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Research Article

# The Effect of Adding of the Enzyme Products Xybeten and Protozin-A to a Diet on Ethological Parameters of Sheep

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**ABSTRACT**

It has been studied the effect of adding synthetic multienzyms *Xybeten* and *Protozin-A* in the ration by examining basic behavioral activities of sheep. The experiment has been carried out using the period's method. During the test period the enzyme products are added at dose 1 g/kg mixture concentrate. Animal behavior is studied through direct individual continuous monitoring for 24 hours. The basic functional activities registered in the ethograms are: feeding, moving and rest as well as markers for presence of discomfort. It was found that the addition of *Protozin-A* and *Xybeten* to ration is not perceived as a stressor and is suitable for use as promoters of the digestive processes in sheep. *Protozin-A* has more significant effect on feeding activity as stimulator. *Xybeten* increases the number of chewing movements ($P<0.01$) and duration of rumination ($P<0.01$) per 1 bolus. The sheep spend most time on the feeding activity, followed by time spent on rest when environmental conditions are controlled by human.

**Key Words*:*** Animal behavior, enzyme, feeding additives, sheep

**ÖZET**

**RASYONA KATILAN XYBETEN VE PROTOZİN-A ENZİM ÜRÜNLERİNİN KOYUNLARDAKİ ETHOLOJİK PARAMETRELER ÜZERİNE ETKİLERİ**

Rasyona katılan sentetik enzimler olan *Xybeten* ve *Protozin-A*'nın koyunlardaki temel davranış faaliyetleri üzerine etkisi incelenmiştir. Deney, periyot yöntemi kullanılarak yapılmıştır. Test dönemi süresince, enzim ürünleri 1 g/kg konsantrasyonunda eklenmiştir. Hayvan davranışları bireysel olarak, 24 saat boyunca devamlı süreyle izlenmiştir. Ethograma kayıtlı temel fonksiyonel aktiviteler: beslenme, hareket ve dinlenme yanı sıra rahatsızlık varlığı belirleyicileridir. Rasyona katılan *Protozin-A* ve *Xybeten* bir stres olarak algılanmamış ve koyunlarda sindirim sistemi düzenleyicisi olarak kullanılmak üzere uygun olduğu saptanmıştır. *Protozin-A*'nın, beslenme aktivitesinin stimülanı olarak daha önemli bir etkisi vardır. *Xybeten*, çiğneme hareketleri sayısını ($P<0,01$) ve her kimus başına ruminasyon süresini ($P<0,01$) artırmıştır. Çevre koşulları insan tarafından kontrol edildiğinde, koyunlar en çok beslenme aktivitesine zaman harcamışlar, geri kalan zamanı dinlenmeye harcamışlardır.

**Anahtar Kelimeler:** Hayvan davranışları, enzim, yem katkı maddeleri, koyun

## Introduction

Researching into the effect of chemical and biological substances on microbe synthesis of an abdomen cavity has recently proved to be in broader perspective. Ideal supplements have to be specific and to remain invariable during the study period; they must not be absorbed by the digestive tract as well as not being toxic to the host animal. In addition, the applied supplements should not have any residual substance in the animal tissue and they have to be capable of being naturally decomposed after excretion. Most of the substances used up to now, such as ionophoric antibiotics, metaninhibitors, growth factors etc. do not meet these requirements.

An enzyme adding to a ration of ruminants has still been regarded with a certain amount of skepticism. However, recent studies, carried out by a sequence of researchers have highlighted some positive findings. Earlier contradictory results might have been influenced by a great deal of different factors, such as: the content of a feeding portion; the type and the quantity of the applied enzymes and finally the way of their application.

A time span of eating and ruminating is closely related to the span of fermentation processes. Food is not allowed to leave the rumen before being ruminated and digested to small pieces of less than 2 mm by the micro organisms' enzymes. Therefore, time needed for fodder digestion in the rumen is regarded as a key factor for the quantity of food portion. Ruminating decreases the size of food particles and increases simultaneously the surface that can be attacked by the micro organisms of the rumen.

After conducting detailed survey on the available literature, it has been established that exogenic enzymes have favourable effect on digestive and reproductive processes of ruminants (Beauchemin et al., 1995 and 1997; Feng et al., 1996; Hristov et al., 1998; Lewis et al., 1995). However, the way of applying the best combination of their factors, aiming at achieving highly-beneficial effect, is still being under discussion.

To the present day, there is not sufficient number of studies on the effect of different enzymes on the livestock in Bulgaria, if there are any they are fragmentary and undeveloped (Grigorova et al., 2010; Radev et al., 2002; Varlyakov and Radev, 1998; Varlyakov et al., 2003 and 2010). There is also comparatively small number of conducted research on exogenic enzymes effect on a sheep nourishing dose/portion. This has given ground for the current study on two enzymes *Xybeten* and *Protozin-A* as modifiers of digestive process in sheep's rumen through researching into their ethological indices.

## Materials and Methods

The research has been conducted in accordance with the method of periods: first period is a control one, while the second and third periods are testing ones. During the Control period animals have been fed with ration, which contents and nutritional value may be seen in Table 1. In the second Test period poly-enzyme *Xybeten* has been added to concentrate mixture forage, and during the 3rd Test period – sheep have been fed with forage with added poly-enzyme *Protozin-A* – 1 g/kg of concentrate mixture dose.

The ration has been given twice a day – at 8 o'clock in the morning and at 1 o'clock in the afternoon.

### Enzyme Products

*Xybeten* is produced by *Trichoderma longibrachiatum Rifai* No TW-1. It is with predominant xylanaze activeness (endo-1,4-beta xylanaze, EC 3.2.1.8.) (minimum of 1 000 U/g). It also has a secondary cellulosic (EC 3.2.1.4) and glucose (EC 3.2.1.6) enzyme activeness.

*Protozin-A* contains amylaze, cellulose, xylanaze, β-glucose, proteinaze, lipase and phitaza. It is a multi-enzyme product with a wide range of enzyme activeness. This product is resistant to high concentration of hydrogen ions.

Both enzyme products mentioned above are produced in Bulgaria and according to their manufacturers they are especially suitable for ruminants.

**Table 1.** Content and nutritive value of compound feeds in the ration.
**Tablo 1.** Rasyon içeriği ve yem bileşiminin besin değeri.

| Components | Dry matter (kg) | ME MJ/kg | Crude protein (g) | Crude fibre (g) | Ca (g) | P (g) |
|---|---|---|---|---|---|---|
| Wheat | 0.350 | 5.0 | 42.44 | 11.56 | 0.24 | 1.48 |
| Meadow hay | 0.762 | 5.4 | 64.35 | 279.04 | 4.85 | 1.83 |
| **Total** | **1.112** | **10.4** | **106.79** | **290.60** | **5.09** | **3.31** |

## Animals

The experiment was based on the behavioural reactions of 5 (five) sheep of same breed, age and physiological conditions. At the beginning of the experiment the animals' weight was 45±2 kg. The experimental animals were reared in closed premises-individual/single boxes with the size of 5.2 $m^2$. Initially the animals underwent an operation - fistulae intervention in the organs of digestive tract for penetrating into the abdomen, aimed at taking the material for testing; we put operatively chronic fistulas on the dorsal rumen sac. Studies began after a fourteen-day recovery period, when the animals with their highly adaptive abilities - established in our previous studies as well as in other authors (Uzunova et al, 2007; Varlyakov et al, 1995) had already created stable dynamic stereotype.

## Ethological Methods

Ethologic observations, carried out at the end of the three periods – one Control and two Test periods, lasted for 24 hours.

Direct, individual and constant monitoring method was used for studying animals' behavior within 24 hours. The following functional activities (FA) were observed and analyzed: feeding, moving and rest through their constituent behavioral acts - eating, rumination, movement and static active condition, lying, which total continuance, activity frequency and twenty-four-hour dynamic were recorded and analyzed.

In addition, the following indices were designed - time span of eating the feeding portion, a span of ruminating, a span from the beginning of eating process to first ruminating act, a number and duration of ruminating periods, a span of ruminating a mouthful and the number of ruminating movements it needs - to measure feeding activeness in more detailed and precise way.

Behavioral stereotypes showing discomfort, abnormal forms of behavior, communication and maintenance the hierarchy were under control.

## Mathematical Methods

The average values of FA are defined with the support of special adapted mathematical model. The arguments for its applying are stated in our previous publications (Varlyakov, 1989; Varlyakov et al., 1995). We deliberately use the formula for calculating the index of a respective functional activity (iFA) to enable the users to make comparison between the results of this and other studies:

**$iFA=\Delta t / t$,**

Where

**iFA** : Index of a respective functional activity

$\Delta$**t** : Sum of recorded periods of the respective FA display (min)

**t** : Total duration of the research (min)

All tests used in our research are in accordance with requirements of Directive for

the protection of vertebrate animals used for experimental and other purposes 86/609 EU, Ordinance 25 for the protection and welfare of laboratory animals (SG.№59/1.07.2003) and the Veterinary Law, Section II, Animals used in experiments (SG.№87/2005).

## Results and Discusson

The results of the conducted experiments illustrating the effect of *Protozin-A* and *Xybeten* enzyme products on the functional activities are shown in Table 2. No considerable mathematical differences have been recorded between the Control Period and the two Test Periods figures, which proves that added enzyme substance is not felt as a stressor by the animals. At the same time an opposite effect of the applied enzyme products on the feeding activity has been observed – *Xybeten* produced a slightly positive effect (+4%), whereas *Protozin-A* led to decrease of approximately 16% in comparison with the Control Period. This is due to some one-way changes of the two FA constituent parts – feeding and ruminating.

**Table 2.** Daily continuance (min) of the feeding activity elements.
**Tablo 2.** Beslenme aktivitesi unsurlarının günlük süresi (dk).

| Type of activity | Control period | | Protozin-A | | Xybeten | |
|---|---|---|---|---|---|---|
| | x | Sx | x | Sx | x | Sx |
| **Feeding activity** | **650.17** | **61.56** | **546.83** | **46.27** | **675.00** | **46.27** |
| Eating | 210.67 | 41.17 | 197.33 | 24.16 | 238.50 | 32.23 |
| Rumination – total | 439.50 | 34.34 | 349.50 | 26.59 | 436.50 | 29.67 |
| *Rumination in standing position* | 42.00 | 13.56 | 27.43 | 9.75 | 44.50 | 7.50 |
| *Rumination in lying position* | 397.50 | 33.95 | 321.57 | 29.62 | 392.00 | 38.47 |

Similar results have been reported by other authors. Krause et al. (1998) established a tendency towards increase in feeding time after the enzyme substances had been added to a ration. Same authors have found that enzymes added to a portion don not have any effect on the rate frequency as well as on the time span of ruminating and ruminating periods' characteristic feature.

Although Yang et al. (2000) recorded an increase in food consumption of the portions with exogenic enzymes, for some reason, there was no change reported in consumption of concentrate mixture with enzymes added to it. The reason why the increased appetite and faster eating was accompanied with decreased amount of time for ruminating is still not clear. Despite the fact that there are some working hypotheses, more studies are needed to be conducted to come to any grounded conclusi-ons.

A dynamic of feeding functional activity (Figure 1) is characterized by two clearly defined peaks connected with the hours of giving the forage. The feeding activity was at its peak during the first 50 min after giving the fodder in the morning and reached 80% during the Control Period. The recorded figures are a normal consequence of our influence (the decided and specific hours for giving the forage) and adapted time reflex by the experimental animals.

Analyzing the data of 24-hours dynamic during both Test periods shows similar tendencies, entirely independent of the type of the enzyme product. They are unevenly shared at different hours of a day – there are high rates for the daylight and hardly any at night. This provides further confirmation of prevalence of daily feeding sheep stereotype, which have already been announced in our and other authors' studies (Lynch et al., 1992, Varlyakov et al., 1995). The duration of feeding was

dropping at early night hours and even more there was scarcely any early in the morning (feeding duration reduced to nil).

Enzyme supplement is the reason for prolonging feeding activeness with approximately 2 hours; that was recorded after the morning and the afternoon food giving. At the same time there is no sufficient number of arguments for making clear and firm statement on what extent the appetite or the speed of eating has been influenced by the enzyme supplement.

As it has already been mentioned, the opposite tendencies for the influence of both enzyme products on FA ruminating were recorded. A clear tendency towards decreasing time of ruminating during the period of enzyme *Protozin-A* use is shown in Table 2, which is with 91 min less (6.32% of a 24 - hour day) than the Control period. No considerable differences were recorded with this behavioral marker when Enzyme product *Xybeten* was added to a ration.

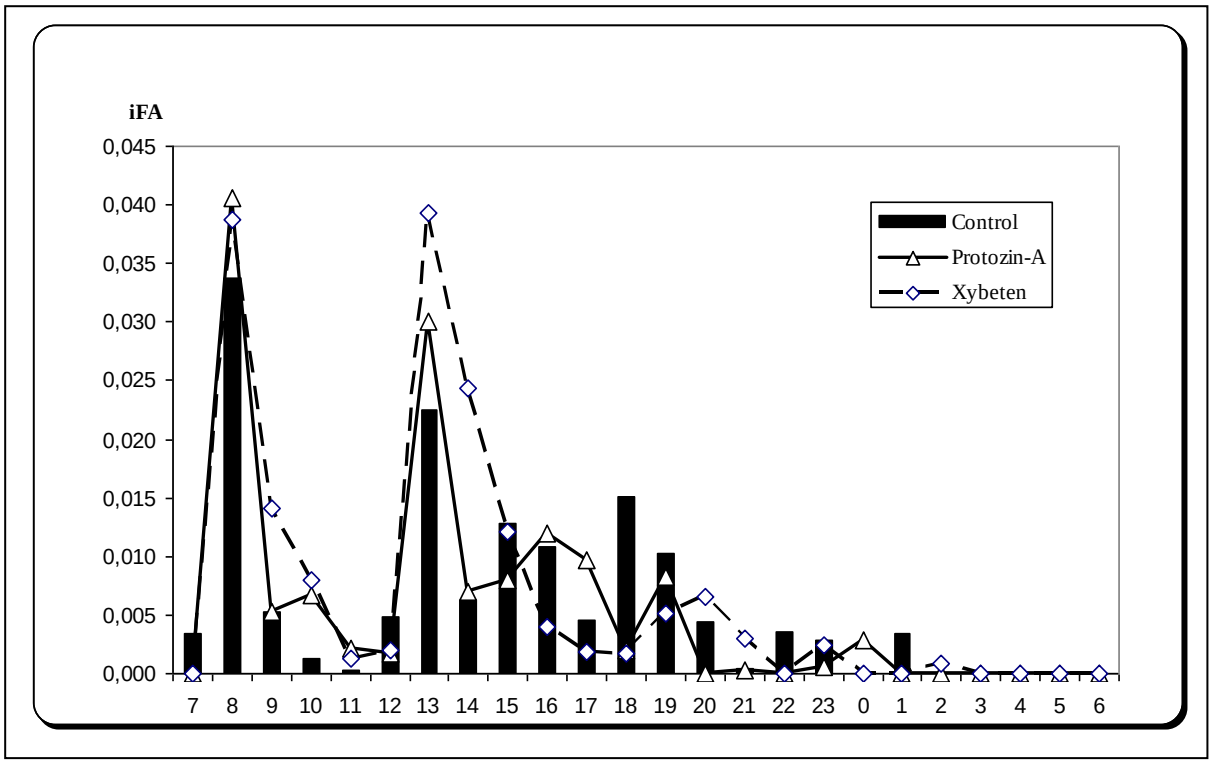


**Figure 1.** Daily Dynamics of functional activity "Eating".
**Şekil 1.** "Yem yeme" fonksiyonel aktivitesinin günlük dinamikleri.

By observing 24-hour dynamic of ruminating activity, it was found that there were 2-3 ruminating periods, with the length of 15 to 20 min, immediately after providing animals with the food. It was also recorded that ruminating activeness is higher during the night hours. 70% of night hours were spent on ruminating, which proved the need for rest to be essential. Ruminating requires a large amount of energy consumption, therefore it is often done in lying position. An increase in ruminating time over 40% in standing position is considered as a marker of discomfort. During the time of our experiment it was recorded 7%-

10% ruminating activity in standing position, which is regarded as normal and indicates that the enzyme supplements did not cause any stress and discomfort with experimental animals. Chewing, naturally followed by ruminating, during the eating time has a crucial role in food digestion and assimilation with ruminants. It is also claimed that there is a relation between the number of chewing movements and the amount of dry matter in a nutritive portion, as well as between the average time span of ruminating and coefficient of assimilation of dry matter. Fifty years ago, Fischer (1963) established that the number of chewing movements per a dry matter of hay is smaller than the number of grass.

The results, indicating the effect of the two enzyme products on the ruminating process markers, are given in Table 3. The period of time from providing animals with food to a start of ruminating is approximately the same in the morning - 117 min (control Period), 116 min (*Protozin-A*) and 134 min (*Xybeten*). In the afternoon after the food supply, the experimental animals during the Control period started ruminating 101 min after eating, while in the Testing periods this time was prolonged due to the enzyme supplements *Protozin-A* and *Xybeten*, with 52 and 56 min respectively. Enzyme supplements had an effect only on the time length of ruminating periods – there was recorded a slight reduction, but not on their number. According to Tossev (1982) the number of ruminating periods and their length depends on the type of forage and its technological processing.

**Table 3.** Effect of *Protozin-A* and *Xybeten* on some elements of rumination process.
**Tablo 3.** Ruminasyon proseslerinin bazı bileşenleri üzerine *Protozin-A* ve *Xybeten* etkisi.

| Parameter | Period | | |
|---|---|---|---|
| | n | x | Sx |
| **Control period** | | | |
| Start of rumination – a.m. (min) | 5 | 117.5 | 16.07 |
| Start of rumination – p.m. (min) | 5 | 101.2* | 38.17 |
| Rumination periods (number) | 5 | 17 | 2.56 |
| Average continuance of rumination period (min) | 86 | 26.9 | 2.33 |
| Chewing movements per 1 ruminated bolus (number) | 601 | 65**a | 0.81 |
| Average continuance for ruminating of 1 bolus (min) | 601 | 56.1**a | 0.73 |
| **First experimental period – Protozin-A (1 g)** | | | |
| Start of rumination – a.m. (min) | 5 | 116.0 | 29.10 |
| Start of rumination – p.m. (min) | 5 | 153.3* | 59.36 |
| Rumination periods (number) | 5 | 16 | 1.51 |
| Average continuance of rumination period (min) | 96 | 21.0 | 1.71 |
| Chewing movements per 1 ruminated bolus (number) | 370 | 66**a | 0.91 |
| Average continuance for ruminating of 1 bolus (min) | 370 | 55**a | 0.69 |
| **Second experimental period –Xybeten (1 g)** | | | |
| Start of rumination – a.m. (min) | 5 | 134.0 | 17.92 |
| Start of rumination – p.m. (min) | 5 | 257.3 | 39.33 |
| Rumination periods (number) | 5 | 16 | 2.12 |
| Average continuance of rumination period (min) | 96 | 24.9 | 2.28 |
| Chewing movements per 1 ruminated bolus (number) | 345 | 70 | 1.10 |
| Average continuance for ruminating of 1 bolus (min) | 345 | 60 | 0.84 |

* : P<0.05, ** : P<0.01

Through the conducted ethological studies, it was found that the enzyme product *Protozin-A* did not affect the number of chewing movements and the time of ruminating of a mouthful, either.

Enzyme *Xybeten* additive genuinely increases the number of chewing movements ($P<0.01$) and the time span of ruminating activity ($P<0.01$) of a food portion. The generally accepted opinion on the elements of ruminating process is that they have strongly individual features, and the established indiscriminate/random effect of *Protozin-A* and *Xybeten* enzyme products in our research needs precise experimental setting, as well as additional further studies.

The recorded results (Table 4) show that animals spend most time on feeding activity, but in controlled conditions of the experimental environment. It can also be seen that the feeding activeness has the lowest figures during the first Test period (37.92% of 24 hours) and the highest ones during the second Test period – 46.88%. The effect of both enzyme products is random, as it has been mentioned before.

**Table 4.** Daily continuance (min) of the studied functional activities.
**Tablo 4.** Araştırılan fonksiyonel aktivitelerin günlük süresi (dk).

| Type of activity | Control period | | Protozin-A | | Xybeten | |
|---|---|---|---|---|---|---|
| | x | Sx | x | Sx | x | Sx |
| Feeding activity | 650.17 | 61.56 | 546.83 | 46.27 | 675.00 | 46.27 |
| Standing | 356.67 | 53.40 | 386.80 | 55.16 | 396.00 | 30.96 |
| Rest | 433.16 | 105.27 | 506.37 | 86.35 | 369.00 | 59.10 |

*Protozin-A* and *Xybeten* addition led to insignificant growth in the figures of standing FA. By ethological observations it was established that this behavioral marker is equally shared throughout the period of 24 hours. The time spent on standing depends on the time used for the other activities. According to Rind and Phillips (1999) it can also be an indicator for discomfort. An increase in time for rest is generally known as a marker for comfort, except for pathologic cases. Recorded changes in standing activity during the Test periods are due to changes in feeding activity (Table 4). A lack of clearly defined peaks is another confirmation of absence of unfavorable environmental factors. It is also a proof that the experiment has been conducted in normal conditions, which makes the recorded results from enzyme products addition to a food portion and its effect on it mostly genuine. Keeping the same ratio during the Test periods is a circumstantial evidence of relatively constant environmental conditions, as well as of lack of considerable individual differences. Regarding some authors (Hargreaves et al., 1990; Iakubowski et al., 1993; Minton et al., 1995), rearing herding animals, such as sheep, in closed space causes some discomfort, thus we consider our experimental conditions suitable for the animals.

## Conclusion

Based on the results of this research, the addition of *Protozin–A* and *Xybeten* to a ration was established not to be a stressor for sheep. An indiscriminate effect of the enzyme products on the feeding activeness has been observed, as the deterrent effect of *Protozin–A* - is more clearly shown. After the final assessment of ethological indices, *Protozin-A* and *Xybeten* addition has been considered as suitable for stimulating sheep digestive process. No case of discomfort has been recorded.

Our previous results have been confirmed - that animals spent most time on feeding activity under controlled environmental conditions, following by the time on rest.

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