The Journal of Animal & Plant Sciences, 29(6): 2019, Page: 1575-1579
ISSN: 1018-7081

EFFECT OF ORAL ADMINISTRATION OF IgY IMMUNOGLOBULINS ON GROWTH RATE AND INCIDENCE OF DIARRHEA IN DAIRY CALVES

M. Kuczaj[1], E. Czerniawska-Piątkowska[2*], I. Kowalewska-Łuczak[3] and M. Pernak[1]

[1]Faculty of Biology and Animal Science, Wrocław University of Environmental and Life Sciences, J. Chełmonskiego 38C, 51-630 Wroclaw, Poland
[2]Department of Ruminant Science, West Pomeranian University of Technology, Klemensa Janickiego 29, 71-270 Szczecin, Poland
[3]Department of Genetics and Animal Breeding, West Pomeranian University of Technology, Piastów Avenue 45, 70-311 Szczecin, Poland
*Corresponding author: ewa.czerniawska-piatkowska@zut.edu.pl

ABSTRACT

Among the most common diseases of calves, digestive system diseases, mainly diarrhea are mentioned. Various researchers indicate that the use of IgY from immunized hens can be effective as a protection against calf diarrhea caused by rotavirus infections. The aim of the study was to evaluate the effect of the lyophilisate from hen egg yolks (IgY) orally administered to calves on in their daily body weight gains and the incidence of diarrhea during the first two months of their lives. The study included 40 Polish Holstein-Frisian calves in the large-scale farm located in the Great Poland Province. The formulation containing immunoglobulins of hen egg yolks was administered orally to calves for 8 weeks. On the 0, 28 and 56 day of the life of calves they were weighed to determine the daily weight gains. The frequency of the incidence of diarrhea, their intensity and duration was being determined. The obtained results were analyzed statistically. The significance of differences was calculated using the univariate analysis of variance and performing the test of multiple Duncan's gap using the Statistica®12.5 PL program. Significant differences ($P \leq 0.05$) in daily weight gains of the calves in both groups of calves during the first 28 days of life were shown. The administration of IgY had a positive effect on the mitigation of the symptoms of diarrhea, on the reduction in the frequency of their incidence (by 10% in relation to the control group) and on shortening the time of their duration (by 8% less than in the control group). It was found that the introduction of immunoglobulin IgY into calf feeding can positively affect their growth and health condition.

Keywords: calves, daily gain, diarrhea, IgY, yolk immunoglobulin.

INTRODUCTION

In the breeding of dairy cattle, the proper rearing of calves is an investment for the future. Animals should enjoy good health, fertility and longevity, which can occur through the use of adequate feeding, awareness of risks, proper selection of rearing techniques and prophylaxis (Razzaque *et al*. 2009). The main purpose of rearing in the first period of 2-3 months of their lives is to prevent the ailments and falls of animals. It has been shown that in calves up to 2. weeks of age the total number of infections was 51.69%, with diarrheas being 63.33% (Korczyński *et al*. 2006). However, in the age group of 2 weeks to 56 days, the proportion of diseases was 93.49%, however, the proportions in the occurrence of diseases have been reversed; the percentage of respiratory diseases of calves increased to 65.20%, and gastrointestinal diseases decreased to 5.3%. According to Cho and Yoon (2014), an important factor in the ethology of diarrhea is stress resulting from transport, changes in feeding, separation from mother or as a result of other stress creating stimuli. Diarrhea with infection background can be caused by viruses, bacteria or protozoa (Cho and Yoon 2014). Most often than not the reduced content of immunoglobulin in the blood of newborn (deficiency of the transfer of passive immunity) has a negative impact on further development of calf increasing in percentage of the incidence of diseases. One way to protect young mammals before the incidence of diseases is the administration of the immunoglobulin of the egg yolk of hen (yolk immunoglobulin, IgY). It has been demonstrated that the functions of the Y immunoglobulin of birds are similar to IgG of mammals, but physical and chemical properties differ strongly (Lee *et al*. 2012). The differences between immunoglobulin IgG and IgY are in favour of IgY as a preparation supporting the immunity of animals (Schade *et al*. 1996; Tambourgi and da Silva 2010). IgY immunoglobulins found prophylactic and therapeutic use in calves against enerotoxic *E. coli* K99, *Salmonella sp*., rotaviruses, coronaviruses and crytosporidia (Vega *et al*. 2015; Arsenopoulos *et al*. 2017; Al-Alo *et al*. 2018).

The aim of the study was to evaluate the effect of lyophilisate from hen egg yolks (IgY) orally administered to calves on in their daily body weight gains

and the frequency of the incidence of diarrhea during the first two months of their lives.

MATERIALS AND METHODS

The research was carried out on 40 calves of the Polish Holstein-Frisian breed of black-and-white variety in the period from birth to the 56th day of their lives on the large-scale farm located in the province of Great Poland. To perform the experiment two groups of calves with 20 individuals were made. In the period analysed, the calves from the experimental group were administered the formulation containing Y immunoglobulin once a day with milk during feeding in the morning. In the individual feeding periods the calves received the following doses of formulation, according to manufacturer's instructions (g/day/pcs.): 1-3 day - 16, 4-7 day - 16, 8-14 day - 4, 15-56 day - 2. The formulation contained 25% of the powder with antibodies (IgY immunoglobulin came from immunized hens), the other 75% is a carrier. The control group of calves was not administered the above mentioned preparation. The experiment was performed in 2017 during the summer (July-September). After birth the calves were kept in individual pens in the cowshed.

The first, primary recommendation in feeding calves is right after birth as soon as possible after the administration of colostrum. This is because of the lack of the innate immunity of the body and the need for its compensation through the acquired immunity. This is done by collecting immunoglobulin and related immune bodies found in colostrum. The maximum efficiency of the penetration of undigested immunoglobulin molecules through the walls of intestines and their absorption in the first hours of the life of calf is about 50%. After 20 hours, it decreases to a few percent. Therefore, during the first 12 hours of life, the calves were administered colostrum (about 7-9 litres of colostrum). Then the calves were moved to individual pens placed outside the cowshed. At the age of about 4 weeks the calves were moved to individual boxes of Canadian type. According to the classical system of feeding calves (Grodzki 2011) on the farm analysed the calves received colostrum in the first 5 days of life, and in the period from 6 to 56 day of life - milk substitute preparation (4-4.5 l/day). The basic condition for the rational rearing of calves is to ensure proper feeding adapted to the rapidly changing alimentary needs. This is especially important because the rearing of calves has a significant impact on the subsequent usefulness of animals and thus on the profitability of production. Keep in mind that economic considerations make the breeders use formulations substituting milk in feeding calves. After a period of 4 to 5 days of administering colostrum there follows a period to give milk and milk substitute formulations with a gradual introduction of fodder. According to the idea, before the calves to become ruminants they should have valuable and easily digestible food, which is rich in protein with high biological value. Milk substitutes constitute this type of food, which can be administered immediately after giving colostrum to drink (Hill *et al*. 2010).

In our study, a complete, full portion formulation contained 25% of crude protein and egg powder comprising immunoglobulin (IgY). Since the 5th day of the life of calves solid feed was introduced. The "concentrated feed prestarter" included CJ feed containing 11.2 MJ, 18.5% of crude protein and 4.5% of crude fibre and the whole corn grain in the ratio of 1:1. Since the first day claves had a constant access to water.

In order to reduce the cases of respiratory and digestive system ailments among calves, immunization of cows using Rotavec Corona and Bovilis Bovipast RSP formulation was used (vaccine for cattle against syncytial virus of the respiratory system of cattle - BRSV) virus of parainfluenza 3 (PI3), against respiratory infections in calves induced by *Mannheimia haemolytica* (*Pasteurella haemolytica*). In addition to the alimentary dose the calves in the first week of life received the Halocur® formulation, counteracting cryptosporoide (Intervet International BV) according to the manufacturer's instructions. At the age of 3-4 weeks, the calves were subjected to disbudding. In order to perform the experiment, the calves were divided into two groups 20 individuals in each group. The animals were subjected to the measurement of their body weight three times: on the day they were born, on the 28th day and the 56th day. On this basis, daily weight gains of calves were calculated. The analysis of the growth of calves was performed on the basis of the formation of body weight, daily weight gains in the whole period of experience, daily consumption of concentrated feed. The occurring types of diarrhea were also taken into account (1 - loose feces, 2 - very loose feces, watery, 3 - loose feces with blood) as well as their duration (measured in days).

The results gained were analysed statistically. Mean values ($\overline{x}$) and standard deviation (SD) was calculated. The significance of differences was calculated using the univariate analysis of variance using the test of multiple Duncan's gap and Statistica®12.5 PL program.

RESULTS AND DISCUSSION

Antibiotics were used to stimulate growth in the animal husbandry long enough, to prevent diseases and of course for treatment, and also many studies and experiments indicated that the use of antibiotics contributed significantly to improve the performance of animals (Meek *et al*. 2015). However, this widespread use of antibiotics led to the incidence of various complications associated with the presence of metabolites

of medicines in animal products and increased bacterial resistance, and therefore the use of sub-therapeutic antibiotics was prohibited. In connection with this the important issue seems to be searching for alternative solutions replacing the existing role of antibiotics (Hedegaard and Heegaard, 2016).

IgG is the main immunoglobulin providing passive immunity of newborn calves (Korhonen *et al*. 2000). In the experiment, the content of immunoglobulin in the blood serum of calves was measured using refractometer. The content of immunoglobulin in the blood of calves in both experimental and control groups did not differ significantly from the point of statistics ($P \leq 0.05$). In the control group the mean value of IgG (g / l) was 62 and 66 in the test group.

The consumption of feed by calves in both groups was analysed and it was found that it was similar and was throughout the experimental period at 246 g for the control group and 300 g for the test group (Table 1). The consumption of feed by calves in the first period of life from birth till 28 day was lower by 3% in the test group, while in the 29-56 day it was 25% higher than in the control group.

Another analysed value was the body weight of the calves in the control group and the test group measured on the day of birth and on the 28th and 56th day. It was found that at the end of the period of 0-28 days the body weight of calves in the test group was 11% higher than in the control group in the second period (28-56 days), the difference was 7% (Table 2).

The daily weight gains of calves in the 0, 28 and 56 day of life were also analysed. Significant differences ($P \leq 0.05$) were observed in daily weight gains of calves during the first period of life (0-28. days) (Table 3) in the control group they reached the level 282 g/day, while in the test group they were 8 g higher. The daily weight gains of calves during the time of experiment were 11% higher in the test group.

The main objective of the study was to assess whether the administration of lyophilisate from the hen yolk (IgY) will have a positive effect on the reduction of the incidence of diarrhea in calves. In Tables 4, 5 and 6 the data on the frequency of the incidence of diarrhea, kinds of diarrhea and duration of diarrhea are summarized. The incidence of diarrhea was higher in the test group (by 10%) compared to the control group (Table 5). The duration of diarrhea was 3 days shorter in the control group. Diarrhea, which lasted 2 days and longer, occurred 19% more often than in the experimental group (Table 4). In the control group 4 cases of diarrhea with blood were recorded and 10 cases of diarrhea in the form of very loose feces. And in the test group 1 case of diarrhea with blood and 9 cases of diarrhea very loose feces was observed (Table 6).

Table 1. Daily feed intake (g) ($\bar{x}$ and SD).

	0-28. days	**29 -56. days**	**0 -56. days**
Control group	63 ± 35.6	396 ± 194.1	246 ± 119.5
Experimental group	61 ± 35.4	496 ± 208.2	300 ± 124.7

Table 2. Calf body weight (kg) measured on days 0. 28 and 56 ($\bar{x}$ and SD).

	day 0.	**28. day**	**56. day**
Control group	39 ± 6 .0	47 ± 8.7	69 ± 10.5
Experimental group	41 ± 4.7	52 4.6	74 ± 7.4

Table 3. Daily gains of calves (g) ($\bar{x}$ i SD).

	0-28.days	**29 - 56. days**	**0 -56. days**
Control group	282 ± 137.8*	767 ± 124.5	524 ± 106.2
Experimental group	370 ± 86.9*	796 ± 154.6	583 ± 105.4

*significant differences $P \leq 0.05$

Table 4. The incidence of diarrhea depending on the duration.

	Duration of diarrhea			**Total**
	0. day	**1. day**	**≥2. days**	**≥1. day**
Control group	99	27	26	53
Experimental group	112	27	21	48

Table 5. The number of cases of diarrhea observed, divided into the type of diarrhea.

	Type of diarrhea, number of cases observed			Total
	1	2	3	
Control group	47	10	4	61
Experimental group	44	10	1	55

Type of diarrhea: 1 - loose feces, 2 - very loose feces, watery, 3 - loose feces with blood.

Table 6. Type and duration of diarrhea.

Age in weeks	Control group				Experimental group			
	Type of diarrhea			Total	Type of diarrhea			Total
	1	2	3	duration	1	2	3	duration
0-7.	3	2		9	2	1	1	5
8-14.	13	6	3	37	9	3		22
15-21.	5	1		11	15	5		39
22-28.	5			5	6	1		9
29-35.	14	1		20	5			7
36-42.	4			5	3			5
43-49.	2			2	3			3
50-56.	2		1	5	1			1

Type of diarrhea: 1 - loose feces, 2 - very loose feces, watery, 3 - loose feces with blood.

Table 6 shows the frequency of the incidence of diarrhea and their duration during each week of the experiment performed. Please note the shift of the intensity of diarrhea incidence in the first month of the life of calves.

In the experiment an additional protection for calves is provided in spite of the measures used (maternal immunization, vaccinations and others), as a formulation including IgY. It was assumed that the addition of IgY immunoglobulin can support the body of calf against the negative effects of diarrhea and prevent the excessive loss of body weight due to disease.

Vega *et al.* (2011) proposed the use of hen egg yolk for the protection of calves against diarrhea caused by rotavirus infections. They have shown that the use of IgY from immunized hens was a promising strategy for the prevention of diarrhea in calves caused by rotaviruses. Moreover, a strong immune response occurred after infection with rotaviruses and after the administration of hen egg yolk (Vega *et al*. 2011).

Also Kuroki *et al*. (1994) in their studies demonstrated the efficacy of IgY action in the oral treatment of diarrhea caused by rotaviruses. Systematic administration of hen egg yolk in powder can be an effective method used in newborn calves to strengthen immunity (Kuroki *et al*. 1994). In other studies, Kuroki *et al*. (1997) demonstrated increased body weight gains and lower virus titre in the feces compared to the control group.

Ikemori *et al*. (1997) in their studies observed the effect of IgY in case of infecting calves with rotaviruses. All calves in the control group (with no immunity) died till the 6 day of experiment. The groups, which received antibodies in the form of powder from hen egg yolks or cattle colostrum survived and had a suitable body weight. The study showed a high degree of IgY protection against rotaviruses comparable to the protection provided by colostrum. The method of the protection of calves presented can be an effective alternative to existing methods. The powder from the whole eggs or hen egg yolk can be an alternative in the prevention and treatment of digestive infections in veterinary medicine. The greatest success with the use of IgY for therapeutic/prophylactic purposes was obtained in the treatment of calves and piglets against *E. coli*, rotaviruses and coronaviruses (Schade *et al*. 2005). Studies were conducted on animal models and as part of the production experiments on the farms of livestock animals. The experiments performed in the field confirmed that the use of IgY as an additive in feeding calves and piglets had a beneficial impact on the health and daily growth gains of animals (Schade *et al*. 2005).

A positive effect of the formulation from chicken egg yolks on the body of calf in the form of increased daily growth gains from birth to 28 day of life was demonstrated in comparison to the control group.

Conclusion: It has been shown that administration of a preparation containing immunoglobulin IgY positively affects the health of calves by reducing the percentage of occurring diarrhea and its duration. The use of immunoglobulin IgY in feeding calves positively influenced their growth and health, especially in the first period of life.

Authors contribution: MK experimental design, proof reading of manuscript, ECP analyzed the data, all

statically analysis, IKL manuscript write-up, analyzed the data, MP all experimental work, helped in manuscript write up.

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