Technology for increasing the bioavailability of feed using quorum sensing inhibitors

K N Atlanderova, A M Makaeva, M Ya Kurilkina, T N Kholodilina and G K Duskaev

Federal Research Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences, Orenburg, Russia

E-mail: atlander-kn@mail.ru

Abstract. The article presents the results of studies on increasing the bioavailability of feed, when using oak bark extract (OBE) and quorum sensing inhibitors (QSI) in the diet of cattle, in particular, the effect on volatile fatty acids, pH, ammonia and the content of nitrogen metabolites. The use of OBE and QSI was accompanied by an increase in the concentration of VFAs by 1.17 and 5.56 % (P ≤ 0.05) three hours after feeding. The studies revealed an increase in the concentration of ammonia in the cicatricial content when adding ECD and IR by up to 2.88 and 8.80 % (P ≤ 0.001) 3 hours after feeding, respectively. The same tendency is observed at a 6-hour exposure, the ammonia level increases by 6.08 % (P ≤ 0.01) and 11.08 % (P ≤ 0.001). The effect of oak bark extract on the bioavailability of the forage substrate in the rumen was accompanied by an increase in the total nitrogen content by 2.10 % (P ≤ 0.05), and in the group using quorum inhibitors, by 4.41 % (P ≤ 0.01). 6 hours after feeding, the content of non-protein nitrogen decreased by 4.56 and 7.45 % (P ≤ 0.01) in the OBE and QSI groups; this indicates a significant rate of nitrogen assimilation by the scar microbiota which converts it into protein.

1. Introduction

In recent years, the approach to creating feed additives was changed, paying attention to measures to create optimal conditions for the functioning of the gastrointestinal tract of farm animals [1] as a system including microbiocenosis of symbiotic microflora [2]. This is true for polygastric, whose life is closely related to microbial communities [3].

Quorum inhibitory (QSI) compounds have been identified from a wide range of natural resources, in particular medicinal plants, edible herbs, fruits and vegetables [4], and spices [5]. Natural products are promising sources of QSI compounds that can inhibit the QS regulation of bacterial colonization and virulence factor production [6]. It is also possible that some antimicrobial properties of phytochemicals can be attributed to QS inhibition, which cannot be associated with inhibition of microorganism growth [7].

Many plants have useful multifunctional properties, and bioactive components can favorably affect the animal’s body [8]. Plant extracts are safe and effective against some bacteria; they are widely used in feeds as stimulants of the growth and productivity of animals [9].

One source of QS inhibitors is oak bark (Quercus robur). Seven components with anti-QS activity were found in the oak bark extract, starting with the greatest effect: pyrogallol, propylresorcinol, coumarin, scopoletin, coniferyl alcohol, vanillin, antiarol [10].
Oak bark shows the most pronounced and stable anti-QS activity, in the absence of obvious antibacterial substances in its composition [11]. This allows the use of quorum sensing inhibitors isolated from oak bark, including in combination with other feed additives [12].

It is known that oak bark extract increases the number of microorganisms that decompose cellulose and other polysaccharides, which stimulates the activity of various hydrolases in the cicatricial fluid of cows [13].

Due to difficulties in using the aqueous extract of oak bark – the unstable chemical composition depending on environmental conditions, growth, and other factors [14], low molecular weight compounds based on the prototype of oak bark extract – quorum sensing inhibitors – were used.

2. Materials and methods
The aim of the article is to study the effect of ECD and IR on the content of nitrogen metabolites, pH, ammonia and volatile fatty acids in the rumen of young cattle.

Animal services and experimental studies were performed in accordance with the instructions and recommendations of Russian Regulations, 1987 (Order No. 755 on 08/12/1977 the USSR Ministry of Health) and “The Guide for Care and Use of Laboratory Animals (National Academy Press Washington, DC 1996). " In carrying out the research, efforts were made to minimize animal suffering and reduce the number of samples used.

In experimental studies, aqueous extract of oak bark (1:10) (OBE) and specially prepared preparation of quorum sensing inhibitors (QSI) were used.

The composition was determined by the liquid chromatography method using a mass selective detector GQCMS 2010 Plus (Shimadzu, Japan) on the HP-5MS column. When developing the ICS preparation, we took into account the ratio of these components in the ECD. The ratio used and the composition of the drug were as follows: coniferyl alcohol – 50 %; antiarol – 20 %; propylresorcinol – 15.5 %; vanillin – 5.9 %; coumarin – 5.3 %; scopoletin – 3.3 %.

An aqueous extract of oak bark was obtained by mixing oak bark powder with water in a ratio of 1:10 and incubation in a boiling water bath (30 min), filtration and centrifugation (centrifuge – OPN-8 (PO Box V-2331, Russia) for 15 minutes at 2000 rpm.

The quorum sensing inhibitor was produced by mixing precise weighed portions of substances with distilled water, dispersion in H2O by ultrasonic treatment on a UZDN-2T disperser (NPP Akadempribor, Russia) for 30 minutes at a frequency of 35 kHz (f-35 kHz, N- 300 W, A-10 μA).

The study was conducted under the conditions of the Federal Scientific Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences (FSC BST RAS), as well as the Center for Collective Use of the FSC.

Physiological studies were conducted at the Pokrovsky Agricultural College production site, a branch of Orenburg State Agrarian University, on red steppe bulls aged 14 months, which were divided into 3 groups by the method of pairs of analogues (n = 12): control – main diet (OR ); experimental group I – OR + OBE 0.64 ml / kg of live weight (LM); experimental group II – OR + IR 0.81 ml / kg of fat. The main diet was created on the basis of feed used by the farm, in accordance with the norms of feeding [15]. The choice of dosages was determined by previous in vitro studies [16].

When studying cicatricial digestion, cicatricial fluid was collected from gobies through the chronic scar fistula in an amount of 350 ml, before feeding, 3 and 6 hours after feeding. Samples were filtered through 4 layers of sterile gauze, the concentration of hydrogen ions (pH) was determined by pH-150MI (LLC “Measuring equipment”, Russia), in accordance with the instructions.

Metabolism of nitrogen metabolites in the cicatricial content was established: residual and total nitrogen — by the Kjeldahl method; ammonia nitrogen – by the microdiffusion method according to Conway. Volatile fatty acids were determined using a Kristallux-4000 gas chromatograph (Russia).

Statistical Processing. Data are expressed as mean values ± standard error of the mean. Statistical analysis was performed using Statistica 10.0 (StatSoft Inc., USA) and Microsoft Excel (Microsoft, USA). Significance of the group differences was estimated using Student’s t-test with p≤0.05 considered as significant.
3. Results
An analysis of the results showed that the introduction of ECD and QSI into the diet of experimental animals affects the degree of intensity of bacterial processes (table 1).

| Exposure, (h) | Group | pH       | Ammonia, mmol / L | VFA, mmol / 100 ml |
|--------------|-------|----------|-------------------|-------------------|
| 3            | Control | 6.98±0.11 | 20.8±0.15         | 7.71±0.06         |
|              | I      | 7.07±0.09 | 21.4±0.32         | 7.80±0.06         |
|              | II     | 7.10±0.06 | 22.6±0.63"***"   | 7.90±0.06"*"     |
| 6            | Control | 6.87±0.07 | 22.0±0.13         | 8.27±0.09         |
|              | I      | 6.77±0.03 | 23.4±0.24"**"    | 8.73±0.12         |
|              | II     | 6.73±0.03 | 24.5±0.26"***"   | 9.00±0.06"***"   |

Significant difference in relation to control; * p ≤ 0.05, ** p ≤ 0.01, *** – Р≤ 0.001

The ion-hydrogen index is associated with enzymatic processes. Therefore, pH is of great importance when studying digestive processes of the scar. The pH shift to a slightly acidic side was accompanied by an increase in the content of volatile fatty acids. Therefore, 3 hours after digestion in experimental groups I and II, the concentration of VFA increased by 1.17 % and 5.56 % (P≤0.05), respectively. 6 hours after, in experimental group II the level of VFA increases by 8.83 % (P≤0.001).

Part of the nitrogen is converted to ammonia, so the nitrogen exchange can be judged by the concentration of ammonia. In our studies, the fact of an increase in the concentration of ammonia in the cicatricial contents of experimental groups I and II by 2.88 % and 8.80 % (P≤0.001) 3 hours after administration was established. The same tendency is observed at a 6-hour exposure, the ammonia concentration in the content of the rumen of animals of experimental group I exceeded the control level by 6.08 % (P≤0.01), in experimental group II – by 11.08 % (P≤0.001).

![Figure 1. The content of nitrogen metabolites in the rumen of animals, after 3 and 6 hours of feeding, mmol / l](image)
Nitrogen metabolism depends on a number of factors: time of taking scar fluid, the diet of experimental animals. Assessment of nitrogen metabolism makes it possible to talk about the nature of the digestive process (Figure 1).

An analysis showed that the introduction of oak bark extract into the diet increases the total nitrogen content by 2.10 % (P \leq 0.05), and the use of quorum sensing inhibitors increases it by 4.41 % (P \leq 0.01). 6 hours after digestion, the highest values were observed in experimental group II (an increase was 6.06 % (P \leq 0.001)).

Three hours after exposure, non-protein nitrogen was lower than in the control group: in experimental group I – by 2.29 %, in experimental group II – by 6.20 % (P \leq 0.05). 6 hours after exposure, in experimental group I, the content of non-protein nitrogen decreased by 4.56 %, in experimental group II – by 7.45 % (P \leq 0.01). This indicates a significant rate of assimilation of nitrogen.

4. Discussion

Plant extracts, known as phytobiotics, are used in animal nutrition, in particular as antimicrobial, anti-inflammatory, antioxidant and antiparasitic agents [17]. Inhibitors of the quorum sensitivity system (QS) are one of the active principles of medicinal plants suppressing opportunistic microflora [18]. Inhibition of “quorum sensing” helps to reduce the load on the microbiota of the body and has a beneficial effect on animal productivity [19].

Digestibility of the feed substrate depends on external factors, feeding diets, and the environment in which the rumen microorganisms are located [20]. The pH shift to a slightly acidic side was accompanied by an increase in the content of volatile fatty acids. Three hours after feeding, in experimental groups I and II, the concentration of VFA increased by 1.17 and 5.56 % (P \leq 0.05).

Changes in microbiological processes are confirmed by the dynamics of the hydrogen index (pH). In all experimental groups, this indicator was higher than in the control one. This is due to the formation of a large amount of ammonia in the rumen. A high concentration of ammonia alkalizes the rumen content associated with improved digestibility [21].

Part of the nitrogen is converted to ammonia; therefore, the nitrogen exchange can be determined by the concentration of ammonia [22]. In experimental group I, the concentration of ammonia exceeded the control indicator by 6.08 % (P \leq 0.01), in experimental group II – by 11.08 % (P \leq 0.001). To a large extent, this result was achieved due to changes in the rumen microbiome.

5. Conclusion

Thus, the use of ECD and IRS in feeding young cattle has a positive effect on digestive processes in the rumen. In particular, a decrease in non-protein nitrogen, helps to accelerate the intensity of nitrogen metabolism.

The results obtained indicate the potential of the additives and require further research.

Acknowledgments

The study was performed in the framework of the project № 0761-2019-0005.

References

[1] Celi P, Cowieson A J, Fru-Nji F et al 2017 Gastrointestinal functionality in animal nutrition and health: New opportunities for sustainable animal production Animal Feed Sci. and Technol. 234 88–100 DOI: 10.1016/j.anifeedsci.2017.09.012

[2] Hillman E T, Lu H, Yao T and Nakatsu C H 2017 Microbial ecology along the gastrointestinal tract Microbes and environments 32(4) 300–13 DOI: 10.1264/jsme2.ME17017

[3] Wanapat M, Cherdthong A, Phesatcha K and Kang S 2015 Dietary sources and their effects on animal production and environmental sustainability Animal nutrit. 1(3) 96–103 DOI: 10.1016/j.aninu.2015.07.004

[4] Husain F M, Ahmad I, Khan F I et al 2018 Seed extract of Psoralea corylifolia and its constituent
bakuchiol impairs AHL-Based Quorum Sensing and biofilm formation in food- and human-related pathogens Frontiers in cellular and infect. Microbiol. 8 351
DOI: 10.3389/fcimb.2018.00351

[5] Chenia H Y 2013 Anti-quorum sensing potential of crude Kigelia africana fruit extracts Sensors (Basel, Switzerland) 13(3) 2802–17 DOI: 10.3390/s130302802

[6] Abreu A C, McBain A J and Simoes M 2012 Plants as sources of new antimicrobials and resistance-modifying agents Nat. Prod. Rep. 29 1007–21

[7] Valenzuela-Grijalva N V, Pinelli-Saavedra A, Muhlia-Almazan A et al 2017 Dietary inclusion effects of phytochemicals as growth promoters in animal production J. Anim. Sci. Technol. 59 8

[8] Delimont N M, Haub M D and Lindshield B L 2017 The Impact of Tannin Consumption on Iron Bioavailability and Status: A Narrative Review Curr. Dev. Nutr. 1(2) 1–12 DOI: 10.3945/cdn.10.000042

[9] Arowolo M A and He J 2018 Use of probiotics and botanical extracts to improve ruminant production in the tropics: A review Anim. Nutr. 4(3) 241–9

[10] Tolmacheva A A, Rogozhin E A and Deryabin D G 2014 Antibacterial and quorum sensing regulatory activities of some traditional Eastern-European medicinal plants Acta Pharmac. 64 173–86

[11] Deryabin D G and Tolmacheva A A 2015 Antibacterial and anti-quorum sensing molecular composition derived from Quercus cortex (oak bark) extract Molecules 20 17093–108

[12] Duskaev G K, Kazachkova N M, Ushakov A S et al 2018 The effect of purified Quercus cortex extract on biochemical parameters of organism and productivity of healthy broiler chickens J. Vet. World 11(2) 235–9

[13] Duskaev G K, Karimov I F, Levakhin G I et al 2019 Ecology of ruminal microorganisms under the influence of quercus cortex extract Int. J. of GEOMATE 16 55 59–66

[14] Dettweiler M, Lyles J T, Nelson K et al 2019 American Civil War plant medicines inhibit growth, biofilm formation, and quorum sensing by multidrug-resistant bacteria Sci. Rep. 9 7692 Retrieved from: https://doi.org/10.1038/s41598-019-44242-y

[15] Klashnikov A P, Fisinin V I, Shcheglov V V and Kleimenov N I 2003 Norms and rations for feeding farm animals Handbook 3rd ed 456 p

[16] Atlanderova K N, Makaeva A M, Sizova E A and Duskaev G K 2019 Stimulation of ruminal digestion of young cattle with oak bark extract (Quercus cortex) IOP Conf. Ser. Earth and Environmental Sci. 341 012059

[17] Trufanov O 2016 Phytobiotics in broiler rations Livest. Breed. Rus. 10 5–7

[18] Atlanderova K, Makaeva A, Miroshnikov S and Ivanishcheva A 2019 Changes in rumen microbiota of cattle with the simultaneous introduction of iron and copper nanoparticles and quorum sensing suppressants FEBS Open Bio. 9(S1) 415–6

[19] Duskaev G K, Deryabin D G, Karimov I F et al 2018 Assessment of (in vitro) Toxicity of Quorum Sensing Inhibitor Molecules of Quercus cortex J. Pharm. Sci. and Res. 10(1) 91–5

[20] Nasrollahi S M, Zali A, Ghorbani G R et al 2019 Short communication: Blood metabolites, body reserves, and feed efficiency of high-producing dairy cows that varied in ruminal pH when fed a high-concentrate diet J. Dairy Sci. 102(1) 672–7

[21] Limin Kung Jr, Shaver R D, Grant R J and Schmidt R J 2018 Silage review: Interpretation of chemical, microbial, and organoleptic components of silages J. of Dairy Sci. 101(5) 4020–33

[22] Brake D W and Swanson K C 2018 Ruminant nutrition symposium: effects of postruminal flows of protein and amino acids on small intestinal starch digestion in beef cattle J. Anim. Sci. 96(2) 739–50 DOI: 10.1093/jas/skx058