EFFECT OF DRIED GRAPE POMACE ADDITION INTO FEED RATION OF SHEEP ON LEUCOCYTES

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Abstract

The aim of this study was to determine the effect of addition of dried grape pomace meal into the feed ration of sheep's on leucocytes characteristics. As an experimental animals eight wethers of Ile de France breed were used. The study was divided to the three time intervals according to feed ration which was fed. First time interval lasted 19 days and feed ration consisted of meadow hay, soy bean meal, wheat meal and mineral supplements. Second time interval lasted 12 days and started immediately after the end of the first time interval and feed ration was the same as during firs time interval, but supplemented with dried grape pomace meal. The addition of dried grape pomace meal was in amount of 1% of feed ration dry matter. Third time interval lasted 12 days and started immediately after the end of the second time interval and feed ration was the same as in the second time interval but the addition of dried grape pomace meal was in amount of 2% of feed ration dry matter. During the last day of each time period the sampling of blood into EDTA tubes was realized. Total white blood cell (WBC), lymphocytes count (LYM), medium size cells count (MID) and granulocytes (GRA) as well as its percentages were determined by hematology analyzer Abacus Junior Vet (Diatron, Austria). Between the first and second blood sampling the increase of WBC and LYM, as well as decrease of GRA was detected. Between the second and third sampling time the decrease of WBC, LYM and GRA was detected. MID was on the same value in all three sampling times. Significant effect of feed ration composition was detected on LYM ($P<0.05$) and GRA ($P<0.01$). Increase of LYM after the addition of 1% of dried grape pomace meal into the feed ration has to be verified in further experiments. If this increase of LYM is confirmed, then it can be used by cows and small ruminants for better passive immunisation of their offspring.

Keywords: *sheep, Ile de France, feed ration, dried grape pomace meal, leucocytes, lymphocytes*

1. INTRODUCTION

Grape by-products are sources of many potential bioactive compounds, which have a potential to be positive in feed ration for nutrients utilisation increasing point of view (Gálik et al. 2018) [1]. In addition, some plant parts can possess biochemical and antioxidant activity and it is most important to know the therapeutic effects on human or animal metabolism (Brindza et al. 2015) [2]. According to Westendarp (2006) [3] tannins have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects. Phytotherapeutically tannin-containing plants are used to treat nonspecific diarrhea, inflammations of mouth and throat and slightly injured skins. Studies with ruminants have also demonstrated that the denaturing properties of tannins can possibly be used to improve protein supply to the small intestine. On the other hand, high tannin concentrations resulted in reduced animal performances and health disorders as well in ruminants as in monogastric animals (Westendarp 2006) [3]. Leucocytes create in immune system a part of the cell mediated immunity. Lymphocytes are responsible for specific immunity. One part of lymphocytes are memory T-cells which protect body against previously encountered pathogens (Kováčik et al. 2015) [4]. Hematological parameters of sheep's blood can be affected by many factors as for example age, sex, seasons, breed, condition and nutrition. Therefore the blood analyze is appropriate tool for monitoring of effect of feed ration changes on physiological reaction of animals (Rolinec et al. 2013) [5]. The aim of this study was to determine the effect of addition of dried grape pomace as a source of nutrients and bioactive compounds into feed ration of sheep's on leucocyte parameters.

2. MATERIALS AND METHODS

Design of this study was prepared for *in vivo* experiment of nutrients digestibility. Nevertheless, after the end of the feeding of each of three experimental feed rations the blood samples from all wethers were collected. The aim of this study was to detect the changes of feed ration supplementation with dried grape pomace meal on leucocyte parameters.

2.1. Animals and housing

This study was realized at Experimental center for farm animals (Slovak University of Agriculture in Nitra). Eight wethers, Ile de France breed with an average weight of 34.50±1.97 kg were included this study. Wethers were obtained from University farm in Žirany (VPPSPU s.r.o. Kolíňany). This study consisted of three time periods. Each time periods had preparatory time period when wethers were housed in two pens per four animals and experimental period when wethers were housed in individual balance cage. During housing in individual balance cage the individual intake and excretion of nutrients were monitored for another experiment. Pens had concrete floor. During the whole study the wethers were housed without bedding. Herewith was assured that wethers ingested only feed ration made for this study.

2.2. Feeding and study design

Concentration of nutrients in feeds used in this study is shown in Table 1. Nutritional value of three experimental feed rations meet the requirement of wethers. During the three time periods wethers were fed with three different feed rations (Table 2). First time period of this study lasted 19 days, wethers were housed 14 days in pen per four animals and 5 days in individual balance cage. Feed ration in first time period consisted from meadow hay, soy bean meal, wheat meal and mineral supplement in form of mineral block lick (Ján Valášek, Slovakia). Blood samples were collected in the morning of 19th day of first time period. Blood samples were taken from *vena jugularis externa* into EDTA tube (Sarstedt, Germany). Second time period with second feed ration followed immediately after the end of the first time period. Second time period of this study lasted 12 days, wethers were housed 7 days in pens per four animals and 5 days in individual balance cage. The feed ration during the second time period consisted from meadow hay, soy bean meal, wheat meal, mineral supplement in form of mineral block lick and dried grape pomace meal in an amount of 1% of total dry matter of feed ration. Blood samples were collected in the morning of the last day of the second time period. Twelve days long third time period of this study started immediately after end of second time period. Wethers were again housed 7 days in pens per four animals and 5 days in individual balance cage. Feed ration during the third time period of this study was similar as in the second time period. The only difference was the supplement of dried grape pomace meal into the feed ration, the supplemented amount was 2% of total dry matter of feed ration. Overall 3 times (1st, 2nd and 3rd sampling) per 8 blood samples were gained. Drinking water was during the whole study *ad libitum*.

Table 1. Average concentration of nutrients in feeds used in the feed rations

	Meadow hay	Wheat meal	Soy bean meal	Dried grape pomace meal
Dry matter (%)	84.5	91.1	89.9	94.2
Crude protein (g.kg^{-1} OM)	55.6	112.3	437.2	99.8
Ether extract (g.kg^{-1} OM)	11.9	14.4	13.4	98.9
Crude fibre (g.kg^{-1} OM)	303.4	28.7	46.9	184.5
Ash (g.kg^{-1} OM)	45.2	17.1	61.7	34.8
NFE (g.kg^{-1} OM)	429.2	738.9	339.5	616.3
Organic matter (g.kg^{-1} OM)	800.1	894.2	836.5	907.3

OM – original matter; NFE – nitrogen free extract.

Table 2. Feed ration composition according to study time period, when was fed

	Meadow hay	Wheat meal	Soy bean meal	Dried grape pomace meal
1st time period (19 days)*	66%	23%	11%	-
2nd time period (12 days)*	66%	22%	11%	1%
3rd time period (12 days)*	65%	22%	11%	2%

* blood samples were collected during last day of each time period.

2.3. Analyses and statistic

All 24 blood samples were analysed within two hours after collecting. Total white blood cell (WBC), lymphocytes count (LYM), medium size cells count (MID), granulocytes count (GRA), lymphocyte percentage (LYM %), medium size cells percentage (MID %) and granulocytes percentage (GRA %) were determined by hematology analyzer Abacus Junior Vet (Diatron, Austria). Gained results were statistically processed by one-way ANOVA. Description statistic, SEM – standard error of mean and P – the effect of feed ration composition on leucocyte parameters was calculated using IBM SPSS v. 20.0. Differences of means between the sampling periods were tested by Tukey HSD test. $P<0.05$ was considered significant.

3. RESULTS

Average values of leucocyte parameters in wethers blood are shown in Table 3. After consumption of feed ration without supplementation of dried grape pomace meal was lymphocyte count the lowest whereas granulocytes count the highest (1st sampling). Twelve days long consumption of feed ration supplemented with 1% of dried grape pomace meal increased the count of white blood cells, as well as lymphocytes (2nd sampling). In comparison with 2nd sampling the following twelve days long consumption of feed ration supplemented with 2% of dried grape pomace meal decreased the count of white blood cells and lymphocytes (3rd sampling). From 1st to 3rd blood sampling count of granulocytes continuously decreased and count of medium size cells did not changed within whole experiment.

Table 3. White blood cells parameters of the blood from wethers during experiment

	1st blood sampling	2nd blood sampling	3rd blood sampling	SEM	P
WBC (x 10^9/L)	9.18	9.63	9.16	0.281	
LYM (x 10^9/L)	6.31	7.11	6.88	0.204	*
MID (x 10^9/L)	0.05	0.05	0.05	0.001	
GRA (x 10^9/L)	2.82	2.47	2.24	0.228	**
LYM (%)	68.83	73.96	75.09	1.701	**
MID (%)	0.50	0.50	0.50	<0.000	
GRA (%)	30.68	25.58	24.48	1.698	**

SEM – standard error of the mean; P – effect of feed ration composition on leucocyte parameters was significant * at $P<0.05$ or ** at $P<0.01$; Significant difference of means between samplings was not detected ($P>0.05$).

Significant effect of feed ration composition, without or with supplementation of 1% or 2% of dried grape pomace meal was found for lymphocyte count ($P<0.05$), granulocyte count ($P<0.05$), lymphocyte

percentage (P<0.05) and granulocyte percentage (P<0.05). On the other hand, significant differences of means between blood samplings were not detected (P>0.05).

4. DISCUSSION

Fundamental prerequisite of sheep performance is the genetic (Gábor et al. 2006) [6], however nutrition is also very important (Gálik et al. 2014 [7]; Šimko et al. 2014 [8]). In feeding of farm animals is a possibility to use some by-products which are produced during processing of food. Nowadays some experiments are aimed to detect the properties of grape by products and to determine the effect of these on farm animal's performance. Due to their concentration of polyphenols grape pomace has become an attractive research field mainly in ruminant feeding. Also Peixoto et al. (2018) [9] declared that grape pomace is a source of phenolic compounds and diverse bioactive properties. Correddu (2014) [10] fed dairy ewes with addition of grape seeds in feed ration and did not find adverse effect on milk production traits and health status and in addition detected an immunomodulatory effect. On the other hand high tannin concentrations could lead to a reduction of animal performances and health disorders as well in ruminants as in monogastric animals (Westendarp 2006) [3]. Positive, or negative effect of changes in feed ration express itself at first in blood of animals (Rolinec at al. 2013) [5]. As it was detected in this study the count of leucocytes and lymphocytes was within the range of values published by other authors. Šoch, Brouček, & Šrejberová (2011) [11] determined WBC in ewes from south Bohemia sampled in different altitude, season and year in interval from 6.47 to 9.57 $x10^9$/L. Binev, Slavova, & Laleva (2006) [12] published WBC in Ile de France breed with similar weight as it was in this study on value 8 $x10^9$/L. After twelve days consumption of feed ration supplemented with dried grape pomace meal came to increase of WBC from 9.18 to 9.63. Similar increase of WBC detected Cash et al. (2016) [13]. They drenched katahdin lambs every 7 day with fermented Pinot Noir grape extract, at the start WBC was 9.95 then after 63 days long experiment increased WBC on 10.26. However WBC in control lambs as well as in lambs drenched every 14^{th} day decreased from 9.56 to 7.97. According to Sehm et al. (2011) [14] the increase of WBC can be influenced by polyphenol rich feeding, which have the potential to positively influence the blood parameters, and WBC mRNA gene expression pattern of immunological marker genes. On the other hand Correddu (2014) [10] published lower WBC (8.5 $x10^9$/L) in dairy sheep with diet containing 300 g of grape seed per day/head compared to sheep in control group (8.9 $x10^9$/L). LYM were in this study in physiological range for sheep. Supplementation of feed ration with 1% of dried grape pomace meal increased LYM from 6.13 to 7.11 $x10^9$/L). But supplementation with 2% of dried grape pomace meal decreased LYM compared to 1% supplementation from 7.11 to 6.88 $x10^9$/L. Cash et al. (2016) [13] detected after drenching of fermented Pinot Noir grape extract to lambs the decrease of LYM. Also Correddu (2014) [10] determined lower LYM percentage by dairy sheep's ingested in feed ration 300 g/day/head of grape seeds compared to control group (53.7 vs 52.3%). However, other farm animals like piglets (Sehm et al. 2011) [14] and broiler chicks (El-Kelawy, Elnaggar & Abdelkhalek 2018) [15] showed increased trend of LYM percentage after dietary inclusion of fruit pomace or grape seeds respectively. The widely accepted explanation for positive effects of condensed tannins, those source are also grape pomaces, on protein digestion and metabolism is that condensed tannins-protein complexes escape ruminal degradation resulting in greater protein availability in the lower tract, for confirmation of this explanation have to be more studied also the complexes between condensed tannins and carbohydrates, endogenous proteins and microbial products and also the degradability of complexes between condensed tannins and proteins by ruminal microbes (Reed, 1995) [16]. But with the dietary protein protection against degradation in the rumen, which is realized by condensed tannins, subsequently increases amino acid supply to abomasum and small intestine, resulting in improved nutritional status of the animals (Min & Hart 2003 [17]; Hoste et al. 2006 [18]). As published Heckendorn et al. (2007) [19] enhanced immune response can be mediated by improved protein availability. This can be the reason of detected increase of WBC and LYM after twelve days long supplementation of wethers feed ration with dried grape pomace meal.

After supplementation of feed ration with 1% or 2% of dried grape pomace increased the LYM percentage over the upper limit of physiological range, which is 70% and even the lymphocyte count remained within the physiological range. Further exploration is needed and it can be hypothesized that

the increased WBC, LYM and LYM percentage during 12 days supplementing of feeds with dried grape pomace meal might result from increased intake of polyphenols which have the potential to positively influence the blood immunological parameters. If this increase of WBC, LYM and LYM percentage is confirmed, then it can be used by cows and small ruminants for better passive immunisation of their offspring.

5. CONCLUSIONS

The supplementation of feed ration of sheep with 1% of dried grape pomace meal increased the WBC, LYM and LYM percentage. Compared to supplementation with 1% the supplementation with 2% of dried grape pomace meal to feed ration of sheep's increased only the percentage of LYM. From the start to the end of this study GRA and GRA percentage decreased continuously and MID together with MID percentage did not changed. From this point of view it seems to be the addition of 1% of dried grape pomace meal in feed ration for sheep's better than 2%. However, further exploration of effect of grape pomace on other sheep performance parameters is needed. Negative effect of dried grape pomace meal ingestion on leucocyte parameters was not detected in this study.

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