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The Effects of Pre-weaning and Post-weaning Feeding Periods on Biochemical Parameters in Terms of Metabolic Profile in Dorper and Lacaune Lambs

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ABSTRACT

Background: This study was conducted to evaluate the effects of feeding on biochemical parameters in terms of metabolic profile in female and male lambs of Dorper and Lacaune breeds at the developmental stages from birth.

Methods: A total of 32 singleton newborn Dorper and Lacaune breed lambs (n=8 for each group; male and female) were used. The trial was continued total of 5 nutritional periods each of 21-day after the first two weeks (0-14 days) suckling period. The biochemical parameters determined were paraoxonase 1 (PON1), ischemia modified albumin (IMA), total protein (TP), albumin (ALB), aspartat amino transferase (AST), alanin amino transferase (ALT), alkaline phosphatase (ALP) and malondialdehyde (MDA).

Result: PON1 activity of all lambs was found to be low during the suckling period (P≤0.001). The highest IMA value was reached in against to the decrease in TP and ALP levels during the weaning period (P≤0.001). There was no difference between the groups in terms of PON1, TP, ALB and ALP. Increases were observed in the AST, ALT, IMA and MDA levels of male Dorpers. (P≤0.05). It was concluded that adaptation periods to solid feeds may cause oxidative damage or variable metabolic activity in male Dorpers.

Key words: Dorper, Lacaune, Lamb feeding, Postweaning, Preweaning.

INTRODUCTION

The healthy development of lambs from birth and their productivity and performance in later ages are closely related to their nutritional status. It is extremely important to evaluate of some biochemical parameters that are useful for revealing the metabolic status and to prevent metabolic diseases, together with their nutrition during growth and development periods. The developmental stages of lambs pre- and post weaning with the neonatal period (28 d post-partum) is a process in which biochemical and metabolic changes are seen abundantly. The neonatal period is one of the most distressed adaptation periods. It may cause oxidant/antioxidant imbalance, lipid peroxidation and oxidative stress formation. The newborn needs establishment of homeostasis, metabolic mechanisms and subsequent performance that are essential for survival and growth depends on rapid adaptations to new environmental and nutritional conditions (Abdel-Fattah *et al.*, 2013; Ognik *et al.*, 2017). Good feeding is an important factor could prevent a major proportion of neonatal and growt phase diseases or death (Piccione *et al.*, 2008). The possibility of developing the immunity of ruminants has been expressed to be attainable through diets (Ognik *et al.*, 2017). Weaning of lambs before 90 days may be successful for optimum rumen development and reducing stress factors, ensuring that they consume adequate solid feed. It allows ewes to prepare earlier for the reproductive state required for accelerated lambing programs (Abdel-Fattah *et al.*, 2013). In this study, the lambs were weaned on the 78th day.

The effects of weaning practices and switching to solid feeds on metabolic biochemical parameters in lambs are not well understood. The current literature does not provide sufficient information on how blood enzymes differ in lambs according to breed and gender. Blood biochemistry studies of Dorper and Lacaune lambs in the neonatal and growing stages are very rare in the literature. This study was conducted to evaluate the effects of feeding on biochemical parameters in terms of metabolic profile in female and male lambs of Dorper and Lacaune breeds at the developmental stages from birth.

MATERIALS AND METHODS

The research was approved by Animal Experiments Local Ethics Committee of Aksaray University with the date and number 31.03.2022/E-24111467-050.01.04-00000707170. It was carried out with 16 Dorper and 16 Lacaune breeds lambs during periods from birth to post weaning period at a local ovine farm in Yenikent County, Aksaray, Turkey (38°25′51″K 33°51′44″D) during the year 2022 in May and June.

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The selected thirty-two (32) newborn singleton healthy lambs with an average weight of 5.307±0.588 kg were used. The lambs were allocated to four treatments according to their live weights homogeneously and randomly. The experimental groups were created with 8 female Dorper (FD), 8 male Dorper (MD), 8 female Lacaune (FL) and 8 male Lacaune (ML). The lambs were kept under the same environmental conditions.

After birth, the lambs were kept in individual birthing pens for about 1 week with their dams and then each group was taken into group pens (5 m × 4 m). Part of the barn were divided into 8 individual partitions with wood and wirefrime (4 trial groups and 4 partitions for their dams). Four compartments adjacent to the compartments belonging to each experimental group and with openings that only lambs could enter and exit were allocated to the dams. The lambs were separated from the ewes during feeding time. During the study, no vaccination or additional drug administration was applied to the lambs.

The lambs were evaluated considering 5 periods according to their feeding period (Table 1). In the 2nd, 3rd and 4th periods, milk consumption by the lambs was carried out by means of a lamb suckling method with 6 hour separation intervals of lambs from their dams (Doney *et al*., 1979). All lambs had free accessed to clean freshwater and their respective *ad-libitum* consantrate feed. The feeders in ewes compartments were disposed at 0.5 m from the floor to avoid lambs consuming these feeds. Feeding was done daily at 06:00 and 18:00 hr.

Ingredients and chemical composition of lamb starter and grower feeds and alfalfa hay given for the lambs at the pre-weaning and post-weaning periods are shown in Table 2. The feeds (Table 1) were prepared in the on-farm feed unit according to the National Research Council recommendations (NRC, 2007) for lambs based on the nutrient requirements.

Procedures described by AOAC (1997) were used to determine the values of dry matter, crude protein (CP), crude cellulose (CC), ether extract (EE) and crude ash (CA). Van Soest (1994) procedure was followed for determining the aencemounts neutral detergent fibre (NDF) and acid detergent fibre (ADF). Organic matter (OM=DM-CA) and non-nitrogen extract [NNE= DM-(CP+CA+EE+CC)] were derived from the analysis results on feed materials through calculation (Table 1).

Blood samples from all lambs were taken from the jugular vein 3 h after morning feeding at the beginning of each period (5 periods). The first blood draw was performed 3 days after lamb births. The samples were collected in flat gel tubes (Becton Dickinson and Company, New Jersey, USA) and all samples were centrifuged at 3000 rpm for 10 minutes. Biochemical parameters were measured colorimetric assay with the help of commercial kits (Relassay, Turkey) and an autoanalyzer (Mindray BS400).

Paraoxonase 1 (PON1) activity was assayed according to Eckerson *et al*. (1983). Ischemia modified albümin (IMA) was measured using the method described by Bar-Or *et al*. (2000); all specimens were determined using a spectrophotometer at 470 nm (Shimadzu, UV-1201V, Japan) and reported in absorbance units (ABSUs). Total protein (TP) was determined using method reported by Doumas *et al.* (1981). Albumin (ALB) was measured following the method of Gustafsson (1978) by a timed endpoint process using bromcresol green. Aspartat amino transferase (AST) was tested by using reagent according to a standardized method reported by Huang *et al.* (2006). Optimized UV kinetic-test according to IFCC (International Federation of Clinical chemistry) was used to determine alanin amino transferase (ALT) (Huang *et al*., 2006). Alkaline phosphatase (ALP) was assayed according to Davidson (1979). The blood plasma malondialdehyde (MDA) level was determined by a method based on the reaction with thiobarbituric acid at 90-100°C (Yagi, 1976).

The data were statistically analyzed by using IBM SPSS Statistics for Windows, Version 20.0. package program. Kruskal Wallis test was used for independent group comparisons, depending on the distributional properties of the data based on groups (according to results of Shapiro Wilk test). When the test statistics is statistically significant for the independent group comparisons, Dunn's post hoc multi comparison test was used to know which group differ from which others. Chi-square test was used for proportions and its counterpart Fisher's Exact test was used when the data were sparse. The difference between groups, time points and the interaction of these two main effects were tested with two way repeated measures of ANOVA. The sphericity assumption was performed by using Mauchly's test sphericity. As, the violation of this assumption, Wilk's Lambda statistic was used as multivariate test results.

RESULTS AND DISCUSSION

Intra-group biochemical parameters

Intra-group PON1 (U/L), IMA (AU), TP (g/dL, ALB (g/dL, AST (U/L), ALT (U/L), ALP (U/L) and MDA (nmol/L) values obtained during the feeding periods of the lambs are shown

Table 1: Feeding treatments for pre-weaning and post-weaning phases.

Periods		Treatments
1 (0-14 day)		Suckling
2 (15-36 day)	Preweaning	Suckling+50 g/lamb alfalfa hay+*ad-libitum* lamb starter feed
3 (36-57 day)	(suckling and creep phase)	Suckling+100 g/lamb alfalfa hay+*ad-libitum* lamb starter feed
4 (58-78 day)		Suckling+200 g/lamb alfalfa hay+*ad-libitum* lamb grower feed
5 (79-100 day)	Postweaning	250 g/lamb alfalfa hay+*ad-libitum* lamb grower feed

in Fig 1. The PON1 has multifunctional roles such as protection against metabolic disorders, inhibition of oxidative damages and lipid peroxidation, innate immunity (Martinelli *et al*., 2013). PON1 activity (Fig 1, panel A) was found to be quite low in the suckling period of all lambs, compared to all other feeding periods (P≤0.001). Some studies have shown that periods of high energy requirement are a metabolic adaptation process and affect the oxidative state, in which case increased lipid peroxidation reduces serum PON1 activity (Deakin and James, 2004; Costa *et al*., 2005). This value increased significantly in the second feeding period of all lambs (P≤0.001).

No studies on IMA have been found in lambs, but it has been suggested that the antioxidant defense mechanism weakens due to increased free radicals in the blood as the level of IMA formed by the modification of albumin under stress conditions. It was determined that the highest IMA value (Fig 1, panel B) was reached in all lambs in the weaning period (P≤0.001). Studies show that; IMA is elevated in ischemic conditions and diseases associated with oxidative stress. In addition, it was stated that IMA levels did not associated with gender or age (Roy *et al*., 2006; Sbarouni *et al*., 2011).

There are studies reporting an inverse relationship between IMA with ALB and TP level (Roy *et al*., 2006; Sbarouni *et al*., 2011). ALB is a protein supports the protein synthesis of the liver in metabolism and acts as an amino acid reserve (Nicholson *et al*., 2000). Looking at the intragroup changes, it can be said that IMA levels increased in parallel with the decrease observed in TP (P≤0.001) values at the 5th period compared to the first three periods. Piccione *et al.* (2013) emphasized TP and ALB concentrations was a significant source of variation in pre-weaning growth and development. TP level (Fig 1, panel C) was not affected in the first three periods of FD, FL and ML groups, but decreased in MD when passing to the third period (P≤0.05). Similarly, it was observed that TP level decreased in general with weaning compared to the first three periods (P≤0.001). In terms of ALB (Fig 1, panel D), the differences observed in the 2nd and 3rd periods of all lambs compared to the other periods were found to be significant (P≤0.001).

The AST (U/L) value of the MD group (Fig 1, panel E) increased significantly at each solid food transition from the suckling (P≤0.001). Intra-group the AST values of MD lambs were compatible with those reported in studies using Dorper lambs (Cruz *et al*., 2017; Souza *et al*., 2020). It is suggested that there was a positive correlation between AST levels with the growth phase of lambs and increase in AST until 120 days of age resulted from the combination of an improvement in muscle activity and in the endogenous

Table 2: Ingredients and chemical composition of the feeds.

Ingredients	Lamb starter feed	Lamb grower feed	Alfalfa hay
Ration formulation (% in DM basis)			
Alfalfa hay	5.0	6.0	
Wheat bran	5.0	6.0	
Barley (steam flaked)	38.0	36.0	
Corn grain (ground)	23.35	24.5	
Soybean meal	12.5	9.76	
Sunflower meal	11.5	11.77	
Molasses	3.35	3.35	
Salt	0.3	0.70	
Limestone	-	0.90	
DCP (Dicalcium phosphate)	-	0.12	
Vitamin- mineral premix*	1	0.90	
Total	100	100	
Chemical composition (% of DM)			
Dry matter	90.44	88.73	93.44
Organic matter	82.74	81.08	82.86
Crude protein	17.00	16.00	9.57
Crude cellulose	7.14	8.10	34.96
Ether extract	3.70	2.89	0.92
Crude ash	7.70	7.65	10.58
Non nitrogen extract	54.90	54.09	37.41
NDF	17.66	29.24	50.09
ADF	5.80	9.70	42.43
ME, Mcal/kg DM	2.83	2.75	1.38

*Contained per kilogram of supplement: Vitamin A, 800,000 IU; vitamin D3, 300,000 IU; vitamin E, 3000 mg; Fe, 4 g; Mn, 4 g; Cu, 0.8 g; Zn, 5 g; Se, 20 mg; I, 70 mg; Co, 40 mg.

**ME (metabolizable energy) was calculated according to NRC, 2007.

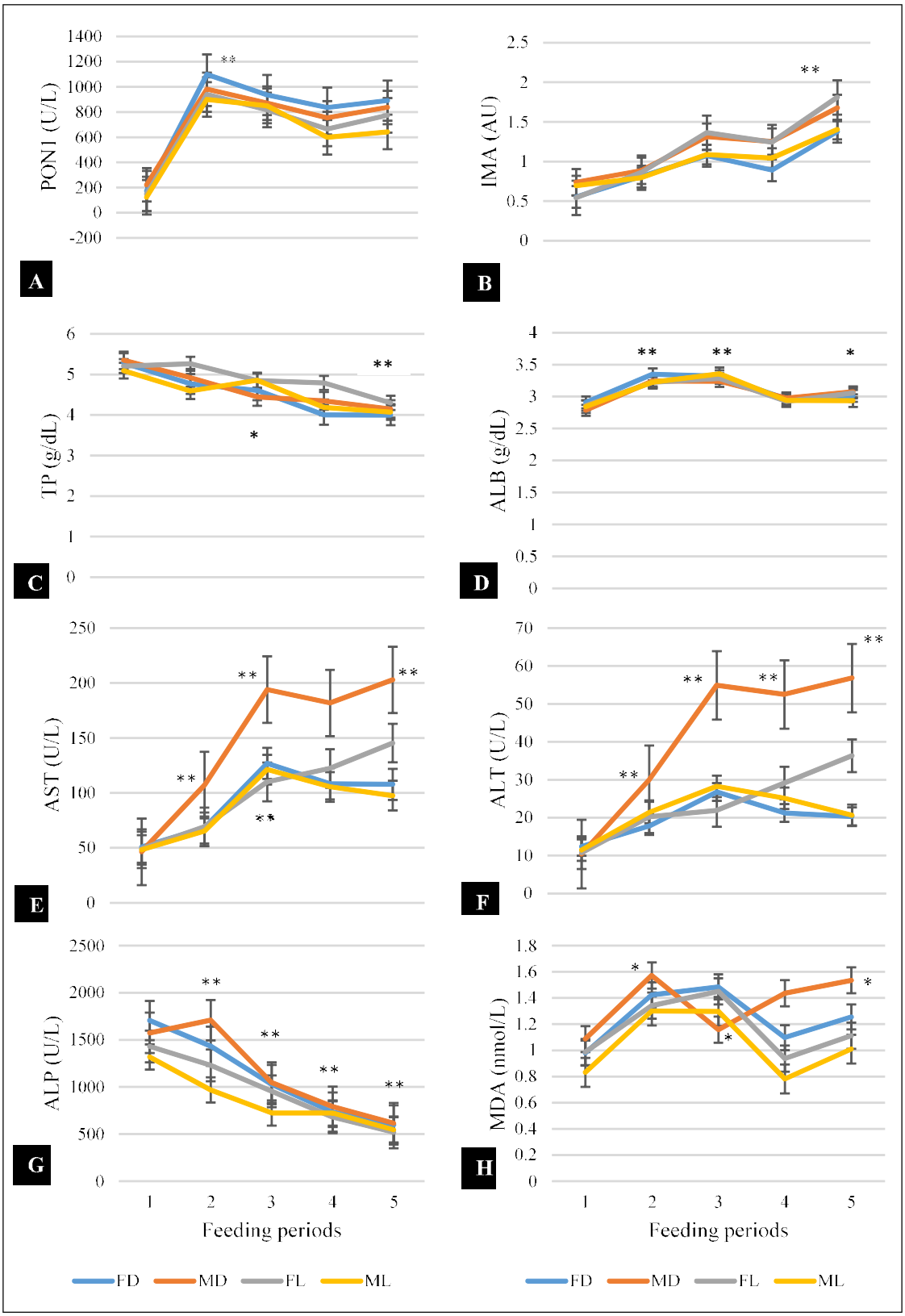

Fig 1: Intra-group biochemical parameters obtained during feeding periods of the lambs. *(P<0.05), **(P≤0.001).

synthesis of AST with the development of the lambs (Feitosa *et al*., 2007; Souza *et al*., 2020). The increase in intra-group AST values of all lambs, especially until the 3rd period (P≤0.001), supports this view. The gradual increase of AST in MD lambs (Fig 1, panel E) can attributed to greater mucle mass, variations in feed management (suckling, weaning, transition of solid feeds), oxidative damage or variable metabolic activity (Fernandes *et al*., 2012). It is possible to explain the variations observed in intra-group ALT values seen in Fig 1, panel F with the same approaches. The ALT value of the MD group (Fig 1, panel F) increased significantly in each period compared to the 1st period, continuously until the end of the trial (P≤0.001). Contrary to studies reporting no difference in ALT (Cruz *et al*., 2017), the increase in ALT in MD lambs in this study is likely to be the result of differences in hepatic and metabolic activity between the sexes (Carlos *et al*., 2015).

ALP (U/L) levels (Fig 1, panel G) gradually decreased within each group until the end of the trial. It was observed that this decrease of each group was statistically significant in the 3rd and 4th periods compared to the first two periods and in the 5th period compared to the first three periods (P≤0.001). It can be said that the decreases observed intra-group ALP values are related to the decrease in the amount of TP in the blood (Fig 1, panel C,G), protein uptake in the tissues and nitrogen evaluability (Dayıoğlu and Doğru, 1996).

It has been stated that feeding a diet containing 17-20% crude protein after the weaning period does not affect the total MDA levels in male lambs (Pelegrin-Valls *et al*., 2020). An increase was observed in the MDA level of the MD group in the 2nd and the 5th period, while a decrease was observed in the transition to the 3rd period (P≤0.05) (Fig 1, panel H). It can be thought that the increase in MDA level observed (Fig 1, panel H) may be related to the adaptation to solid feed or oxidative damage (Ognik *et al*., 2017; Wang *et al*., 2019).

Inter-group biochemical parameters

Average PON1 (U/L), IMA (AU), TP (g/dL), ALB (g/dL, AST (U/L), ALT (U/L), ALP (U/L) and MDA (nmol/L) values obtained from lambs during fattening period and the differences between groups of these values are shown in Table 3. There was no statistical difference between the group averages of PON1, TP, ALB and ALP values of female and male Dorper and Lacaune lambs throughout the fattening period (P≥0.05). It was emphasized that the lack of difference in TP and ALB levels was due to the adequate of milk and normal protein metabolism (Soriano *et al*., 2015). On this contrary, also it has been confirmed that the lower values for TP verified after a while probably represent the transition period in the immunoglobulin content in the bloodstream which is characterized by the end of the degradation process of immunoglobulins passively received via colostrum (Silva *et al*., 2010). However, although there was a significant increase in IMA in the MD and FL groups (P≤0.05) compared to the other two groups throughout the experiment, ALB did not change. This may be due to the liver's ability to synthesize ALB and lambs to become proficient in the use of nitrogenous compounds from the feeds (Souza *et al*., 2020). The mean ALP values obtained from the groups were contradicts to the results of the research claiming that the ALP enzyme was higher in lambs younger than 74 days old due to the high amount of bone isoenzymes released into the bloodstream (Thrall *et al*., 2007, Madureira *et al*., 2013).

It has been suggested that the transition from liquid (milk/milk replacer) to solid feeds (starter or grass) during weaning requires a functional rumen development and that the negative effects of stress factors may not be encountered when the structure and function of the rumen are fully developed (Wang *et al*., 2019). In this study, the reason for the increased IMA levels in MD and FL groups (P≤0.05) may be due to inadequate energy from feeds ,thus the occurrence of oxidative stress in each group, regardless of race or gender (Roy *et al*., 2006). This result may also mean that the lambs do not yet have the necessary rumen development for weaning.

During the total fattening period, the increase in the mean AST and ALT levels of the MD group was found to be significantcompared to the other groups (P≤0.05). This situation can be explained by the occurrence of variable

Table 3: Inter-group average values ofbiochemical parameters obtained from lambs during the fattening period.

1-5 period	Groups					P values
	FD	MD	FL	ML	SEM	
PON1, (U/L)	785.88	732.20	668.37	622.40	45.235	
IMA, (AU)	0.940[a]	1.173[b]	1.164[b]	1.005[a]	0.025	*
TP, (g/dL)	4.531	4.641	4.882	4.559	0.072	
ALB, (g/dL)	3.110	3.064	3.069	3.060	0.017	
AST (U/L)	92.35[a]	146.44[b]	99.17[a]	87.49[a]	6.489	*
ALT (U/L)	19.689[a]	40.920[b]	23.667[a]	21.364[a]	2.364	*
ALP (U/L)	1098.88	1146.90	962.00	856.32	43.246	
MDA (nmol/L)	1.248	1.357[a]	1.165	1.044[b]	0.040	*

*Difference among the group averages shown with different lower case in the same line are significant (P≤0.05).

SEM: Standard error of mean.

metabolic activity and/or oxidative damage during the adaptation of MD lambs to feeding (Carlos *et al*., 2015). The mean MDA level increased in the MD group compared to the ML group during the total fattening period ($P \leq 0.05$). According to Yonny *et al*. (2016), blood MDA a lipid oxidation end product is expected to increase, due to the production of free radicals that can damage rumen and intestinal tissue. The fact that MD lambs had higher levels of MDA than ML lambs in the 4th and 5th periods and throughout the entire trial in this study may be related to lipid peroxidation. Nevertheless, in this study, there were no differences in MDA between Lacaune groups in any phase ($P \geq 0.05$). Therefore, management practices related to 16-17% protein content in male/female Lacaune lamb diets seemed here not to affect the interplay between nutrition and immune function (Ognik *et al*., 2017).

In the study the interaction between the effects of dietary treatment, breed and gender were insignificant.

CONCLUSION

At the end of the study, it was observed that the AST and ALT levels in the transitions to solid feeds increased in male Dorpers. They had the IMA levels higher than female Lacaune lambs and the MDA levels than male Lacaune lambs. It was concluded that adaptation periods to solid feeds may cause oxidative damage or variable metabolic activity in male Dorpers. However, there is a need for researches on the determination the effects of nutrition on the antioxidant defens mechanism in small ruminants from birth.

Conflict of interest

All authors declared that they have no conflict of interest.

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