii zagotivli sinazhu na vtraty syrogo proteinu ta iogo fraktsiinyi sklad uprodovzh zberigannya. Kormi i kormovirobnitstvo. Mizhvidomchii tematichnii naukovii zbirnik (Vinnitsya), 2010, 66: 274-280.
- 57. Moran J.P., Owen T.R. The effect of bacterial inoculant on the fermentation of lucerne silage. *Proc. KHI International Silage Conference*. Aberystwyth, 1996: 166-167.
- Fijałkowska M., Pysera B., Lipiński K., Strusińska D. Changes of nitrogen compounds during ensiling of high protein herbages — a review. *Ann. Anim. Sci.*, 2015, 15(2): 289-305 (doi: 10.1515/aoas-2015-0008).
- 59. Ilyaletdinov N.K., Akhmediev A.N. *Izvestiya AN SSSR. Seriya biologicheskaya*, 1979, 3: 427-434 (in Russ.).
- 60. Rigy E., Zsédely E., Tóth T., Schmidt J. Ensiling alfalfa with hydrolyzed corn meal additive and bacterial inoculant. *Acta Agronomica Óvariensis*, 2011, 53(2): 15-23.
- Lynch J.P., Jin L., Lara E.C., Baah J., Beauchemin K.A. The effect of exogenous fibrolytic enzymes and a ferulic acid esterase producing inoculant on the fibre degradability, chemical composition and conservation characteristics of alfalfa silage. *Anim. Feed Sci. Tech.*, 2014, 193: 21-31 (doi: 10.1016/j.anifeedsci.2014.03.013).
- Tabacco E., Borreani G., Crovetto G.M., Galassi G., Colombo D., Cavallarin L. Effect of chestnut tannin on fermentation quality, proteolysis, and protein rumen degradability of alfalfa silage. J. Dairy Sci., 2006, 89(12): 4736-4746 (doi: 10.3168/jds.S0022-0302(06)72523-1).
- 63. Kurtoglu V., Coskum B. Effect of bacterial adding alfalfa silage on milk yield and milk composition of dairy cattle. *Revue Mèd. Vèt.*, 2003, 154(12): 755-762.
- 64. Mohammed R., Stevenson D.M., Beauchemin K.A., Muck R.E., Weimer P.J. Changes in ruminal bacterial community composition following feeding of alfalfa ensiled with a lactic acid bacterial inoculant. *J. Dairy Sci.*, 2012, 95(1): 328-339 (doi: 10.3168/jds.2011-4492).
- Silva V.P., Pereira O.G., Leandro E.S., Da Silva T.S., Ribeiro K.G., Mantovani H.C., Santos S.A. Effects of lactic acid bacteria with bacteriocinogenic potential on the fermentation profile and chemical composition of alfalfa silage in tropical conditions. *J. Dairy Sci.*, 2016, 99(3): 1895-1902 (doi: 10.3168/jds.2015-9792).
- 66. Tao L., Zhou H., Zhang N., Si B., Tu Ya., Ma T., Diao Q. Effects of different source additives and wilt conditions on the pH value, aerobic stability, and carbohydrate and protein fractions of alfalfa silage. *Anim. Sci. J.*, 2017, 88(1): 99-106 (doi: 10.1111/asj.12599).
- 67. Lück E. Chemische Lebensmittelkonservirung. Berlin, Heidelberg, NY, Tokyo, 1985.
- 68. CHukanov N.K., Popenko A.K. *Mikrobiologiya konservirovaniya trudnosilosuemykh rastenii* Microbiology of preservation of plants which are hard to be silage]. Alma-Ata, 1986 (in Russ.).
- 69. McDonald P. Biokhimiya silosa [Silage biochemistry]. Moscow, 1985 (in Russ.).